

# Analysis of genetic diversity among indigenous landraces from sesame (*Sesamum indicum* L.) core collection in China as revealed by SRAP and SSR markers

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## Abstract

The molecular genetic diversity of 404 indigenous landraces from sesame core collection in China were evaluated by 11 SRAP and 3 SSR markers, 175 fragments were generated, of which 126 were polymorphic with an average polymorphism rate of 72%. Jaccard's genetic similarity coefficients ( $GS=0.7130$ ), Nei's gene diversity ( $h=0.2418$ ) and Shannon's Information index ( $I=0.3847$ ) were calculated, a dendrogram of the 404 landraces was made, landraces from various zones were distributed throughout the dendrogram, accessions from different agro-ecological zones were indistinguishable by cluster analysis, geographical separation did not generally result in greater genetic distance, a similar pattern was obtained using principal coordinates (PCO) analysis. As to seven agro-ecological zones, the maximum Nei's gene diversity ( $h = 0.2613$ ) and Shannon index ( $I = 0.3980$ ) values in zone VII indicated that they were genetically more diverse than those in other zones, while the least genetically diverse region was zone III ( $h = 0.1772$ ,  $I = 0.2858$ ). Nei's genetic identity and genetic distance among landraces from seven agro-ecological zones were also analyzed, the genetic relationship of seven zones was inferred using the UPGMA method. This study demonstrated that SRAP and SSR markers were appropriate for evaluation of sesame genetic diversities. There existed extensive genetic diverse among indigenous landraces and the abundance of genetic diversity of landraces in different agro-ecological zones was various. Understanding of these characteristics of indigenous landraces in China can provide theoretical foundation for further collection, effective protection and reasonable

utilization of these sesame landraces in breeding.

**Keywords** Core collection; SSR; Genetic diversity; Landraces; Sesame; SRAP

## Introduction

Sesame (*Sesamum indicum* L.) is one of the four major oil crops (rapeseed, soybean, peanut and sesame) with nutritional value in China. It has been cultivated for about 2200 years, while China has been identified as one of the five sesame diversity centres in classical studies (Zeven and Zhukovsky, 1975; Hawkes, 1983).

A sesame core collection containing 453 accessions (404 indigenous landraces, 18 indigenous released cultivars, 31 exotic accessions) was established from 4251 accessions (Zhang et al., 2000). The 404 indigenous landraces were from seven agro-ecological zones (29 provinces) in China, which were divided according to climatic and geographic characteristics, as well as the planting system. The 404 indigenous landraces in this sesame core collection represent well the genetic variability of the whole indigenous sesame landraces in China.

Though the quantity of indigenous sesame landraces is considerable in China, the diversities in morphological traits, phenotypic traits, agronomic traits, quality traits and resistance to stress or diseases among them have been evaluated, three sesame germplasm catalogues were edited and published (Chen, 1981; Feng, 1992, 1997). Dong (1990) identified resistance to *Fusarium oxysporium* of several Chinese sesame germplasm, Li et al. (1991) identified some Chinese sesame germplasm of their resistance to *Macrophomina phaseolina*, Feng et al. (1991) identified and evaluated tolerance to water-logging stress of several Chinese sesame germplasm, which were

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**Table 1.** Description of 404 accessions used in this study

| Pop. code | Acc. No. | Varietal type | Origin                                    | Annotation               |
|-----------|----------|---------------|---|--------------------------|
| A         | 42       | Landrace      | Northeast and northwest of China          | agro-ecological zone I   |
| B         | 78       | Landrace      | Northern China                            | agro-ecological zone II  |
| C         | 55       | Landrace      | The Yellow River and Huai River valley    | agro-ecological zone III |
| D         | 69       | Landrace      | The Yangtze and Han River valley          | agro-ecological zone IV  |
| E         | 56       | Landrace      | Middle and lower of Yangtze valley        | agro-ecological zone V   |
| F         | 57       | Landrace      | South-central of China and southern China | agro-ecological zone VI  |
| G         | 47       | Landrace      | Southwest of China                        | agro-ecological zone VII |

