Analysis of DNA Polymorphism and Genetic Relationships in Cultivated Peanut (Arachis hypogaea L.) Using Microsatellite Markers

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Abstract   Eleven SSR primer pairs were used to amplify the genomic DNA isolated from 24 peanut genotypes including all 4 market types. Four primers could detected polymorphism and produced a total of 33 alleles. The average number of alleles per locus was 8.25 , and up to 13 alleles were found at one locus. The genetic similarity (GS) indexes among 24 cultivated peanut accessions were calculated based on the data from these SSR markers. The value of GS was varied from 0.2 to 1.0 , with an average of 0.4788. The results of UPGMA indicated that the majority of 24 accessions could be divided into two groups by subspecies , and most genotypes were clustered by market types. It is no doubt that microsatellites are very useful DNA markers to analyze DNA polymorphism and genetic relationship in cultivated peanut.

Key words   Microsatellites ; Peanut ; Polymorphism ; Genetic relationship

SSR

1   2 , *   3   1   1 *

([1]   ,   530007 ;[2]   ,   530007 ;[3]
    ,   530005)

11   SSR   24   (   )   PCR   ,   4   ,
33   ,   5 13 ,   8.25   24
21   0.2 1.0 ,   0.4788   UPGMA   ,
                    (   ) ;   ,   ,SSR
          DNA
SSR ;   ;   ;
    : S565

Cultivated peanut or groundnut (*Arachis hypogaea* L.) is an important crop for oil and protein source. In China , two major market types are widely grown , Virginia in the North and Spanish in the South. The other two types , Valencia and Runner are rarely scattered across small area. Despite the existence of substantial diversity among cultivated peanut genotypes for various morphological , physiological and agronomic traits , very little DNA variations have been detected by using protein- or DNA-based markers[1 5]. However , recent studies showed that AFLP , RAPD and SSR markers could detect considerable polymorphism in a limited number of accessions of cultivated peanut[6 14] , which hold a promise for marker-based genetic improvement of peanut , as in most other crops.

Microsatellites , also known as simple sequence repeats or SSRs , are small array of one to six tandemly arranged bases spread throughout the genomes. Microsatellites as markers are advantageous over many other markers mainly because they are highly polymorphic , highly abundant , co-dominant inheritance , analytical simple and readily transferable. SSRs have now been recommended

as standard markers to be used in the construction of highly saturated maps , and in some cases , in marker-assisted selection. Level of polymorphism detected by microsatellites is generally high in almost all plants assayed. In peanut , however , SSR markers were not available until He *et al*. succeeded in developing SSR primers in large scale through microsatellite-enriched library[15] . The objectives of this study were to detect DNA variation in Chinese peanut germplasm and to study genetic relationship among closely-related cultivated peanut genotypes using their developed SSR markers.

1　Materials and Methods

1.1　Plant materials

　　Twenty-four peanut genotypes representing four market types from the bank of Guangxi peanut germplasm were used for the analysis. Among them , five were breeding lines , 11 were local germplasms and 8 were introduced accessions (Table 1) . Genomic DNA was extracted from leaves of the 15-day-old seedlings by a modified CTAB method.

Table 1　Peanut accessions used for study

No.	Accession	Origin	Type	No.	Accession	Origin	Type
1	BC$_2$-420-21	Guangxi , China	Spanish	13	Lipu fanguidou (　　)	Guangxi , China	Virginia
2	Xie kang qing (　　)	Hubei , China	Spanish	14	Pingguo da huasheng (　　)	Guangxi , China	Virginia
3	Hei huasheng (　　)	Hubei , China	Spanish	15	Baise da huasheng (　　)	Guangxi , China	Virginia
4	Guang liu (　)	Guangxi , China	Spanish	16	Yangshuo fuli yangdou (　　)	Guangxi , China	Virginia
5	BC$_2$-454-20	Guangxi , China	Spanish	17	Parapoto	India	Virginia
6	5084	Guangxi , China	Spanish	18	Miandian	Burma	Virginia
7	Xiao huasheng (　　)	Guangxi , China	Runner	19	NCAC17090	India	Valencia
8	Ningming wuqu zhihang (　　)	Guangxi ,China	Runner	20	PI341879	USA	Valencia
9	Beiliu qindou (　　)	Guangxi ,China	Runner	21	Tifrust-12	USA	Valencia
10	Long' an baowan huasheng (　　)	Guangxi , China	Runner	22	Rwanda huasheng (　　)	Rwanda	Valencia
11	Mashan hequn huasheng (　　)	Guangxi , China	Runner	23	Tifrust-6	USA	Valencia
12	Shanglin tangjiang huasheng (　　)	Guangxi , China	Runner	24	ICG1703	India	Valencia

