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Genetic diversity of Chinese summer soybean germplasm revealed by SSR markers

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Abstract **There are abundant soybean germplasm in China. In order to assess genetic diversity of Chinese summer soybean germplasm, 158 Chinese summer soybean accessions from the primary core collection of *G. max* were used to analyze genetic variation at 67 SSR loci. A total of 460 alleles were detected, in which 414 and 419 alleles occurred in the 80 Huanghuai and the 78 Southern summer accessions, respectively. The average number of alleles per locus was 6.9 for all the summer accessions, and 6.2 for both Huanghuai and Southern summer accessions. Marker diversity (*D*) per locus ranged from 0.414 to 0.905 with an average of 0.735 for all the summer accessions, from 0.387 to 0.886 with an average of 0.708 for the Huanghuai summer accessions, and from 0.189 to 0.884 with an average of 0.687 for the Southern summer accessions. The Huanghuai and Southern summer germplasm were different in the specific alleles, allelic-frequencies and pairwise genetic similarities. UPGMA cluster analysis based on the similarity data clearly separated the Huanghuai from Southern summer soybean accessions, suggesting that they were different gene pools. The results indicate that Chinese Huanghuai and Southern summer soybean germplasm can be used to enlarge genetic basis for developing elite summer soybean cultivars by exchanging their germplasm.**

Keywords: Chinese summer soybean (*Glycine max* (L.) Merr), SSR markers, Genetic diversity.

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Soybean originated from China where the cultivation of soybean has a long history of more than 5000 years. Because of a long-term of natural and artificial selection, abundant soybean germplasm have been accumulated. So far, more than 23000 soybean accessions have been collected and conserved at the National Crop Germplasm Bank of China. However, Gai et al.[1] reported that out of the 308 ancestors, 38 had provided approximately 54.18% and 56.84% of nuclear and cytoplasmic genetic basis, respectively, of 651 soybean cultivars released during 1923 to 1995 in China, respectively. It indicated that the genetic basis of these released cultivars is rather narrow and there is much potential to enlarge the genetic basis for developing elite cultivars.

Simple sequence repeat (SSR) markers have been widely used in plant genetic diversity analysis, molecular mapping, gene tagging, and pedigree analysis because of their simplicity, co-dominance and abundance in plant genomes. In soybean, high levels of length polymorphism have been reported for a number of alleles[2—4]. So far, studies at the molecular level for genetic diversity of soybean germplasm have been mainly focused on Northern America improved cultivars and their ancestral introductions using SSR markers[5—7]. Only Abe et al.[8] analyzed genetic diversity of Asian soybean accessions using SSR markers. As the comprehensive resources of the original gene pool of the world, Chinese soybean accessions were only evaluated based on morphological and agronomic traits[9—11]. Genetic diversity analysis of these accessions at genomic DNA level has never been thoroughly and systematically conducted; therefore, it has been difficult for soybean breeders to utilize them.

Chinese soybean germplasm are classified into three ecotypes, named spring, summer and autumn according to their planting systems. Among them, summer soybean germplasm consists of Huanghuai summer and Southern summer soybean germplasm[12]. In this study, 158 Chinese Huanghuai summer and Southern summer accessions were selected from the primary core collection of *G. max* to determine the genetic diversity of Chinese summer soybean germplasm by 67 SSR markers for improving their conservation and utilization.

1 Materials and methods

(ⅰ) Plant materials. One hundred and fifty-eight Chinese summer soybean (*Glycine max* (L.) Merr) accessions selected from the primary core collection of *G. max* were used (Table 1). Most of them were landraces from different collection sites, and the rest were improved cultivars. Seeds were obtained from the National Crop Germplasm Bank of China.

(ⅱ) Genomic DNA preparation. To avoid the possibility of selecting a single contaminating seed, fresh young trifoliate leaf tissue from at least twenty plants per accession was bulked for genomic DNA preparation. DNA was extracted using the CTAB method[13].

(ⅲ) SSR loci selection. Sixty-seven SSR loci used in the study (Table 2) had been previously mapped on the integrated genetic linkage map of soybean[14]. These SSR loci were evenly distributed on 20 genetic linkage groups (LGs) of soybean with an average of 3 loci per LG, ranging from 2 to 6. The primer sequences of these SSR loci were obtained from the SoyBase, the USDA-ARS sponsored genome database (http://129.186.26.94/SSR.html).

(ⅳ) PCR amplifications and detections of alleles. PCR amplifications were performed in a volume of 20 μL containing 50 ng genomic DNA, 10 mmol/L

Table 1 Chinese Huanghuai and Southern summer soybean accessions used in the study