the prior studies of sesame accessions resistance to disease or stress. Xiao et al. (1992) analyzed the main characteristics of 372 black seed coat sesame accessions in China, including morphological traits, agronomic traits, quality traits and resistances, Feng et al. (1996) carried out further characterization and evaluation of 225 pre-selected sesame accessions in China, with 3 repeats at 14 experimental stations in different ecological regions from 1992 to 1994. They screened for a set of accessions with certain excellent agronomic traits or resistance characters which could be used as parents material in breeding. Zhang et al. (2001) evaluated resistance to *Macrophomina phaseolina* and *Fusarium oxysporium* of 85 sesame accessions primarily selected, elucidated resistance and application potential of disease resistant accessions. They also found that there were isoenzyme bands similarity and differences between disease resistant and susceptible accessions in some zone. Although such many traits among Chinese sesame germplasm have been studied, morpho-agronomic markers may not reflect true genetic identities and diversities (Ferdinandez et al., 2001). Information on Chinese sesame germplasm genetic diversity at molecular level is limited and evaluation of molecular genetic diversity among the whole indigenous sesame landraces is especially absent. However, molecular markers are simple, informative, and portable compared to morphological and phenotypic traits. The utilization of molecular markers have been effective in evaluating genetic variation within species, because they are not affected by the environmental conditions in which the plants are grown (Powell et al., 1996).

This paper represents the latest study of the molecular genetic diversity among indigenous landraces from sesame core collection in China. The objectives of this investigation were to explore the characteristics of genetic diversity and their relationships among landraces from different agro-ecological zones in China, then provide theoretical foundation for utilizing these landraces effectively and carrying out the molecular biological studies purposefully.

## Materials and Methods

### Plant Materials

All of the 404 indigenous landraces from the sesame core collection in China were used in this study (Table 1). They were divided into 7 populations (with population code A to G) according to the 7 agro-ecological zones (I to VII) in China (Zhang et al., 2000). Young healthy leaves from plants of each accession were collected and conserved under  $-80^{\circ}\text{C}$  until DNA extraction.

DNA extraction, polymerase chain reactions (PCR) amplification and electrophoresis

Total genomic DNAs of 404 accessions were prepared from young leaves according to CTAB method (Doyle and Doyle, 1987) with some modification.

A total of 36 SRAP primer combinations and 10 SSR primer pairs were used (Table 2). The SRAP and SSR primer sequences were referred to Li et al. (2001) and Dixit et al. (2005), respectively. PCR of SRAP markers were conducted in 20  $\mu\text{L}$  solution containing 80 ng of DNA, 50 ng of forward primers, 50 ng of reverse primers, 1  $\times$  buffer (MBI), 4 mmol of  $\text{Mg}^{2+}$ , 0.40 mmol of dNTPs, and 1 U Taq polymerase (MBI). The PCR profile was an initial denaturation of  $94^{\circ}\text{C}$  for 2 min, followed by 4 cycles of  $94^{\circ}\text{C}$  for 1 min,  $35^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 1 min, then 34 cycles of  $94^{\circ}\text{C}$  for 1 min,  $50^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 1 min, and a final incubation at  $72^{\circ}\text{C}$  for 5 min, then  $4^{\circ}\text{C}$  thereafter. According to Zhang et al. (2008), PCR of SSR markers were conducted in 20  $\mu\text{L}$  solution containing 25 ng of DNA, 4  $\mu\text{mol}$  of forward primers, 4  $\mu\text{mol}$  of reverse primers, 1  $\times$  buffer (MBI), 2 mmol of  $\text{Mg}^{2+}$ , 0.25 mmol of dNTPs, and 0.80 U Taq polymerase (MBI). The PCR profile was an initial denaturation of  $94^{\circ}\text{C}$  for 3 min, followed by 34 cycles of denaturing at  $94^{\circ}\text{C}$  for 50 sec, annealing at appropriate temperature for 45 sec, extension at  $72^{\circ}\text{C}$