1.2　PCR and electrophoresis

　　SSR primers were developed and kindly provided by Dr. Guohao He (Tuskegee University , USA) . PCR amplification was performed in 10 μL reaction mixture containing 1 unit of *Taq* DNA-polymerase , 10 × PCR buffer containing 1. 5 mmol/L MgCl$_2$, 100 μmol/L of each dNTPs , 1. 5 pmol/L of forward and reverse primers and 50 ng of genomic DNA. Amplification was carried out on a Biometra *T1* Thermocycler using the following program : an initial denaturation of 4 min at 94　, 30 cycles of 1 min at 94　, 1 min at 55　, 2 min at 72　, and final extension at 72　for 10 min. The PCR products were separated by 6 % denatured polyacrylamide gel electrophoresis and visualized by silver staining.

1.3　Data Analysis

　　Genetic similarity (*GS*) was calculated according to the formula : $GS_{ij} = 2N_{ij}/(N_i + N_j)$, where N_{ij} was the band number that appeared in both genotypes i and j. N_i and N_j were the total band number of genotypes i and j , respectively. Genetic diversity (*GD*) was calculated as : $GD_{ij} = 1 - GS_{ij}$. Clustering analysis was carried out according to unweighted pair group method arithmetic averages (UPGMA) by using SAS System Software.

2　Results and Discussion

2.1　Polymorphism among cultivated peanut detected by SSR markers

　　Eleven SSR primers were screened and four of them were able to detect 33 alleles among 24 peanut genotypes. For the four polymorphic loci , a range of 5 - 13 alleles were obtained , with an average of 8. 25 per locus (Table 2) . One primer pair , PM-36 , detected as many as 13 al-

leles among the cultivated peanut with diversified origin （Fig. 1）. Even within the same market type , DNA fingerprints were different （Fig. 2）. By employing the four polymorphic markers , 21 out of the 24 peanut genotypes could be clearly differentiated. The results indicated that the number of alleles detected by peanut SSR primers could be comparable to many other plants , such as soybean , rice , barley and cucumber , and microsatellites could be one of the most powerful tools for differentiating closely-related peanut cultivars. However , more than half of the primers failed to detect any polymorphism , while these primers could identify genetic variation among the

peanut accessions collected from worldwide[15] , which indicated the genetic base of Chinese peanut germplasm was narrow. More informative SSR markers are apparently needed to be developed for the construction of highly saturated maps and marker-assisted selection in cultivated peanut.

Table 2　　Number of alleles detected by four SSR primers

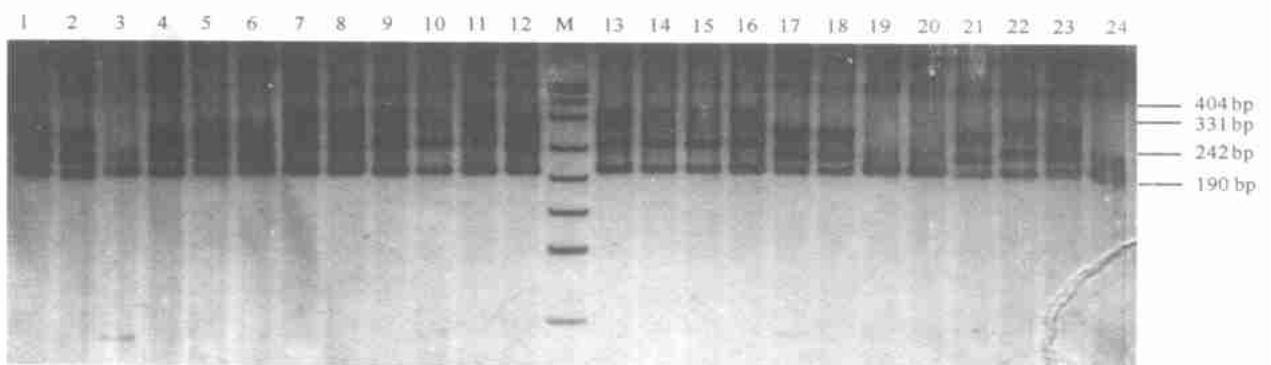| Primer	Annealing temperature	Number of alleles detected
PM-50	55	5
PM-15	55	7
PM-36	55	13
PM-31	52	8