Ref No.	Name	Collection site	Ref No.	Name	Collection site
H01	Yilichuandahuangdou	Leting, Henan	N01	Nannong 493-1	Nanjing Agricultural College (former)
H02	7599	Baxia Institute of Agricultural Research of Zhangjiakou	N02	Sudou 1	Jiangsu Academic of Agricultural Research
H03	Pingdinghuang	Renqiu, Hebei	N03	Jinda 332	Jinling University (former)
H04	Pingdingkaibaihua	Quyang, Hebei	N04	Lishuizhongzihuangdouyi	Lishui,Jiangsu
H05	Xinhuangdou	Shandong Academic of Agricultural Research	N05	Chalukou 1	Institute of Agricultural Research of East China (former)
H06	Qihuangyihao	Shandong Academic of Agricultural Research	N06	Jiangdouwanqiudou	Jiangdu, Jiangsu
H07	Zaohuangsanhao	Yantai Institute of Agricultural Research	N07	Nantonghuangyoudou	Nantong, Jiangsu
H08	Yuejinsihao	Heze Institute of Agricultural Research	N08	Liuyuebao	Taihu, Anhui
H09	Wenfengqihao	Jining Institute of Agricultural Research	N09	Houzimao	Tongcheng, Anhui
H10	Yanhuang 1	Hanzhou, Shandong	N10	Pudongdahuangdou	Shanghai
H11	Pingdinghuang	Linju, Shandong	N11	Fengxiasuidaohuang	Fengxian, Shanghai
H12	Dalihuang	Fushan, Shandong	N12	Anluhonghuangdou	Anlu, Hubei
H13	Pingdingsi	Yexian, Shandong	N13	Wuchangdonghuangdou	Wuchang, Hubei
H14	Yiwohou	Pingdu, Shandong	N14	Huangpohouzimao	Huangpo, Hubei
H15	Pingdinghuang	Yidu, Shandong	N15	Tianmendazihuang	Tianmen, Hubei
H16	Gulihun	Boshan, Shandong	N16	Puqihuangsedou	Puqi, Hubei
H17	Liuyuexian	Pingyi, Shandong	N17	E'shizaobaihuangdou	Eshi, Hubei
H18	Wuyezi	Lunan, Shandong	N18	Wuchangqingpidou	Wuchang, Hubei
H19	Tian'edan	Mengyin, Shandong	N19	Zeiwuyao	Deqing, Zhejiang
H20	Pingdingwu	Zaozhuang, Shandong	N20	Maobaidou	Yuhang, Zhejiang
H21	Bayuezha	Laiwu, Shandong	N21	Dongdou	Tiantai, Zhejiang
H22	Diaosiguidouzi	Jiyang, Shandong	N22	Maodou	Sanmen, Zhejiang
H23	Liuyejian	Linqing, Shandong	N23	Baipi	Lanxi,Zhejiang
H24	Tiejiaopi	Shouzhang, Shandong	N24	Bayueba	Hangzhou, Zhejiang
H25	Dahuapi	Jining, Shandong	N25	Dakehuang	Xincheng, Guangxi
H26	Tiejiaohuang	Caoxian, Shandong	N26	1138-2	Nanjing Agricultural college (former)
H27	Luanchuanbayuezhabaidou	Lunchuan, Henan	N27	Qiyuezao	Wufeng, Hubei
H28	Linxiancaohuangdou	Linxian, Henan	N28	Lushuibai	Yunxian, Hubei
H29	Minquanniumaohuang	Minquan, Henan	N29	Chihuangdou-3	Fengdu, Sichuan
H30	Chenliuniumaohuang	Kaifeng, Henan	N30	Hepinghuangdou	Zigong, Sichuan
H31	Neihuangniumaohuang	Neihuang, Henan	N31	Huayaodou	Beichuan, Sichuan
H32	Wuzhihongmaohuang	Wuzhi, Henan	N32	Meizaodou	Xichang, Sichuan
H33	Biyangniumaohuang	Bi yang,Hen Biyang Henan	N33	Hongyanmao'erhui	Huidong, Sichuan
H34	Yongchengdahuangjianke	Yongcheng, Henan	N34	Daqingpidou	Jianyang, Sichuan
H35	Luyitian'edan	Luyi, Henan	N35	Hongdou	Xuyong, Sichuan
H36	Wangshanhou	Zhenba, Shanxi	N36	Huayaozi	Emei, Sichuan
H37	Shanzibai	Luoyang, Shanxi	N37	Tiandou (1)	Shengxian, Zhejiang
H38	Fengxianxihecao	Fengxian, Jiangsu	N38	Qiyuedou	Dexing, Jiangxi
H39	Peixiandabaijiao	Peixian, Jiangsu	N39	Baihuadouzi	Ningdu, Jiangxi
H40	Tongshantian'edan	Tongshan, Jiangsu	N40	Daqingsi	Shangrao, Jiangxi
H41	Pixianruantiaozhi	Pixian, Jiangsu	N41	Huarongchongyangdoujia	Huarong, Hubei
H42	Xinyihuangdou	Xinyi, Jiangsu	N42	Yueyangniumaohong	Yueyang, Hubei

(*to be continued on the next page*)

(*Continued*)

Ref No.	Name	Collection site
H43	Donghaibaihuacao	Donghai, Jiangsu
H44	Ganyuqishuiwangdou	Ganyu, Jiangsu
H45	Xudou 1	Xuzhou Institute of Agricultural Research
H46	Binhaidabaihuajia	Binhai, Jiangsu
H47	Guanyundasili	Guanyun, Jiangsu
H48	58-161	Jiangsu Academic of Agricultural Research
H49	Zaofeng 6	Institute of Genetics, CAS (former)
H50	Baxiandadou	Baxian, Hebei
H51	Ludou 2	Jining Institute of Agricultural Research,
H52	Ludou 4	Shandong Academic of Agricultural Research
H53	Yuejin 5	Heze Institute of Agricultural Research
H54	Kefeng 1	Zhengzhou, Henan
H55	Zhengzhou 135	Henan Academic of Agricultural Research
H56	Zheng 77249	Henan Academic of Agricultural Research
H57	Shuibaidou	Fengxian, Shanxi
H58	Bayuezha	Liantian, Shanxi
H59	Huichabayuezha	Zhen'an, Shanxi
H60	Nibadou	Langao, Shanxi
H61	Guoyangdajianke	Guoyang, Anhui
H62	Wuhedadou	Wuhu, Anhui
H63	Fuyang 335	Fuyang Institute of Agricultural Research
H64	Lingbixiaoyoudou	Lingbi, Anhui
H65	Suxianpingdingwu	Suxian, Anhui
H66	Daoshuhuang	Chuxian, Anhui
H67	Zhongdou 19 (83-19)	Institute of Oil Research, CAAS
H68	Zhonghuang 1	Institute of Crop Research, CAAS
H69	Qianjin 2	Cangxian, Hebei
H70	Shirengouzaohuangdou	Fengning, Hebei
H71	Huangben 13	Lingshou, Hebei
H72	Shizhuangdalihuang	Anping, Hebei
H73	Wangchengdabayuexian	Laixi, Shanxi
H74	Luoyehuang	Jimo, Shandong
H75	Bengjiehuang	Haiyang, Shandong
H76	Ludou 6	Weifang Institute of Agricultural Research
H77	Yudou 12	Henan Academic of Agricultural Research
H78	Zheng 133	Henan Academic of Agricultural Research
H79	Shanzibai	Ningshan, Shanxi
H80	Chuxiu	Huaiyin Institute of Agricultural Research