**Table 2.** Sequences of primers used in this study.

| Primer code    | Forward primer          |                | Reverse primer          |             |
|----------------|-------------------------|----------------|-------------------------|-------------|
|                | Primer sequence (5'–3') | Primer code    | Primer sequence (5'–3') | Primer code |
| Me01           | TGAGTCCAAACCGGATA       | Em01           | GACTGCGTACGAATTAAT      |             |
| Me02           | TGAGTCCAAACCGGAGC       | Em02           | GACTGCGTACGAATTTGC      |             |
| Me03           | TGAGTCCAAACCGGAAT       | Em03           | GACTGCGTACGAATTGAC      |             |
| Me04           | TGAGTCCAAACCGGACC       | Em04           | GACTGCGTACGAATTTGA      |             |
| Me05           | TGAGTCCAAACCGGAAG       | Em05           | GACTGCGTACGAATTAAC      |             |
| Me06           | TGAGTCCAAACCGGTAA       | Em06           | GACTGCGTACGAATTGCA      |             |
| Me07           | TGAGTCCAAACCGGTCC       | Em07           | GACTGCGTACGAATTCAA      |             |
| Me08           | TGAGTCCAAACCGGTGC       | Em08           | GACTGCGTACGAATTCTG      |             |
| Me09           | TGAGTCCAAACCGGACG       | Em09           | GACTGCGTACGAATTCGA      |             |
| Me10           | TGAGTCCAAACCGGACT       | Em10           | GACTGCGTACGAATTCAG      |             |
| Me11           | TGAGTCCAAACCGGAGG       | Em11           | GACTGCGTACGAATTCCA      |             |
| GBssr-sa-05-F  | TCATATATAAAAAGGAGCCCAAC | GBssr-sa-05-R  | GTCATCGCTTCTCTCTTCTTC   |             |
| GBssr-sa-08-F  | GGAGAAATTTTCAGAGAGAAAAA | GBssr-sa-08-R  | ATTGCTCTGCCTACAAATAAAA  |             |
| Sesame-09-F    | CCCAACTCTTCGTCTATCTC    | Sesame-09-R    | TAGAGGTAATTGTGGGGGA     |             |
| GBssr-sa-33-F  | TTTCTCTGAATGGCATAGTT    | GBssr-sa-33-R  | GCCCAATTTGTCTATCTCCT    |             |
| GBssr-sa-72-F  | GCAGCAGTTCCGTCTTGG      | GBssr-sa-72-R  | AGTGCTGAATTTAGTCTGCATAG |             |
| GBssr-sa-108-F | CCACTCAAAATTTTCACTAAGAA | GBssr-sa-108-R | TCGTCTTCTCTCTCCTCC      |             |
| GBssr-sa-123-F | GCAAACACATGCATCCCT      | GBssr-sa-123-R | GCCCTGATGATAAAGCCA      |             |
| GBssr-sa-173-F | TTTCTTCTCGTTGCTCG       | GBssr-sa-173-R | CCTAACCAACCACCCTCC      |             |
| GBssr-sa-182-F | CCATTGAAAACCTGCACAAA    | GBssr-sa-182-R | TCCACACACAGAGAGCCC      |             |
| GBssr-sa-184-F | TCTTGCAATGGGGATCAG      | GBssr-sa-184-R | CGAATATAGATAATCACTTGGAA |             |

for 1 min, and a final incubation at 72°C for 10 min, then 4°C thereafter. PCR were carried out in a 96 well plate in a PTC-100 thermocycler (MJ Research, Watertown, MA).

PCR products were size-separated on 6% denaturing polyacrylamide gels. The electrophoresis parameters and silver staining of gels were based on the protocols of Lin et al. (2005).

#### Data analysis

After silver staining, all major DNA band sizes were recorded using 100 bp DNA ladder as the reference. The amplified DNA fragments were recorded as either 1 or 0, respectively, representing presence or absence of the band.

Pairwise genetic similarity coefficient (GS) was calculated using Jaccard's coefficient by the SIMQUAL program of the NTSYS-pc software package version 2.1 (Rohlf, 2000). Afterwards, a dendrogram (Sokal and Michener, 1958) was constructed based on the genetic similarity matrix by the UPGMA algorithm and generated by the SHAN clustering program (Sneath and Sokal, 1973) of the NTSYS-pc. Subsequently, principal coordinate (PCO) analysis was carried out using principal component analysis programs such as

DCENTER and EIGEN of the NTSYS-pc and based on genetic similarity matrices. Finally a scatter plot was obtained. Nei's gene diversity ( $h$ ), Shannon's information index ( $I$ ), Nei's genetic identity and genetic distance were estimated using POPGENE version 1.32 (Yeh and Boyle, 1997). Duncan's multiple range test was performed by SPSS software. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

## Results

### Level of polymorphism

Total of 36 SRAP primer combinations and 10 SSR primer pairs were employed randomly to screen polymorphism between 12 accessions (typical accessions from population A to G). Among of them, 11 SRAP primer combinations and 3 SSR primer pairs (Table 3) amplified abundant, clear and repeatable fragments, then they were employed to evaluate the genetic diversity of 404 landraces.

A total of 175 amplified fragments were detected and 126 of them were polymorphic, the polymorphism rate was 72%.

