Genetic diversity of nuts traits and fatty acids of pecan (*Carya illinoinensis*) germplasm resources

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Abstract

Pecan (*Carya illinoinensis*) is the most economically valuable nut tree growing in many countries of the world. 10 nut quantitative traits and 15 fatty acid components of 112 pecan accessions were determined to analyze the morphometric and fatty acids genetic diversity in this study. The measured nuts traits of single nut mass, nut transverse, longitudinal and lateral diameter, nut aspect ratio, single nuts kernel mass, kernel yield and shell thinness were found highly variable. 15 fatty acids were detected among 36 tested fat acids in the nut kernel of pecan, and 14 fatty acids were found high variation except for the C12:0. Plenty of these traits are significant economic importance and could be used as breeding targets to improve the pecan variety. The positive correlations were observed between each pair of single nut mass, nut transverse diameter, nut longitudinal diameter and nut lateral diameter. Single nuts kernel mass is significantly positively correlated with single nut mass, nut transverse diameter, nut longitudinal diameter and nut lateral diameter. The 2D PCA plot successfully grouped the samples according to their phenotypic resemblance and morphological characteristics. 112 accessions were grouped into 4 and 3 major clusters according to the nut quantitative traits and fatty acids components and contents, respectively. Based on these results, we suggest that multidisciplinary research team should be set for genetic breeding of pecan to promote the conservation of local genetic diversity and improve the nuts production and commercialization in China.

Introduction

Pecan (*Carya illinoinensis* (Wangenh.) K. Koch), which is native from north America and below to *Juglandaceae* family, is one of the most economically valuable nut trees in the world (Grauke et al., 2016; Poletto et al.,2015). Breeding promoted the development of improved varieties of pecan. Pecan growers have conquered grafting technology and established the first commercial orchard through grafting ‘Centennial’ pecans in 1846, improving exciting pecan culture (Taylor, 1905). Plenty of pecan varieties of the world were bred in USA (Bentley et al., 2019). The geographic range of species distribution reflects broad adaptation. Regional constraints factors including short growing seasons, soil acidity-alkalinity, disease pressure associated with increased moisture, and so on, should be considered in the cultivation of pecan. To date, the pecan trees were grown in many climate zones, including subtropical, tropical and temperate regions.

Pecan trees are large, longer lived, outcrossing, highly heterozygous, and slow-to-bear. The trees are monoecious and dichogamy. There are two types, protandrous and protogynous. The population manifests heterodichogamy, to maximize cross pollination and outbreeding between individuals. Previous report showed that dichogamy is controlled by a single locus with simple dominance (Thompson 1985). This pattern of floral biology increases heterozygosity in natural stands (Bentley et al., 2019), thus, pecan germplasm resources have complicated genetic background and have many traits with genetic diversity. The pecan average breeding cycle need 35 years from initial cross to release due to the long juvenility (Bentley et al., 2019).

The pecan nut oil is rich in unsaturated fatty acids (UFA) and considered as a healthy oil (Villarreal-Lozoya et al., 2007; Ros and Mataix 2006). The content of monounsaturated fatty acids (MUFA) was higher than that of polyunsaturated fatty acids (PUFA) in pecan. Many studies have shown that eating of MUFA could lesser low-density lipoprotein (LDL) cholesterol, guards against coronary heart disease (CHD), controls the blood pressure, and might have valuable effects on inflammation (Sujatha et al., 2001;Alonso et al., 2006). PUFA have antithrombotic and antiatherosclerotic properties (Simopoulos 1991), prevent the development of diseases like arterial hypertension and insulin resistance (Manco et al., 2004), protective against diabetic renal disease (Garman et al., 2009). Fatty acids components and their contents were different among different pecan varieties (Toro-Vazquez et al., 1999; Lara et al., 2001; Bouali et al., 2013).

Pecan has been introduced to China for more than 110 years, and this species has been cultivated in several provinces, including Jiangsu, Zhejiang, Anhui, and so on. Some cultivars were introduced from the United States by breeders in 20th century. And small number of varieties were selected from seedlings. 42 cultivars and 70 advanced breeding selections of pecan with very large range of nuts shapes and sizes were collection and conservation in Nanjing Lvhe District pecan fine variety base of Jiangsu Provincial. Morphological measurements are relatively valuable tools used in systematic analysis and cultivars identification (Rubini Pisano et al., 2018; Igbari et al., 2019). 10 nut quantitative traits and 36 fatty acid components of 112 pecan accessions were determined to analyze the morphometric and fatty acids genetic diversity in this study.

Materials And Methods

Materials

42 cultivars and 70 advanced breeding selections of pecan were evaluated in this study (Table 1). Experiments were carried out in 2020 on 9 to 11 -year-old pecan trees trained with standard horticultural practices at the experiment farm of at Nanjing (Nanjing Lvhe District pecan fine variety base of Jiangsu Provincial, *Nanjing Green Universe Pecan Science & Technology Co. Ltd*) with plant spacing 6 m□8m. The site of the experimental orchard is located at 32°27'N latitude and 118°34'E longitude. Average temperatures were 21 to 29 and 19 to 27 in summer or autumn, respectively. Total annual precipitations were 351mm and 403 mm in summer or autumn, respectively. Soil characteristics were including: Soil Texture=clay loam, pH value=7. Drip irrigation system was performed to guarantee abundant water for pecan growth and development throughout the growing season. The nuts were harvested upto pericarp dehiscence and dried at low temperature (30 oC) in oven for 24 hours for investigating their characters. Evaluation for each species was performed separately based on a randomized complete blocks design (RCBD) with three replications and 30 nuts in each replicate.

