Assessment of Genetic Diversity Among Chinese High-Oleic Peanut Genotypes Using Miniature Inverted-Repeat Transposable Element Markers

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Short Report

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Abstract

As compared with normal-oleic peanut, high-oleic peanut proved to be heart-healthier, and had prolonged shelf life and extended seed longevity. However, there have concerns about the genetic diversity of present-day high-oleic peanut cultivars, which relied heavily on high-oleic donors with F435 type *FAD2* mutations. In the study, a total of 104 high-oleic peanut cultivars/lines/mutants from main breeding teams in China were used to assess their genetic diversity with *Ah*MITE markers. Of all the 31 cultivars tested, those from CTW team had the highest genetic variability. Again, of all the 73 lines studied, those from CTW team ranked first in genetic diversity. As compared with cultivars from CTW and C&Y teams, greater genetic diversity was detected in new lines of both teams, indicating that recent breeding efforts had resulted in improved genetic diversity in high-oleic peanut.

Introduction

High-oleic peanut is advantageous over its normal-oleic counterpart, having prolonged shelf life, extended seed longevity and several health benefits. Up to now, much progress has been made in breeding high-oleic peanut cultivars. In China, related programs were mainly carried out at Shandong Peanut Research Institute (SPRI), Kaifeng Academy of Agriculture and Forestry (KFAAF) and Hebei Academy of Agriculture and Forestry Sciences (HBAAFS). Some high-oleic peanut cultivars were developed by breeders from Henan Academy of Agricultural Sciences (HNAAS) and Chinese Academy of Agricultural Sciences (CAAS) Oil Crops Research Institute (OCRS) (Wang and Zhu 2017).

As far as we know, only four research papers (in Chinese) on high-oleic peanut genetic diversity have been published, using high-oleic peanut genotypes with F435 type *FAD2* mutations (G448A in *FAD2A*, 442insA in *FAD2B*). Hu et al. (2013) utilized 12 SSR (simple sequence repeat) primer pairs in the analysis of 5 high-oleic peanut genotypes along with a normal-oleic cultivar (Huayu 22). Similarity coefficient was found to be 0.325–0.750, averaging 0.655. Yu et al. (2017) used 31 SSR primer pairs to assess the genetic diversity of 41 high-oleic peanut cultivars/lines. Similarity coefficient varied from 0.333 to 0.889, with an average of 0.626. In the study by Wang et al. (2020), genetic distance of 25 high-oleic peanut cultivars/lines, as revealed by 140 SSR primer pairs, ranged from 0.057 to 0.624, with a mean value of 0.451. Guo et al. (2020) used 217 SSR primer pairs to study the genetic diversity of 8 high-oleic peanut cultivars bred at KFAAF and found that similarity coefficient was 0.3438–0.9688, with a mean value of 0.6149.

As the output of continuous breeding efforts, more and more high-oleic peanut cultivars, lines and mutants are currently available. Under such conditions, there still have concerns about the genetic diversity of present-day high-oleic peanut cultivars, which relied heavily on F435 type high-oleic donors.

The aim of this study was to compare the degree of genetic diversity in high-oleic peanut cultivars/lines from different teams in China, and to find out if gene basis for high-oleic peanut has been broadened by

recent breeding efforts, through assessment of the levels of genetic diversity in representative high-oleic peanut genotypes.

Material And Methods

Peanut material

Totally 104 high-oleic peanut cultivars/lines/mutants from main breeding teams in China were used in the study. These included 12 cultivars from CTW, 3 cultivars from C&Y, 6 cultivars from JZG, 3 cultivars from YRL, 7 cultivars from other teams, 32 lines from CTW, 16 lines from C&Y, and 10 mutants from CTW (Table 1).

