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Full Length Research Paper

Genetic diversity of Shaanxi soybean landraces based on agronomic traits and SSR markers

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Genetic diversity of a primary core collection of 91 soybean landraces from Shaanxi Province, China, was analyzed using simple sequence repeat (SSR) markers and agronomic traits. A total of 250 alleles were detected in the 91 soybean accessions, with a mean of 7.14 alleles per locus. The mean of polymorphism information content (PIC) was 0.26, ranged from 0.11 for Satt184 to 0.60 for Satt242. UPGMA cluster analysis and PCA analysis clearly showed that, 91 accessions formed two major clusters which generally correspond to geographic origin. Cluster I contained 76 soybean landraces and it was further separated into five subgroups (I-1 to I-5). Cluster II (northern group) included 15 accessions from northern Shaanxi. Group I-1 (Guanzhong group) contained 19 landraces, with 16 from Guanzhong, 3 from northern Shaanxi. Group I-2 (southern group I) composed of 13 accessions from southern Shaanxi and 2 from Guanzhong. Group I-3 (mixture group) contained 18 landraces, with 10 landraces from Guanzhong and 8 from southern Shaanxi. Group I-4 (southern group II) contained 21 accessions, of which 20 from southern Shaanxi and 1 from northern Shaanxi. Group I-5 (southern group III) included only 2 southern Shaanxi landraces. AMOVA analysis showed that, a significant proportion of variance (94.28%) was due to variation within populations.

Key words: Soybean (*Glycine max*(L.) *Merr*), landraces, genetic diversity, simple sequences repeat (SSR).

INTRODUCTION

Soybean originated in China and has been cultivated for more than 3000 years (Fukuda, 1933; Vavilov, 1951; Lu, 1978; Hymowitz and Newell, 1981; Wang, 1985; Dong, 1998; Zhou et al., 1998; Xu, 1993; Xu and Gai, 2002). It is widely distributed in China and has been cultivated in diverse geographical regions. The long history of cultivation in different environments has contributed to the evolution of many genetically distinct soybean types in China. Chinese soybean production is generally divided into Northern, Huanghuai Valley and Southern production regions. Within these three primary regions, soybean production can be further divided into seven ecotypes according to ecogeographic regions of origin and planting system, including Northeastern spring soybean (NEsp), Northern spring soybean (Nsp), Huanghuai Valley spring soybean (Hsp), Huanghuai Valley summer soybean (Hsu), Southern spring soybean (Ssp), Southern summer soybean (Ssu) and Southern autumn soybean (Sau) (Qiu et al., 2003; Wang et al., 2006). Shaanxi province is situated in the middle of Yellow River Valley, between latitudes 31° and 40°N and longitudes 105° and 112°E and with altitudes ranging from 300 to 3000 m above sea

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Abbreviations: NEsp, Northeastern spring soybean; **Hsp,** Huanghuai valley spring soybean; **Hsu,** Huanghuai valley summer soybean; **Ssp**, Southern spring soybean; **Ssu,** Southern summer soybean; **Sau,** Southern autumn soybean; **SSRs,** simple-sequence repeats; **CTAB,** cetyl trimethyl ammonium bromide; **PIC,** polymorphism information content; **SMC,** simple matching coefficients; **SHAN,** sequential, hierarchical and nested clustering; **PCA,** principal component analysis; **NA,** number of alleles.

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level, possessing diverse environments which cover three major different climate zones: southern subtropical moist and semi-moist climate, temperate semi-moist climate and temperate arid and semi-arid climate that correspond to three different geographic regions from south to north: Southern Shaanxi, Guanzhong (which is located in the middle of Shaanxi) and Northern Shaanxi. The diverse climates and geographic regions have determined the diverse cultivated soybean grown in Shaanxi province. More than 1000 *Glycine max* accessions have been collected *in situ* in Shaanxi, representing five of the seven ecotypes in China: Nsp, Hsp, Hsu, Ssp and Ssu. However, the genetic diversity of soybean landraces in Shaanxi has not been extensively investigated. A better understanding of the genetic diversity of Shaanxi soybean will help us to rationally manage and effectively utilize them in the breeding program.

Genetic diversity studies have been carried out for soybean from China. Wang et al. (2006) determined genetic diversity of 129 soybean accessions which represented Chinese soybean collection using 60 mapped SSR markers and deduced that, accessions from the lower regions of the Yellow River Valley possessed the greatest allelic richness, suggesting that the Yellow River Valley may be center for Chinese cultivated soybean. Li et al. (2008) further studied 1863 Chinese soybean landraces from 29 provinces in China using 59 SSR markers and came to the same conclusion as that of Wang et al. (2006). Chen and Nelson (2005) estimated the relationship between geographical origin and genetic diversity of soybean accessions collected from four geographically diverse provinces in China using 31 selected decamer primers and found that, primitive cultivars of China were generally genetically isolated in relatively small geographical areas. Wang et al. (2010) characterized genetic diversity among 40 soybean accessions from Shanxi province using 40 SSR primer pairs and showed that, wild soybeans and landraces possessed greater allelic diversity than cultivars. On the basis of agronomic trait variation, Dong et al. (2001) defined three diversity centers of Chinese wild soybean: the northeast, the Yellow River and the coastal region of Southeast China. Dong et al. (2004) suggested that, the phenotypic diversity center of cultivated soybean was the Yellow River region.

Significant advances in molecular genetics and genomics recently have resulted in many DNA markers which are stable and have proven to be genetically informative and useful for genotype identification and genetic diversity assessment (Keim et al., 1992; Li et al., 2001; Li and Nelson, 2001, 2002; Xu and Gai, 2003; Chen and Nelson, 2004, 2005; Wang et al., 2006; Nichols et al., 2007). Microsatellites or simple-sequence repeats (SSRs) have become useful markers for genetic analysis, because they are co-dominant with a high level of allelic diversity, easily and economically to operate (McCouch et al., 1997) and available to high throughput genotyping of a large number of accessions. They are nearly unlimited in numbers, presumably selectively neutral and can be organized into linkage maps (Cregan et al., 1999). SSR markers have been used to evaluate genetic diversity and domestication of crops (Fu et al., 2007; Yoon et al., 2009; Li et al., 2010; Guo et al., 2010), develop genetic map (McCallum et al., 2006) and assistant selection in plant breeding (Kelley et al., 2006). However, morphological and agronomic characters are still useful in valuating genetic diversity because of their visualization and easy to get.

Because it is wildly planted in different ecogeographic regions in Shaanxi, soybean has evolved into various ecotypes following long-term natural and artificial selection. Using the sampling strategy proposed by Qiu et al. (2003), we established a primary core collection of soybeans from the 1035 entire soybean germplasm collection of Shaanxi province (Liu et al., 2006), this accessions were selected based on their ecotypes and phenotypic variation for morphological and agronomic traits from the 1035 Shaanxi accessions held ex situ. In the present study, genetic diversity of this primary core collection of soybeans was investigated using agronomic traits and SSR markers. The objective of this study is to understand the genetic diversity of soybean germplasm from Shaanxi province. This information will be very useful for rational management and allow breeders to better understand the evolutionary relationships among accessions and to develop strategies to integrate useful diversity into their breeding programs.

MATERIALS AND METHODS

Plant material

According to the sampling strategy proposed by Qiu et al. (2003), a primary core collection of 91 soybean landraces was established from the 1035 entire soybean germplasm collection of Shaanxi province to represent phenotypic variability for 15 agronomic and morphological traits, including 43 landraces from southern Shaanxi, 28 from Guanzhong and 20 from northern Shaanxi (Liu et al., 2006; Table 1). The geographic distribution of all these 91 soybean accessions from Shaanxi is shown in Figure 1. All seeds were obtained from the Chinese National Crop Germplasm Conservation Center *(*http://icgr.caas.net.cn , verified 6 January, 2006).

Agronomic characters

The field experiment was carried out using a randomized complete block design with three replications in two crop seasons 2005 and 2006 at Yangling, Shaanxi, People's Republic of China. The seeds were sown in 3 row plots, with 2.0 m in length and 0.6 m between rows and 0.2 m between plants. All the accessions were sown on April 15 each year.

The date of flowering and maturity were investigated during the growth period and then, the days to flowering and the days after flowering were counted. At the maturity stage, ten adjacent plants were randomly sampled from the middle of each plot for evaluating 9 agronomic characters: plant height, number of branches, number of nodes in the main stem, number of pods in the main stem, number of pods in branches, number of seeds per plant, seed

Table 1. The code number, the geographic regions and the assigned cluster based on SSR markers of the 91 soybean accessions used in the present investigation.