Table 1
112 pecan germplasm collections.

Accession	Cultivar's or seedling line's name	Accession	Cultivar's or seedling line's name	Accession	Cultivar's or seedling line's name
1	El mart	39	Western Schley	77	SD35
2	Elliott	40	Shawnne	78	SD36
3	Odom	41	Shoshoni	79	SD37
4	Oconee	42	Zhongshan 25	80	SD38
5	Barton	43	SD01	81	SD39
6	Podsednik	44	SD02	82	SD40
7	Pawnee	45	SD03	83	SD41
8	Moneymaker	46	SD04	84	SD42
9	Success	47	SD05	85	SD43
10	Devore	48	SD06	86	SD44
11	Desirable	49	SD07	87	SD45
12	Forkert	50	SD08	88	SD46
13	Gracross	51	SD09	89	SD47
14	Starking	52	SD10	90	SD48
15	Jackson	53	SD11	91	SD49
16	Jinhua	54	SD12	92	SD50
17	Caddo	55	SD13	93	SD51
18	Cape Fear	56	SD14	94	SD52
19	Kanza	57	SD15	95	SD53
20	Colby	58	SD16	96	SD54
21	Creek	59	SD17	97	SD55
22	Lvzhou 1	60	SD18	98	SD56
23	Mahan	61	SD19	99	SD57
24	meyer	62	SD20	100	SD58
25	Melrose	63	SD21	101	SD59
26	Mohawk	64	SD22	102	SD60
27	Nacono	65	SD23	103	SD61
28	Cheyenne	66	SD24	104	SD62
29	Choctaw	67	SD25	105	SD63
30	Sauber	68	SD26	106	SD64
31	Seven	69	SD27	107	SD65
32	Shaoxing	70	SD28	108	SD66
33	Schley	71	SD29	109	SD67
34	Stuart	72	SD30	110	SD68
35	Sioux	73	SD31	111	SD69
36	Tejas	74	SD32	112	SD70
37	Wichita	75	SD33		
38	Waco	76	SD34		

Nut quantitative traits evaluation

The following ten nut quantitative traits were studied in this study: single nut mass (SNM), nut transverse diameter (NTD), nut longitudinal diameter (NLOD), nut lateral diameter (NLAD), nut shape index (NSI), ratio of transverse longitudinal (RTO), single nuts kernel mass (SNKM), kernel yield (KY), shell thinness (ST) and oil yield (OY). Nut shape index= nut longitudinal diameter/ nut transverse diameter, kernel yield (%) = nuts kernel mass/ nut mass x 100%.

Pecan oil was extracted from nuts kernel with Soxhlet extraction method using petroleum ether (Toro-Vazquez et al., 1999; Jabar et al., 2015). 10 g of minced nuts kernel per collections was put separately in a Soxhlet apparatus with 220 ml of petroleum ether. Oil was extracted during 12 hours at 75◦C. Oil was obtained by solvent evaporation in a Rotavapor at 70◦C and then measured. The oil was stored at 4◦C until analyses. Oil yield (%) = solvent free oil/kernel mass x 100%. The oil was stored at 4◦C until analyses.

Fatty acids componenst and content determination

Pecan fatty acids were determination by gas chromatography mass spectrometry (GCMS) using internal standard method (El Riachy et al., 2019). Extracted oil samples (1 mg), which were after saponification and methyl esterification, was analyzed by GC-MS using an Agilent 7890A, 5975C, using an HP-5MS column (30m×0.25mm, 0.25µm; Agilent). Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The column heat gradient was 60 ^{o}C to 200 ^{o}C at 8 ^{o}C /min, followed by 3 ^{o}C /min gradient to 280 ^{o}C, which was held for 5 min. A 1 µl injection was used for each sample analyzed by GC-MS. 36 fatty acids were measured in this study (Supplementary Table S1).

Statistical analysis

Data processing, using the below statistical analyses, was performed using the SPSS Statistics version 17. The data were subjected to basic statistics and an ANOVA (analysis of variance) was executed to provide the significant differences of characters between accessions. Duncan's multiple range tests were used to compare means for each trait. A correlation analysis was performed to study the relationship between traits. Principal component analysis (PCA) was done to study patterns of variation in a set of interrelated traits through the identification of subsets of these traits. Ward's cluster analysis using the squared euclidean distance was performed to classify the genotypes (Crossa and Franco, 2004). Genetic similarity computing and the construction of respective 2D plots were also performed.

Results

Statistics and correlations for the 10 nut quantitative traits variables

The 10 nut quantitative traits of 112 *C. illinoinensis* germplasm were determined and the descriptive statistics of means, mean standard error, variance, range, maxima, minima, standard deviations and coefficients of variation (CV) are analyzed in this study (Table 2, Supplementary Table S2). The results showed that some nut traits had high CVs, representing extensive morphological variability. These included single nuts kernel mass (32.75%), single nut mass (28.80%), shell thinness (25.03%). The remaining traits showed comparatively low CV values (<20%). Oil yield and ratio of transverse longitudinal had the lowest CVs of 5.38% and 2.91%, respectively. The ANOVA showed that accessions effects in pecan were significant on all nut quantitative traits.

Table 2
Analysis of diversity for 10 nut quantitative traits in 112 pecan germplasm.