**Table 3** Amplification of primers used in this study

| Primer       | Loci size (bp) | Total loci | Monomorphic loci No. | Polymorphic loci No. | Polymorphism rate (%) |
|--------------|----------------|------------|----------------------|----------------------|-----------------------|
| Me01Em01     | 440-750        | 10         | 1                    | 9                    | 90.00                 |
| Me02Em02     | 450-540        | 11         | 5                    | 6                    | 54.55                 |
| Me02Em03     | 350-640        | 8          | 2                    | 6                    | 75.00                 |
| Me03Em02     | 220-1300       | 14         | 3                    | 11                   | 78.57                 |
| Me06Em05     | 390-700        | 10         | 6                    | 4                    | 40.00                 |
| Me07Em06     | 480-900        | 26         | 14                   | 12                   | 46.15                 |
| Me08Em04     | 150-610        | 14         | 4                    | 10                   | 71.43                 |
| Me08Em05     | 210-590        | 23         | 6                    | 17                   | 73.91                 |
| Me09Em06     | 180-650        | 14         | 4                    | 10                   | 71.43                 |
| Me09Em08     | 150-650        | 16         | 2                    | 14                   | 87.50                 |
| Me10Em10     | 180-920        | 14         | 2                    | 12                   | 85.71                 |
| GBssr-sa-123 | 290-360        | 5          | 0                    | 5                    | 100.00                |
| GBssr-sa-173 | 250-270        | 2          | 0                    | 2                    | 100.00                |
| GBssr-sa-182 | 250-550        | 8          | 0                    | 8                    | 100.00                |
| Total        | 150-1300       | 175        | 49                   | 126                  | 72.00                 |

The number of fragments detected by each primer ranged from 2 (GBssr-sa-173) to 26 (Me07Em06), with an average of 12.5. The number of polymorphic fragments detected by each primer ranged from 2 (GBssr-sa-173) to 17 (Me08Em05), with an average of 9. Product sizes ranged from 150 to 1300 bp (Table 3).

#### Genetic diversity analysis

Genetic similarity coefficient (GS), Nei's gene diversity ( $h$ ) and Shannon's information index ( $I$ ) for variation were estimated for all agro-ecological zones (Table 4). The average pairwise GS (0.7865) was the highest in zone III (also with the highest minimum pairwise GS 0.5405), indicated that genetic relationship between landraces in this zone was the

**Table 4.** Description of genetic diversity of each agro-ecological zone

| Zone  | Max. GS* | Min. GS | Aver. GS | na*    | ne*    | $h^*$  | $I^*$     |
|-------|----------|---------|----------|--------|--------|--------|-----------|
| I     | 0.9892   | 0.4426  | 0.7447   | 1.8333 | 1.3319 | 0.2119 | 0.3397bcd |
| II    | 0.9765   | 0.4370  | 0.7339   | 1.9444 | 1.3294 | 0.2138 | 0.3408bcd |
| III   | 0.9697   | 0.5405  | 0.7865   | 1.7857 | 1.2731 | 0.1772 | 0.2858d   |
| IV    | 0.9789   | 0.4344  | 0.7214   | 1.8968 | 1.3593 | 0.2293 | 0.3600ab  |
| V     | 0.9892   | 0.4000  | 0.7030   | 1.8333 | 1.4193 | 0.2511 | 0.3829ab  |
| VI    | 0.9789   | 0.4359  | 0.7208   | 1.8570 | 1.3770 | 0.2320 | 0.3626ab  |
| VII   | 0.9592   | 0.4711  | 0.6857   | 1.8333 | 1.4375 | 0.2613 | 0.3980a   |
| I-VII | 0.9892   | 0.3937  | 0.7130   | 2.0000 | 1.3792 | 0.2418 | 0.3847    |

Letters marked with \* were abbreviations: GS indicates Genetic similarity coefficient; Na indicates Observed number of alleles; Ne indicates Effective number of alleles;  $h$  indicates Nei's gene diversity;  $I$  indicates Shannon's Information index.