Table 1
High-oleic peanut genotypes used in this study and their sources

High-oleic cultivar/line	Source	High-oleic cultivar/line	Source
Huayu 961	CV-CTW	20L19	L-CTW
Huayu 962	CV-CTW	20L17	L-CTW
Huayu 963	CV-CTW	20L15	L-CTW
Huayu 964	CV-CTW	20L16	L-CTW
Huayu 965	CV-CTW	20L18	L-CTW
Huayu 966	CV-CTW	20L24	L-CTW
Huayu 661	CV-CTW	20L23	L-CTW
Huayu 662	CV-CTW	20L86	L-CTW
Huayu 663	CV-CTW	20L104	L-CTW
Huayu 664	CV-CTW	20L87	L-CTW
Huayu 666	CV-CTW	20L114	L-CTW
Huayu 667	CV-CTW	20L125	L-CTW
Huayu 917	CV-C&Y	20L123	L-CTW
Huayu 32	CV-C&Y	20L129	L-CTW
Huayu 910	CV-C&Y	20L127	L-CTW
Kainong 301	CV-JZG	20L124	L-CTW
Kainong 61	CV-JZG	20L111	L-CTW
Kainong 71	CV-JZG	20L163	L-CTW
Kainong 176	CV-JZG	20L162	L-CTW
Kainong 1715	CV-JZG	20L168	L-CTW
Kainong 1768	CV-JZG	20L192	L-CTW
Jihua 11	CV-YRL	20S18	L-CTW
Jihua 16	CV-YRL	20S80	L-CTW
Jihua 18	CV-YRL	20S85	L-CTW

Note: CV = cultivar, L = line, M = mutant, CTW = Dr. Chuan Tang Wang team, SPRI, C&Y = Dr. Xiao Yuan Chi and Shan Lin Yu team, SPRI, JZG = Dr. Jian Zhong Gu team, KFAAF, YRL = Dr. Yu Rong Li team, HBAAFS, BSL = Dr. Bo Shou Liao team, OCRS, CAAS, DWZ = Mr. Dian Wen Zhang team, Rizhao, Shandong, JC = Dr. Jing Chen team, SPRI, XYZ = Dr. Xin You Zhang team, HNAAS.

High-oleic cultivar/line	Source	High-oleic cultivar/line	Source
Zhonghua 24	CV-Other-BSL	20S77	L-CTW
Rihua OL1	CV-Other-DWZ	20S78	L-CTW
Rihua OL2	CV-Other-DWZ	Huayu 618	L-C&Y
Huayu 51	CV-Other-JC	Huayu 9118	L-C&Y
Huayu 52	CV-Other-JC	Huayu 9121	L-C&Y
DF05	CV-Other-XYZ	Huayu 9128	L-C&Y
Yuhua 37	CV-Other-XYZ	Huayu 9130	L-C&Y
Huayu 960	L-CTW	Huayu 9131	L-C&Y
Huayu 967	L-CTW	Huayu 617	L-C&Y
Huayu 968	L-CTW	Huayu 9115	L-C&Y
Huayu 969	L-CTW	Huayu 9116	L-C&Y
Huayu 660	L-CTW	Huayu 9117	L-C&Y
Huayu 665	L-CTW	Huayu 9118	L-C&Y
Huayu 668	L-CTW	Huayu 9119	L-C&Y
Huayu 669	L-CTW	Huayu 9121	L-C&Y
Huayu 9620	L-CTW	Huayu 9123	L-C&Y
Huayu 9621	L-CTW	Huayu 9124	L-C&Y
Huayu 9622	L-CTW	Huayu 9125	L-C&Y
Huayu 9623	L-CTW	CTWE	Mutant-CTW
Huayu 9624	L-CTW	FB4	Mutant-CTW
Huayu 9625	L-CTW	20L74	Mutant-CTW
Huayu 6620	L-CTW	20L174	Mutant-CTW
20L2	L-CTW	ADHO	Mutant-CTW
20L4	L-CTW	20HES-18	Mutant-CTW
20L3	L-CTW	15L46HOA	Mutant-CTW

