# Analysis and evaluation of seed kernel oil content and fatty acid components of Acer truncatum in Central China

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## Abstract

In order to screen out the high-oil Acer truncatum germplasm resources so as to improve the yield and value of seed oil, this experiment was carried out by analyzing and evaluating a total of 70 samples from natural populations of A. truncatum in nine Central Plains of China, the results were subjected to nested ANOVA and correlation analyses, as well as a comprehensive evaluation of the various groups using principal component analysis and the affiliation function method. The findings demonstrated that the 70 samples ranged in seed kernel oil content from 14.43% to 50.11% (average 33.11%), and that the major fatty acid fractions had coefficients of variation that ranged from 24.13% to 33.40%, with differences between them being significant. According to correlation analysis, latitude, temperature, and precipitation had a greater impact on the seed kernel oil content than they did on the relative content of fatty acid fractions. Altitude, temperature, and precipitation were the primary causes of changes in the content of fatty acid fractions. The principal component analysis reduced the nine indexes to two principal components with a cumulative contribution rate of 84.29%, and ranked them according to the comprehensive evaluation value obtained from the principal component analysis and the transformation of the affiliation function. The analysis screened out the ten germplasm samples with the highest comprehensive quality and the three samples with the highest comprehensive evaluation value.

# Analysis and evaluation of seed kernel oil content and fatty acid components of *Acer truncatum* in Central China

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Abstract: In order to screen out the high-oil Acer truncatum germplasm resources so as to improve the yield and value of seed oil, this experiment was carried out by analyzing and evaluating a total of 70 samples from natural populations of A. truncatum in nine Central Plains of China, the results were subjected to nested ANOVA and correlation analyses, as well as a comprehensive evaluation of the various groups using principal component analysis and the affiliation function method. The findings demonstrated that the 70 samples ranged in seed kernel oil content from 14.43% to 50.11% (average 33.11%), and that the major fatty acid fractions had coefficients of variation that ranged from 24.13% to 33.40%, with differences between them being significant. According to correlation analysis, latitude, temperature, and precipitation had a greater impact on the seed kernel oil content than they did on the relative content of fatty acid fractions. Altitude, temperature, and precipitation were the primary causes of changes in the content of fatty acid fractions. The principal component analysis reduced the nine indexes to two principal components with a cumulative

contribution rate of 84.29%, and ranked them according to the comprehensive evaluation value obtained from the principal component analysis and the transformation of the affiliation function. The analysis screened out the ten germplasm samples with the highest comprehensive quality and the three samples with the highest comprehensive evaluation value.

**Keywords:** Acer truncatum; oil content; fatty acid components; neuric acid; correlation analysis

#### Introduction

Acer truncatum Bunge is a widely used ornamental tree species throughout the world, with lovely leaves and graceful posture. In recent years, A. truncatum has also been found to have high medicinal value; its seeds contain nearly 27% of the protein and eight essential amino acids, and its oil is rich in unsaturated fatty acids, with a content of more than  $90\%^{[1-3]}$ . Neurotic acid, also known as shark's acid, is a long-chain unsaturated fatty acid found in seed kernel oil that has been shown to improve cellular vitality, restore the activity of brain cell endings, and have a therapeutic effect on memory loss, cerebral palsy, and other cerebral disorders, as well as a skin whitening effect<sup>[4-6]</sup>. Since it is difficult to extract, expensive, and low yield, neurotic acid is typically only obtained from the deep-sea brains of sharks and other mammals. However, A. truncatum seed oil has a high concentration of neurotic acid (up to 4%–9%), which represents a significant new source for the sustainable use of neurotic acid [7-9].

Liu Xiangyi et al  $^{[10]}$  analyzed 12 fatty acids from A. truncatum seeds and concluded that the unsaturated fatty acid content of A. truncatum oil reached more than 92%, making it suitable for use as a high-quality raw material in the manufacturing of nutritional and therapeutic oils. Wei Ming et al  $^{[11]}$  found 44.3% oil and 16 fatty acids in the seed kernels of A. truncatum in Jiangyou, Mianyang. Qiao Q et al  $^{[12]}$  examined 138 A. truncatum seed oils from 14 A. truncatum populations around China and found an average oil content of 28.57% and a fatty acid level of 5.76%. Wu Longkun et al  $^{[13]}$  collected and analyzed A. truncatum seed oil from the Changwu area of Liaoning province, found 12 fatty acids, and evaluated the optimal experimental conditions, yielding the greatest oil production of 30.5%. By using fatty acid spectrum profiling and physicochemical testing, Ke Zhang et al  $^{[14]}$  were able to identify the constituents and quality of A. truncatum oil. They were able to identify 18 fatty acids, of which the neurotic acid concentration may reach 8%, and 3.51% of flavonoids that can be employed as anti-alcohols. During the maturity phase of A. truncatum seeds, a total of 17 fatty acid components were found by Dai Yanman  $^{[15]}$  et al. They came to the conclusion that by testing the seeds at various times throughout the year, the A. truncatum seed kernels would achieve their peak oil and fatty acid concentration by the end of October.

There are currently more studies on woody oilseed species with high oil content and unique components, but there are few studies comparing the oil content and fatty acid composition of seed kernels among populations of A. truncatum. In order to lay a theoretical foundation for the selection and breeding of high-oil A. truncatum germplasm, samples from natural A. truncatum populations in the Central Plains of China were collected and their variations in oil content and fatty acid components of seed kernels were analyzed. By providing a theoretical framework for the selection and breeding of high-oil A. truncatum genetic resources, this study aims to advance the growth of the A. truncatum oil industry.

## Materials and Methos

## Plant Materials

A total of 70 samples were obtained from nine natural populations of A. truncatum in the provinces of Shandong, Jiangsu, and Shaanxi through field surveys of the species' range. The samples were obtained in October when the fruits of the A. truncatum became ripe and yellowish-brown, and they were then allowed to naturally dry in the shade for three to five days after harvesting, and then peeled off the skin by hand to obtain pure seeds for the analysis and determination of seed kernel oil content as well as fatty acid fractions. Each sampling site's latitude, longitude, and altitude were noted, and the remaining meteorological factors were confirmed with the local meteorological agency while the samples were gathered were numbered and catalogued (Table 1).