Ref No.	Name	Collection site
N43	Anhuachihuangdou (Jia)	Anhua, Hubei
N44	Baishuidou	Luodian, Guizhou
N45	Xiaohuangpidou-2	Shicheng, Guizhou
N46	Mimidou-13	Yanhe, Guizhoug
N47	Baishuidou-2	Yinjiang, Guizhou
N48	Huangdouzi-4	Yuping, Guizhou
N49	Xiaolvpidou-2	Liping, Guizhou
N50	Zaodou-3	Songtao, Guizhou
N51	Huangkewudou	Chengmai, Guangzhou
N52	Jiwodou	Yongfu, Guangxi
N53	Bayueqing	Nandan, Guangxi
N54	Jingxihuangdou 1	Jingxin, Guangxi
N55	Changpingdahuangdou	Mengshan, Guangxi
N56	Longzhouhuangdou	Longzhou, Guangxi
N57	Meiwanhuangdou	Ningming, Guangxi
N58	Baituqingdou	Hechi, Guangxi
N59	Debaoqing	Debao, Guangxi
N60	Bayueheipidou	Guizhou, Guangxi
N61	Chongzuohedou	Chongzuo, Guangxi
N62	Dajiaodou	Zhenxiong, Yunnan
N63	Xiaohuangdou	Huize, Yunnan
N64	Xihuangdou	Wenshan, Yunnan
N65	Dabaidou	Honghe, Yunnan
N66	Xibaidou	Qujing, Yunnan
N67	Daqingdou	Weishan, Yunnan
N68	Heidou	Mojiang, Yunnan
N69	Songzihuangdou	Zhaotong, Yunnan
N70	Heipiluosidou	Jinhu, Jiangsu
N71	Yixingwanhuangdou	Yixing, Jiangsu
N72	Wandouzao	Zhuxi, Hubei
N73	Aijiaohuang	Kangding, Sichuan
N74	Zaohuangdou	Jiande, Zhejiang
N75	Xundou-1	Zhouning, Fujian
N76	Bayuehuang-5	Taining, Fujian
N77	Qingpidou	Shunchang, Fujian
N78	Xinqiaohuangdou	Dayong, Hubei

Table 2 Number of alleles and marker diversity (*D*) of the Huanghuai (H) and Southern (S) summer soybean germplasm

Locus(LG)	No. of alleles						Marker diversity (*D*)		
	Total	H	S	Shared	Specific to H	Specific to S	Total	H	S
Satt236(A1)	7	7	7	7	0	0	0.775	0.779	0.764
Satt300(A1)	6	4	6	4	0	2	0.414	0.451	0.368
Satt187(A2)	5	5	5	5	0	0	0.630	0.594	0.586
Satt390(A2)	6	5	5	4	1	1	0.703	0.679	0.707
Satt409(A2)	13	13	13	13	0	0	0.905	0.886	0.874
Satt429(A2)	6	6	6	6	0	0	0.766	0.790	0.684
Satt197(B1)	10	8	9	7	1	2	0.858	0.831	0.799
Satt415(B1)	4	3	4	3	0	1	0.654	0.653	0.631
Satt453(B1)	5	5	5	5	0	0	0.687	0.519	0.742
Satt168(B2)	6	5	6	5	0	1	0.763	0.648	0.692
Satt556(B2)	6	5	6	5	0	1	0.433	0.387	0.473
Satt577(B2)	8	8	7	7	1	0	0.805	0.747	0.752
Satt180(C1)	4	4	4	4	0	0	0.719	0.685	0.563
Satt194(C1)	5	4	5	4	0	1	0.737	0.737	0.684
Satt565(C1)	7	7	6	6	1	0	0.713	0.766	0.602
Sat_130(C2)	3	3	3	3	0	0	0.608	0.593	0.615
Satt277(C2)	12	11	12	11	0	1	0.870	0.831	0.846
Satt281(C2)	12	12	11	11	1	0	0.874	0.880	0.841
Satt286(C2)	6	5	6	5	0	1	0.780	0.736	0.738
Satt307(C2)	7	6	7	6	0	1	0.770	0.735	0.702
Satt371(C2)	5	5	4	4	1	0	0.684	0.601	0.686
Satt184(D1a+Q)	6	5	6	5	0	1	0.743	0.746	0.723
Satt203(D1a+Q)	6	6	5	5	1	0	0.741	0.726	0.643
Satt267(D1a+Q)	3	3	2	2	1	0	0.608	0.535	0.488
Satt005(D1b+W)	15	10	15	10	0	5	0.874	0.826	0.882
Satt216(D1b+W)	9	7	8	6	1	2	0.848	0.804	0.707
Satt542(D1b+W)	9	8	8	7	1	1	0.743	0.687	0.767
Satt002(D2)	9	8	8	7	1	1	0.834	0.754	0.838
Satt226(D2)	7	5	7	5	0	2	0.826	0.765	0.832
Satt386(D2)	4	4	4	4	0	0	0.521	0.468	0.560
Sat_112(E)	5	5	4	4	1	0	0.590	0.470	0.670
Satt230(E)	4	4	2	2	2	0	0.569	0.617	0.426
Satt268(E)	8	8	4	4	4	0	0.733	0.739	0.488
Satt146(F)	6	4	6	4	0	2	0.668	0.682	0.617
Satt334(F)	7	6	5	4	2	1	0.791	0.809	0.718
Satt586(F)	12	12	9	9	3	0	0.890	0.863	0.856
Sct_188(F)	3	2	3	2	0	1	0.509	0.500	0.511
Satt012(G)	13	10	13	10	0	3	0.890	0.840	0.884
Satt309(G)	5	5	3	3	2	0	0.534	0.494	0.493
Satt352(G)	8	8	8	8	0	0	0.853	0.864	0.800
Satt279(H)	8	7	8	7	0	1	0.809	0.827	0.708
Satt434(H)	5	4	4	3	1	1	0.640	0.487	0.679
Satt442(H)	7	6	7	6	0	1	0.763	0.747	0.758
Satt239(I)	6	6	6	6	0	0	0.771	0.734	0.724
Satt571(I)	6	6	3	3	3	0	0.646	0.778	0.189
Sct_189(I)	7	7	7	7	0	0	0.827	0.796	0.830
Satt414(J)	8	6	7	5	1	2	0.647	0.757	0.385
Satt431(J)	7	7	7	7	0	0	0.840	0.808	0.835
Satt596(J)	6	6	5	5	1	0	0.800	0.789	0.791
Sct_001(J)	6	6	4	4	2	0	0.729	0.715	0.615
Satt001(K)	8	7	7	6	1	1	0.756	0.786	0.673
Satt242(K)	7	6	7	6	0	1	0.811	0.775	0.802
Satt588(K)	7	6	7	6	0	1	0.777	0.715	0.823
Sat_099(L)	8	7	7	6	1	1	0.751	0.631	0.784
Satt373(L)	7	7	7	7	0	0	0.774	0.736	0.698
Satt462(L)	12	12	10	10	2	0	0.886	0.869	0.856
Satt346(M)	5	5	5	5	0	0	0.694	0.631	0.701
Satt590(M)	6	5	6	5	0	1	0.762	0.687	0.712
Satt022(N)	7	7	6	6	1	0	0.808	0.811	0.772
Satt339(N)	6	5	6	5	0	1	0.776	0.653	0.757
Satt387(N)	3	3	3	3	0	0	0.577	0.607	0.490
Satt530(N)	6	5	5	4	1	1	0.770	0.774	0.684
Satt243(O)	5	5	4	4	1	0	0.700	0.702	0.626
Satt259(O)	6	4	6	4	0	2	0.666	0.616	0.699
Satt345(O)	7	7	6	6	1	0	0.816	0.825	0.730
Satt487(O)	7	6	7	6	0	1	0.772	0.724	0.788
Satt592(O)	5	5	5	5	0	0	0.740	0.744	0.677
Total	460	414	419	373	41	46			
Average	6.9	6.2	6.2				0.735	0.708	0.687