Trait	Mean	Mean SE	Sd.	Variance	CV%	Range	Max.	Min.
Single nut mass (g)	6.14	0.18	1.77	3.16	28.80	8.68	10.62	1.94
Nut transverse diameter (mm)	21.67	0.23	2.43	5.81	11.19	12.35	26.37	14.02
Nut longitudinal diameter (mm)	38.50	0.58	6.23	38.28	16.18	31.98	54.38	22.40
Nut lateral diameter (mm)	20.59	0.22	2.38	5.64	11.54	12.12	25.86	13.74
Nut aspect ratio (Nut shape index)	1.78	0.02	0.26	0.07	14.51	1.34	2.43	1.09
Ratio of transverse longitudinal	1.05	0.03	0.03	0.00	2.91	0.13	1.13	1.00
Single nuts kernel mass (g)	3.16	0.10	1.03	1.08	32.75	5.22	6.28	1.06
Kernel yield (%)	51.22	0.54	5.70	32.60	11.13	31.72	62.85	31.13
Shell thinness (mm)	0.78	0.02	0.20	0.04	25.03	1.36	1.77	0.41
Oil yield (%)	65.92	0.32	3.54	11.41	5.38	15.19	73.82	55.36

El mart had the highest single nut mass (10.62 g), followed by Choctaw (10.28 g), SD07 (9.82 g) and Mohawk (9.61g). The lightest single nut mass was SD09 (1.94g), followed by SD38 (2.25 g) and SD08 (2.43 g). The maximum nut transverse diameter was SD07 (26.37 mm), followed by Jackson (26.29 mm), Choctaw (25.56 mm) and Success (25.43 mm). Maximum nut longitudinal diameter was observed in Mahan (54.38 mm), followed by Nacono (50.38 mm), El mart (50.11 mm) and Western (49.77 mm). SD09 had the minimum nut longitudinal diameter (22.40 mm). Maximum nut lateral diameter was observed in SD07 (25.86 mm), followed by Jackson (25.21 mm), Tejas (25.08 mm) and Choctaw (24.62 mm). SD38 had the minimum nut longitudinal diameter (13.74 mm). Nut aspect ratio (Nut shape index) indicated the shape of the nuts. The maximum of nut aspect ratio was Sauber (2.43), followed by Mahan (2.32) and Shawnne (2.30 mm). SD04 had the minimum nut aspect ratio of 1.09. Cape Fear had the thinnest shell thinness (0.41 mm), followed by SD02 (0.45 mm) and SD01 (0.47 mm). The thickest shell thinness was recorded in SD04 (1.77 mm), followed by SD13 (1.38 mm) and SD47(1.25 mm). The highest kernel yield was Sioux (62.85%), followed by SD02 (62.25%) and Mahan (61.36%), and the lowest kernel yield was SD04 (31.13%). The highest oil yield was SD05 (73.82%), followed by Wichita (72.44%) and Creek (72.03%), and the lowest oil yield was SD41 (55.36%).

Statistically significant correlations among all the 10 nut traits were analyzed (Table 3). The positive correlations were observed between each pair of single nut mass, nut transverse diameter, nut longitudinal diameter and nut lateral diameter. Nut shape index is significantly positively correlated with nut

longitudinal diameter, but negatively correlated with nut transverse diameter and nut lateral diameter. Single nuts kernel mass is significantly positively correlated with single nut mass, nut transverse diameter, nut longitudinal diameter and nut lateral diameter. There is a very significant negative correlation between shell thinness and kernel yield (-0.793). Oil yield is significantly positively correlated with single nut mass, single nuts kernel mass and kernel yield.

Table 3
Correlation coefficients among 10 nut quantitative traits in 112 pecan germplasm.

	SNM	NTD	NLOD	NLAD	NSI	RTO	SNKM	KY	ST	OY
SNM	1									
NTD	**0.792****	1								
NLOD	**0.706****	**0.485****	1							
NLAD	**0.807****	**0.962****	**0.460****	1						
NSI	0.126	**-0.273****	**0.701****	**-0.272****	1					
RTO	-0.107	0.045	0.048	**-0.214***	-0.005	1				
SNKM	**0.937****	**0.700****	**0.679****	**0.703****	0.160	-0.043	1			
KY	0.083	-0.038	0.061	-0.072	0.070	0.175	**0.414****	1		
ST	0.190	0.146	0.065	**0.214***	-0.031	**-0.257***	-0.103	**-0.793****	1	
OY	**0.386****	0.135	0.181	0.187	0.084	-0.176	**0.418****	**0.262****	-0.032	1

Abbreviation: SNM, Single nut mass; NTD, Nut transverse diameter; NLOD, Nut longitudinal diameter; NLAD, Nut lateral diameter; NSI, Nut shape index; RTO, Ratio of transverse longitudinal; SNKM, Single nuts kernel mass; KY, Kernel yield; ST, Shell thinness; OY, Oil yield. ** indicated that correlation is significant at the 0.01 level; *indicated that correlation is significant at the 0.05 level.

Principal component analysis (PCA) of 10 nut quantitative traits variables

10 pecan nut quantitative traits were put into four components that explained 89.078% of the total variation through PCA (Table 4). The first component, which accounted for 41.031% of the total variation, included single nut mass, nut transverse diameter, nut longitudinal diameter, nut lateral diameter and single nuts kernel mass. The second component, accounting for 23.498% of the total variation, included kernel yield and shell thinness. The third component, which explained 13.51% of the total variation, included nut shape index only. The fourth component, explaining 11.039% of the total variation, included ratio of transverse longitudinal and oil yield.