Table 1 Geographical location and climatic conditions of 9 collection sites of Acer truncatum

| Seed<br>sources                 | Code | $\begin{array}{c} {\rm Altitude} \\ /{\rm m} \end{array}$ | $\begin{array}{c} \text{Longitude} \\ / {\rm ^{\circ}E} \end{array}$ | $\begin{array}{c} {\rm Latitude} \\ / {\rm ^{\circ}N} \end{array}$ | Annual average temperature | $\begin{array}{c} {\rm January} \\ {\rm average}/ \end{array}$ | July average / | Annual precipitation /mm | Frost-free season /d |
|---------------------------------|------|---|--|--|----------------------------|--|----------------|--------------------------|----------------------|
| Taian,                          | STA  | 219   | 117°15'  | 36°12′   | 12.9                       | -2.6   | 26.4           | 697                      | 195                  |
| Shandong<br>Jinan,<br>Shandong  | SJN  | 43  | 116°77'  | 36°68'   | 13.6                       | -3.2   | 27.2           | 614                      | 235                  |
| Haiyang,                        | SHY  | 140   | 120°89'  | 36°63'   | 12.0                       | -0.6   | 23.5           | 696                      | 207                  |
| Shandong<br>Yiyuan,<br>Shandong | SYY  | 400   | 117°92'  | 35°89'   | 13.6                       | -0.5   | 26.5           | 730                      | 198                  |
| Zaozhuang,                      | SZZ  | 184   | 116°98'  | 34°73'   | 13.5                       | -0.2   | 26.7           | 875                      | 196                  |
| Shandong<br>Sishui,<br>Shandong | SSS  | 169   | 117°20'  | 35°38'   | 13.4                       | -0.2   | 27.3           | 755                      | 200                  |
| Xian,                           | SXA  | 425   | 108°45'  | 33°94'   | 13.4                       | -0.6   | 26.5           | 621                      | 226                  |
| Shannxi<br>Xuzhou,              | JXZ  | 28.8  | 117°31'  | 34°01′   | 14.0                       | 2.8  | 28.2           | 865                      | 210                  |
| Jiangsu<br>Wuhu,<br>Anhui       | AWH  | 10  | 117°92'  | 30°77'   | 15.5                       | 3.0  | 28.9           | 1200                     | 230                  |

Determination of seed kernel oil content and fatty acid composition

Each plant's seeds weighed about 10g, and calibration samples of pure A. truncatum oil with known oil content were taken. The oil content of the calibration samples was quickly determined by utilizing the SPEC-PMR pulsed nuclear magnetic resonance instrument oil and water analyzer, and each copy was repeated three times<sup>[16, 17]</sup>.

The fatty acid fraction of A. truncatum seed kernel was determined based on the external standard method specified in GB 5009.168-2016. After methyl esterification of A. truncatum seed oil samples in sulfuric acid medium, the supernatant was obtained, and the fatty acid standard assay solution and the sample to be measured were separately determined on the machine, and then the chromatograms were obtained by GC analysis. GC/MS conditions: DA-2560 capillary column (100 m  $\times$   $\Phi$ 0.25 mm  $\times$  0.2  $\mu$ m); high purity nitrogen carrier gas; inlet temperature of the injector is 270, the Detector temperature was 280; the initial column temperature was 100 (lasted 13 min),  $100^{\sim}180$  (heating rate 10 /min, lasted 6 min),  $180^{\sim}200$  (heating rate 1 /min, lasted 20 min),  $200^{\sim}230$  (heating rate 4 /min, lasted 10.5 min). The fatty acid content was obtained by peak area normalization  $[^{18-20}]$ , and the composition obtained from the determination is shown in Table 2.

Table 2 Fatty acid composition of samples

| No. | Name of fatty acid | Fatty acid abbreiation | No. | Name of fatty acid    | Fatty acid abbreiation |
|-----|--------------------|------------------------|-----|-----------------------|------------------------|
| 1   | Myristic acid      | C14:1                  | 11  | Eicosatetraenoic acid | C20:1                  |
| 2   | Palmitic acid      | C16:0                  | 12  | Eicosapentaenoic acid | C20:2                  |
| 3   | Palmitoleic acid   | C16:1                  | 13  | Behenic acid          | C22:0                  |
| 4   | Heptadecanoic acid | C17:0                  | 14  | Erucic acid           | C22:1n9                |
| 5   | Stearic acid       | C18:0                  | 15  | Eicosapentaenoic acid | C23:0                  |

| No. | Name of fatty acid       | Fatty acid abbreiation | No. | Name of fatty acid      | Fatty acid abbreiation |
|-----|--------------------------|------------------------|-----|-------------------------|------------------------|
| 6   | Oleic acid               | C18:1n9c               | 16  | Docosadienoic acid      | C22:2                  |
| 7   | Linoleic acid            | C18:2n6c               | 17  | XXIV Carbonic Acid      | C24:0                  |
| 8   | Arachidic acid           | C20:0                  | 18  | Neuronic acid           | C24:1                  |
| 9   | γ-linolenic acid         | C18:3n6                | 19  | Methyl docosahexaenoate | C22:6n3                |
| 10  | $\alpha$ -linolenic acid | C18:3n3                |     |                         |                        |

## Statistical analysis

Using SPSS 23.0, data were analyzed using nested design ANOVA, multiple comparison analysis, correlation analysis, and principal component analysis; and oil content and fatty acid fractions were thoroughly investigated utilizing the method of affiliation function and principal component analysis<sup>[21]</sup>.

The affiliation function method was used for evaluation: the affiliation function value of each index was calculated, and the average value of the affiliation function value of each index was used as the evaluation standard; the larger the average value of the affiliation function, the better the effect, and the overall quality of A. truncatum seed oil.

$$R(X_j) = \frac{X_j - X_{\min}}{X_{\max} - X_{\min}}$$

Where:  $X_j$  is the measured index value,  $X_{min}$  and  $X_{max}$  refer to the minimum and maximum values of all measured values, respectively.