Accession number	Code number	Geographic region (County)	Cluster
N01	ZDD10255	Northern Shaanxi (Fugu)	Outlier
N02	ZDD10170	Northern Shaanxi (Huangling)	I-1
M03	ZDD10207	Guanzhong (Tongchuan)	I-1
M04	ZDD10199	Guanzhong (Changwu)	I-1
M05	ZDD10250	Guanzhong (Yaoxian)	I-1
M06	ZDD10315	Guanzhong (Tongchuan)	I-1
N07	ZDD10297	Northern Shaanxi (Yichuan)	I-1
N08	ZDD10299	Northern Shaanxi (Huanglong)	I-1
M09	ZDD10309	Guanzhong (Xunyi)	I-1
M10	ZDD10337	Guanzhong (Longxian)	I-1
M11	ZDD10371	Guanzhong (Linyou)	I-1
M12	ZDD03614	Guanzhong (Chang'an)	I-1
M13	ZDD03618	Guanzhong (Fuping)	I-1
M14	ZDD10408	Guanzhong (Lantian)	I-1
M15	ZDD10413	Guanzhong (Fuping)	I-1
M16	ZDD10418	Guanzhong (Chang'an)	I-1
M17	ZDD10386	Guanzhong (Baoji)	I-1
M18	ZDD10379	Guanzhong (Baoji)	I-1
M19	ZDD10646	Guanzhong (Lantian)	I-1
M20	ZDD10636	Guanzhong (Baoji)	I-1
M21	ZDD03669	Guanzhong (Baoji)	I-3
M22	ZDD03671	Guanzhong (Fengxiang)	I-3
M23	ZDD03666	Guanzhong (Qishan)	I-3
M24	ZDD10730	Guanzhong (Fufeng)	I-3
M25	ZDD10742	Guanzhong (Pucheng)	I-3
M26	ZDD24061	Guanzhong (Dali)	I-3
M27	ZDD10809	Guanzhong (Lantian)	I-3
M28	ZDD03700	Guanzhong (Baishui)	I-3
M29	ZDD10802	Guanzhong (Fufeng)	I-3
M30	ZDD03717	Guanzhong (Pucheng)	I-3
S31	ZDD10184	Guanzhong (Taibai)	I-3
S32	ZDD10517	Southern Shaanxi (Ningqiang)	I-3
S33	ZDD10458	Southern Shaanxi (Shangnan)	I-3
S34	ZDD10460	Southern Shaanxi (Shangnan)	I-3
S35	ZDD10422	Southern Shaanxi (Luonan)	I-3
S36	ZDD10509	Southern Shaanxi (Liuba)	I-3
S37	ZDD10473	Southern Shaanxi (Shanyang)	I-3
S38	ZDD10373	Guanzhong (Fengxian)	I-5
S39	ZDD10459	Southern Shaanxi (Shangnan)	I-4
S40	ZDD10376	Guanzhong (Fengxian)	I-4
S41	ZDD10609	Southern Shaanxi (Ningshan)	I-5
S42	ZDD10445	Southern Shaanxi (Danfeng)	I-2
S43	ZDD19503	Southern Shaanxi (Ningshan)	I-2
S44	ZDD19497	Southern Shaanxi (Baihe)	I-2
S45	ZDD19463	Southern Shaanxi (Zhen'an)	I-2
S46	ZDD10442	Southern Shaanxi (Shanxian)	I-2
S47	ZDD10454	Southern Shaanxi (Shangnan)	I-2
M48	ZDD03629	Guanzhong (Fufeng)	I-2
M49	ZDD24060	Guanzhong (Yangling)	I-2
S50	ZDD10600	Southern Shaanxi (Shiquan)	I-2

Table 1. Continued.

S51	ZDD10656	Southern Shaanxi (Luonan)	I-2
S52	ZDD10661	Southern Shaanxi (Shangxian)	I-2
S53	ZDD10699	Southern Shaanxi (Langao)	I-2
S54	ZDD10664	Southern Shaanxi (Danfeng)	I-2
S55	ZDD10694	Southern Shaanxi (Mianxian)	I-2
S56	ZDD10698	Southern Shaanxi (Langao)	I-2
S57	ZDD10683	Southern Shaanxi (Zhashui)	I-4
S58	ZDD10757	Southern Shaanxi (Danfeng)	I-3
S59	ZDD10762	Southern Shaanxi (Shangnan)	I-4
S60	ZDD10796	Southern Shaanxi (Baihe)	I-4
S61	ZDD10775	Southern Shaanxi (Mianxian)	I-4
S62	ZDD10777	Southern Shaanxi (Langao)	I-4
S63	ZDD10768	Southern Shaanxi (Zhen'an)	I-4
S64	ZDD10825	Southern Shaanxi (Shangxian)	I-4
S65	ZDD10868	Southern Shaanxi (Xunyang)	I-4
S66	ZDD10841	Southern Shaanxi (Zhashui)	I-4
S67	ZDD10845	Southern Shaanxi (Zhen'an)	I-4
S68	ZDD10828	Southern Shaanxi (Shangxian)	I-4
S69	ZDD10883	Southern Shaanxi (Shiquan)	I-4
S70	ZDD19536	Southern Shaanxi (Zhenba)	I-4
S71	ZDD03691	Southern Shaanxi (Shangnan)	I-4
S72	ZDD03706	Southern Shaanxi (Shangnan)	I-4
S73	ZDD10927	Southern Shaanxi (Shanyang)	I-4
S74	ZDD10907	Southern Shaanxi (Luonan)	I-4
S75	ZDD10912	Southern Shaanxi (Shangxian)	I-4
N76	ZDD10226	Northern Shaanxi (Suide)	II
N77	ZDD10230	Northern Shaanxi (Zizhou)	II
N78	ZDD10237	Northern Shaanxi (Zhidan)	II
N79	ZDD10242	Northern Shaanxi (Huanglong)	II
N80	ZDD10134	Northern Shaanxi (Fugu)	II
N81	ZDD10143	Northern Shaanxi (Jingbian)	II
N82	ZDD10260	Northern Shaanxi (Yulin)	II
N83	ZDD10274	Northern Shaanxi (Fugu)	II
N84	ZDD10329	Northern Shaanxi (Wubu)	II
N85	ZDD10332	Northern Shaanxi (Zizhou)	II
N86	ZDD10135	Northern Shaanxi (Fugu)	II
N87	ZDD10239	Northern Shaanxi (Luochuan)	I-4
N88	ZDD10263	Northern Shaanxi (Yulin)	II
N89	ZDD10294	Northern Shaanxi (Luochuan)	II
N90	ZDD10252	Northern Shaanxi (Fugu)	II
N91	ZDD10270	Northern Shaanxi (Dingbian)	II

weight per plant, 100-seed weight and index of seed shape (index of seed shape=seed length×seed width / seed thickness2, the bigger the index is, the more flat the seed is and the smaller the index is the round the seed is). When seeds had been dried, the seed protein and fat content were detected by near infrared spectroscopy DA7200 (Sweden Perten instruments Company).

DNA extraction and SSR marker analysis

Genomic DNA of each accession was extracted from dry seeds by a modified cetyl trimethyl ammonium bromide (CTAB) method (Keim et al., 1988). The DNA concentration of all samples was calculated by spectrophotometer readings at wavelengths of 260/280 and work solution of all DNA samples was adjusted to a concentration of 10 ng /µl.

Simple sequence repeat (SSR) markers were chosen based on Wang et al. (2003) and Xie et al. (2003), then thirty-five SSR loci covering 20 soybean chromosomes were used in this study. All 35 SSR loci were shown in Supplementary Table 1. The sequences of SSR primers were obtained from soybase (http://soybase.org/resources/ssr.php). All primers were synthesized