Note: CV = cultivar, L = line, M = mutant, CTW = Dr. Chuan Tang Wang team, SPRI, C&Y = Dr. Xiao Yuan Chi and Shan Lin Yu team, SPRI, JZG = Dr. Jian Zhong Gu team, KFAAF, YRL = Dr. Yu Rong Li team, HBAAFS, BSL = Dr. Bo Shou Liao team, OCRS, CAAS, DWZ = Mr. Dian Wen Zhang team, Rizhao, Shandong, JC = Dr. Jing Chen team, SPRI, XYZ = Dr. Xin You Zhang team, HNAAS.

High-oleic cultivar/line	Source	High-oleic cultivar/line	Source
20L5	L-CTW	20L185	Mutant-CTW
20L12	L-CTW	15L46HOB	Mutant-CTW
20L10	L-CTW	20A18M5N2	Mutant-CTW
Note: CV = cultivar, L = line, M = mutant, CTW = Dr. Chuan Tang Wang team, SPRI, C&Y = Dr. Xiao Yuan Chi and Shan Lin Yu team, SPRI, JZG = Dr. Jian Zhong Gu team, KFAAF, YRL = Dr. Yu Rong Li team, HBAAFS, BSL = Dr. Bo Shou Liao team, OCRS, CAAS, DWZ = Mr. Dian Wen Zhang team, Rizhao, Shandong, JC = Dr. Jing Chen team, SPRI, XYZ = Dr. Xin You Zhang team, HNAAS.			

Primer pairs

38 *Ah*MITE (Miniature Inverted-repeat Transposable Element) primer pairs producing discernable, reproducible and polymorphic bands were utilized to study the genetic diversity among the populations and groups (Table 2, Table 3).

Table 2
List of the 38 *Ah*MITE primer pairs used

Primer name	Forward primer (5'→3')	Reverse primer (5'→3')
AhTE0001	aatgtccatggctccttgtc	accccatccttcagacaca
AhTE0004	tgaggctcacctcacatcac	gcactacgcctatgctgtca
AhTE0007	ccaacttgttttatttcatttcct	ttgtcggatttcaaattatttct
AhTE0010	gccgtgtcattccacaaag	tggacaatgctgtttgtcgt
AhTE0016	tgctgctgttatgaagaca	accgacagcctctagcgatt
AhTE0017	tccgccaagaagcatagact	caccggtgagtagcctttctctt
AhTE0018	agggtgcctagccttgtttt	ggagaagctctccactgacg
AhTE0019	ccttgctccttcttggtgtca	aaaagcttgctggagggtga
AhTE0020	ttcatcatcaaccctaactattttg	tgcttggtgatgattgaaaaa
AhTE0021	agtcccaagtggacaccaag	ttagaccaattcatgtaagggtcac
AhTE0023	ctgcactgctccaaattcaa	tcaaccgaaggacaaatcaa
AhTE0024	acagtacaccacaccacga	cgtcctcttgcttctgctct
AhTE0025	ttctccctgctctccgtaaa	cgaatcttcattttattaggtgatgg
AhTE0028	cgatcaattcgggtcggtttt	cctgcagctccaatttaatca
AhTE0031	tcaaggctgctttacttttgc	ctgagccctttggtgttgat
AhTE0033	aaatgtccgcagacaaaaat	gaatgccttaagccagggtg
AhTE0035	tctgcgcaaaattatgagatt	ccggggattcattgttacc
AhTE0036	acccgacgttggaataagt	acgactttgcgagttcgttt
AhTE0038	agtcaaactcgaaggcaciaa	cgattatattccggccaagg
AhTE0039	aaaccgatccatgaaagtgc	aaggggatttctccaagcat
AhTE0046	tcccaccttttcagtttagca	tggtgccactcaagttgtct
AhTE0047	aaaattcggtcatgacctaaagca	gggtgccgttcaaaagttgat
AhTE0048	ctatcccttcaccacatgc	caattgtgtttcttgactgggtg
AhTE0049	atcacgccacctccttetta	ccttaaggggggtcattccat
AhTE0055	aaattacatggaagaccataattgc	agaatcatattcatatgtgagcaaa
AhTE0056	tggcaatttcagatcatcca	gtcccaccagattccagaga
Source: http://marker.kazusa.or.jp/Peanut/marker/list/		