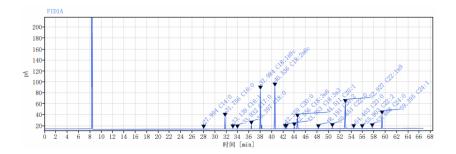
## Results

Seed Kernel Oil Content and Fatty Acid Fractions in Inter- and Intra-population Groups

In Table 3, the findings from the analysis of 70 samples from 9 populations to determine the oil content and main fatty acid components of A. truncatum seed kernels are shown. According to Table 3, the seed kernel oil content of differed greatly among different populations, with AWH-5 having the highest oil content (50.11%) and SJN-14 having the lowest oil content (14.43%), a difference of about three times. According to 58.6% of the total experimental material, 41 of the 70 samples had oil content above average, showing that the distribution of A. truncatum germplasm resources was quite even in terms of seed kernel oil content level. AWH (46.77%), SX (36.95%), and JXZ (33.47%) were the three populations with higher-than-average oil content among populations, and SJN had the lowest seed kernel oil content (28.35%). The average oil content of the nine populations ranged from 28.35% to 46.77%, and the average oil content among populations was 33.26%. For populations and sample people, the areas corresponding to the extremes of seed kernel oil concentration were essentially the same. The average coefficient of variation of seed kernel oil content was 21.64%, indicating that the oil content of seed kernels of A. truncatum has a rich degree of variation, which can be used as a basis for the screening of A. truncatum 's high-oil germplasm resources.

In 70 samples of A. truncatum, 37 fatty acid fractions were experimentally identified; the chromatograms of these samples are presented in Fig. 1 (where the chromatogram of the STA-1 sample is used as an example). A total of 19 fatty acid fractions were found, but 6 of them—myristic acid, heptadecanoic acid, eicosanoid, docosapentaenoic acid, palmitoleic acid, and methyl docosahexaenoate—had concentrations that could be ignored and were less than 0.1%, thus they were not included in the table. According to Table 3's findings, the average amounts of each fatty acid portion in the Genboldia seed kernel are, in descending order: linoleic acid (21.11%) > oleic acid (17.45%) > erucic acid (14.28%) > eicosapentaenoic acid (6.36%) > neuronic acid (5.54%) > palmitic acid (3.44%) > stearic acid (1.70%) >  $\alpha$ -linolenic acid (1.13%) > Behenic acid (0.72%) > eicosanoic acid (0.35%) > gamma-linolenic acid (0.33%) > eicosadienoic acid (0.19%) > arachidic acid (0.19%). The neuronic acid content of the 70 samples ranged from 2.78% to 10.80%, with a mean value of 5.54%; the sample with the highest content was STA-4 (10.80%), followed by SJN-9 (10.35%) and

STA-7 (10.26%), and the sample with the lowest content was SXA-6 (2.78%), with a rise of up to 288%; the neuronic acid content of the 9 populations was also examined. The extreme difference was 4.45%, with STA having the greatest percentage (7.74%) and SXA having the lowest (3.29%). It appears that the distribution of variance in oil content and fatty acid fractions of A. truncatumseed kernels was reasonably even since the sites corresponding to the extremes of oil content and neuronic acid concentration of the samples and populations were the same. The coefficients of variation of the components ranged from 24.13% to 38.20%, with an average coefficient of variation of 31.21%, and the larger coefficients of variation indicated that the fatty acid fractions of A. truncatum seed kernels were more rich in variation; the coefficients of variation for oleic acid were the smallest among the fatty acid fractions (24.13%), and eicosapentaenoic acid was the largest (38.20%), suggesting that oleic acid variations are relatively stable, whereas The coefficient of variation of eicosadienoic acid was 33.40%, indicating that the test material possessed the material basis for screening the high neuronic acid-specific germplasm.



Since the contents of behenic acid, eicosapentaenoic acid,  $\gamma$ -linolenic acid, eicosadienoic acid, arachidic acid, palmitoleic acid, seventeen-carbonic acid, eicosapentaenoic acid, myristic acid, and docosa-2-dioenoic acid accounted for less than 1% of the total fatty acid content of the test material, the above 10 fatty acid fractions will not be evaluated in the subsequent analyses.

Fig.1 Chromstogram of the fatty acid composition of STA-1

**Table 3.** Analysis of seed kernel oil content and fatty acid components in each population of *Acer truncatum* 

| Code  | Oil content | Fatty acid fraction(m,%) | Fatty acid fraction(m,%) | Fatty acid fraction(m,%) | Fatty acid frac |
|-------|-------------|--------------------------|--------------------------|--------------------------|-----------------|
|       |             | C16:0                    | C18:0                    | C18:1n9c                 | C18:2n6c        |
| STA-1 | 34.71       | 3.76                     | 1.57                     | 16.98                    | 20.26           |
| STA-2 | 29.05       | 5.05                     | 2.09                     | 22.10                    | 27.13           |
| STA-3 | 25.94       | 5.96                     | 2.45                     | 25.82                    | 34.45           |
| STA-4 | 30.27       | 5.16                     | 2.23                     | 22.73                    | 30.22           |
| STA-5 | 27.49       | 5.65                     | 2.30                     | 25.13                    | 29.33           |
| STA-6 | 26.23       | 4.72                     | 1.92                     | 13.86                    | 17.40           |
| STA-7 | 29.87       | 5.11                     | 3.09                     | 29.20                    | 35.96           |
| STA-8 | 30.69       | 3.07                     | 1.31                     | 13.17                    | 19.66           |
| STA-9 | 23.30       | 3.18                     | 1.81                     | 14.14                    | 19.06           |
| SJN-1 | 27.61       | 3.46                     | 1.22                     | 13.92                    | 18.36           |
| SJN-2 | 27.45       | 3.25                     | 1.39                     | 14.61                    | 19.96           |
| SJN-3 | 33.97       | 2.89                     | 0.80                     | 10.96                    | 15.43           |
| SJN-4 | 30.76       | 2.01                     | 1.25                     | 11.57                    | 13.30           |
| SJN-5 | 30.20       | 2.21                     | 1.21                     | 11.25                    | 13.20           |
| SJN-6 | 30.91       | 2.23                     | 0.98                     | 9.31                     | 13.66           |

