



## Genetic diversity analysis of okra (*Abelmoschus esculentus* L.) by inter-simple sequence repeat (ISSR) markers

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**ABSTRACT.** Okra (*Abelmoschus esculentus* L.) is not only a nutrient-rich vegetable but also an important medicinal herb. Inter-simple sequence repeat (ISSR) markers were employed to investigate the genetic diversity and differentiation of 24 okra genotypes. In this study, the PCR products were separated by electrophoresis on 8% nondenaturing polyacrylamide gel and visualized by silver staining. The 22 ISSR primers produced 289 amplified DNA fragments, and 145 (50%) fragments were polymorphic. The 289 markers were used to construct the dendrogram based on the unweighted pair-group method with arithmetic average (UPGMA) cluster analysis. The dendrogram indicated that 24 okras were clustered into 4 geographically distinct groups. The average polymorphism information content (PIC) was 0.531929, which showed that the majority of primers were informative. The high values of allele frequency, genetic diversity, and heterozygosity showed that primer-sample combinations produced measurable fragments. The mean distances ranged from 0.045455 to

0.454545. The dendrogram indicated that the ISSR markers succeeded in distinguishing most of the 24 varieties in relation to their genetic backgrounds and geographical origins.

**Key words:** Okra; Inter-simple sequence repeat (ISSR); Genetic diversity

## INTRODUCTION

Okra, a dicotyledoneae ephemeral herb, belongs to the Mallow family. It is valued for its edible green seed pods. The geographical origin of okra is disputed, with supporters of South Asian, Ethiopian, and West African origins. The plant is cultivated in tropical, subtropical, and warm temperate regions around the world. Currently, okra are cultivated and distributed throughout the world. China is a large agricultural nation with a long history of okra cultivation (de Sousa Ferreira Soares et al., 2012), with a more than 100-year history of planting in Pingxiang, Jiangxi Province. Okra evolved from potatoes and the spicy pepper a hundred years ago. It was considered a minor crop until more attention was paid to its recent genetic improvements (Schafleitner et al., 2013), especially its rapid growth cycle, easy cultivation, resistance to pests, high yield, and high nutritional value (Calisir et al., 2005). The whole body of the okra is a treasure. The leaves and seeds are considered a valuable traditional medicine (Gul et al., 2011). Okra seeds contain abundant mineral elements, including iron, potassium, calcium, and manganese. It is also an oil and protein source, which can be used as a coffee additive (i.e., in place of drinking coffee). The fruit is a popular vegetable, which was supplied in the 2008 Beijing Olympic Games because it contains abundant vitamins, pectin, and minerals (Oyelade et al., 2003). The big and beautiful flowers of okra can be used as an ornamental plant. Okra is an allopolyploid plant with considerable variation in its chromosome number and ploidy level. The chromosome number ranges from 56 for *Abelmoschus angulosus* to 200 for *A. caillei*, an amphipolyploid between *A. esculentus* and *A. caillei* (Ford, 1938; Siemonsma, 1982).

The availability of a variety of DNA marker systems such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeat (SSR) (Gupta and Varshney, 2000) are limited by the low reproducibility of RAPD, high cost of AFLP, and the need to know the flanking sequences to develop species-specific primers for SSR polymorphism (Reddy et al., 2002). Inter-simple sequence repeat (ISSR) was employed to investigate genetic diversity and differentiation. ISSR has a few advantages because ISSR primers anneal directly to simple sequence repeats and, thus, unlike SSR markers, no prior knowledge of a target sequence is required (Wang et al., 2012).

ISSR-polymerase chain reaction (PCR) is an alternative strategy that has been devised to reduce the time invested in microsatellite isolation and significantly increase yield (Babaei et al., 2012). In particular, ISSR markers allow more stringent annealing temperatures and reveal more polymorphic fragments. They can be highly variable within a species. The cost of the ISSR analysis is relatively lower than that of AFLP (Esselman et al., 1999) and displays acceptable reproducibility. Therefore, it is widely used in current studies of population genetics [e.g., bermudagrass (Farsani et al., 2012), *Houttuynia cordata* (Wei and Wu, 2012), and cultivated bromeliad (Zhang et al. 2012)]. In this paper, ISSR markers were used to analyze the genetic diversity and genetic structure of okra populations from the different regions in China. The population-level genetic diversity of okra was analyzed in the present study for the first time to determine the genetic differentiation between populations from the

different regions in China. It will provide invaluable information for future okra conservation and management programs.