Different letters show significant difference by Duncan's multiple range test ( $P < 0.05$ ) for the values within the eighth column.

nearest. The followings were average pairwise GS in zone I (0.7447), zone II (0.7339), zone IV (0.7214), zone VI (0.7208), zone V (0.7030) and zone VII (0.6857), the pairwise GS of the 404 landraces in all of the seven zones was ranged from 0.3937 to 0.9892, with an average of 0.7130. For all of the 404 landraces, the average Nei's gene diversity and Shannon index for the 175 loci was 0.2418 and 0.3847, respectively. The maximum Nei's gene diversity ( $h = 0.2613$ ) and Shannon index ( $I = 0.3980$ ) values among the landraces in zone VII indicated that they were genetically more diverse than those in other zones, while the least genetically diverse region was zone III ( $h = 0.1772$ ,  $I = 0.2858$ ). The  $h$  and  $I$  values of the other five zones decreased ordinally from zone V to zone VI, IV, II and I (data shown in Table 4). The trend of this variation consistent with that of pairwise GS above, showing that genetic diversity of the seven zones were various, and the abundant of genetic diversity were decreased from zone VII to zone V, VI, IV, II, I and III.

Duncan's multiple range test of the  $I$  values from seven zones was performed (Table 4). Although differences between zone I, II and III, and difference between zone IV, V, VI and VII were not significant, zone III differed significantly from zone IV, V, VI and VII, and zone VII differed significantly from zone I, II and III.

#### Clustering analysis based on UPGMA method

The UPGMA-based dendrogram obtained from the SRAP and SSR data was shown in Figure 1, the distribution of landraces (origin from population A to G) on the dendrogram was shown

**Table 5.** Description of the distribution of accessions in the dendrogram.

| Cluster  | Group    | Sub-group | Frequency |        |        |        |        |        |        | Total No. of Acc |
|----------|----------|-----------|-----------|--------|--------|--------|--------|--------|--------|------------------|
|          |          |           | Pop. A    | Pop. B | Pop. C | Pop. D | Pop. E | Pop. F | Pop. G |                  |
| I        | I-1      | I-1-1     | 23        | 30     | 38     | 37     | 24     | 30     | 22     | 204              |
|          |          | I-1-2     | 7         | 29     | 13     | 15     | 12     | 11     | 5      | 92               |
|          |          | I-1-3     | .         | .      | .      | 1      | .      | .      | .      | 1                |
|          |          | I-1-4     | .         | 2      | .      | .      | .      | .      | 1      | 3                |
|          |          | I-1-5     | .         | .      | 1      | .      | .      | .      | .      | 1                |
|          |          | I-1-6     | 4         | .      | .      | .      | 1      | .      | .      | 5                |
|          |          | I-1-7     | .         | 1      | 1      | .      | 1      | .      | .      | 3                |
|          |          | I-1-8     | .         | .      | .      | .      | 3      | 2      | 4      | 9                |
|          | Subtotal | 34        | 62        | 53     | 53     | 41     | 43     | 32     | 318    |                  |
|          | I-2      | I-2-1     | 1         | 1      | .      | 2      | .      | 2      | .      | 6                |
|          |          | I-2-2     | .         | 2      | 1      | 2      | 1      | .      | .      | 6                |
|          |          | I-2-3     | 1         | .      | .      | .      | .      | .      | .      | 1                |
| I-2-4    |          | 1         | .         | .      | .      | .      | .      | .      | 1      |                  |
| Subtotal | 3        | 3         | 1         | 4      | 1      | 2      | .      | 14     |        |                  |
| Subtotal | 37       | 65        | 54        | 57     | 42     | 45     | 32     | 332    |        |                  |
| II       | II-1     | II-1-1    | 5         | 2      | .      | 2      | 9      | 5      | 2      | 25               |
|          |          | II-1-2    | .         | .      | .      | .      | .      | .      | 5      | 5                |
|          |          | II-1-3    | .         | 1      | 1      | 1      | .      | 1      | 2      | 6                |
|          |          | II-1-4    | .         | 3      | .      | 5      | 4      | 4      | 1      | 17               |
|          | Subtotal | 5         | 6         | 1      | 8      | 13     | 10     | 10     | 53     |                  |
|          | II-2     | II-2-1    | .         | 2      | .      | .      | .      | .      | .      | 2                |
|          |          | II-2-2    | .         | 3      | .      | 1      | .      | .      | .      | 4                |
|          |          | II-2-3    | .         | 1      | .      | 2      | .      | 1      | .      | 4                |
|          |          | II-2-4    | .         | .      | .      | .      | .      | .      | 3      | 3                |
|          | Subtotal | .         | 6         | .      | 3      | .      | 1      | 3      | 13     |                  |
|          | II-3     | .         | .         | .      | 1      | 1      | 1      | 2      | 5      |                  |
|          | II-4     | .         | 1         | .      | .      | .      | .      | .      | 1      |                  |
| Subtotal | 5        | 13        | 1         | 12     | 14     | 12     | 15     | 72     |        |                  |