| Code         | Oil content | Fatty acid fraction(m,%) | Fatty acid fraction(m,%) | Fatty acid fraction(m,%) | Fatty acid fractio |
|--------------|-------------|--------------------------|--------------------------|--------------------------|--------------------|
| SJN-7        | 30.67       | 2.15                     | 0.96                     | 9.23                     | 13.54              |
| SJN-8        | 36.11       | 3.34                     | 1.67                     | 14.10                    | 20.23              |
| SJN-9        | 28.82       | 4.88                     | 2.09                     | 21.35                    | 26.56              |
| SJN-10       | 31.08       | 3.97                     | 2.04                     | 18.41                    | 21.14              |
| SJN-11       | 33.36       | 3.65                     | 2.51                     | 17.79                    | 25.32              |
| SJN-12       | 19.15       | 3.98                     | 1.94                     | 18.21                    | 23.21              |
| SJN-13       | 19.81       | 4.02                     | 1.65                     | 21.04                    | 16.57              |
| SJN-14       | 14.43       | 6.30                     | 2.41                     | 26.08                    | 34.51              |
| SJN-15       | 30.97       | 2.69                     | 1.18                     | 12.01                    | 15.36              |
| SHY-1        | 31.49       | 3.43                     | 1.75                     | 17.44                    | 20.59              |
| SHY-2        | 29.39       | 2.89                     | 1.37                     | 19.33                    | 17.84              |
| SHY-3        | 34.06       | 2.33                     | 1.26                     | 13.54                    | 15.67              |
| SHY-4        | 35.92       | 2.41                     | 0.97                     | 11.17                    | 13.84              |
| SHY-5        | 30.95       | 3.33                     | 1.25                     | 15.84                    | 17.84              |
| SYY-1        | 35.06       | 3.54                     | 1.80                     | 15.48                    | 24.22              |
| SYY-2        | 30.69       | 4.06                     | 2.33                     | 18.03                    | 25.84              |
| SYY-3        | 34.10       | 3.78                     | 2.14                     | 18.07                    | 23.81              |
| SYY-4        | 23.01       | 5.74                     | 3.17                     | 25.30                    | 33.98              |
|              |             |                          | 1.72                     |                          |                    |
| SYY-5        | 32.26       | 3.75                     |                          | 12.11                    | 25.08              |
| SYY-6        | 27.01       | 4.71                     | 2.37                     | 17.07                    | 30.72              |
| SYY-7        | 27.65       | 4.30                     | 2.55                     | 17.96                    | 31.60              |
| SZZ-1        | 30.49       | 2.55                     | 1.16                     | 10.17                    | 14.75              |
| SZZ-2        | 29.93       | 2.43                     | 1.02                     | 9.97                     | 14.49              |
| SSS-1        | 31.35       | 2.22                     | 1.87                     | 22.59                    | 24.23              |
| SSS-2        | 32.48       | 3.99                     | 2.22                     | 20.95                    | 26.94              |
| SSS-3        | 28.13       | 3.70                     | 1.62                     | 19.90                    | 19.48              |
| SSS-4        | 36.44       | 3.56                     | 2.39                     | 22.72                    | 22.83              |
| SSS-5        | 35.55       | 3.79                     | 1.91                     | 18.28                    | 23.59              |
| SSS-6        | 31.87       | 3.30                     | 1.76                     | 18.90                    | 21.52              |
| SSS-7        | 36.45       | 3.75                     | 2.27                     | 18.80                    | 25.59              |
| SSS-8        | 34.28       | 3.16                     | 1.97                     | 16.67                    | 22.07              |
| SSS-9        | 29.42       | 3.87                     | 1.66                     | 20.98                    | 20.23              |
| SSS-10       | 29.91       | 4.10                     | 1.85                     | 20.65                    | 21.35              |
| SXA-1        | 40.48       | 2.64                     | 1.19                     | 19.07                    | 15.83              |
| SXA-2        | 35.27       | 2.78                     | 1.28                     | 20.13                    | 17.10              |
| SXA-3        | 33.78       | 2.66                     | 1.39                     | 19.33                    | 15.54              |
| SXA-4        | 34.57       | 2.81                     | 1.24                     | 19.09                    | 16.29              |
| SXA-5        | 40.11       | 2.75                     | 1.24                     | 19.38                    | 16.92              |
| SXA-6        | 37.51       | 2.53                     | 1.09                     | 17.22                    | 15.01              |
| JXZ-1        | 32.05       | 2.84                     | 1.25                     | 20.16                    | 16.07              |
| JXZ-2        | 28.42       | 2.96                     | 1.30                     | 20.65                    | 17.38              |
| JXZ-3        | 25.35       | 3.12                     | 1.26                     | 19.13                    | 20.08              |
| JXZ-4        | 42.38       | 2.81                     | 1.13                     | 16.92                    | 18.48              |
| JXZ-5        | 32.57       | 3.10                     | 1.23                     | 18.85                    | 20.08              |
| JXZ-6        | 30.78       | 2.96                     | 1.23                     | 17.74                    | 19.53              |
| JXZ-7        | 42.77       | 2.57                     | 1.08                     | 15.17                    | 16.46              |
| AWH-1        | 48.79       | 2.66                     | 1.62                     | 14.24                    | 18.88              |
| AWH-2        | 45.57       | 2.70                     | 1.67                     | 14.31                    | 18.83              |
| AWH-3        | 46.35       | 3.19                     | 1.90                     | 17.07                    | 22.78              |
| AWH-4        | 47.52       | 3.16                     | 1.98                     | 17.13                    | 22.50              |
| 7 7 4 4 1T-A | 11.02       | 0.10                     | 1.00                     | 11.10                    | 22.00              |

| $\operatorname{Code}$ | Oil content | Fatty acid fraction( $m,\%$ ) | Fatty acid fraction( $m,\%$ ) | Fatty acid fraction( $m,\%$ ) | Fatty acid fractio |
|-----------------------|-------------|-------------------------------|-------------------------------|-------------------------------|--------------------|
| AWH-5                 | 50.11       | 2.83                          | 1.74                          | 14.71                         | 20.10              |
| AWH-6                 | 44.78       | 3.39                          | 1.99                          | 17.69                         | 23.74              |
| AWH-7                 | 42.89       | 3.08                          | 1.87                          | 16.65                         | 21.89              |
| AWH-8                 | 46.87       | 3.20                          | 1.94                          | 17.35                         | 22.49              |
| AWH-9                 | 48.05       | 2.79                          | 1.64                          | 14.67                         | 19.67              |
| Mean                  | 33.11       | 3.44                          | 1.70                          | 17.45                         | 21.11              |
| CV                    | 21.64       | 27.91                         | 30.32                         | 24.13                         | 26.21              |