Primer name	Forward primer (5'→3')	Reverse primer (5'→3')
AhTE0057	gctgcttcgctcttgatttc	ggaaaaggggttttcaaagg
AhTE0059	cgatctgccaatttcaaacc	tggcattgctgtctgtgct
AhTE0060	gggtgttatgctgcatgggt	tcagctcaaatacgtggaatg
AhTE0067	cgttgcttgaattgcagatg	gccctctcaataccgacttg
AhTE0089	tcgggggtcaaatttcaagag	ccatcctggatcagaatttca
AhTE0091	gccatggattgcatttggtt	caatttcacatcggttgctg
AhTE0101	ggggatgaattctcctacca	aaaatttcttttatggtaacctatgc
AhTE0463	accgtatgagtggcttgctt	ccgcatcaatttcaggagt
AhTE0615	tgtcctcccaagtttcggt	cccgttcccaaccttaaaat
AhTE0660	aaagatttcgccacgtgtatc	taaccggtttaattccgacg
AhTE0696	ctttgtttgttagccacacttt	cggatattgccgaatgctta
AhTE0797	acccttcatactgtgcctg	tgtggccaaattaataaatcaca
Source: http://marker.kazusa.or.jp/Peanut/marker/list/		

Table 3

Statistical analysis of genetic diversity in high-oleic peanut from different breeding teams in China

Group/population	Sample size	na	ne	h	i	np	pp	G _{st}
CV-CTW + CV-C&Y	15	1.8933	1.4760	0.2884	0.4392	67	89.33	0.2640
CV-CTW	12	1.6800	1.4221	0.2461	0.3663	51	68.00	
CV-C&Y	3	1.5200	1.3486	0.2010	0.2969	39	52.00	
CV-JZG + CV-YRL + CV-Other	16	1.8267	1.4227	0.2615	0.4017	62	82.67	0.1826
CV-JZG	6	1.6267	1.3508	0.2096	0.3178	47	62.67	
CV-YRL	3	1.5200	1.3416	0.1985	0.2942	39	52.00	
CV-Other	7	1.6667	1.3855	0.2285	0.3442	50	66.67	
L-CTW + L-C&Y + Mu-CTW	73	2.0000	1.4567	0.2839	0.4410	75	100.00	0.1127
L-CTW	47	1.9467	1.4432	0.2730	0.4211	71	94.67	
L-C&Y	16	1.7867	1.4256	0.2468	0.3724	59	78.67	
Mu-CTW	10	1.6667	1.3706	0.2215	0.3357	50	66.67	
Total	104	2.0000	1.4665	0.2910	0.4518	75	100.00	0.0540
Note: na = observed number of alleles, ne = effective number of alleles, h = Nei's (1973) gene diversity, i = Shannon's information index, np = number of polymorphic loci, pp = percentage of polymorphic loci.								

DNA extraction, PCR and electrophoresis

DNA was extracted from cotyledonary slices of peanut seeds according to a previously reported protocol (Yu et al. 2010). A 20 µl of PCR mixture consisted of 2 X *Taq* PCR Master Mix 10µl (Tiangen Biotech, Beijing), forward and reverse primers 1µl each (10 µM), template DNA 1 µl, and sterile double distilled water 7 µl. Thermal cycling profile was 5 min at 94°C for pre-denaturation, 35 cycles of 94°C denaturation for 30 sec, 55°C annealing for 30 sec, 72°C extension for 40 sec, followed by a final extension step of 5 min. PCR was performed on a thermocycler (Model Tc-s-B, Bioer, Inc.), and amplification products were resolved on 1% (w/v) agarose gels with GelGreen dye (Biotium, California) under 1 X TAE buffer, 120 V and 20 min electrophoresis conditions.