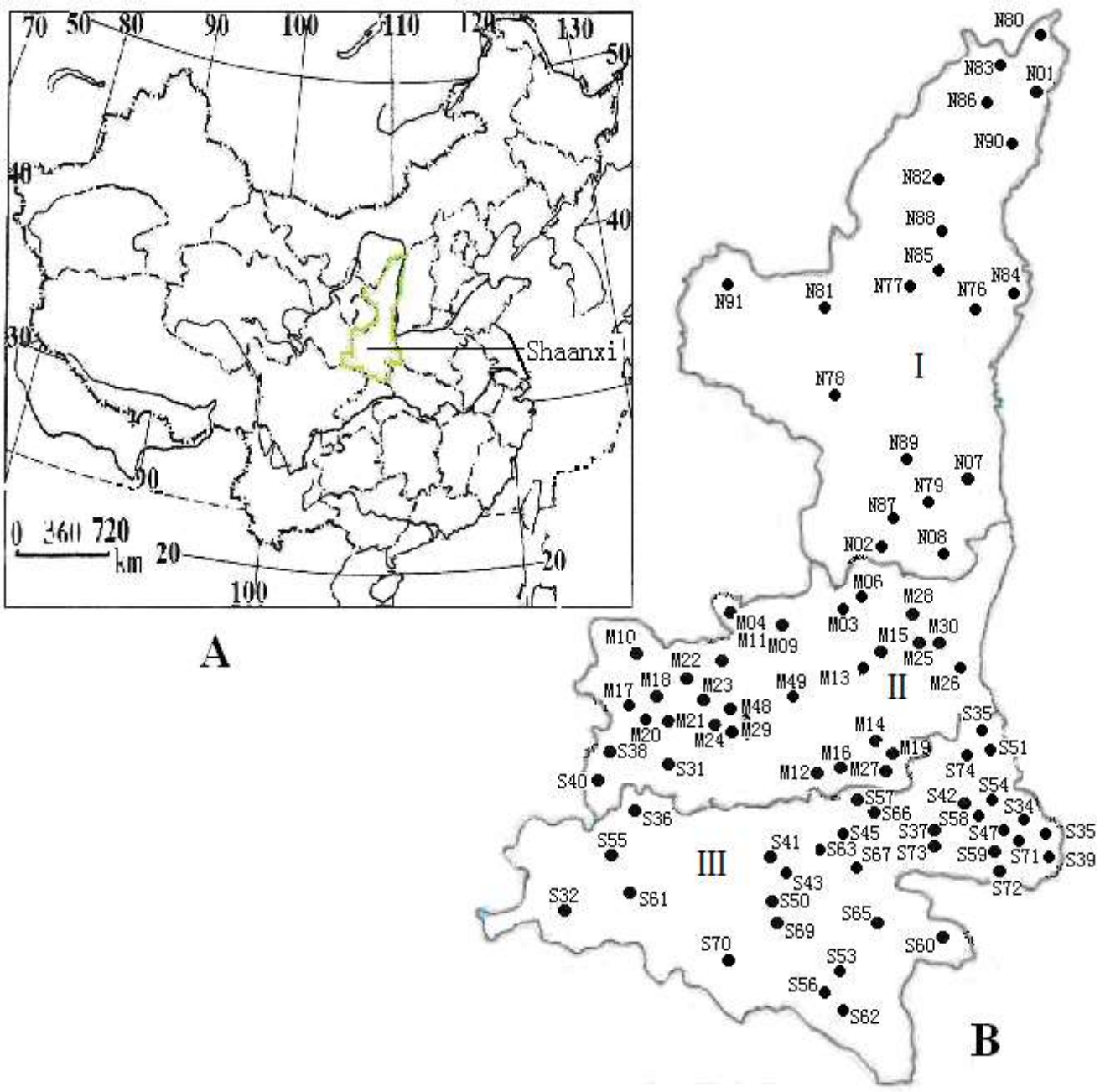

Figure 1. Geographic distribution of sampling localities of soybean landraces in the present study. A, Map of China, showing the location of Shaanxi province; B, map of Shaanxi province, in which "I" stands for north Shaanxi region, "II" stands for Guanzhong region and "III" stands for south Shaanxi region.

by Shanghai Sangon Biotechnology Company in China. Polymerase chain reaction (PCR) reactions were performed in a total volume of 20 µl, containing 60 ng of genomic DNA, 3 mM of MgCl2, 0.15 µM of each primer, and 300 µM of each dNTP, 1.25 units of Taq polymerase and 1 × PCR buffer. The PCR program (PTC-100, Bio-rad Co. Ltd. America) was as follows: 3 min pre-denaturation at 95°C, 40 s denaturation at 94°C 40 s, annealing at from 42 to 58°C (depending on the primer used) and 40 s extension at 72°C for 35 cycles, finally 5 min incubation at 68°C. The PCR products were separated by 8% PAGE (W/V) gel in 1×TBE and visualized by silver staining.

Data statistics and analysis

Agronomic data from three replications were averaged and each trait was divided into 10 levels based on it's mean value ($\bar{X}$) and standard deviation (σ), from the first grade ($x_i < (\bar{X} - 2\sigma)$) to 10th grade ($x_i \geq (\bar{X} + 2\sigma)$), every 0.5 standard deviation formed a single grade. Then, these continuous agronomic data were transformed into 0, 1 quality data based on the above 10 levels, the data was recorded as 1 where the agronomic trait value was at the level, the

Table 2. The average NA, PIC and SMC of each population from geographic region.

Geographic region	Average number of alleles per locus	Average PIC	Average pairwise SMC
Northern Shaanxi	5.40	0.67	0.802
Guanzhong	5.54	0.68	0.811
Southern Shaanxi	5.91	0.71	0.809

data was recorded as 0 where the agronomic trait value was not at the level. The frequency of different levels was calculated, then the genetic diversity of different agronomic traits in northern Shaanxi, Guanzhong, southern Shaanxi as well as the whole province was estimated by using the formula:

$$H' = \sum_{i=1}^{n} p_i \ln p_i ,$$

Where P_i is the frequency of the *i*th level and *n* is the total number of levels.

Alleles were scored as either present (1) or absent (0) for each SSR locus for statistical analysis The polymorphism information content (PIC) value of the single locus (i) was calculated by the following formula (Weir, 1990):

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} p_{ij}^2,$$

Where p_{ij} is the frequency of the *j*th allele of the *i*th marker locus and *n* is the total number of allele.

The original data matrix was formed including both SSR data and agronomic data and entered into the NTSYS program (Rohlf, 1992). The data were analyzed using the qualitative routine to generate simple matching coefficients (SMC), calculated as SMC = *a/(n-d)*, where *a* is the number of bands in common between two accessions, *n* is the number of bands in the matrix and *d* is the number of bands absent in both accessions (Sokal and Michener, 1958). Simple matching coefficients were used to construct a dendrogram by the unweighted pair-group method with arithmetic mean (UPGMA) and the sequential, hierarchical and nested clustering (SHAN) routine in the NTSYS program. Principal component analysis (PCA) was performed with the same program using the Decenter and Eigen procedures.

The components of variance attributable to differences geographic regions of Shaanxi province and among individuals within regions were estimated from the genetic distance matrix, as specified in the analysis of molecular variance (AMOVA) procedure in ARLEQUIN version 3.1 (http://cmpg.unibe.ch/software/arlequin3/). A non-parametric permutation procedure with 3000 permutations was used to test the significance of variance components associated with the different possible levels of genetic structure in this study (Excoffier et al., 1992). The pairwise Fst values, a value of F statistic analogs computed from AMOVA, were used to compare genetic distances between any two groups.

RESULTS

Agronomic traits diversity

The value of 13 agronomic traits of each accession and the maximum, minimum, average, standard deviation and coefficient of variance (CV) for each trait were shown in Supplementary Table 6. The genetic diversity indices of 13 agronomic traits of Shaanxi soybean were between 1.65 (seed shape index) to 2.06 (protein content). Genetic diversity indices were different from regions or traits. However, genetic diversity indices of whole province were the highest in 10 of the 13 agronomic traits (Figure 2). Among different regions, 7 of 13 agronomic traits (plant height, no. of pods in the main stem, number of pods in branches, number of seeds per plant, seed weight per plant, index of seed shape and fat content) displayed highest genetic diversity indices in Guanzhong population, whereas 3 traits (number of nodes in the main stem, protein content and days after flowering) showed the highest genetic diversity indices in Southern Shaanxi population. Number of branches, 100-seed weight and days to flowering displayed the highest genetic diversity indices in the Northern Shaanxi population. Although, genetic diversity was different from trait to trait, the average genetic diversity index of 13 traits was highest in Guanzhong, followed by Southern and Northern Shaanxi, respectively. For example, genetic diversity index of seed shape index was much higher in Northern Shaanxi or Guanzhong than that in Southern Shaanxi. Figure 2 also indicated that, most morphological traits (plant height, no. of main stem, etc.) in Guanzhong population varied greatly, while growth traits (days to flowering and days after flowering) varied little compared with other 2 populations.