Meanwhile, according to the results of nested ANOVA (Table 4), there were highly significant differences in all indicators among A. truncatum populations, with the greatest variability in seed kernel oil content; only seed kernel oil content and palmitic acid showed highly significant differences among A. truncatum populations, while stearic acid, oleic acid, and linoleic acid showed significant differences, and -linolenic acid showed no significant differences. The results showed that, for A. truncatum, under different habitat conditions, the oil content of seed kernels and major fatty acid fractions among M. metabolica populations were richly variable and genetically unstable; under similar habitat conditions, the differences in A. truncatum 's major fatty acid fractions were small, and the degree of variation was not rich enough, but A. truncatum 's oil content of seed kernels and palmitic acid content showed highly similitude. However, independent of habitat similarities, the oil content and palmitic acid content of A. truncatum seed kernels exhibited highly significant variances within and among populations, indicating that these two metrics are highly variable.

**Table 4.** Variance analysis of seed kernel oil content and main fatty acid components in interpopulation and intra-population of *Acer truncatum* 

| Indicators  | Mean square       | Mean square        |               | F value           | F value            |
|-------------|-------------------|--------------------|---------------|-------------------|--------------------|
|             | Among populations | Within populations | Random errors | Among populations | Within populations |
| Oil content | 297.68            | 41.041             | 14.203        | 20.959**          | 3.298**            |
| C16:0       | 3.511             | 0.973              | 0.498         | 7.049**           | 2.083**            |
| C18:0       | 1.166             | 0.223              | 0.129         | 9.028**           | 1.815*             |
| C18:1n9c    | 47.554            | 20.626             | 12.325        | 3.858**           | 0.077*             |
| C18:2n6c    | 121.724           | 27.6               | 16.695        | 7.291**           | 1.727*             |
| C18:3n3     | 0.489             | 0.119              | 0.082         | 5.989**           | 1.506              |
| C20:1       | 10.51             | 2.863              | 1.808         | 5.814**           | 1.647              |
| C22:1n9     | 86.795            | 11.537             | 9.19          | 9.444**           | 1.277              |
| C24:1       | 15.658            | 1.626              | 1.867         | 8.386**           | 0.863              |

Note: \* mean significant different at 0.05 level; \*\* mean significant different at 0.01 level.

## Correlation Analysis

Analysis of the correlation between the seed kernel oil content and the main fatty acid fractions of A. truncatum (Table 5) showed that the seed kernel oil content showed highly significant negative correlation with palmitic acid, eicosapentaenoic acid, erucic acid and neuronic acid, with the correlation coefficients of -0.482, -0.404, -0.406, and -0.527, and significant negative correlation with oleic and linoleic acid, with the correlation coefficients of -0.265, -0.246, and the correlation with the rest of the fatty acids was not significant, -0.246, and the correlation with the rest of the fatty acid components was not significant, which indicates that as the oil content of the seed kernel increases, the increment of unsaturated fatty acids is significantly higher than that of saturated fatty acids, implying that the higher the oil content of the seed kernel, the higher its food value; saturated fatty acids compared with unsaturated fatty acids, although the two saturated fatty acids of stearic acid and palmitic acid showed a highly significant positive correlation with the rest of which

were the main fatty acid components. Although the two saturated fatty acids, stearic acid and palmitic acid, showed a highly significant positive correlation with the other major fatty acid fractions, the average content of these two fatty acids only accounted for 5.14% of the total fatty acids, while the unsaturated fatty acids, such as oleic acid, linoleic acid,  $\alpha$ -linolenic acid, eicosapentaenoic acid, erucic acid, and neuronic acid, accounted for 65.87% of the total fatty acids, and the correlations between the unsaturated fatty acids also showed a highly significant positive correlation. The correlation between unsaturated fatty acids also showed highly significant positive correlation. Combined with the results of the analysis of the correlation between the oil content of the seed kernel and the main fatty acid fractions, it can be seen that the increment in the proportion of unsaturated fatty acids was significantly higher than that of the saturated fatty acids, which indicated that it was of great significance for the development and utilization of the edible value of A. truncatum seed oil.

**Table 5.** Correlation analysis between seed kernel oil content and main fatty acid components of *Acer truncatum* 

| Indicators  | Oil content | C16:0   | C18:0   | C18:1n9c | C18:2n6c | C18:3n3 | C20:1   | C22:1n9 | C24:1 |
|-------------|-------------|---------|---------|----------|----------|---------|---------|---------|-------|
| Oil content | 1           |         |         |          |          |         |         |         |       |
| C16:0       | -0.482**    | 1       |         |          |          |         |         |         |       |
| C18:0       | -0.177      | 0.788** | 1       |          |          |         |         |         |       |
| C18:1n9c    | -0.265*     | 0.689** | 0.658** | 1        |          |         |         |         |       |
| C18:2n6c    | -0.246*     | 0.857** | 0.906** | 0.700**  | 1        |         |         |         |       |
| C18:3n3     | -0.173      | 0.702** | 0.743** | 0.402**  | 0.829**  | 1       |         |         |       |
| C20:1       | -0.402**    | 0.858** | 0.846** | 0.888**  | 0.883**  | 0.642** | 1       |         |       |
| C22:1n9     | -0.406**    | 0.780** | 0.792** | 0.769**  | 0.839**  | 0.680** | 0.923** | 1       |       |
| C24:1       | -0.527**    | 0.808** | 0.657** | 0.649**  | 0.773**  | 0.689** | 0.826** | 0.897** | 1     |

Note: \* mean significant different at 0.05 level; \*\* mean significant different at 0.01 level.