## MATERIAL AND METHODS

### Total DNA extraction

Okra leaves were collected from different locations in China. Genomic DNA was extracted from 0.5 g fresh leaves following the improved CTAB protocol (Jie et al., 2008). The DNA quality was determined using 1.0% agarose gels. The purified genomic DNA was quantified using a BioRad SmartSpec 3000 UV-Vis Spectrophotometer.

### ISSR PCR amplification

ISSR markers were produced by PCR using the genomic DNA and ISSR primers. PCRs were conducted using 22 primers (Table 1) to characterize the 24 okra varieties. The 20- $\mu$ L mixture contained 10 ng template DNA, 2.0  $\mu$ L 10X PCR buffer, 1.8 mM MgCl<sub>2</sub>, 0.1 mM dNTPs (TaKaRa, Dalian, China), 2% formamide, 100 nM of each primer, 1.5 U *Taq* polymerase (TaKaRa, Dalian, China), and double-distilled water. Amplifications were performed using an ABI 2720 Thermal Cycler (Applied Biosystems, USA) with the following PCR program: 5 min of initial denaturing at 94°C, 40 cycles of 94°C for 45 s, 1 min for annealing at the primer-specific melting temperature, and 72°C for 90 s, followed by a final extension of 5 min at 72°C. First, the PCR products were analyzed by electrophoresis on 2.0% agarose gel with 0.5X TBE buffer. Then, the PCR products were electrophoretically separated using 8.0% nondenaturing polyacrylamide gels buffered with 0.5X TBE, run at 250 V (constant) for 2.5 h, and visualized by silver staining (Mortz et al., 2001). A 100-bp DNA ladder was used as a size marker.

**Table 1.** ISSR primers with annealing temperature (T<sub>m</sub>) used in this study and the amplified results as the number of total bands (TB), number of polymorphic bands (PB), and % of polymorphic bands (PPB).

Primer	Sequence 5'-3'	T <sub>m</sub> (°C)	TB	PB	PPB (%)
809	AGAGAGAGAGAGAGAGG	47	12	2	17
811	GAGAGAGAGAGAGAGAC	47	21	10	47
817	CACACACACACACAA	45	10	6	60
818	CACACACACACACAG	47	15	11	73
823	TCTCTCTCTCTCTCC	47	7	3	43
825	ACACACACACACACT	45	10	4	40
829	TGTGTGTGTGTGTGTC	47	9	2	22
830	TGTGTGTGTGTGTGG	47	7	3	43
834	AGAGAGAGAGAGAGACT	47	12	7	58
835	AGAGAGAGAGAGAGCC	49	7	3	43
840	GAGAGAGAGAGAGACT	49	16	10	63
841	GAGAGAGAGAGAGACC	49	13	3	23
842	GAGAGAGAGAGAGATG	49	15	9	60
846	CACACACACACACAAT	49	14	13	93
847	CACACACACACACAAC	51	20	6	30
848	CACACACACACACAAG	51	21	20	95
855	ACACACACACACACCT	49	9	7	78
857	ACACACACACACACCG	49	17	14	82
884	ACTAGAGAGAGAGAGAG	47	18	2	11
887	GCATCTCTCTCTCTC	49	7	2	29
889	ACTACACACACACAC	49	14	5	36
891	ACTTGTGTGTGTGTG	49	15	3	20
Average			13	7	50
Total			289	145	