Statistical analysis

8 populations inclusive of CV-CTW, CV-C&Y, CV-JZG, CV-YRL, CV-Other, L-CTW, L-C&Y, and Mu-CTW and 3 groups including CV-CTW + CV-C&Y, CV-JZG + CV-YRL + CV-Other, and L-CTW + L-C&Y + Mu-CTW were used in genetic diversity analysis (Table 3).

Each polymorphic band was manually scored as present (1) or absent (0) for each individual peanut samples. Observed number of alleles, effective number of alleles, Nei's (1973) gene diversity, Shannon's information index, number of polymorphic loci, and percentage of polymorphic loci were calculated using the Popgene 1.31 software (Yeh et al. 1999). Analysis of molecular variance (AMOVA) was conducted using GenAlex (Peakall and Smouse 2012). Cluster analysis of populations and genotypes was done with Popgene 1.31 software using Nei's (1972) genetic distance and UPGMA (unweighted pair group method with arithmetic mean) method and DPS 14.50 package using Jacard distance and flexible group average cluster method (Tang and Zhang 2013), respectively. Similarity coefficient was calculated with NTSYSpc 2.10e (Applied Biostatistics, Inc., 2000).

Results And Analysis

AhMITE polymorphism

38 AhMITE primer pairs generated a total of 75 polymorphic loci (1.97 polymorphic loci per primer pair). Among the 8 populations, the mean percentage of polymorphic loci (pp), Nei's (1973) gene diversity (h), and Shannon's Information index (i) were 100%, 0.2910 and 0.4518, respectively. Lines from CTW (CV-CTW) had the highest level of variability, with pp, h and i being 94.67%, 0.2730 and 0.4211, followed by lines from C&Y (L-C&Y), with pp, h and i being 78.67%, 0.2468, 0.3724, respectively. Cultivars from CTW ranked third (pp, h and i were 68.00%, 0.2461 and 0.3663). CV-Other and Mu-CTW were in the fourth and fifth position respectively. CV-JZG, CV-C&Y and CV-YRL had relatively low levels of variability (Table 3). Among the 3 groups, Group 3 (L-CTW + L-C&Y + Mu-CTW) and Group 1 (CV-CTW + CV-C&Y) possessed high levels of variability, and Group 2 (CV-JZG + CV-YRL + CV-Other) occupied the third position.

Genetic structure of populations

The coefficient of genetic differentiation (G_{st}) between the cultivars of CTW and C&Y teams were 0.2640 (26.40% of total variation resided between, and 73.60% within the 2 populations) (Table 3). Among the cultivars from JZG, YRL and other teams, G_{st} was 0.1826. Among the lines from CTW and C&Y and mutants from CTW team, G_{st} was 0.1127. G_{st} between the 3 above-mentioned groups was 0.0540, indicating that merely 5.40% genetic variation resided between these groups (Table 3).

Likewise, when the 8 populations were used in AMOVA, the results revealed that of the total genetic diversity, 9.2% was attributed to among population diversity and the rest (90.8%) to within populations (Table 4). Despite that among population variation was significant, the vast majority of *AhMITE* variation can be explained by differences within populations.

Table 4
AMOVA of 104 peanut genotypes of 8 populations using 38 *Ah*MITE primer pairs

Source of variation	degree of freedom	Sum of squares	Variance component	% total variance
Among populations	7	157.799	1.082	9.2%**
Within populations	96	1021.557	10.641	90.8%
Total	103	1179.356	11.724	100%
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As shown in Fig. 1, high-oleic peanut lines and mutants from CTW team grouped together, and cultivars from YRL and JZG teams also had a close relationship. Cultivars from C&Y team were distantly related to other genotypes, however (Fig. 1). As compared with cultivars and lines from C&Y team, cultivars from CTW team were more closely related to the rest genotypes (Fig. 1).