# $Correlation\ Analysis\ with\ Geographic\ Factors$

Meanwhile, from the results of correlation analysis of oil content and major fatty acid fractions of A. truncatum seed kernel with geographic factors (Table 6), it can be seen that the oil content of seed kernel showed highly significant positive correlation with annual mean temperature, January mean temperature, July mean temperature and annual precipitation, and highly significant negative correlation with altitude, indicating that A. truncatum in the southern plain area of China, which is at a lower elevation, with higher temperatures and sufficient precipitation, is more a seed source of high oil content, and its climatic conditions are are more suitable for the accumulation of A. truncatum seed oil. Among the saturated fatty acids, both palmitic acid and stearic acid showed significant positive correlation with altitude, palmitic acid showed highly significant negative correlation with January mean temperature and frost-free period, and stearic acid only showed significant negative correlation with frost-free period; among the unsaturated fatty acids, eicosapentaenoic acid showed highly significant positive correlation with altitude, oleic and linoleic acid showed significant positive correlation with altitude, and the correlation between saturated fatty acids and unsaturated fatty acids and altitude was similar, and the content increased with the increase of altitude. The correlation between saturated fatty acids and unsaturated fatty acids with altitude was similar, and the content increased with increasing altitude; α-linolenic acid showed a highly significant positive correlation with longitude, while eicosapentaenoic acid and erucic acid showed a strong negative correlation with mean annual temperature, erucic acid also had a strong negative correlation with January mean temperature, meanwhile, neuronic acid showed highly significant negative correlation with mean annual temperature, mean January temperature, annual precipitation and frost-free period, indicating that the relative content of neuronic acid was lower in the seed source with high temperature and sufficient precipitation; and there was a significant or highly significant negative correlation between the frost-free period and the main saturated fatty acid fractions.

which indicated that the relative content of fatty acid fractions of A. truncatum was more in the case of lower temperature and shorter growing period.

In summary, the oil content of A. truncatum seed kernels was more affected by latitude, temperature and precipitation, and altitude, temperature and precipitation were the main reasons for the geographic variations of fatty acid fractions, and the results of the correlation analyses also reflected A. truncatum 's habit of preferring cooler and wetter temperatures, and being more drought-tolerant.

**Table 6.** Correlation analysis between seed kernel oil content, main fatty acid components and geographical factors of *Acer truncatum* 

|            | Latitude      | Longitude     | Altitude | Average<br>tempera-<br>ture | January    | July       | Precipitation | n Frost-fr |
|------------|---------------|---------------|----------|-----------------------------|------------|------------|---------------|------------|
| Indicators | $(^{\circ}N)$ | $(^{\circ}E)$ | (m)      | ()                          | average () | average () | (mm)          | season     |
| Oil        | -0.202        | -0.055        | -0.382** | 0.629**                     | 0.654**    | 0.422**    | 0.722**       | 0.230      |
| content    |               |               |          |                             |            |            |               | ,          |
| C16:0      | 0.257*        | 0.128         | -0.005   | -0.204                      | -0.334**   | -0.118     | -0.209        | -0.330**   |
| C18:0      | 0.263*        | 0.177         | -0.139   | 0.034                       | -0.128     | 0.031      | 0.044         | -0.304*    |
| C18:1n9c   | 0.237*        | -0.152        | -0.258*  | -0.122                      | 0.014      | 0.004      | -0.089        | -0.282*    |
| C18:2n6c   | 0.268*        | 0.183         | -0.157   | -0.014                      | -0.113     | 0.012      | 0.005         | -0.354**   |
| C18:3n3    | 0.155         | 0.329**       | -0.102   | -0.007                      | -0.096     | -0.021     | 0.048         | -0.315**   |
| C20:1      | 0.335**       | 0.039         | -0.133   | -0.242*                     | -0.229     | -0.135     | -0.217        | -0.390*    |
| C22:1n9    | 0.219         | 0.150         | -0.065   | -0.257*                     | -0.276*    | -0.092     | -0.231        | -0.422**   |
| C24:1      | 0.137         | 0.222         | 0.072    | -0.356**                    | -0.417**   | -0.179     | -0.327**      | -0.391**   |

Note: \* mean significant different at 0.05 level; \*\* mean significant different at 0.01 level.

## Principal Component Analysis

The oil content of seed kernels and the main fatty acid fractions of 70 A. truncatum samples from nine populations were subjected to principal component analysis, and two principal components were extracted by analyzing the degree of contribution of each index, screening out independent variables with dominant roles, and using an eigenvalue greater than one as the principle (the results are shown in Table 7). As can be seen in Table 7, the cumulative contribution of the first 2 principal components is as high as 84.29%, which has been able to explain most of the information. In the 1st principal component, palmitic acid, stearic acid, oleic acid, linoleic acid, eicosapentaenoic acid, erucic acid, and neuronic acid contents were more highly correlated with PC1, and the absolute value of the loading was higher, all of which were greater than 0.8, so the 1st principal component can be summarized as the fatty acid component playing a major contributing role; in the 2nd principal component, the oil content of the seed kernel of A. truncatum had the largest absolute value of the loading, and thus the 2nd principal component mainly contained the seed oil content. In summary, the difference between the oil content of A. truncatum seed kernels and the major fatty acid fractions was large, which was the same as the results of the previous analysis of variance, and the contribution of the major fatty acid fractions was more than that of the oil content of the seed kernels, indicating that the fatty acid fractions played a major contributing role in the variation of the seed oil of A. truncatum.

**Table 7.** Principal component analysis of seed kernel oil content and main fatty acid components in *Acer truncatum* 

| Indicators | Principal component | Principal component |
|------------|---------------------|---------------------|
|            | PC1                 | PC2                 |

| Indicators                | Principal component | Principal component |
|---------------------------|---------------------|---------------------|
| Oil content               | -0.435              | 0.859               |
| C16:0                     | 0.916               | -0.089              |
| C18:0                     | 0.883               | 0.301               |
| C18:1n9c                  | 0.802               | -0.011              |
| C18:2n6c                  | 0.941               | 0.229               |
| C18:3n3                   | 0.782               | 0.3                 |
| C20:1                     | 0.964               | -0.018              |
| C22:1n9                   | 0.939               | -0.046              |
| C24:1                     | 0.895               | -0.215              |
| Eigen value               | 6.56                | 1.027               |
| Contributive percentage/% | 72.884              | 84.293              |