Tris-HCl(pH 9.0), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L each dNTP, 0.2 μmol/L forward and reverse primers, and 1 U Taq DNA polymerase (Promega) by the following program: 94℃ for 4 min; 94℃ for 30 s, 47℃ for 30 s, 35 cycles; 72℃ for 30 s, with a final extension step at 68℃ for 10 min. Primers were synthesized by Sangon Biotechnology Ltd Co. (Shanghai, China).

The PCR reaction was mixed with half volume of formamide loading-buffer (98% formamide, 10 mmol/L of EDTA(pH 8.0), 0.25% bromophenol blue, and 0.25% xylene cyanol FF), then heated at 95℃ for 5min. 5 μL of the denatured mixture was loaded and separated on 6% (w/v) denaturing polyacrylamide gels containing 8 mol/L Urea in 0.5×TBE buffer. The DNA bands were visualized by silver staining. The sizes of the alleles were estimated by comparison to pBR322 DNA/*Msp* Ⅰ marker. The same gel was used to separate SSR alleles from different PCR reactions for time- and money- saving. According to the size range of PCR amplifications, the reactions producing SSR alleles with low molecular weight were firstly loaded, and then those with higher molecular weight after an interval of 15 min. Electrophoresis was conducted for 2 h at a constant power of 100 W.

(ⅴ) Data analysis. The number of alleles per locus was counted. Marker diversity (*D*) for each SSR locus was estimated using the formulas: $Di = n(1-\Sigma P_{ij}^2)/n-1$[6], where *n* is the number of accessions analyzed, and P_{ij} the frequency of the *j*th allele for the *i*th locus summed across all alleles at the locus. Average marker diversity (*D*) was estimated as $D = \Sigma Di/r$, where *r* was the number of loci analyzed. To generate a binary data matrix, the presence and absence of an allele per locus for each accession was coded 1 and 0, respectively. The genetic similarity was calculated using the Jaccard coefficients from the alleles across all the loci in the 158 summer accessions according to the following formula: $J = N_{ij}/(N-N_{00})$, where N_{ij} was the number of shared alleles in both accessions *i* and *j*, *N* was the number of all alleles across all summer accessions used, the N_{00} was the number of alleles present neither in accession *i* nor in accession *j*. To visualize the relationship among any two accessions, the dendrogram was constructed on the basis of the similarity coefficients with the unweighted pair-group method with arithmetic averages (UPGMA). χ^2 tests were done to evaluate if there was significant difference in allelic-frequencies between the Huanghuai summer and Southern summer soybean germplasm. *D* value and χ^2 test were calculated by Microsoft Excel. *J* and UPGMA were performed with NTSYS-pc software package.

2 Results

(ⅰ) Genetic variation. A total of 460 alleles across 67 SSR loci were detected in the 158 accessions (Table 2), in which 414 alleles occurred in 80 Huanghuai summer accessions and 419 alleles in 78 Southern summer accessions. The number of alleles per locus ranged from 3 to 15 with an average of 6.9 across all summer accessions, from 2 to 13 with an average of 6.2 in the Huanghuai summer accessions, and from 2 to 15 with an average of 6.2 in the Southern summer accessions.