Table 4
First 4 components from the PCA analysis of 10 nut quantitative traits in 112 pecan germplasm.

Traits	F1	F2	F3	F4
Single nut mass	**0.967**	-0.095	0.058	-0.012
Nut transverse diameter	**0.847**	-0.347	-0.223	0.290
Nut longitudinal diameter	**0.772**	0.334	0.506	0.126
Nut lateral diameter	**0.847**	-0.436	-0.181	0.100
Nut shape index	0.206	0.611	**0.756**	-0.086
Ratio of transverse longitudinal	-0.088	0.396	-0.170	**0.692**
Single nuts kernel mass	**0.946**	0.199	-0.089	-0.051
Kernel yield	0.225	**0.805**	-0.454	-0.123
Shell thinness	0.005	**-0.830**	0.367	-0.077
Oil yield	0.375	0.108	-0.245	**-0.696**
Eigenvalue of correlation matrix	4.103	2.350	1.351	1.104
Explained proportion of total variance %	41.031	23.498	13.510	11.039
Cumulative proportion of total variance %	41.031	64.529	78.039	89.078

Because the first two PCs explained 64.5% of the total variation among the cultivars (Table 4), the approximation of the real multivariate diversity of the cultivars on the 2-PC axis is quite acceptable for the most important discriminating (contributing) traits. The scatter plot of the first two principal components (Fig. 1) shows geometrical distances among the cultivars in the plot that reflect a genetic similarity for the 10 measured nut traits and the relations between the four groups delivered by the cluster analysis. According to phenotypic resemblance and morphological characteristics, the samples were plot grouped. For example, accessions SD08, SD09, SD38, SD50 and Kanza had the smallest nut transverse diameter and lateral diameter, minimum single nut mass and nuts

kernel mass. SD58, SD59 and SD60 had the smaller single nut mass, nuts kernel mass and lateral diameter. SD04, SD13, SD14 and Zhongshan 25 had the thickest shell thinness (> 1.0mm) and minimum kernel yield. SD07, Choctaw, Jackson, Kiowa, Success and Waco had the biggest nut lateral diameter and nut transverse diameter, bigger nut fruit weight, higher single nuts kernel mass and medium shell thinness. Mahan, Wichta, Melrose, Nacono, Pownee, Western shelly, Desirable, Mohawk and El mart had largest nut longitudinal diameter and single nuts kernel mass, biggest nut weight, thinner shell thinness. These results demonstrate that single nut mass, nut transverse diameter, nut longitudinal diameter, nut lateral diameter, single nuts kernel mass, kernel yield and shell thinness are highly positively correlated and as a result, these morphological traits led to the highest loading factors in this PCA analysis. The distribution of the cultivars in Fig. 1 showed that their rich genetic diversity (profiles) for the studied traits.

Fatty acid components and content analysis, statistics and correlations for the 15 fatty acids variables

36 fatty acids (Supplementary Table S1) were measured in the oil of 112 pecan germplasm, and 15 fatty acids were detected (Table 5, Supplementary Table S3). The highest fatty acid is octadecenoic acid (252.485 mg/g), followed by octadecadienoic acid (133.952 mg/g), hexadecanoic acid (26.393mg/g), and octadecatrienoic acid (7.233 mg/g), decanoic acid (0.014 mg/g) and octadecanoic acid (0.014 mg/g) were the lowest fatty acid. Ratio of UFA was up to 93.55%. Unsaturated fat is broken down into two subgroups known as mono- and polyunsaturated. These are the healthy forms attributed to cholesterol reduction and heart health. Ratio of mono- and polyunsaturated fatty acid were 64.54% and 35.46% respectively. The maximum hexadecanoic acid was detected in Pawnee (50.579 mg/g), followed by SD49 (42.780 mg/g), SD39 (38.701 mg/g) and SD64 (38.651 mg/g). The lightest hexadecanoic acid was detected in SD45 (0.408 mg/g). SD49 had the highest octadecenoic acid (401.802 mg/g), followed by Pawnee (379.83 mg/g) and Oconee (323.365 mg/g). SD49 had the highest octadecadienoic acid (244.335 mg/g) and octadecatrienoic acid (17.742 mg/g), followed by SD42 (231.960 and 12.685 mg/g, respectively) and SD64 (227.208 and 12.509 mg/g, respectively). SD49 had the highest Eicosenoic acid (3.477 mg/g) and tricosanoic acid (0.416 mg/g). SD49 had the highest total of fatty acids (713.911 mg/g) and total of unsaturated fatty acids (668.853 mg/g), followed by Pawnee (620.707 mg/g and 568.167 mg/g) and SD64 (587.681 mg/g and 547.813 mg/g). Ratio of unsaturated fatty acids in all Carya illinoinensis germplasm were more than 91%, and the highest was SD45 (99.64%). The maximum of mono- (406.776 mg/g) and polyunsaturated fatty acids (262.077 mg/g) were in SD49, followed by Pawnee (384.170 mg/g) and Oconee (326.585 mg/g) for monounsaturated fatty acids, and by SD42 (244.645 mg/g) and SD64 (239.717 mg/g) for polyunsaturated fatty acids. The maximum of monounsaturated fatty acid ratio was Forkert (80.88%), followed by Sauber (78.24%) and SD04 (77.74%).