## Synthesized Assessment

According to the correlation analysis results, each fatty acid component in the seed oil of A. truncatum has a complex correlation, and it is difficult to use a particular indicator to determine the value of the population between the high and low or not, so the composite scores of each indicator between the 70 A. truncatum were calculated using the method of principal component analysis and the method of the affiliation function. There were 70 experimental materials in this experiment, and the samples with the top 10 scores were screened from high to low according to the size of the comprehensive evaluation value, and the evaluation results obtained are shown in Tables 8 and 9. According to Table 8, it can be seen that the two analytical methods resulted in the 10 highest comprehensive evaluation values were basically the same except for individual samples, and the 10 germplasm with the highest comprehensive evaluation values were STA-7, STA-3, SZB-4, and SJA-14, respectively, STA-4, STA-5, SJA-9, SZB-6, SZB-7 and SJN-2; as can be seen from Table 9, after the comprehensive evaluation of the nine populations in the two analytical methods, the resulting rankings in addition to the SJA and AWH slightly different, the rest of the population rankings do not differ, the preliminary assumption is that the habitat conditions are different resulting from the same time do not rule out the possibility of individual variation and thus affecting the scoring results. However, in general, the evaluation results were still reliable and accurate, with STA having the highest overall evaluation value among the nine A. truncatum populations, followed by SZB and SJN; the rankings of individual samples were similar to those of the populations, which further proved the accuracy of the overall evaluation. The results of this experiment provide a scientific and theoretical basis for the breeding work of screening high oil A. truncatum germplasm and genetic improvement.

**Table 8.** Comprehensive evaluation of seed kernel oil content and main fatty acid components of 70 samples of *Acer truncatum* 

| Code   | Principal component analysis   | Principal component analysis | $\operatorname{Code}$ | Memnership function method | Mem  |
|--------|--------------------------------|------------------------------|-----------------------|----------------------------|------|
|        | Comprehensive evaluation value | Rank                         |                       | Membershiop function value | Rank |
| STA-7  | 4.70                           | 1                            | STA-7                 | 0.83                       | 1    |
| STA-3  | 4.19                           | 2                            | STA-3                 | 0.77                       | 2    |
| SJA-14 | 4.18                           | 3                            | SZB-4                 | 0.74                       | 3    |
| SZB-4  | 4.10                           | 4                            | STA-4                 | 0.74                       | 4    |
| STA-5  | 3.66                           | 5                            | SJA-14                | 0.73                       | 5    |
| STA-4  | 3.66                           | 6                            | STA-5                 | 0.73                       | 6    |
| SJA-9  | 2.77                           | 7                            | SJA-9                 | 0.65                       | 7    |
| SZB-6  | 2.65                           | 8                            | SZB-6                 | 0.62                       | 8    |
| SZB-7  | 2.57                           | 9                            | SZB-7                 | 0.61                       | 9    |
| SJN-2  | 2.04                           | 10                           | SJN-2                 | 0.58                       | 10   |

**Table 9.** Comprehensive evaluation of seed kernel oil content and main fatty acid components of *Acer truncatum* in 9 populations

| Code | Principal component analysis   | Principal component analysis | Code | Memnership function method | Memner |
|------|--------------------------------|------------------------------|------|----------------------------|--------|
|      | Comprehensive evaluation value | Rank                         |      | Membershiop function value | Rank   |
| STA  | 1.82                           | 1                            | STA  | 0.55                       | 1      |
| SZB  | 1.65                           | 2                            | SZB  | 0.53                       | 2      |
| SJN  | 1.13                           | 3                            | SJN  | 0.49                       | 3      |
| SJA  | -0.43                          | 4                            | AWH  | 0.36                       | 4      |
| AWH  | -0.66                          | 5                            | SJA  | 0.32                       | 5      |
| JXZ  | -1.01                          | 6                            | JXZ  | 0.29                       | 6      |
| SYT  | -1.08                          | 7                            | SYT  | 0.27                       | 7      |
| SXA  | -1.58                          | 8                            | SXA  | 0.24                       | 8      |
| SZZ  | -2.44                          | 9                            | SZZ  | 0.13                       | 9      |

### Discussion

By screening the germplasm resources and selecting the appropriate variants, the selection and breeding of good varieties of A. truncatum can be initially realized. Earlier studies on phenotypic traits of A. truncatumproved that it has abundant variation, which provides a theoretical basis for screening the genes for good traits in ornamental trees, and this experiment proved that the oil content and fatty acid content of A. truncatum seed kernels also have a high degree of variation. One of the important reference indexes for screening A. truncatum germplasm with high oil content and high quality is the oil content. From the results, it can be seen that the oil content of the seed kernels of the 70 test materials ranged from 14.43% to 50.11%, with an increase of 247%, and the average oil content of the seed kernels was 33.11%; the average oil content of the seed kernels of the interspecific populations was 33.26%, with a variation range of 28.35% to 46.77%, with an increase of 65%, compared with the average oil content of the seed kernels of the populations of 33.26%. with an increase of 65%, which is more stable compared to the results of the individual samples. The results of this measurement are more similar to the results of other studies that have been seen [12, 22]. The coefficient of variation of seed kernel oil content was 21.64%, and the coefficients of variation of major fatty acid fractions ranged from 24.13% to 38.20%, with larger coefficients of variation for each index, which shows that there is abundant variation among the populations of A. truncatum, which is of great significance for the screening of high-oil A. truncatum single plants and their use in breeding of good varieties, so as to increase the A. truncatum oil production and oil value, and lay an important theoretical foundation for the improvement of the benefits of A. truncatum economic forests in the future. It is of great significance to improve the yield and oil value of maple oil, and to lay an important theoretical foundation for the future improvement of economic benefits of maple forest. A. truncatum oil can not only be used as high-quality health care edible oil, but also has high medicinal value. In this experiment, the fatty acid fractions with contents above 1% were tested and analyzed, and the measured average content results were as follows: linoleic acid (21.11%) > oleic acid (17.45%) > erucic acid (14.28%) > eicosapentaenoic acid (6.36%) > neuronic acid (5.54%) >palmitic acid (3.44%) > stearic acid (1.70%) >  $\alpha$ -linolenic acid (1.13%), in which the content of neuronic acid was similar to the determination of neuronic acid content of A. truncatum in Yunnan region by Liu Xiangyi<sup>[23]</sup> et al. (5.25%) and the content of neuronic acid in the seed oil of A. truncatum in the whole country by Qiaoqian [12] et al. (5.76%), but was lower than that of the content of neuronic acid in Shandong province by Wang Yinhua<sup>[22]</sup> et al. (6.78%), which was more similar to the results of the present experiment It can be seen that the neuronic acid content of A. truncatum is higher in Shandong Province, which is more valuable for selection and breeding.