in Table 5. The dendrogram divided the 404 landraces into two clusters (cluster I and cluster II) at genetic similarity of 0.59. Cluster I was a robust cluster (with 332 landraces), which was more than three times larger than cluster II (with 72 landraces). It could be grouped into two groups (I-1, I-2) at genetic similarity of 0.66, and consisted of twelve sub-groups (I-1-1 to I-1-8, I-2-1 to I-2-4). Cluster II could be grouped into four groups (II-1, II-2, II-3, II-4) at genetic similarity of 0.66, and group II-1 and II-2 consisted of eight sub-groups (II-1-1 to II-1-4, II-2-1 to II-2-4).

The obvious character of the dendrogram was that group I-1 in cluster I was represented by the largest number (78.71%) of accessions, but this sub-group exhibited the least amount of genetic diversity. The sub-group I-1-1 comprised 204 landraces (more than 50%) out of 404 landraces analyzed, which

were landraces from zone II, III and IV. There were 92 landraces in sub-group I-1-2 and it was also mainly consisted of landraces from zone II, III and IV, as well as including some landraces from zone II. The other sub-groups in cluster I comprised of only a small quantity of landrace each. By summed up all the distribution of 332 landraces in cluster I, it is definite that most of landraces from zone II, III and IV were included in cluster I, accounting for 83.33%, 98.18% and 82.61% of landraces respectively. There were 25 landraces in sub-group II-1-1, nearly 36% of them were from zone V. Sub-group II-1-2 was consisted of only 5 landraces from zone VII. Except for sub-group II-1-4 comprising 17 landraces, the remaining sub-groups and group II-3, II-4 of cluster II comprised of only a few of accessions each. Summarized the distribution of 72 landraces in cluster II, it was mainly consisted of landraces

**Table 6.** Unbiased measures of identity and genetic distance among seven agro-ecological zones of sesame landraces.

|          | Zone I | Zone II | Zone III | Zone IV | Zone V | Zone VI | Zone VII |
|----------|--------|---------|----------|---------|--------|---------|----------|
| Zone I   |        | 0.9786  | 0.9750   | 0.9770  | 0.9721 | 0.9696  | 0.9621   |
| Zone II  | 0.0216 |         | 0.9755   | 0.9835  | 0.9727 | 0.9672  | 0.9571   |
| Zone III | 0.0253 | 0.0248  |          | 0.9780  | 0.9652 | 0.9720  | 0.9524   |
| Zone IV  | 0.0233 | 0.0166  | 0.0222   |         | 0.9863 | 0.9799  | 0.9694   |
| Zone V   | 0.0283 | 0.0276  | 0.0354   | 0.0138  |        | 0.9809  | 0.9794   |
| Zone VI  | 0.0309 | 0.0333  | 0.0284   | 0.0204  | 0.0193 |         | 0.9785   |
| Zone VII | 0.0387 | 0.0439  | 0.0488   | 0.0311  | 0.0208 | 0.0217  |          |

from zone V and VII. Accessions from various zones were distributed throughout the dendrogram.

#### Principal coordinates (PCO) analysis

The PCO analysis indicated that, the first and the second principal component accounted for 22.06% and 10.09% of the total variation (Fig. 2), respectively, while the third principal component accounted for an additional 4.59%. The contributions of the remaining principal components were less than 3.18% individually. The scatter plot of the first two principal components is presented in Figure 2. The scattering pattern in Figure 2 agrees closely with the clustering displayed in the dendrogram (Fig. 1). Accessions from the major cluster in the dendrogram seemed to form a very close cluster in the PCA scatter plot (indicated by ellipses numbered with A to F). Accessions clustered in ellipses A, B, C, D, E and F were basically from subgroup I-1-1, I-1-2, I-1-3 to I-1-8 and group I-2, II-1, II-2 to II-4 of the dendrogram correspondingly.

#### Analysis of Nei's genetic identity and genetic distance among seven agro-ecological zones

Nei's genetic identity and genetic distance among seven agro-ecological zones of sesame landraces were analyzed and summarized in Table 6. Genetic distances among agro-ecological zones are very low, zone IV and zone V pursued the lowest genetic distance (0.0138) as well as the highest genetic identity (0.9863), while zone III and zone VII pursued the highest genetic distance (0.0488) as well as the lowest genetic identity (0.9524).