SSR allelic diversity

A total of 250 alleles were detected in the 91 accessions at 35 SSR loci. Supplementary Figure 5 showed the amplification result of all 91 accessions by the primer Satt259. The number of alleles (NA) at a locus varied from 4 for Satt230 to 12 for Satt259 and satt590, with a mean of 7.14 alleles per locus. 15 of the 250 alleles (6.0%) were unique and detected in only one accession. 20 alleles were specific to northern Shaanxi landraces and southern Shaanxi landraces, respectively, 8 alleles only detected in Guanzhong landraces. The PIC ranged from 0.40 for Satt242 to 0.89 for Satt184 with a mean of 0.74 per locus. The number of alleles per locus and PIC value for each locus of all 91 accessions were presented in Supplementary Table 5. The mean NA was the highest

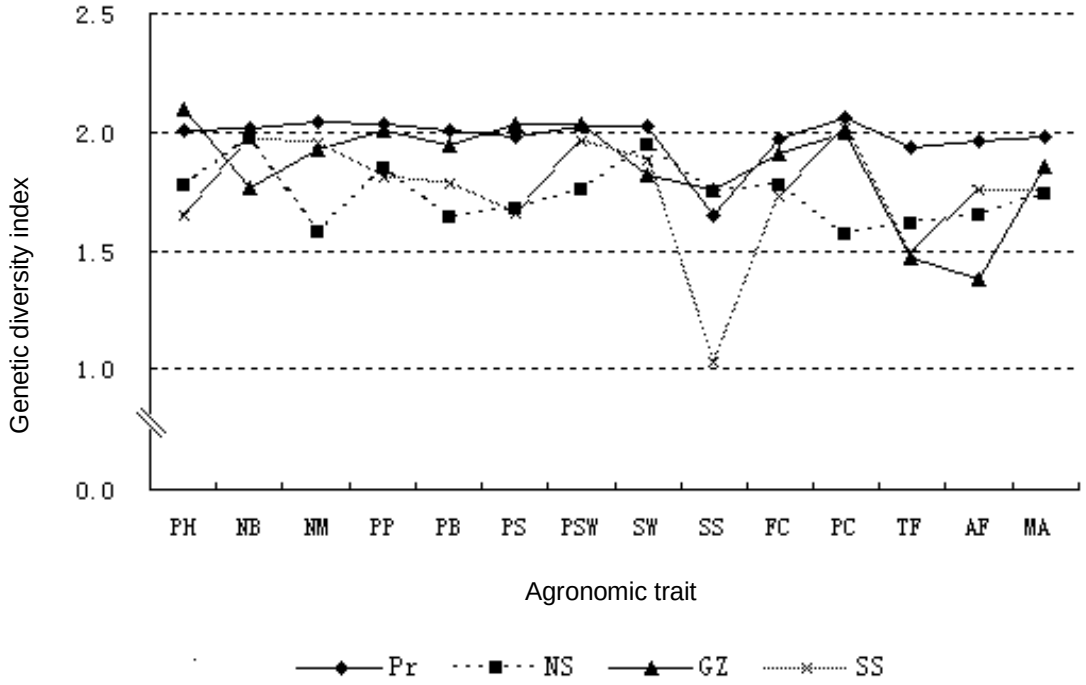

Figure 2. Genetic diversity index of 13 agronomic traits in whole province and different regions. Plant height (PH); no. of branches(NB); no. of nodes in main stem(NM); no. of pods in main stem(PP); no. of pods in branches(PB); no. of seeds per plant (PS); seed weight per plant (PSW); 100-seed weight (SW); index of seed shape (SS); fat content (FC); protein content (PC); days to flowering (TF); days after flowering (AF); average genetic diversity index of 13 traits (MA). Whole province (Pr), Northern Shaanxi (NS), Guangzhong (GZ) and Southern Shaanxi (SS).

in southern Shaanxi landraces (5.91), followed by Guanzhong landraces (5.54) and northern Shaanxi landraces (5.40). The mean PIC value showed similar trends as mean NA (Table 2).

Supplementary Figure 6 showed the genetic diversity indices of 35 SSR loci detected by the stated 35 primers. Genetic diversity indices of whole province were the highest in 12 of the 35 SSR loci. Among different regions, genetic diversity indices of Southern Shaanxi were the highest in 17 of the 35 SSR loci, followed by that of Northern Shaanxi (11 loci) and Guanzhong (7 loci). The highest average genetic diversity index of 35 loci was found in South Shaanxi population, followed by North Shaanxi and Guanzhong population, respectively, but the difference between any 2 populations was not significant. 16 SSR loci showed the lowest genetic diversity and 11 SSR loci displayed the highest genetic diversity in Northern Shaanxi population.

Cluster analysis and principal component analysis

The simple matching coefficients (SMC) among 91 soybean accessions ranged from 0.740 (between S43 from southern Shaanxi and N82 from northern Shaanxi) to 0.920 (between M17 and M18, both landraces from the same geographic region of Shaanxi, Guanzhong), with an average of 0.801. The averaged SMC of each geographic group was 0.802 for northern Shaanxi, 0.811 for Guanzhong, 0.809 for southern Shaanxi (Table 2). The averaged SMC between any 2 geographic groups was 0.802 for Guanzhong-Southern Shaanxi, 0.793 for Guanzhong-Northern Shaanxi and 0.791 for Northern-Southern Shaanxi. The population from northern Shaanxi has a higher level diversity than the soybean landraces from Guanzhong and southern Shaanxi.

The dendrogram based on SMC between accessions showed that, 91 accessions formed two major clusters, which generally corresponded to ecotype (Figure 3): cluster I contained 76 soybean landraces, of which 43 were from southern Shaanxi, 28 landraces from Guanzhong, the remaining 5 from northern Shaanxi. Cluster II included 15 accessions from northern Shaanxi. Cluster I was further separated into five subgroups (I-1 to I-5) and one outlier (from North Shaanxi). Group I-1 included 19 16 landraces from Guanzhong and 3 from northern Shaanxi and it was named as Guanzhong Group. Group I-2 contained 13 landraces from Southern Shaanxi, 2 from Guanzhong, thus, it was designed as Southern Shaanxi Group I. Group I-3 contained 18 landraces, with 10 landraces from Guanzhaong and 8 from southern Shaanxi, so it was called Guanzhong-Southern Shaanxi Group. Group I-4 contained 20 landraces from southern Shaanxi and 1 from northern Shaanxi and it was named

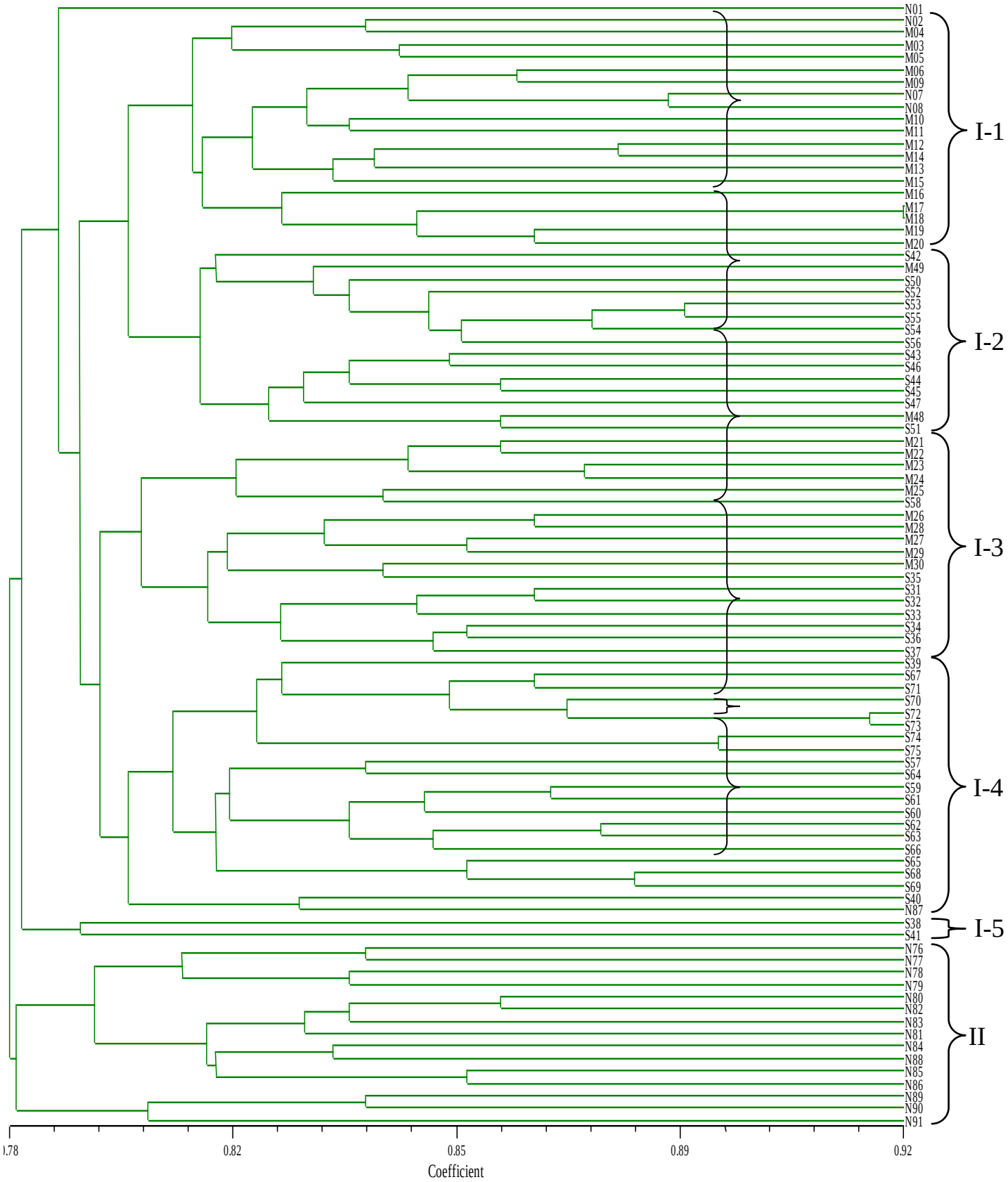

Figure 3: Dendrogram produced using UPGMA cluster analysis based on sample match coefficients demonstrating the association among 91 soybean accessions. The number of each accession was shown in table 1.