Table 5
Analysis of diversity for 15 fatty acids contents in 112 pecan germplasm.

Symbol	Fatty acids	Mean	Mean SE	Sd.	Variance	CV	Range	Max.	Min.
C10:0	Decanoic acid (mg/g)	0.014	0.000	0.004	0.000	31.80	0.03	0.037	0.006
C12:0	Dodecanoic acid(mg/g)	0.159	0.000	0.005	0.000	2.93	0.04	0.188	0.151
C14:0	Tetradecanoic acid (mg/g)	0.077	0.004	0.044	0.002	57.50	0.26	0.260	0
C15:0	Pentadecanoic acid (mg/g)	0.096	0.002	0.024	0.001	24.58	0.11	0.167	0.055
C16:0	Hexadecanoic acid (mg/g)	26.393	0.537	5.660	32.268	21.45	50.17	50.579	0.408
C16:1	Hexadecenoic acid (mg/g)	0.450	0.014	0.151	0.023	33.64	0.66	0.852	0.188
C17:0	Heptadecanoic acid (mg/g)	0.135	0.005	0.051	0.003	37.47	0.34	0.344	0
C17:1	Heptadecenoic acid (mg/g)	0.361	0.007	0.078	0.006	21.57	0.57	0.758	0.184
C18:0	Octadecanoic acid (mg/g)	0.014	0.004	0.004	0.000	27.34	0.03	0.032	0.006
C18:1	Octadecenoic acid (mg/g)	252.485	3.723	39.118	1557.053	15.49	236.74	401.801	165.063
C18:2	Octadecadienoic acid (mg/g)	133.952	3.151	33.336	1112.203	24.89	185.70	244.335	58.635
C18:3	Octadecatrienoic acid (mg/g)	7.233	0.215	2.290	5.173	31.65	15.61	17.742	2.130
C20:0	Eicosanoic acid (mg/g)	0.351	0.013	0.132	0.018	37.67	1.00	0.999	0
C20:1	Eicosenoic acid (mg/g)	1.623	0.041	0.434	0.192	26.75	2.80	3.477	0.675
C23:0	Tricosanoic acid (mg/g)	0.171	0.035	0.037	0.001	21.63	0.31	0.416	0.102
Total of fatty acids (mg/g)		423.514	5.964	62.747	3984.974	14.82	462.66	713.911	251.251
Total of NFA (mg/g)		396.105	5.525	58.104	3419.001	14.67	432.97	668.853	235.884
Ratio of NFA (%)		93.55	0.072	0.76	0.576	0.81	8.10	99.64	91.54
Total of MNFA (mg/g)		254.919	3.779	39.646	1599.351	15.55	240.54	406.776	166.234
Ratio of MNFA (%)		64.54	0.574	6.09	36.913	9.43	33.22	80.88	47.67
Total of PNFA (mg/g)		141.185	3.337	35.318	1247.150	25.02	201.31	262.077	60.765
Ratio of PNFA (%)		35.46	0.574	6.09	36.913	17.17	33.22	52.33	19.12

The ANOVA showed that accessions effects in pecan were significant on all detected 15 fatty acids components. The descriptive statistics of means, mean standard error, variance, range, maxima, minima, standard deviations and CV of 15 fatty acids are also analyzed (Table 5). The results showed that some fatty

acids had high CVs, representing extensive fatty acid component variability. These included eicosanoic acid (37.67%), heptadecanoic acid (37.47%), hexadecenoic acid (33.64%), decanoic acid (31.8%) and octadecatrienoic acid (31.65%). The remaining fatty acids showed comparatively low CV values (<30%). Ratio of unsaturated fatty acids had the lowest CV of 0.81%.

Statistically significant correlations among all the 15 fatty acids contents were analyzed (Table 6). The positive correlations were observed between each pair of C12, C14, C15 and C16. The positive correlations were observed between each pair of C18:1, C18:2, C18:3, C20, C20:1 and C23, except for the pair of C18:2 and C23. C17 was positive correlations with other fatty acids components except for the C14, C16:1 and C18. C17:1, C18:1 and C20:1 were positive correlations with other fatty acids components except for the C16:1 and C18. C18:2 was positive correlations with other fatty acids components except for the C10, C16:1, C18 and C23. C18:3 was positive correlations with other fatty acids components except for the C10, C16:1 and C18. However, C16:1 and C18 were not correlations with other fatty acids components.

Table 6
Correlation coefficients among 15 fatty acids contents in 112 pecan germplasm.

	C10	C12	C14	C15	C16	C16:1	C17	C17:1	C18	C18:1	C18:2	C18:3	C20	C20:1
C10	1													
C12	**0.533****	1												
C14	-0.114	**0.510****	1											
C15	-0.122	**0.235***	**0.691****	1										
C16	0.045	**0.344****	**0.710****	**0.657****	1									
C16:1	0.124	0.084	-0.007	-0.058	0.032	1								
C17	**0.381****	**0.270****	0.144	**0.271****	**0.445****	-0.005	1							
C17:1	**0.412****	**0.653****	**0.516****	**0.456****	**0.604****	0.114	**0.453****	1						
C18	0.102	0.01	-0.047	-0.005	0.01	-0.102	-0.025	-0.064	1					
C18:1	**0.391****	**0.578****	**0.426****	**0.288****	**0.547****	0.137	**0.499****	**0.787****	-0.082	1				
C18:2	-0.105	**0.289****	**0.611****	**0.769****	**0.659****	-0.012	**0.225***	**0.508****	-0.12	**0.190***	1			
C18:3	0.001	**0.299****	**0.450****	**0.682****	**0.577****	-0.049	**0.420****	**0.473****	-0.137	**0.229***	**0.857****	1		
C20	**0.465****	**0.338****	0.065	**0.239***	**0.303****	-0.068	**0.707****	**0.345****	0.014	**0.452****	**0.215***	**0.482****	1	
C20:1	**0.312****	**0.563****	**0.507****	**0.413****	**0.567****	0.085	**0.525****	**0.746****	-0.098	**0.894****	**0.350****	**0.366****	**0.462****	1
C23	**0.569****	**0.385****	0.083	0.162	**0.327****	0.078	**0.353****	**0.565****	0.153	**0.587****	0.161	**0.227***	**0.456****	**0.524**