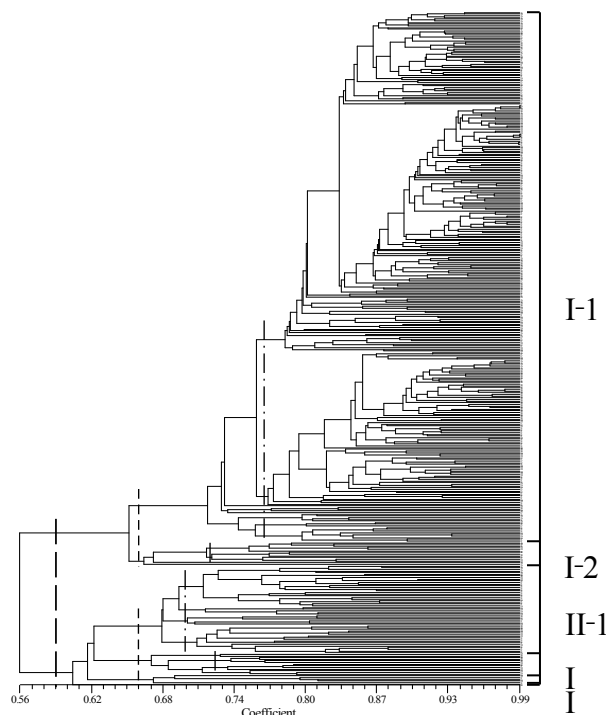
According to the Nei's genetic distance shown in Table 6, the genetic relationship of landraces in seven agro-ecological zones was inferred using the UPGMA method, while optimal dendrogram was shown in Figure 3. It could be grouped into two clusters, the first cluster comprised landraces from Northern China (zone I and zone II), as well as the Yellow River and Huai River valley (zone III), while the second clus-

ter was consisted of landraces from the Yangtze River valley (zone IV and zone V), Southern China (zone VI), and Southwest of China (zone VII). Two distinct zones, zone III and zone VII, formed two separate branches. However, zones with similar eco-geographical factors were grouped into the same or adjacent groups, such as zone I and II, as well as zone IV, V, and VI.

## Discussion

#### Advantages of markers used in this study

DNA markers are powerful and reliable tools for evaluating genetic variation within or between populations (Powell et al., 1996; Qamaruz et al., 1998). Genetic variability in sesame has been previously studied by isozymes (Isshiki and Umezake, 1997) and many DNA markers, including RAPD (Bhat et al., 1999; Ercan et al., 2004; Pham et al., 2009), ISSR (Kim et al., 2002) and AFLP (Hernan and Petr, 2006). However, this is the latest study of utilization of the SRAP and SSR markers on sesame genetic diversity. SRAP is a promising technique for the characterization of genetic diversity because it possesses a high degree of reproducibility and discriminatory



**Figure 1.** Dendrogram for indigenous landraces in sesame core collection. Dendrogram was constructed by UPGMA method based on SRAP and EST-SSR markers. Accessions were showed only by their population code according to Table 1

power, as well as high polymorphism rate. SSR can be developed easily at a lower cost, often have putative functions, and are more valuable for genetic diversity analysis, comparative mapping and marker assisted selection (MAS) breeding since they are derived from transcripts. SRAP and SSR markers have been successfully applied to many cultivated and wild plants, however, the application of them on sesame was scarce. This study was the latest report of estimating genetic diversity in sesame by SRAP and SSR markers, the polymorphic fragments amplified by each primer was with an average of 9, the polymorphism rate of amplification reached 72%, so they represented as excellently as in other plants.

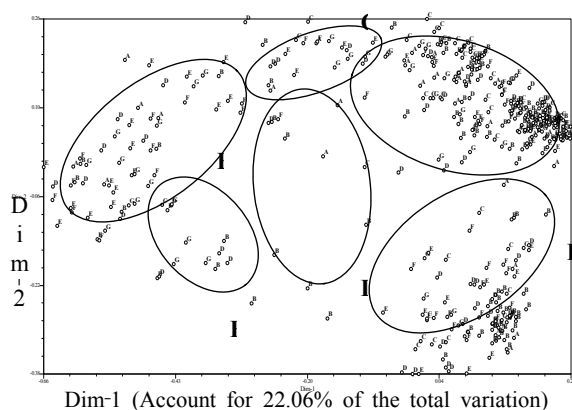
#### Analysis of various genetic diversity characteristics in zone III and zone VII