In order to compare Huanghuai with Southern summer soybean germplasm, specific alleles were recorded and listed in Table 2. Of the total number of alleles detected, 373 alleles (81%) were shared, 41 alleles (9%) at 29 loci were typical of the Huanghuai summer germplasm, and 46 alleles (10%) at 33 loci were typical of the Southern summer germplasm. There were 328 alleles (79%) in the Huanghuai summer germplasm and 344 alleles (82%) in the Southern summer germplasm with a frequency of 0.25 or lower. Only one allele in the Huanghuai summer germplasm and 3 alleles in the Southern summer germplasm occurred with a frequency of 0.75 or higher.

D (Marker diversity) value per locus ranged from 0.414 to 0.905 with an average of 0.735 for all summer germplasm (Table 2), from 0.387 to 0.886 with an average of 0.708 for the Huanghuai summer germplasm and from 0.189 to 0.884 with an average of 0.687 for the Southern summer germplasm. *D* value was higher in the Huanghuai summer germplasm than that of the Southern summer germplasm at 37 loci, and was lower in the Southern summer germplasm than that of the Huanghuai summer germplasm at 30 loci.

χ^2 test indicated that the differences in allelic-frequencies between the Huanghuai and Southern summer germplasm at 53 loci (79.10%) were significant at the level of $P_{0.05}$, 49(73.13%)of which were significant at the $P_{0.01}$ level (Table 3).

J value (Jaccard coefficient) estimated by the 67 loci was used to analyze genetic similarity between all pairs of the 158 summer accessions (data no shown). *J* value ranged from 0.101 to 0.672 with an average of 0.321 for the Huanghuai/Huanghuai summer comparisons and from 0.125 to 0.716 with an average of 0.298 for the Southern/Southern summer comparisons. The average *J* value ranged from 0.225 to 0.337 between any Huanghuai summer accession and all other Huanghuai summer accessions, and from 0.254 to 0.342 between any Southern summer accession and all other Southern summer accessions. The average *J* value ranged from 0.179 to 0.293 between a Huanghuai summer accession and all Southern summer accessions, and 0.174 to 0.272 between any Southern summer accession and all Huanghuai summer accessions. The average *J* value was 0.236 for all Huanghuai/Southern summer comparisons.

(ⅱ) Genetic relationships. UPGMA cluster analysis of the similarity data divided all 158 summer accessions into three groups (Fig. 1): Group Ⅰ included most of Huanghuai summer accessions; Group Ⅱ included only two accessions, "7599" (H02) from Zhangjiakou,

Table 3 χ^2 test for significant differences of allelic-frequencies between the Huanghuai and Southern summer soybean germplasm[a)]

Locus	χ^2	Locus	χ^2	Locus	χ^2
Satt236	5.707	Satt267	54.977**	Sct_189	9.840
Satt300	6.617	Satt005	27.848*	Satt414	37.742**
Satt187	19.745**	Satt216	83.434**	Satt431	16.983**
Satt390	16.130**	Satt542	13.946	Satt596	8.868
Satt409	29.632**	Satt002	30.814**	Sct_001	44.951**
Satt429	15.225**	Satt226	30.062**	Satt001	29.369**
Satt197	47.688**	Satt386	2.676	Satt242	29.168**
Satt415	9.112*	Sat_112	15.784**	Satt588	12.058
Satt453	26.396**	Satt230	24.794**	Sat_099	32.766**
Satt168	61.823**	Satt268	70.694**	Satt373	40.488**
Satt556	8.556	Satt146	33.939**	Satt462	27.484**
Satt577	52.606**	Satt334	33.493**	Satt346	13.448**
Satt180	27.880**	Satt586	35.061**	Satt590	35.078**
Satt194	16.543**	Sct_188	1.251	Satt022	9.451
Satt565	22.524**	Satt012	38.157**	Satt339	47.421**
Sat_130	0.700	Satt309	19.496**	Satt387	9.518**
Satt277	33.653**	Satt352	24.755**	Satt530	38.080**
Satt281	14.264	Satt279	31.075**	Satt243	31.798**
Satt286	32.377**	Satt434	30.969**	Satt259	11.901*
Satt307	30.763**	Satt442	12.400	Satt345	33.247**
Satt371	22.486**	Satt239	30.555**	Satt487	13.644*
Satt184	6.471	Satt571	82.559**	Satt592	15.765**
Satt203	29.971**				

a) "*" and "**" represent significant difference at the level of $P_{0.05}$ and $P_{0.01}$, respectively.

Hebei Province and "Heidou" (N68) from Mojiang, Yunnan Province, which belong to Huanghuai summer and Southern summer germplasm, respectively; Group Ⅲ included most of the Southern summer accessions except for two Huanghuai summer accessions: "Huichabayuezha" from Zhen'an, Shanxi Province and "Ludou 2" from Shandong Province.

Group Ⅰ was distinctly classified into five sub-groups as follows:

The first sub-group (labeled 1) mainly consisted of cultivars in the middle and north part of Hebei Province, such as "Yilichuandahuangdou" (H01), "Pingdinghuang" (H03), "Baxiandadou" (H50). However, "Shubaidou" and "Bayuezha" from Shanxi Province, which were closely related to each other, were also found in the sub-group. The reason of their close relation to these pre-mature summer accessions from Hebei Province needed to be further explored.

The second sub-group (labeled 2) included only one accession, "Yiwohou" (H14) from Shandong Province. This accession showed genetic uniqueness here.