Differences in yield and quality traits of crops are the result of gene-environment interactions, meaning that different environments can lead to differences among seed sources<sup>[24, 25]</sup>. In the correlation analysis, there were mostly significant or highly significant negative correlations between the oil content of A. truncatum

seed kernels and the major fatty acids, indicating that the fatty acid fractions of A. truncatum seed kernels with high oil content were on the contrary lower, combined with the results of the correlation analysis with the geographic factors, it can be seen that A. truncatum has more seed sources of high oil content in the southern plains where the elevation is low, the air temperature is high, and the precipitation is sufficient; the major fatty acid fractions mainly showed a significant or highly significant positive correlation with the longitude, and significant or highly significant negative correlation with the frost-free period, which is more suitable for the fatty acid accumulation within the seed oil of A. truncatum in the northern part of the country where the longitude is high and the air temperature is low. The oil content of seed kernel and fatty acid fraction showed different correlations with geographic factors, which was similar to the results of the correlation analysis between the two, the oil content of A. truncatum seed kernel is mainly affected by altitude, temperature and precipitation, while the fatty acid fraction is mainly affected by longitude, temperature and growth period, and the results of the correlation analysis also reflected the A. truncatum 's preferences for cool and humid, and the drought-tolerant habit, which is also in line with the current recognized opinion. The correlation results also reflected that A. truncatum is cool, humid and droughttolerant, and also agreed with the current accepted opinion that the accumulation of seed oil in the seed kernel of A. truncatum is more favorable with the increase of altitude [26, 27]. The A. truncatum samples in this experiment were only collected from the Central Plains, with little variation in latitude, longitude and climatic factors, which led to the limitations of the study. In the subsequent study, it is necessary to further expand the screening range of A. truncatum population collection sites, to eliminate the limitations of the sampling locations, and to draw more accurate and reliable conclusions.

After the oil content and major fatty acid fractions of A. truncatum seed kernels from nine populations with a total of 70 samples were analyzed separately by principal component analysis with dimensionality reduction, the nine indicators were simplified into two representative principal components, the first principal component included eight major fatty acid fractions of palmitic acid, stearic acid, oleic acid, linoleic acid, eicosapentaenoic acid, erucic acid and neuraminic acid, with a contribution rate of 72.884\%, and the second principal component only encompassed the oil content, with a contribution of 11.409%. Quality evaluation is an important part of the genetic breeding work of forest trees, and the comprehensive evaluation by the commonly used principal component analysis and affiliation function method can ensure the scientificity and accuracy of the evaluation results<sup>[28]</sup>. After the comprehensive evaluation of the oil content and major fatty acid fractions of the seed kernels of A. truncatum, the results obtained by the two evaluation methods were basically the same except for individual populations, and their evaluation results were accurate and reliable. Based on the evaluation results, 10 germplasm with high scores in the comprehensive quality evaluation were screened out: STA-7, STA-3, SZB-4, SJA-14, STA-4, STA-5, SJA-9, SZB-6, SZB-7, and SJN-2, and three excellent populations, STA, SZB, and SJN, with high relative contents of major fatty acids were also screened out, especially ST -4 had a neuronic acid content as high as 10.8%, which was in line with the requirements of the selection and breeding objectives of high oil Yuanbao maple.

As a high-value plant, selecting and breeding excellent germplasm with high oil and high yield is the top priority in the future research direction of A. truncatum, so in order to better and more accurately screen the excellent A. truncatum germplasm, further expanding the scope of the collection site, and at the same time combining with molecular marker technology to find the target genes with more precision, is the key to break the obstacles of A. truncatumselection and breeding work in the future. In future studies, components other than fatty acids within the seed kernels of A. truncatum, such as proteins, vitamin E3 and soluble sugars, which are also of high medical value, can also be explored<sup>[3, 29]</sup>. In addition, emerging technologies such as SSR molecular marker technology can be used for genetic breeding of forest trees. Chun Li<sup>[30]</sup> and others used SSR molecular markers to correlate the oil content of sesame seed kernels with the protein quality, and detected 19 SSR loci that were significantly correlated with the two traits, which provided an important theoretical basis for molecular marker-assisted breeding of sesame oil, the results provide an important theoretical basis for molecular marker-assisted breeding of sesame oil, Qiuyue Ma <sup>[31]</sup> and others designed a high-quality draft genome assembly that predicted at least 28,438 genes, providing new insights into the biosynthesis of very long-chain monounsaturated fatty acids, in addition to identifying three KCS genes that

may contribute to the regulation of neuronic acid biosynthesis, which advances functional genetics research on Genus metacrisis as well as providing theoretical foundations for the study of the molecular mechanisms affecting the production of neuronic acid. In summary, the identification of high oil *A. truncatum* asexual lines at the molecular level can effectively avoid individual errors caused by the traditional methods of identifying plant traits, so as to find the key genes more accurately, and to make an important contribution to the work of selecting and breeding high oil and high yielding *A. truncatum* excellent germplasm resources.

#### Conclusions

Compared with other oilseed woody species, A. truncatum has higher seed kernel oil content (33.11%) and neuronic acid content (5.54%), and has rich variability. It is worth noting that the data obtained in this experiment showed opposite trends in the increment of seed kernel oil content and fatty acid fractions; the variations of seed kernel oil content and fatty acid fractions were strongly influenced by geographic and ecological factors: the southern plains with lower elevations, higher temperatures, and sufficient precipitation had higher seed kernel oil content, and the northern part of the country with high longitude and low temperatures was more suitable for the accumulation of fatty acids. Therefore, if the derived research results can be used to guide the introduction and selection of excellent A. truncatum germplasm (e.g., high oil content and high neuronic acid), the development of its oil industry can be further enhanced. Finally, ten high-quality individuals, STA-7, STA-3, SZB-4, SJA-14, STA-4, STA-5, SJA-9, SZB-6, SZB-7, and SJN-2, as well as three high-quality populations, STA, SZB, and SJN, were selected by the principal component analysis and the affiliation function method. This work provides a scientific and theoretical basis for improving the yield and quality of the oil and lays the foundation for its application in edible and medicinal uses.

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