Fig. 1　　Profile of amplification products from genomic DNAs of 24 genotypes using SSR primer PM-36
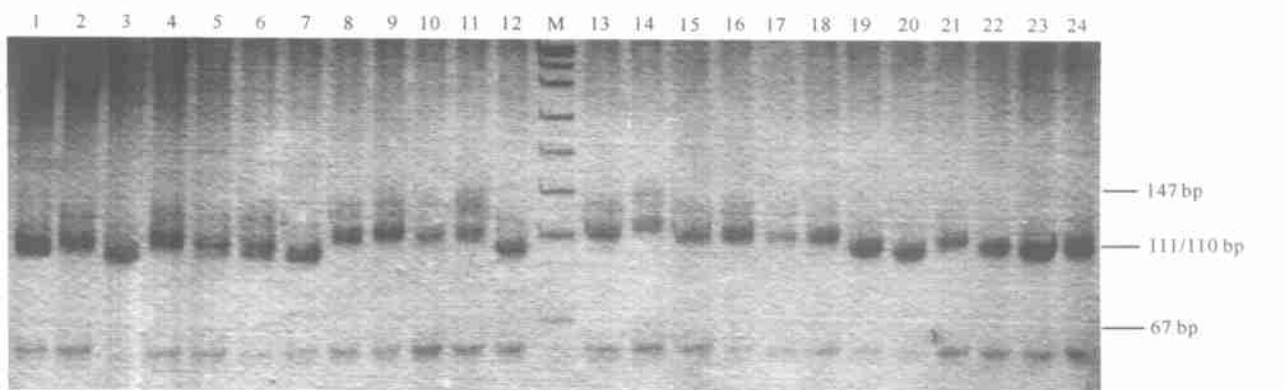

Fig. 2　　Profile of amplification products from genomic DNAs of 24 genotypes using SSR primer PM-50

2. 2　Genetic similarity and cluster analysis

　　GS values among 24 genotypes based on SSR analysis ranged from 0. 2 to 1. 0 （Table 3）, with an average of 0. 48. Genotype 13 , 15 and 16 had the highest values of *GS* （ *GS* = 1） between each other , and all of

them were Virginia. The lowest genetic similarity showed between genotype 23 vs. 7 , 9 , 13 , 14 , 15 and 16 respectively with *GS* value of 0. 2 , where genotype 23 is of ssp. *fastigiata* Waldron but the other accessions are ssp. *hypogaea* .

Table 3   Genetic similarity among 24 accessions based on SSR analysis

Material	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1																							
2	0.381	1																						
3	0.700	0.526	1																					
4	0.818	0.476	0.700	1																				
5	0.800	0.421	0.556	0.800	1																			
6	0.667	0.600	0.632	0.667	0.842	1																		
7	0.400	0.211	0.444	0.400	0.444	0.316	1																	
8	0.696	0.364	0.667	0.696	0.571	0.455	0.667	1																
9	0.400	0.211	0.333	0.400	0.444	0.316	0.889	0.381	1															
10	0.500	0.316	0.444	0.500	0.556	0.421	0.778	0.286	0.889	1														
11	0.696	0.455	0.667	0.696	0.571	0.455	0.476	0.583	0.571	0.667	1													
12	0.400	0.211	0.333	0.300	0.444	0.421	0.667	0.286	0.667	0.556	0.381	1												
13	0.300	0.316	0.333	0.300	0.333	0.316	0.667	0.286	0.778	0.667	0.571	0.667	1											
14	0.300	0.316	0.333	0.300	0.333	0.316	0.667	0.286	0.667	0.556	0.476	0.667	0.889	1										
15	0.300	0.316	0.333	0.300	0.333	0.316	0.667	0.571	0.778	0.667	0.571	0.667	1.000	0.889	1									
16	0.300	0.316	0.333	0.300	0.333	0.316	0.667	0.571	0.778	0.667	0.571	0.667	1.000	0.889	1.000	1								
17	0.696	0.273	0.571	0.783	0.667	0.545	0.286	0.667	0.381	0.476	0.667	0.190	0.286	0.190	0.286	0.286	1							
18	0.571	0.300	0.421	0.667	0.737	0.600	0.316	0.273	0.421	0.526	0.545	0.211	0.316	0.211	0.316	0.316	0.909	1						
19	0.526	0.444	0.471	0.632	0.588	0.444	0.353	0.400	0.353	0.471	0.500	0.235	0.235	0.235	0.235	0.235	0.700	0.778	1					
20	0.800	0.316	0.667	0.700	0.667	0.526	0.333	0.667	0.333	0.444	0.667	0.333	0.222	0.222	0.222	0.222	0.762	0.632	0.706	1				
21	0.556	0.353	0.500	0.667	0.750	0.706	0.375	0.632	0.500	0.625	0.526	0.375	0.500	0.375	0.500	0.500	0.632	0.706	0.400	0.375	1			
22	0.353	0.375	0.400	0.471	0.400	0.375	0.267	0.333	0.267	0.400	0.333	0.267	0.267	0.267	0.267	0.267	0.333	0.375	0.571	0.400	0.462	1		
23	0.636	0.381	0.700	0.545	0.500	0.571	0.200	0.174	0.200	0.300	0.522	0.300	0.200	0.200	0.200	0.200	0.435	0.286	0.316	0.600	0.333	0.471	1	
24	0.700	0.421	0.778	0.600	0.556	0.632	0.222	0.571	0.222	0.333	0.571	0.333	0.222	0.222	0.222	0.222	0.571	0.421	0.471	0.778	0.375	0.800	0.800	1