Principal component analysis (PCA) for the fatty acids variable

PCA put the 15 fatty acids, total of fatty acids, total and ratio of UFA and total and ratio of MUFA and PUFA into 5 components which explained 86.201% of the total variation (Supplementary Table S5). The first component included C12, C14, C15, C16, C16:1, C17:1, C18:1, C18:2, C18:3, C20:1, total of fatty acids, total of UFA, total of MUFA, total of PUFA, accounting for 44.595% of the total variation. The second component included C10, ratio of MUFA and PUFA, accounted for 22.123% of the total variation. The third, fourth and fifth component included C20, ratio of UFA and C18, respectively, accounting for 7.757%, 6.888% and 5.024% of the total variation, respectively.

A 2D PCA plot was made according to the first two components (Fig. 2). The samples were plot grouped according to fatty acids content. For example, accessions SD49, Pawnee, SD46, Oconee, SD67, SD37, SD65, SD 68, SD39 and SD64 had the most mount of C14, C15, C16, C16:1, C17:1, C18:1, C18:2, C20:1 and the total of fatty acids, total of UFA and total of MUFA. Accessions SD42, Jinhua, SD36, Wichita, SD38, SD69, and Odom had the most mount of C14, C15, C16, C16:1 and C18:2, the secondly of C17:1, and the total of fatty acids, total of UFA, the less of ratio of MUFA. Accessions SD25, SD13, SD16, SD18, SD04, Elliot and Forkert had minimum of C14, C15, C16, C16:1, C17:1, C18:2, C18:3, and the total of fatty acids, total of UFA and total of PUFA. Accessions SD54, SD40, SD08, Shaoxing and Caddo had the minimum of C10 and C18:1, lesser of C17:1, and the total of fatty acids and total of UFA, and the highest of ratio of PUFA. These results demonstrate that C10, C14, C15, C16, C16:1, C17:1, C18:1, C18:2, C20:1, total of fatty acids, total of UFA, total of MUFA, total of PUFA and ratio of PUFA were highly positively correlated, and as a result, these fatty acids compositions and content led to the highest loading factors in this PCA analysis.

Cluster analysis

Two dendrograms were obtained from the Ward's method using the euclidean distance based on the 112 accessions nut quantitative traits (Fig. 3A) and fatty acids content (Fig. 3B). Based on the nut quantitative traits, the 112 accessions were grouped in four distinct groups (Fig. 3A). C1 contained 31 accessions which had larger relatively singer nut mass and higher singer nuts kernel mass. C2 contained 12 accessions which had maximum singer nut mass and singer nut kernel mass, the longer nut transverse and lateral diameter. C3 contained 28 accessions which had minor singer nut mass and singer nut kernel mass, shorter nut transverse and longitudinal diameter. C4 contained 41 accessions which had miner singer nut mass and singer nut kernel mass, shorter nut longitudinal diameter, miner nut shape index, and thicker shell thinness.

Based on the 15 fatty acids contents, the 112 accessions were grouped in three major clusters (Fig. 3B). Cluster D1 included 32 accessions which had higher contents of C16, C18:1, C20:1, total of fatty acids, total of UFA and total of MUFA. Cluster D1 included two accessions which had highest contents of C16, C18:1, C20:1, total of fatty acids, total of UFA and total of MUFA. However, cluster D3 included 78 accessions, which had lower contents of C16, C18:1, C20:1, total of fatty acids, total of UFA and total of MUFA.

Discussion

Nut quantitative traits and fatty acids components and contents of 112 pecan accessions were analyzed to determine patterns of diversity, characterize cultivars, and identify plants with commercial or nutritional potentials. Comparing to the walnuts (*Juglans regia* L.) (Hou et al., 2014), our study showed that pecan nuts present larger variation for all morphometric traits. Poletto et al also reported that pecan nuts present larger variation for all morphometric traits through studding pecan nuts of 60 accessions growing in southern Brazil (Poletto et al., 2019). The mean values of the kernel yield and nut shape index in our study are similar to that obtained by Poletto et al. but, the mean values of some measured nut traits are not the same with those obtained in the present study, maybe due to more samples selected in our study especially the seedling lines. Wells and Conner study showed that nineteen North American cultivars of pecan presented lower variance for morphometric of the nuts (Wells et al., 2015). Thus, our results showed that pecan present genetic diversity, suggesting that these resources have desirable characteristics for commercial purpose and the results of the study were used to justify the preservation of the genetic resources of this cultivar through conservation-by-use. Morphometric measures of nuts were employed for the comparison of the cultivar with nonselected naturally occurring trees. The results of study were used to demonstrate the cultivar with interesting characteristics for commercial purpose could be as the genetic resources for preservation. Morphometric dissimilarities were important sources of genetic variation and could be used breeding in pecan in future.