Genotype grouping

Based on *Ah*MITE profiling, the 104 high-oleic peanut genotypes fell into two categories (I and II). Each category was further divided into two sub-categories (I-a, I-b, II-a, and II-b) (Fig. 2). Most of the lines from C&Y team were found in category I, whereas all the cultivars and most of the lines from CTW team were found in category II. All the cultivars from CTW team were in II-b, and 5 of the 6 cultivars from JZG team were in I-b. The mutants from CTW team could be found in 3 of the 4 sub-categories (3 in I-b, 5 in II-a, and 2 in II-b). Cultivars from YRL team and lines from CTW team were in 3 of the 4 sub-categories (Fig. 2). Lines from CTW and C&Y teams were more genetically diversified than cultivars from both teams.

Conclusions And Discussion

Genetic similarity coefficient of the 104 high-oleic peanut genotypes in the present study, as calculated by NTSYSpc 2.10e ranged from 0.4400 to 0.9333, with a mean value of 0.6953 (detailed data unshown), comparable to that in a previous study using *Ah*MITE markers to analyze 115 peanut cultivars/lines (mean value = 0.6902) (Wang et al. 2013). In earlier reports on high-oleic peanut, where only SSR markers were exploited, the minimum similarity coefficient was in the range of 0.3250–0.3438, and the average similarity coefficient ranged from 0.6149–0.6550 (Hu et al. 2013; Yu et al. 2017; Guo et al. 2020). However, in contrast to co-dominant markers, dominant markers tended to underestimate genetic diversity (Qian and Ge 2001), thus making comparison between level of genetic diversity in the present study using *Ah*MITE markers with that in the previous studies using SSR markers difficult. But in the present study, it was still possible to assess genetic diversity of high-oleic peanut genotypes from representative breeding teams at gene, population and group levels.

In this study, of all the 8 populations, lines from CTW ranked first in genetic variability, followed by lines from C&Y, and cultivars from CTW occupied the third position. CTW team and C&Y team have succeeded in broadening the gene base in high-oleic peanut, as new lines from both teams had higher levels of genetic diversity. Over 90% of the total genetic diversity could be ascribed to within population diversity. Like the situation in the 8 populations, as shown by G_{st} of individual groups, for each group, within populations variation predominated (over 70% of total).

With the identification/creation of new high-oleic peanut mutants in CTW team (Nkuna et. 2021; Wang and Zhu 2017), more and more natural and induced mutants were used in breeding. Also, more exotic germplasm lines were used in hybridization in the development of these lines. These were the reasons why the lines from CTW team had the highest genetic diversity and why the lines and mutants from the team had a close relationship. Since all of the 3 cultivars from YRL used the same high-oleic peanut donor (Kaixuan 01–6) from JZG team (Wang and Zhu 2017), cultivars from YRL and JZG were also closely related.

To summarize, much variability resided within populations in this study. Of the high-oleic peanut cultivars, those from CTW team had the highest genetic variability. Of all the high-oleic peanut lines tested, those from CTW team ranked first in genetic diversity. As compared with high-oleic cultivars from CTW and C&Y teams, greater genetic diversity was detected in new lines of both teams, indicating that recent breeding efforts were effective in improving genetic diversity of high-oleic peanut.

Declarations

Conflict of interest All of the authors of “Assessment of genetic diversity among Chinese high-oleic peanut genotypes using Miniature Inverted-Repeat Transposable Element Markers” here declare that there is no conflict of interest.

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Figures

Figure 1

Dendrogram of 8 populations using Nei's (1972) genetic distance and UPGMA method

Figure 2

Dendrogram of 104 high-oleic peanut genotypes using Jacard distance and flexible group average cluster method