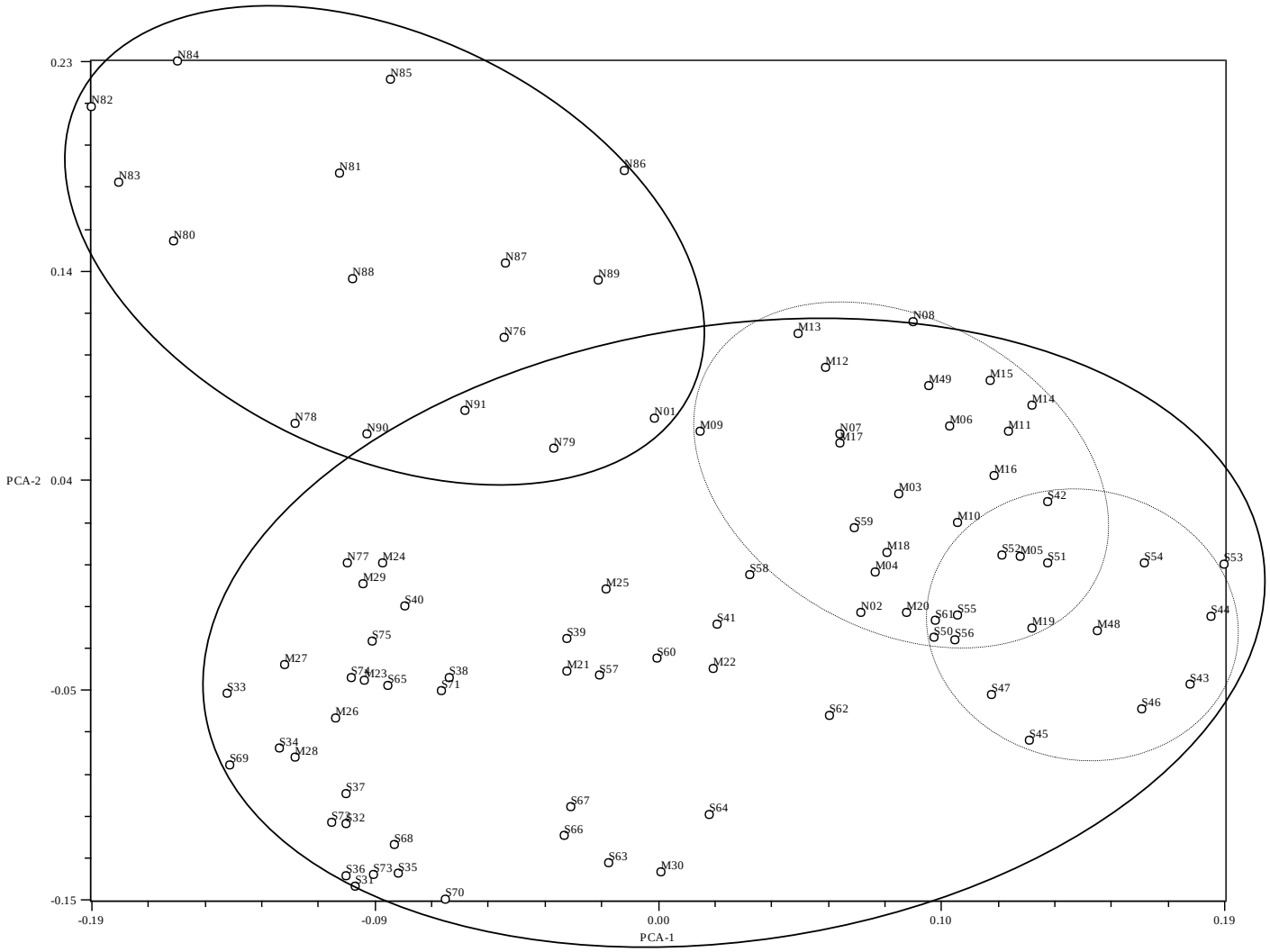

Figure 4. Association between 91 soybean accessions on the basis of the first two principal coordinates obtained from a principal coordinate analysis of sample match coefficients based on SSR and agronomic traits data. The number of each accession was shown in Table 1.

as southern Shaanxi Group II. Group I-5 included only 2 southern Shaanxi landraces, therefore, it was named as Southern Shaanxi Group III.

The PCA analysis (Figure 4) corresponded well with results of clustering by UPGMA. The first principal component accounted for 27.3% of the total variation and the second principal component explained 8.5% of the total variation. These results were consistent with that from the cluster analysis.

AMOVA to partition genetic diversity among the populations

Analysis of molecular variance indicated that, 5.72% of the variance was due to differences among populations of geographic regions and 94.28% was due to difference of individuals within geographic regions (Table 3). However, despite the small value for variation among populations, it was statistically significant ($P < 0.0001$).

The pairwise F_{st} values for the landraces from three geographic regions of Shaanxi ranged from 0.0428 to 0.0707 between geographic regions, these values are all significantly different from zero (Table 4).

DISCUSSION

Li et al. (2008) studied genetic diversity of 1863 Chinese soybean landraces with 59 SSR loci. The results indicated that, the average number of alleles per locus was 19.7. Li et al. (2010) genotyped a sample of 303 accessions of domesticated soybean and wild soybean with 99 microsatellite markers, they found the SSR loci averaged 21.5 alleles per locus. Guan et al. (2010)

Table 3. Analysis of molecular variance for 91 soybean samples from three different geographic regions of Shaanxi province.

Source of variation	Degree of freedom	Sum of squares	Variance component	Percentage of variation	P value
Among regions	2	207.74	2.292	5.72	<0.0001
Individuals within regions	88	3325.05	37.79	94.28	<0.0001
Total	90	3532.80	40.08		
Fixation index (F_{st})=0.057					

Table 4. Pairwise F_{st} values for 91 soybean accessions from different geographic regions of Shaanxi province.

	Southern Shaanxi	Guangzhong	Northern Shaanxi
Southern Shaanxi	0.0000		
Guangzhong	0.0428*	0.0000	
Northern Shaanxi	0.0707*	0.0663*	0.0000

* Indicating significant at 0.01 probability level.

Table 5. Thirty-five SSR loci, the number of alleles per locus and PIC value for each locus of all 91 accessions in this study.

Locus (linkage block)	Alleles	PIC	Locus (linkage block)	Alleles	PIC
Satt300(A1)	9	0.73	Satt146(H)	7	0.82
Satt390(A2)	5	0.74	Satt568(F)	5	0.73
Satt197(B1)	9	0.64	Satt012 (G)	7	0.72
Satt415(B1)	6	0.72	Satt434(H)	6	0.78
Satt168(B2)	7	0.67	Satt442 (H)	7	0.74
Satt577(B2)	5	0.69	Satt571(I)	7	0.82
Satt194(C1)	5	0.72	Satt414(J)	9	0.80
Satt565(C1)	10	0.82	Sct_001(J)	6	0.85
Satt371(C2)	5	0.71	Satt001 (K)	8	0.77
Satt286 (C2)	7	0.75	Satt242(K)	8	0.40
Satt267(D1a+Q)	5	0.66	Sat_099(L)	6	0.78
Satt184(D1a+Q)	11	0.89	Satt462(L)	10	0.87
Satt005(D1b+w)	6	0.76	Satt590 (M)	12	0.84
Satt216(D1b+w)	9	0.71	Satt022(N)	6	0.71
Satt226(D2)	8	0.75	Satt339(N)	6	0.80
Satt002(D2)	6	0.78	Satt259(O)	12	0.72
Satt230 (E)	4	0.73	Satt345(O)	6	0.80
Satt268 (E)	5	0.60			

compared the genetic diversity of 205 Chinese landraces, which represented the 7 different soybean ecotypes in China, with 39 Japanese soybean accessions from various regions. Total of 745 alleles were detected in 46 SSR loci with an average of 16.2 alleles per locus. In this study, we genotyped 91 Shaanxi soybean landraces with 35 SSR loci and obtained 7.14 alleles per locus which was much lower than that in the previous studies. It might be that our samples were obtained only from one province, whereas the earlier mentioned studies included accessions from all over the China and worldwide or wild soybean. However, our result corresponded well with the study of Wang et al. (2010), who analyzed 40 soybean accessions including cultivars, landraces and wild soybean accessions using 40 SSR primer pairs and found 6.55 alleles per locus. Our result indicated that, there was relatively abundant allelic diversity among Shaanxi soybean landraces.