Different shapes, sizes, and chemical composition of pecan nuts may be used in later for different purpose in the industry. Nutrition and health food were development depending on their chemical composition. The pecan accessions evaluated in this study revealed oil yield (65.92%) higher than that (62.6%) obtained by Poletto et al. (2019). Comparing with the hazel nuts (49.2%), macadamia (59.2%), pine nut (58.8%), peanut (37.9%), walnut (50.8%), and almond (40.8%) (Maguire et al., 2004), pecan nut yield the greatest percentage of oil. Pecans and walnuts contain different amount of UFA, even have similar amount of total fat acids. pecans contain over 74.07% fat acids, while walnuts contain about 65.26%. Pecans contain 66.67% MUFA of the total UFA, however, walnuts contain 16.13% only (Prasad 2003). The fatty acid profile of the five nuts (brazil, pecan, pine, pistachio and cashew nuts) were determined by capillary-column GC, and the results showed that 11 fatty acid compositions were detected in pecan nuts and ratio of unsaturated/ saturated fatty acids of pecan (13.54) higher than those in brazil (2.79), pine (6.81), pistachio (9.75) and cashew (3.92) nuts (Ryan et al., 2006). The ratio of unsaturated fatty acids (93.55%) of pecan in our study was similar to that obtained (93.66%) in the percent study (Ryan et al., 2006). 15 fatty acids (Table 5, Supplementary Table S3) were detected among 36 tested fat acids in the nuts kernel of 112 pecan germplasm (Supplementary Table S1), and significant variations exist in many fatty acid components except for the C12:0. And the UFA, MUFA, PUFA contents variation coefficients were 14.67, 15.55 and 25.02 respectively, indicating these fatty acids components were genetic variation among different pecan.

Pecan is an exotic species in China, the acreage areas have been continued increase in Jiangsu, Anhui and other provinces of our country. Pecan morphometric and fatty acids genetic diversity were a very large range in this study. New pecan individuals were originated from directed or spontaneous crossing among different cultivars during the past mor than one century cultivating in China. Such new individuals were usually not registered, however, have unique valuable one or more characters, for instance, SD49 had the highest total of fatty acids (713.911 mg/g), total of UFA (668.853 mg/g), MUFA (406.776 mg/g) and PUFA (262.077 mg/g). SD02 (0.45 mm) had the thinnest shell thinness and highest kernel yield (62.25%). SD05 had the highest oil yield (73.82%). These individuals with excellent characterizations should be conservated and used for pecan development and breeding in future. Multidisciplinary research team should be set for genetic breeding of pecan to promote the conservation of local genetic diversity and the traits neglected, improve the nuts production and commercialization in China.

In conclusion, this study consolidated that pecan present genetic diversity in nut quantitative traits and fatty acids components and contents. Pecan nut kernel contains 15 fatty acids components. The ratio of unsaturated fatty acids was up to 93.55%, and the ration of MUFA was higher than that of PUFA. And the UFA, MUFA, PUFA contents were genetic variation among different pecan. Favorable fatty acids and morphometric characteristics should be considered in pecan breed.

Declarations

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Zhang Ji-Yu, Wang Tao, Zhang Fan, Li Yong-Rong, Liu Yong-Zhi, Wang Gang and Wang Xiao-Yong. The first draft of the manuscript was written by Zhang Ji-Yu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest: The author declares no conflict of interest.

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Figures

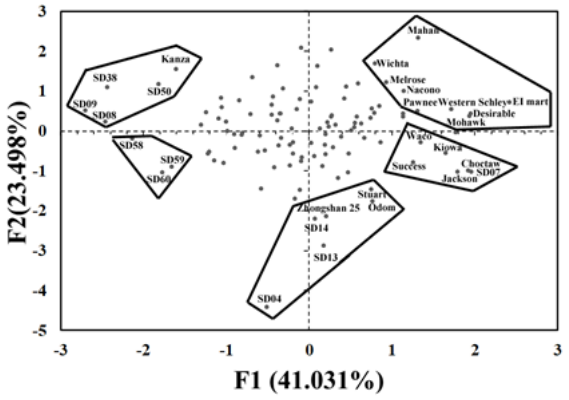

Figure 1

Two dimensional PCA plot based on the first two components for 10 nut quantitative traits of 112 pecan germplasm.