The third sub-group (labeled 3) included more accessions and was classified into three clusters. The accessions were mostly from Shandong Province in the first cluster, which were further classified into two smaller clusters: One included some released cultivars with the pedigree relations to "Qihuang 1" and "Juxuan 23", such as "Yuejin 4" (H08), "Wenfeng 7" and "Yanhuang 1", which all were progenies of "Juxuan 23", while "Wenfeng 7" was a progeny of "Juxuan 23" and "Qihuang 1". In addition, a landrace (H14) from Shandong Province, and a landrace (H04) from Quyang, Hebei Province were in this smaller cluster; the other included some landraces from Shandong Province, such as "Pingdinghuang" (H15) from Yidu, as well as two landraces (H27 and H28) from Henan Province. The second cluster were also further classified into two smaller clusters: one included four cultivars (H16, H23, H24 and H26) from Shandong Province, several cultivars belonging to "Niumaohuang" type, such as "Chenlinniumaohuang" (H30), "Minquanniumaohuang" (H29), "Neihuangniumaohuang" (H31), and "Wuzhiniumaohuang" (H32), and some landraces from Xuzhou such as "Fengxianxihecao" (H38), "Pixianruantiaozhi" (H41), "Peixiandabaijiao" (H39), "Tongshantian'edan" (H40), "Xinyipingdinghuang" (H42) and "Donghaibaihuacao" (H43). All these cultivars from Xuzhou were widely planted in the 1960s. The other smaller cluster included three more closely related cultivars, "Zhengzhou 135" (H55), "Zaofeng 1" (H54) and "Zheng 77249" (H56). "Zaofeng 1" was the father of "Zhengzhou 135" and "Zhengzhou 135" was the mother of "Zheng 77249". Two cultivars (H61 and H63) from the north of Anhui Province and "Nibadou" (H60) from the south to Qinling, Shanxi Province were also found in the smaller cluster. The third cluster mainly included cultivars from the geographically closed regions of the south-west of Shandong Province,

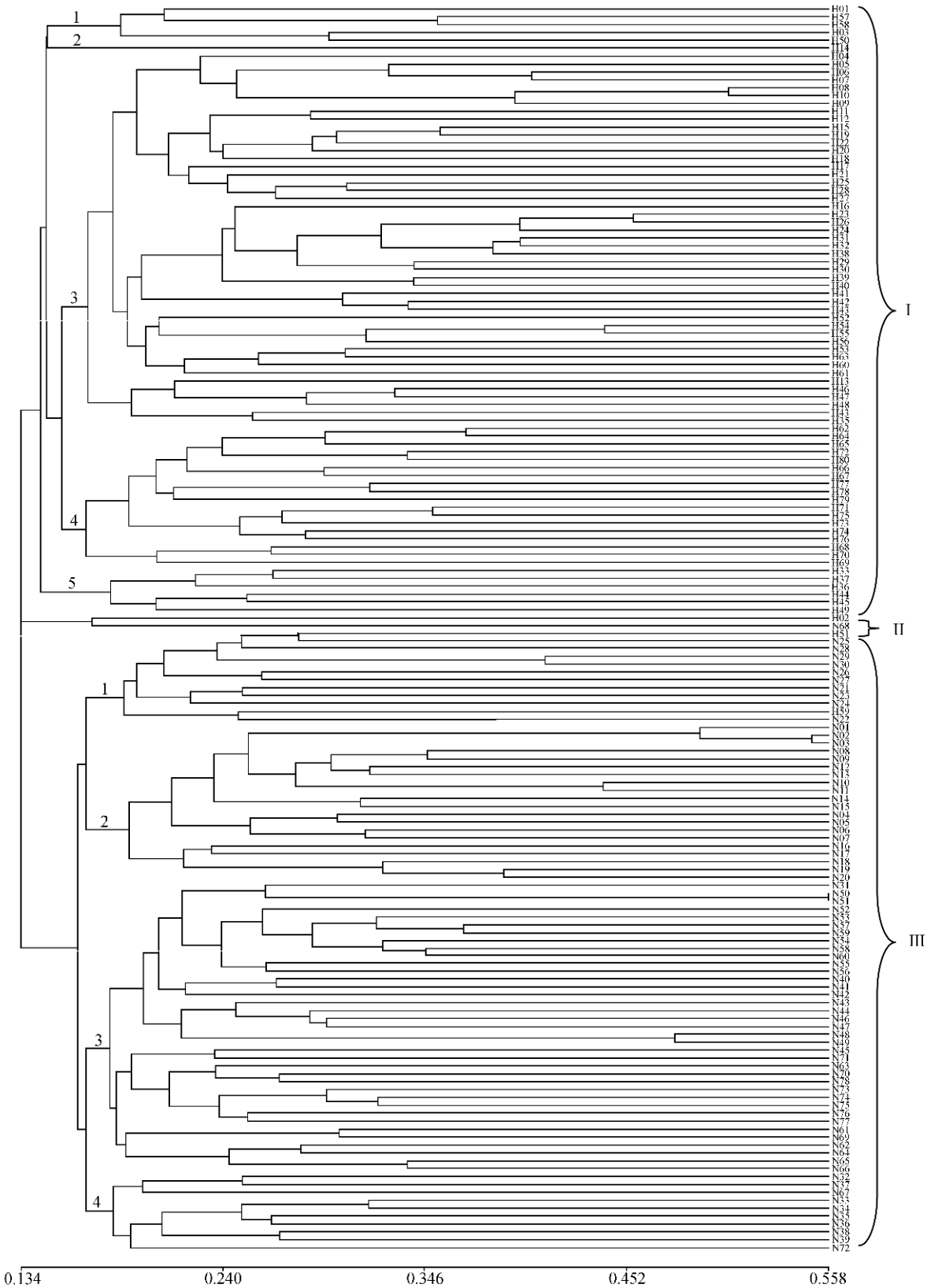

Fig. 1. UPGMA dendrogram of genetic relationship based on genetic similarity among Chinese summer soybean accessions. Ref. No per accession was indicated as Table 1.

the north of Anhui Province, and the east of Henan Province, such as "Pingdingsi" (H13), "Binhaidabaihua" (H46), "58-161" (H48), "Guanyundasili" (H47), "Yongcheng Dahuangjianke" (H34) and "Luyitian'edan"(H35). Of these cultivars, "58-161" was selected from "Binhaidabaihua". Yongcheng and Luyi are both located in the north of Henan Province; Binhai and Guanyun are geographically very close.