Genetic diversity in Southern Shaanxi population is higher than that in either Guanzhong or Northern Shaanxi

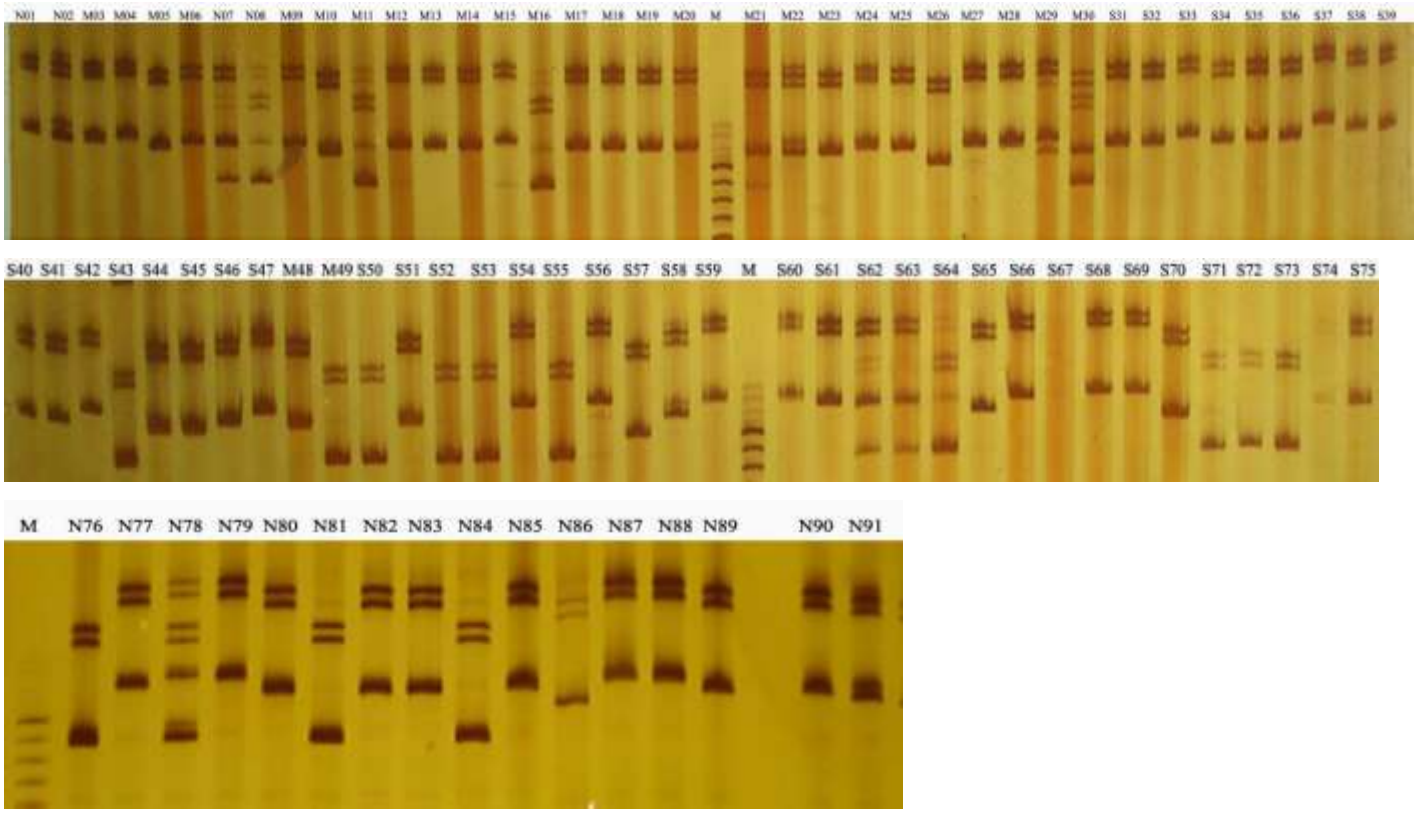

Figure 5. Patterns of polymorphic amplification in all 91 soybean accessions at SSR locus Satt259. The number of each accession was shown in Table 1; M,DNA marker.

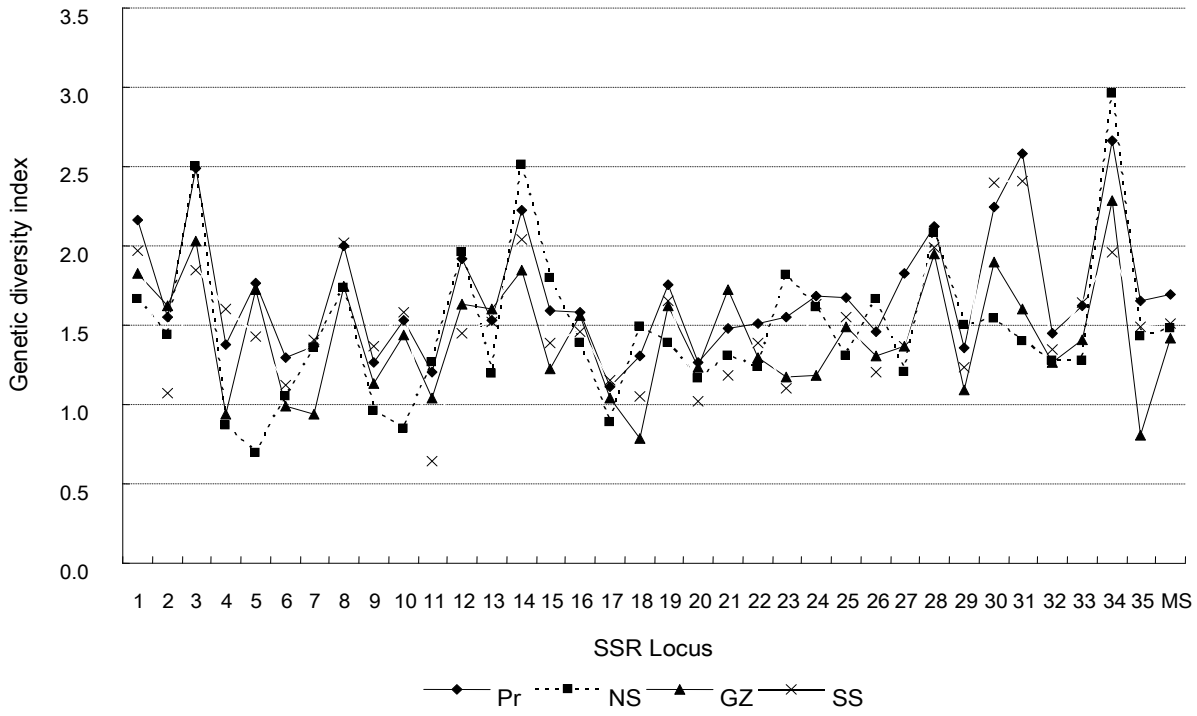

Figure 6. Genetic diversity indices of 35 SSR loci in whole province and different regions. The number 1-35 stand for different SSR primer which could be seen in Table S1. MS=Average genetic diversity index of 35 loci; whole province (Pr), Northern Shaanxi (NS), Guangzhong (GZ) and Southern Shaanxi (SS).

Table 6. Thirteen agronomic traits and their max, min, average, standard deviation and CV for each trait.