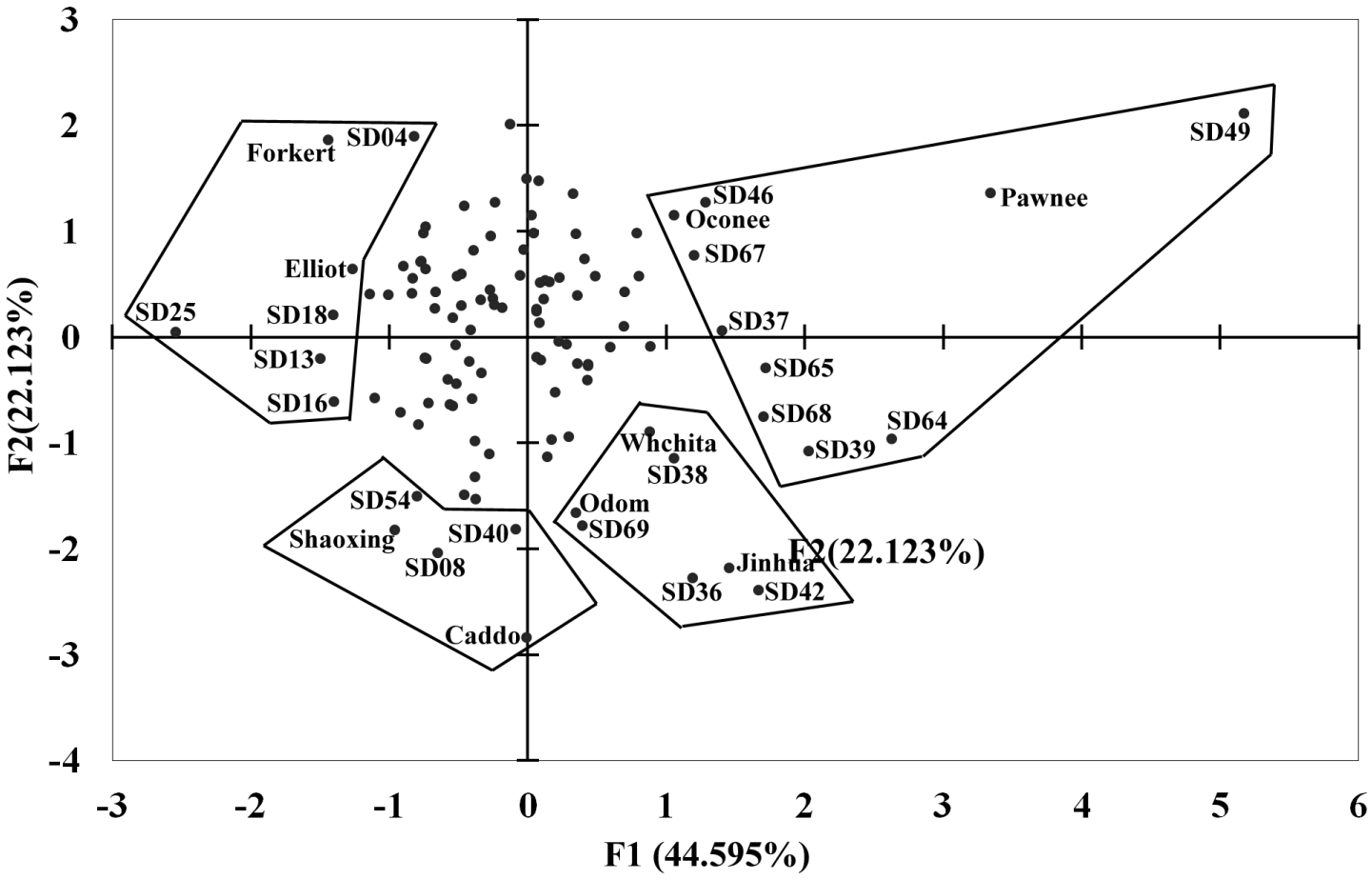

Figure 2

Two dimensional PCA plot based on the first two components for 15 fatty acids contents in 112 pecan germplasm.

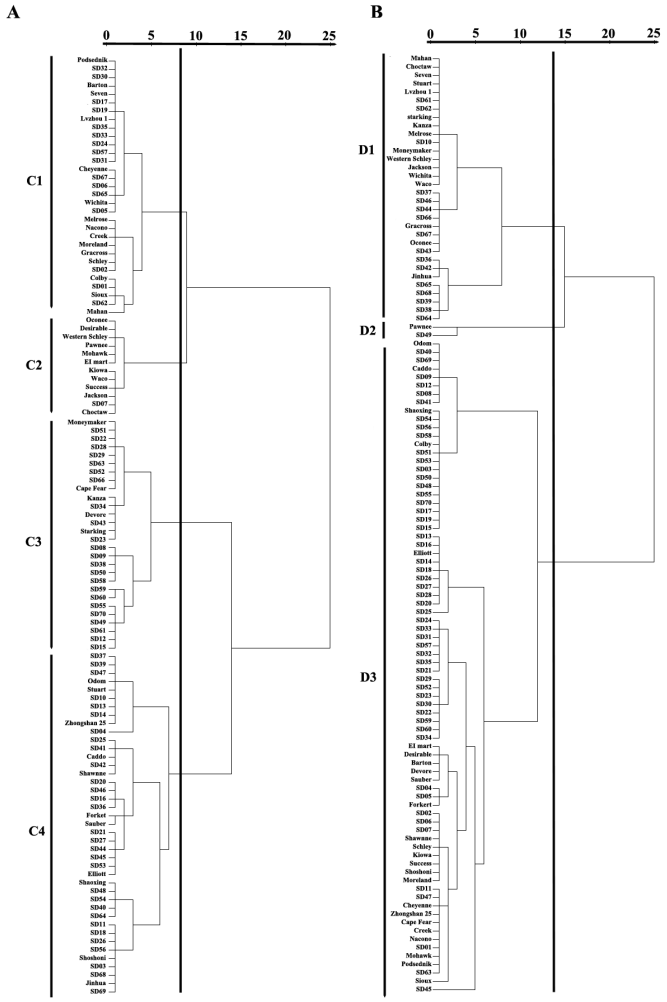

Figure 3

Dendrograms of 112 pecan germplasm based on the 10 nut quantitative traits (A) and 15 fatty acids contents (B) using the SPPS software.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplTableS1.xlsx
- suppltableS2.xlsx
- suppletableS3.xlsx