The fourth sub-group (labeled 4) was divided into two clusters: one mainly included cultivars from the north of Anhui Province and the peninsula area of Shandong Province, such as "Wuhedadou" (H62), "Zhongdou 19" (H67) and "Yudou 12" (H77), which are suitably planted in the north of Anhui Province, "Yudou 12" (H77) and "Zheng 133" (H78) with identical cytoplasm, as well as "Haiyangbengjiehuang" (H75), "Wangchengdabayuexian" (H73), "Luohuangye" (H74), "Ludou 6" (H76). Additionally, "Huangben 13" (H71), which is an extremely pre-maturing summer cultivar, "Shi zhuangdahuangli" (H72), "Shanzibai" (H79) from Ningshan, Shanxi Province, and "Chuxiu" (H80) from Jiangsu Province. The other cluster included "Zhonghuang 1" (H68) from the mid-north of Hebei Province, an extremely pre-maturing summer cultivar, "Shirengouzaohuangdou" (H70) from Fengning, and "Qianjin 2" (H69).

The fifth sub-group (labeled 5) was classified into two clusters, mainly including late-maturing cultivars from the south of Huanghuai region. One cluster comprised "Wangshanhou" (H36) from Zhenba of Shanxi Province, and "Shanzibai" (H37) and "Niumaohuang" (H33) from Qinyang, Henan Province; The other cluster comprised "Ganyuqishuiwangdou" (H44) and "Xudou 1" (H45) from the north of Jiangsu Province. "Kefeng 6" (H49) was placed in this cluster because of it had pedigrees of "58-161" and "Xudou 1".

Group Ⅲ was distinctly classified into four sub-groups as following:

The first sub-group (labeled 1) was complex, including 3 accessions (N21, N23 and N24) from Zhejiang Province, which were clustered together, 2 accessions (N29 and N30) from Sichuan Province, as well as 2 accessions (N27 and N28) from Hubei Province, one accession (N22) from Zhejiang Province and one accession (N25) from Zhuang Autonomous Region of Guangxi. Cultivar "1138-2" (N26), which is selected from "Fengxiandao" were also placed in the sub-group, obviously distinct from its parents. What is more, the sub-group also included 2 accessions of Huanghuai summer soybean, "Huichabayuezha" (H59) from Zhenan, Shanxi Province and "Ludou 2" (H51) from the south-west of Shandong Province mentioned above. The two accessions showed genetic uniqueness.

The second sub-group (labeled 2) was further divided into two clusters. In the first cluster, three accessions were closely related, "Nannong 493-1" (N01), "Su ou1" (N02) which was one progeny of "Nannong 493-1", and "Jinda 322" (N03) were clustered together. Two cultivars from the same site, such as N08 and N09 from Anhui Province, and N10 and N11 from Shanghai, were clustered together; four accessions (N04, N05, N06 and N07) from Jiangsu Province were classified into a smaller cluster; two accessions from Hubei Province, "Anlu honghuangdou" (N12) and "Wuchangdonghuangdou" (N13), and two accessions from Hubei Province, "Huangpohouzihuang" (N14) and "Tianmendazihuang" (N15) were also assembled together. The other cluster included three accessions (N16, N17 and N18) from Hubei Province and two accessions (N19 and N20) from Zhejiang Province.

The third sub-group (labeled 3) comprised three clusters. One cluster was further divided into three smaller clusters. The first smaller cluster included nine accessions from Zhuang Autonomous Region of Guangxi besides three accessions (N31, N55 and N51) from Sichuan, Guizhou and Guangdong provinces, respectively. The second smaller cluster included two accessions (N41 and N42) from Hunan Province and an accession (N40) from Jiangxi Province. The third smaller cluster included 5 accessions from Guizhou Province besides for one accession (N43) from Hunan Province. One cluster comprised five accessions from Yunnan Province and one accession (N61) from Zhuang Autonomous Region of Guangxi. The last group included most of the accessions from Fujian Province such as N75, N76 and N77, two accessions (N70 and N71) from Jiangsu Province, and four accessions (N45, N73, N74 and N78) from Guizhou, Sichuan, Zhejiang and Hunan Province, respectively.

The fourth sub-group (labeled 4) included most of accessions (N32, N33, N34, N35 and N36) from Sichuan Province, two accessions (N38 and N39) from Jiangxi Province, one accession (N37) from Zhejiang Province, one accession (N67) from Yunnan Province and one accession (N72) from Hubei Province.

From the above analysis of Group Ⅲ, we might find that the first and second sub-group included most of the accessions from the middle and lower reaches of Yangzi such as from Jiangsu, Zhejiang and Hubei provinces. The accessions in the third and fourth sub-groups were mostly from Sichuan, Guizhou, Yunnan and Guangxi provinces. Therefore, these results supported Wang's opinion to a certain extent, who regarded Yunnan and Guizhou as a separate cultivated region in China[1)].