Code no.	Accession no.	PH	NB	NM	PP	PB	PS	SW	PSW	SS	TF	AF	PC	FC
N01	ZDD10255	67.5	3.0	17.9	22.1	27.8	90.9	16.3	24.7	2.8	52.7	102.3	44.0	13.2
N02	ZDD10170	66.1	5.7	17.9	30.8	76.9	195.7	11.4	22.0	2.2	54.7	79.7	43.8	18.1
M03	ZDD10207	133.5	8.7	25.3	37.1	105.2	247.1	21.2	47.0	1.6	68.3	97.3	43.3	18.5
M04	ZDD10199	94.3	7.1	15.5	16.6	126.5	258.2	11.8	28.7	2.1	67.7	81.7	44.8	17.2
M05	ZDD10250	72.3	6.0	17.0	33.5	96.3	245.2	13.9	35.3	1.7	71.7	90.0	40.7	19.0
M06	ZDD10315	123.2	8.2	21.4	23.4	199.4	402.5	8.5	42.0	3.2	65.0	90.0	38.9	16.2
N07	ZDD10297	129.4	5.5	15.2	17.2	142.2	305.5	7.8	23.1	3.5	68.0	81.0	41.9	16.1
N08	ZDD10299	67.0	7.3	16.0	44.3	137.1	305.2	10.7	24.3	3.5	64.0	90.3	40.8	15.0
M09	ZDD10309	148.6	4.8	23.4	56.9	175.0	444.9	8.8	41.0	2.9	58.3	93.3	41.6	16.1
M10	ZDD10337	182.5	6.9	23.8	40.8	216.2	462.3	8.8	36.3	2.2	68.3	91.7	39.1	18.0
M11	ZDD10371	44.1	4.1	16.0	47.8	91.9	259.3	8.3	17.7	1.6	66.3	83.3	39.4	18.3
M12	ZDD03614	92.2	4.8	22.4	23.2	58.9	138.2	18.0	22.0	1.4	62.0	87.3	46.0	17.6
M13	ZDD03618	113.9	6.1	19.3	41.5	149.7	292.3	10.8	43.7	2.2	62.0	94.3	46.0	16.4
M14	ZDD10408	78.7	5.6	17.4	18.3	53.8	112.7	17.7	22.3	1.5	61.7	87.7	46.1	17.4
M15	ZDD10413	73.8	4.7	17.8	37.3	79.3	195.3	20.4	35.0	1.7	67.7	85.7	42.8	20.2
M16	ZDD10418	52.5	5.6	15.9	44.4	106.7	251.5	13.1	34.0	1.5	66.3	88.0	44.2	18.0
M17	ZDD10386	137.4	6.5	21.5	27.2	85.3	215.3	20.1	40.7	1.6	67.0	91.3	44.6	18.5
M18	ZDD10379	69.1	5.8	18.7	41.7	130.2	302.4	12.3	44.0	1.7	67.0	96.7	43.0	18.4
M19	ZDD10646	115.3	4.0	24.1	39.8	81.6	220.5	19.8	40.3	1.8	75.0	94.0	43.8	18.4
M20	ZDD10636	138.7	6.9	23.0	31.1	134.7	265.6	11.4	24.3	2.2	53.0	94.0	44.1	16.5
M21	ZDD03669	177.3	6.3	20.5	33.2	186.1	372.8	9.2	63.3	3.6	67.3	87.7	41.0	17.1
M22	ZDD03671	92.9	4.8	21.3	73.4	148.1	390.5	7.5	22.0	3.2	67.0	82.0	41.5	16.1
M23	ZDD03666	180.7	6.8	21.7	33.0	184.5	420.5	7.2	35.3	2.9	62.0	94.3	39.0	17.8
M24	ZDD10730	85.3	5.3	20.0	75.3	180.5	448.3	7.7	32.7	3.2	64.0	87.3	41.5	15.7
M25	ZDD10742	151.2	5.3	20.8	33.8	146.7	333.0	8.6	26.7	3.3	64.0	87.3	42.7	16.0
M26	ZDD24061	90.9	4.6	19.1	37.4	47.4	197.1	20.0	41.3	1.4	49.7	71.0	45.8	20.1
M27	ZDD10809	66.8	5.4	18.5	28.9	58.4	149.1	17.5	16.0	1.9	52.3	79.3	46.4	17.3
M28	ZDD03700	180.0	5.9	19.7	25.3	189.7	385.8	7.0	21.3	2.6	58.3	88.3	41.3	17.6
M29	ZDD10802	83.3	4.9	21.1	29.8	57.6	138.8	17.9	22.0	1.7	61.3	88.0	46.1	16.5
M30	ZDD03717	146.0	8.0	23.1	19.1	131.8	289.0	16.3	47.0	2.5	68.3	91.7	40.6	18.1
S31	ZDD10184	81.1	4.5	19.3	46.0	98.3	260.7	19.8	18.3	1.8	67.0	79.7	47.5	17.1
S32	ZDD10517	58.2	3.9	14.0	44.5	56.3	172.1	15.2	23.3	1.7	54.7	62.0	47.0	18.1
S33	ZDD10458	84.1	5.0	19.7	32.3	55.1	131.6	19.3	20.7	1.8	67.0	80.3	48.2	16.5
S34	ZDD10460	95.6	5.3	20.2	26.8	85.1	192.8	20.8	31.7	1.6	62.7	96.7	45.4	18.0
S35	ZDD10422	159.1	9.4	25.3	26.5	142.1	271.5	21.9	46.0	2.2	74.3	96.0	45.8	17.7
S36	ZDD10509	87.7	5.4	18.6	28.4	92.3	208.3	17.3	38.3	1.8	69.0	90.7	45.9	18.5
S37	ZDD10473	77.2	5.4	16.9	33.0	84.1	189.5	18.1	28.3	1.9	66.3	93.3	41.9	18.6
S38	ZDD10373	91.5	7.4	22.6	50.2	114.0	273.7	14.6	37.0	1.9	68.0	89.7	43.4	19.0
S39	ZDD10459	81.2	5.5	23.7	42.1	92.9	208.7	13.3	30.7	1.5	69.0	88.3	45.7	17.7
S40	ZDD10376	108.7	7.4	21.6	42.1	118.5	240.8	15.4	33.3	2.2	69.0	97.3	42.4	19.1
S41	ZDD10609	125.7	7.7	23.9	55.5	236.5	449.7	6.4	27.7	1.8	77.7	84.0	48.1	16.4
S42	ZDD10445	69.2	4.4	18.9	48.7	55.8	192.8	13.0	21.0	1.7	50.3	76.7	43.7	19.6
S43	ZDD19503	94.3	10.0	21.4	31.9	144.3	321.6	11.2	32.7	1.7	78.0	85.7	43.3	19.2
S44	ZDD19497	113.7	6.7	26.9	55.0	153.3	322.8	16.0	50.7	1.8	77.0	99.3	44.5	18.7
S45	ZDD19463	138.5	8.7	24.7	25.2	122.5	248.9	17.3	43.7	2.1	75.0	96.3	44.3	18.6
S46	ZDD10442	80.2	7.5	16.8	60.6	146.6	337.8	13.5	40.0	1.9	74.3	94.7	42.1	19.2
S47	ZDD10454	89.0	5.3	20.4	68.0	97.8	300.9	11.9	44.1	1.6	75.7	86.7	42.9	18.8
M48	ZDD03629	82.5	5.7	20.3	46.8	128.7	266.2	10.2	20.0	1.7	61.0	88.3	44.1	17.2
M49	ZDD24060	51.0	4.7	13.1	31.7	45.4	145.3	19.7	24.0	1.6	50.3	82.3	44.6	20.6
S50	ZDD10600	97.1	6.6	22.1	60.5	163.9	347.9	8.2	26.7	1.9	77.0	94.7	45.6	17.7
S51	ZDD10656	84.9	5.4	20.1	26.0	52.8	121.1	14.9	15.7	1.7	58.0	92.0	47.1	17.4

Table 6. Continued.