The diverse abundant of genetic diversity of landraces in different agro-ecological zones of China resulted from many reasons, including ecogeographic factors and sesame cultivation history and so on. China is famous for its vast territory, therefore various planting systems were formed in different agro-ecological zones with different climatic, ecologic and geographic characters. Thus it is probable that main crops and planting systems in different provinces from the same agro-ecological zone were diverse. Sesame has been cultivated in China for about 2200 years. The agro-ecological zone III, which comprised of Henan province and Anhui province, was the main area of sesame production in China from old. The eco-geographic factors were really suitable for sesame growth, its sesame sowing area in zone III to produce nearly half of the total sesame production in China. The crop planting system in zone III was two-maturity per year and sesame was summer planting, which was highly popular in this zone. Farmers recip-

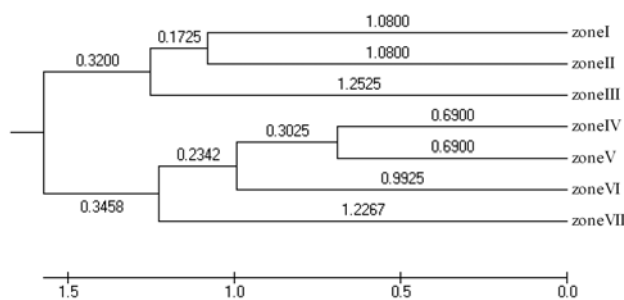
rocaly introduced landraces frequently, this could deeply decrease the genetic diversity of landraces there. Since agro-ecological zone III was the main area of sesame production in China, cultivar improvement was regarded and started from the 1950s, many excellent released cultivars such as “786” and “332” which suited for the production of that time were bred, and consequently some landraces were substituted gradually by released cultivars. In China the indigenous sesame germplasm resources were collected since the 1950s and 3200 accessions were assembled up to the year of 1963. It is a pity that only less than half of them were conserved by the end of the 1970s, subsequently large scale of sesame germplasm further collection was carried out with support of the Chinese government, but many old landraces (especially those from zone III) had disappeared then, so the genetic diversity of landraces from zone III was limited ( $h=0.1772$ ,  $I=0.2858$ ). In contrast with it in zone III, landraces in zone VII displayed prevalence of higher diversity ( $h=0.2613$ ,  $I=0.3980$ ). The vast area from sichuan, Guizhou, Yunnan to Tibet, the particularity and complexity of geographical and climatic factors in zone VII could partly account for the higher genetic diversity there, diverse planting system were formed. Summer planting, spring planting and autumn planting of sesame existed simultaneously in different regions. Furthermore, since sesame was not the main crop in zone VII, cultivar improvement was not regarded as much, many old landraces had been being cultivated, while the higher genetic diversity could be retained.

#### Impact of geographical factors on sesame landraces in China

In this study, accessions from various zones were distributed throughout the dendrogram (Fig. 1), indicating accessions from different agro-ecological zones were indistinguishable by cluster analysis, which agreed with others in showing that geographical separation did not generally result in greater genetic distance (Ercan et al., 2004; Hernan and Petr, 2006; Venkataramana et al., 1999). This could be ascribed to the long cultivation history of sesame in China, landraces introduce, exchanged, or traded from different agro-ecological zones. This study also analyzed characteristics about the genetic diversity, genetic identity and genetic distance among seven agro-ecological zones in China (Table 4, Table 6 and Figure 3), which displayed a decrease trend of genetic diversity from southwest region to southern region and northern region. These results provided some information in DNA level, which may be helpful reference in future study on the exploration the origin, the genetic diversity centre and the spreading of sesame landraces in China.



**Figure 2.** The scatter plot of principal coordinates analysis for indigenous landraces in sesame core collection. The scatter plot was constructed based on SRAP and EST-SSR markers. The major clusters were indicated by ellipses numbered with A to F.



**Figure 3.** Dendrogram showing genetic relationships among sesame landraces from the seven agro-ecological zones in China.

#### Theoretical significance for sesame germplasm and breeding

As the depth of genetic diversity of landraces in different agro-ecological zones of China was various, and geographical separation did not generally result in greater genetic distance, it is necessary to reinforce the collection and protection of sesame germplasm resources from agro-ecological zones with higher diversity, such as southwest region of China. Furthermore, parent materials of sesame breeding could be selected from the regions mentioned above, genetic distance of parents should be considered firstly, while geographic distance is not so important.

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