3 Discussions

(ⅰ) Genetic diversity of Chinese summer soybean

1) Wang, Y. S., Studies on the maturity groups ecological regions and responses to day length and temperature of soybean varieties, Nanjing: Nanjing Agricultural University Dissertation, 1999.

germplasm. This study analyzed genetic diversity of Chinese summer soybean germplasm using SSR markers. There existed higher genetic variation in Chinese summer soybean germplasm in comparison to Northern American improved cultivars and introductions [5,6]. Of 67 SSR loci tested in the study, four (Satt001, Satt005, Satt002 and Satt012) of them were examined by Diwan and Cregan[5] and 17 (Satt187, Satt390, Satt409, Satt168, Satt577, Satt194, Satt307, Satt184, Satt005, Satt002, Satt146, Satt012, Satt309, Satt001, Satt242, Satt588 and Satt022) were examined by Narvel et al.[6]. In the above four loci examined, Diwan and Cregan found that there was a total of 37 alleles and an average marker diversity of 0.805 in the 35 ancestral introductions accounting for more than 95% of the genetic basis of soybean in North America, while we detected a total of 45 alleles and an average marker diversity of 0.839 in the 158 Chinese summer soybean accessions, a total of 37 alleles and an average marker diversity of 0.802 for the 80 Huanghuai summer accessions, and a total of 43 alleles and an average marker diversity of 0.820 for the 78 Southern summer accessions. In the above 17 loci examined, Narvel et al.[6] reported a total of 110 alleles and an average marker diversity of 0.659 in the 79 Northern American elite cultivars and introductions, while we detected a total of 133 alleles and an average marker diversity of 0.765 in the 158 of Chinese summer accessions, a total of 114 alleles and an average marker diversity of 0.732 in the 80 Huanghuai summer accessions, and a total of 126 alleles and an average marker diversity of 0.735 in the 78 Southern summer accessions. Although the number of alleles (80) at 7 loci (Satt236, Satt197, Satt180, Satt203, Satt002, Satt431 and Satt001) reported in the 132 soybean cultivars from 14 Asian countries by Abe et al.[8] was higher than ours in the 158 summer accessions, the average marker diversity(0.756)in these cultivars was lower than that (0.789) of all Chinese summer accessions.

There were many specific alleles detected both in Huanghuai and Southern summer germplasm (Table 2). Significant differences in allelic-frequencies at most loci between Huanghuai and Southern summer germplasm also have been found (Table 3). The average genetic similarity was lower for Huanghuai/Southern summer comparisons (*J* value of 0.236 averaged) than that of Huanghuai/Huanghuai summer comparisons (*J* value of 0.321 averaged) or Southern/Southern summer comparisons (*J* value of 0.298 averaged). Huanghuai and Southern summer accessions also tended to form different groups (Fig. 1). All these results suggest that Huanghuai and Southern summer germplasm should be divided into different gene pools. However, Abe et al.[8] found no difference between Huanghuai and Southern germplasm. Our conclusion, which is different from that of Abe et al.[8] was mainly due to the differences on materials and SSR loci. Since the soybean accessions and SSR loci in our study were purposefully selected and were more representative, the results were more reliable.

Chinese soybean germplasm include spring, summer and autumn germplasm, in which summer soybean germplasm are further divided into the two types of Huanghuai and Southern summer germplasm according to their geographic origins; similarly, spring soybean germplasms also are classified into North, Huanghuai and Southern spring germplasm. The analysis of summer soybean germplasms can provide insight into the diversity of spring and autumn soybean germplasm. So our results suggest that there might be abundant genetic diversity and differences not only among spring, summer and autumn soybean germplasm, but also among North spring, Huanghuai spring and Southern spring soybean germplasm.

(ii) Clues provided by genetic diversity of soybean germplasm for further study. Allelic variation of SSR markers proved to be valuable for assessment of genetic relationship between soybean cultivars [6,8]. The results in this study demonstrated not only genetic diversity, but also genetic relationship of Chinese summer soybean germplasm using SSR markers. Some accessions were genetically closely related. For instance, "Zaofeng 1", "Zhengzhou 135" and "Zheng 77249" from Henan Province were clustered together; some accessions of the same origin were clustered together, such as cultivars from Shandong Province or Xuzhou City; some accessions with similar genotypes and phenotypes were clustered together such as "Minquanniumaohuang" and "Chenliuniumaohuang". Therefore, all these accessions should be further identified for morphological and agronomic performance as well as isozyme and molecular diversity in order to provide the exact information about soybean breeding. On the contrary, some cultivars related in the pedigree showed great differences. For example, both "Zheng 133" and "Yuejin 4" derived from the progenies of "Juxuan 23" × "5905" were clustered in two different sub-groups. Since "Zheng 133" was introduced into Henan Province in the fourth progeny of the cross, could the environmental influence and directional selection result in the difference from "Yuejin 4"? Similarly, the majority of U.S germplasm ancestral lines of modern soybean cultivars were introduced from China; they also d significantly genetically differ from Chinese accessions by RAPD markers [15,16]. Soybean cultivars grown in Korea and Japan were presumably originated from China. However, soybean germlasm in these two countries showed more similarity, but were clearly different from those in China by SSR markers [17]. Additionally, SSR analysis indicated that Japanese and Chinese soybean germlasm were different gene pools [8]. Were they selected according to different utilizations and needs under different environmental conditions that lead to significant genetic differences? All questions addressed in this study need to be further studied by modern molecular biology techniques.

(iii) Development of EST-SSR markers to enrich the soybean SSR marker pool. So far, more than 1000 SSR markers have been developed and most of them were distributed in intergenic regions, and therefore there has a low chance for these markers to associate with functional genes. With the remarkable progress in functional genomic projects, ESTs (expressed sequence tags) become a primary resource for development of novel DNA markers. EST-SSR markers derived from transcribed regions of the DNA have recently been intensively used for molecular mapping, genetic diversity and phylogenetic analysis in different plant species, such as grape [18], rice [19], durum wheat [20], rye [21], barley [22], bread wheat [23] and almond [24]. In soybean, 3383 SSRs were detected in 56147 unigenes assembled from 314254 soybean ESTs [25]. The development and utilization of EST-SSR markers, especially in evaluating soybean germplasm are likely to shed insight into genetic diversity on the level of functional genes in the future for marker-assisted selection (MAS) in soybean breeding.

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