S52	ZDD10661	88.4	5.3	18.9	29.8	73.7	178.1	19.8	31.7	1.8	69.7	91.7	45.5	18.1
S53	ZDD10699	87.7	4.1	23.1	59.2	62.1	178.5	12.0	19.0	1.8	76.3	86.7	43.9	18.0
S54	ZDD10664	91.4	4.8	19.1	51.8	94.5	212.2	19.9	36.3	1.6	67.7	94.3	44.3	17.4
S55	ZDD10694	96.5	6.6	27.2	52.9	107.3	269.9	13.4	30.7	1.8	69.7	90.7	46.1	17.9
S56	ZDD10698	92.1	6.1	27.5	35.8	74.9	172.0	20.7	32.0	2.0	94.0	82.0	45.7	18.1
S57	ZDD10683	73.7	5.4	18.8	54.7	116.4	279.5	15.2	35.0	2.0	64.0	97.3	46.7	17.6
S58	ZDD10757	154.0	5.2	20.5	28.9	104.5	227.9	10.8	24.0	3.5	62.7	85.7	40.8	16.0
S59	ZDD10762	116.8	7.6	22.9	22.5	93.1	191.5	24.0	36.0	1.8	80.0	87.7	42.8	16.7
S60	ZDD10796	116.4	4.0	22.3	55.1	68.4	204.3	16.1	31.3	1.9	84.7	76.7	43.8	16.4
S61	ZDD10775	84.2	7.5	18.3	27.4	103.0	191.7	14.7	29.7	2.3	73.0	89.0	43.1	17.0
S62	ZDD10777	106.8	8.7	24.5	28.4	110.7	222.1	13.3	25.7	1.8	88.0	86.0	45.3	16.7
S63	ZDD10768	105.7	7.1	26.3	42.7	120.6	245.7	16.5	42.0	2.2	81.0	96.7	42.1	17.1
S64	ZDD10825	83.8	5.5	20.4	62.3	114.6	335.5	12.7	40.7	2.1	61.3	92.0	40.0	18.1
S65	ZDD10868	150.4	7.6	24.3	16.3	170.8	293.6	15.3	40.7	2.0	82.3	97.3	42.0	17.3
S66	ZDD10841	108.2	6.6	19.1	18.9	97.7	206.9	17.2	33.7	1.7	67.7	85.7	42.5	17.8
S67	ZDD10845	96.9	6.6	21.5	31.7	91.7	200.7	15.7	28.0	1.7	68.3	93.3	42.2	17.9
S68	ZDD10828	135.4	8.1	22.4	16.8	168.9	315.7	14.6	39.0	2.1	66.7	98.7	42.2	17.1
S69	ZDD10883	128.7	9.2	20.8	16.4	241.5	462.8	11.5	47.3	2.0	84.7	84.0	43.6	17.1
S70	ZDD19536	92.9	5.6	18.5	21.9	84.0	180.7	20.0	37.3	1.9	71.7	91.0	39.9	18.6
S71	ZDD03691	98.1	6.3	20.1	27.1	91.9	216.7	17.9	31.3	1.7	69.7	87.7	41.3	18.7
S72	ZDD03706	88.4	5.6	19.7	39.9	121.4	273.9	11.4	26.3	2.1	69.0	85.3	44.2	17.8
S73	ZDD10927	90.0	6.6	17.3	17.5	128.1	233.1	20.1	39.7	2.0	68.0	97.3	41.4	17.9
S74	ZDD10907	104.1	5.7	18.9	27.9	69.4	163.3	16.9	25.0	1.9	68.3	80.7	46.1	17.9
S75	ZDD10912	102.9	6.9	23.2	35.6	88.8	204.6	19.1	35.0	1.9	73.0	99.7	42.6	18.9
N76	ZDD10226	62.4	4.6	17.9	21.3	65.8	136.5	18.3	33.9	2.2	62.0	73.0	44.3	16.3
N77	ZDD10230	67.8	6.3	17.8	27.0	121.5	324.5	17.0	45.3	2.3	53.0	84.0	44.5	16.7
N78	ZDD10237	75.5	6.3	17.5	28.3	80.3	234.0	17.3	37.3	2.1	49.0	86.0	42.6	18.7
N79	ZDD10242	97.2	9.0	17.2	20.8	118.0	243.4	14.1	32.1	1.8	49.0	81.0	44.8	18.0
N80	ZDD10134	67.0	7.2	15.8	18.4	108.4	228.8	17.0	31.9	2.0	45.0	60.0	42.3	17.6
N81	ZDD10143	65.2	3.6	13.8	23.0	24.2	109.8	19.6	18.7	1.7	39.0	60.0	46.5	17.6
N82	ZDD10260	47.3	3.2	15.5	15.2	35.1	95.1	18.2	22.7	2.0	44.0	81.0	45.7	17.8
N83	ZDD10274	136.4	6.6	21.4	12.0	71.6	175.8	17.2	33.5	2.1	53.0	85.0	46.7	18.1
N84	ZDD10329	109.6	5.8	20.4	15.8	91.2	205.2	14.7	35.7	2.3	67.0	63.0	45.5	16.4
N85	ZDD10332	105.9	6.9	19.8	17.3	68.8	146.1	22.8	36.1	1.7	61.0	74.0	46.4	17.4
N86	ZDD10135	103.3	3.6	15.8	27.6	39.6	152.0	14.0	15.8	2.3	47.0	62.0	48.6	16.1
N87	ZDD10239	91.3	6.8	14.3	19.5	94.5	179.5	14.9	28.6	1.9	50.0	59.0	45.8	17.3
N88	ZDD10263	141.4	5.0	20.2	39.0	137.0	324.4	7.0	23.5	4.6	49.0	56.0	45.8	15.8
N89	ZDD10294	94.2	6.4	19.8	26.8	78.0	193.8	7.3	18.7	3.4	64.0	54.0	45.0	16.0
N90	ZDD10252	121.6	6.0	19.4	7.4	79.4	181.8	9.5	16.5	4.6	62.0	56.0	46.4	15.3
N91	ZDD10270	104.0	7.8	19.0	23.3	177.3	477.3	8.1	29.3	4.0	53.0	56.0	46.5	16.3
Max		182.5	10.0	27.5	75.3	241.5	477.3	24.0	63.3	4.6	94.0	102.3	48.6	20.6
Min		44.1	3.0	13.1	7.4	24.2	90.9	6.4	15.7	1.4	39.0	54.0	38.9	13.2
Average		101.0	6.1	20.1	34.2	108.6	248.5	14.6	31.7	2.2	65.2	85.3	43.9	17.5
Stand deviation		31.2	1.5	3.1	14.6	46.7	90.7	4.4	9.4	0.7	10.3	11.31	2.3	1.19
CV		0.31	0.24	0.16	0.43	0.42	0.36	0.30	0.29	0.31	0.16	0.13	0.05	0.07

Plant height (PH); number of branches (NB); number of nodes in main stem(NM); number of pods in main stem (PP); number of pods in branches (PB); number of seeds per plant(PS); 100-seed weight (SW); seed weight per plant (PSW); index of seed shape (SS); days to flowering (TF); days after flowering (AF); protein content (PC); fat content (FC).

germplasm. The higher number of alleles and PIC value present in the Southern Shaanxi population than those in Guanzhong and Northern Shaanxi population suggested that, Southern Shaanxi population is a potential source

for new alleles for use in soybean breeding programs. Obviously, the alleles that was only detected in one geographic region was much higher in Northern and Southern Shaanxi than in Guanzhong, indicating that Northern or Southern Shaanxi population had much more unique alleles. This could be explained by various climate and landforms in southern and northern Shaanxi region, while environment in Guanzhong region is quite uniform.

The highest SSR diversity was detected in the Southern Shaanxi soybean population, followed by Northern Shaanxi and Guanzhong populations. However, the average genetic diversity of agronomic traits in Guanzhong soybean population is highest, followed by Southern and Northern Shaanxi populations. One explanation is that, SSR loci reflected the non-coding DNA sequence which did not control phenotypic traits, the second explanation is that, phenotypic traits were easily influenced by environment, the field test was performed in Yangling, Guanzhong region, which was benefit for landraces from Guanzhong to show their phenotypic diversity, while other landraces from north or south Shaanxi were rather difficult to adapt the environment in Guanzhong, which could affect the phenotype value of each trait from these two populations. South Shaanxi germplasm had more SSR alleles per locus and PIC value, whereas North Shaanxi population had the lowest average pairwise SMC which means a more genetic diversity. This reflected different genetic variation of south and north Shaanxi soybean population. South Shaanxi collection contained more allelic variation which was consistent with its environment, whereas accessions from north Shaanxi showed low similarity coefficient, which means there were large genetic distance among accessions. As we know, south Shaanxi region are mountain area and the huge mountain, Qinling covered most of south Shaanxi area. Different mountain ranges created many little valleys, basin or flatlands which were relative isolated from the each other and each has its micro-climate, which could cause new gene variation. While north Shaanxi was hilly region and also had diverse landforms and climate. Genetic diversity of soybean in this region might be higher than what we already know. As we can see from this study, landraces from north Shaanxi, although small in number, were separated into 2 main groups and widely spread on PCA plot, while landraces from Guanzhong and south Shaanxi were only put into the group 2. The degree of genetic similarity of North Shaanxi landraces were lower than those in other 2 populations, similar to the SMC of Guanzhong-south Shaanxi population, this suggested that there would be more genetic diversity in north Shaanxi region, which had not been reported before. Since it was a famous arid area in China, soybean landraces from north Shaanxi may be specifically adapt to adverse conditions such as salt and drought and may have useful genes to tolerate these abiotic stresses. More attention should be paid to collection, evaluation, conservation and utilization of the landraces and genes in north Shaanxi region.

In summary, a primary core collection of 91 soybean landraces was established from the entire soybean germplasm collection of Shaanxi province. Previous data showed that, this core collection of 91 accessions is good representative (Liu et al., 2006). UPGMA cluster analysis and PCA analysis based on SSR markers and agronomic data clearly showed that, 91 accessions formed two major clusters and that clusters generally corresponded to geographic origin. AMOVA analysis showed that, a significant proportion of variance (94.28%) was due to differences within populations.

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