Dendrogram constructed from SSR data showed that 24 peanut accessions were divided into two clusters Ⅰ and Ⅱ distinctly, corresponding to the two subspecies *hypogaea* and *fastigiata*, with exception of 4 genotypes (Fig. 3). Cluster Ⅰ contained 8 peanut accessions which belong to ssp. *hypogaea*, and most of them were formed into two subgroups (Ⅰ‐1 and Ⅰ‐2) by botanical varieties. The average genetic diversity (*GD*) was similar in subgroup Ⅰ‐1 and subgroup Ⅰ‐2, with the value of 0.167 and 0.148, respectively. Cluster Ⅱ consisted of 16 accessions, 12 of them from ssp. *fastigiata* and the others from ssp. *hypogaea*. The accessions in this cluster could also be divided into two subgroups (Ⅱ‐1 and Ⅱ‐2), which primarily complied with their botanical types. Five of all 6 Spanish accessions were in subgroup Ⅱ‐1 and half of the Valencia accessions were in Ⅱ‐2. Comparing with the cluster Ⅰ, the average genetic diversity (*GD*) value in cluster Ⅱ was higher, with an average value of 0.4405. This was probably because that the five breeding lines from hybridization, 8 introduced accessions from foreign countries included in this cluster might be far related.
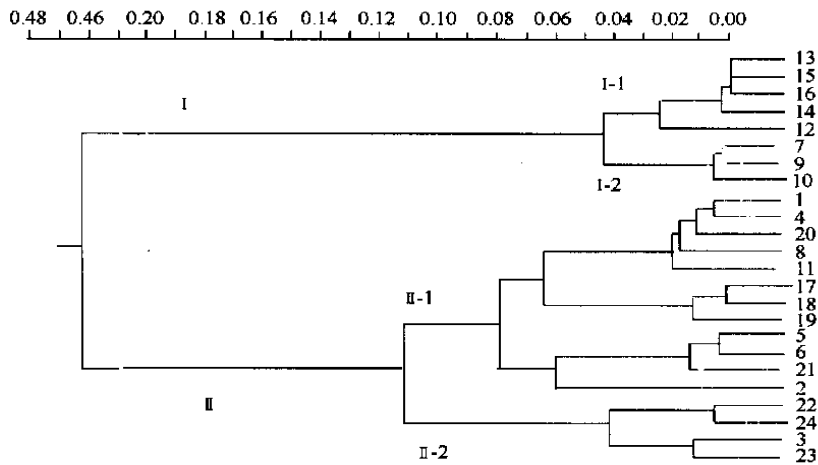

Fig. 3   Dendrogram showing the genetic relations among 24 cultivated peanut genotypes

The results indicated that SSRs were very effective DNA markers on genetic similarity and cluster analysis in cultivated peanut. By using 4 primer pairs, 24 accessions could be divided into two clusters and four groups corre‐

sponding to two subspecies and four market types. In con-
clusion , the genetic relationships between accessions can
be revealed by SSR markers. More SSR markers should
be developed , not only for diversity studies , but also for
high-resolution genetic mapping , evolutionary studies and
marker-assisted selection in peanut.

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