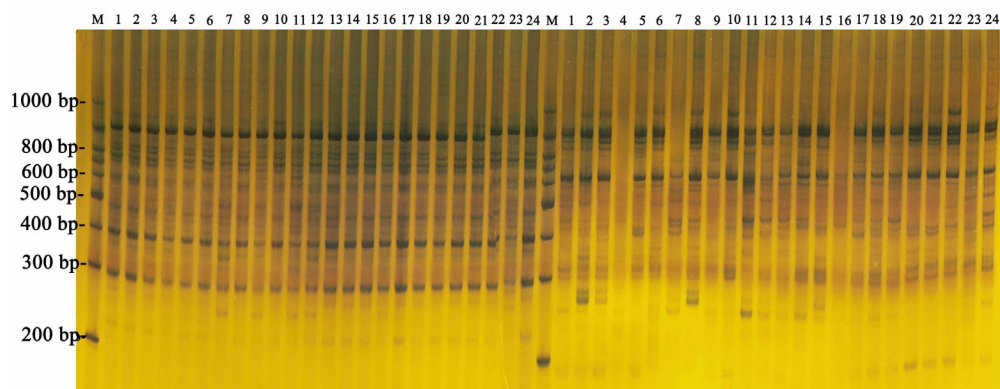
## Data analysis

All ISSR bands were scored as present (1) or absent (0). The obtained genetic distance matrix was used to perform the cluster analysis and construct the unweighted pair-group method with arithmetic average (UPGMA) dendrogram using the PowerMarker software (Liu and Muse 2005). We used the PowerMarker software to calculate polymorphism information content (PIC), allele frequency, genetic diversity, genetic distance, and heterozygosity for each primer. The principal coordinated analysis (PCoA) was performed with the modules DCENTER and EIGEN implemented in NTSYS-pc, and the 2 principal coordinates were used to visualize the dispersion of accessions in a two-dimensional array of eigenvectors. Bayesian assignment tests were applied to estimate the number of genetic clusters and evaluate the degree of admixture among them using Structure v2 (Pritchard et al., 2000). Structure was run with a “burn-in” setting of 100,000 followed by 20,000 MCMC iterations using the admixture model with sampling localities as prior population assignment and allelic frequencies correlated among populations. Ten runs were performed for each value for  $K$  ranging from 2 to 7. The most likely value for  $K$  was calculated with STRUCTURE HARVESTER (Earl and vonHoldt, 2012) using the statistic  $K$ , which represents the greatest rate of change between each subsequent  $K$  value (Evanno et al., 2005).

## RESULTS

### ISSR polymorphism

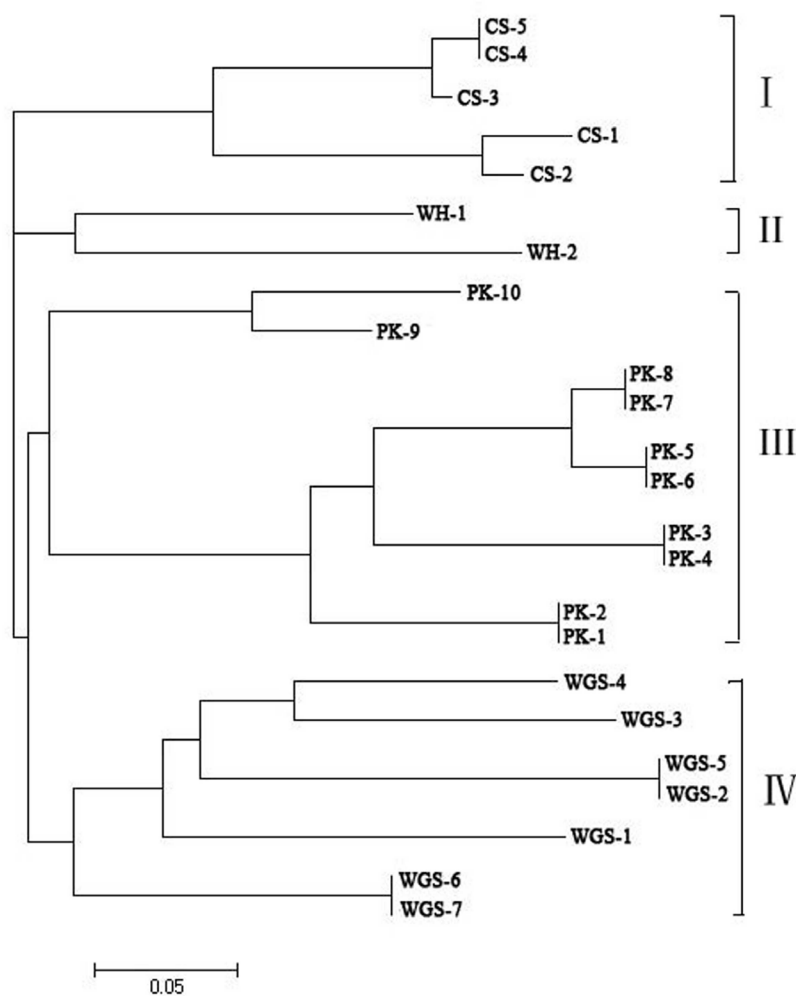
The 22 selected ISSR primers produced 289 fragments, with an average of 13 bands per primer. There were 145 polymorphic fragments ranging from 200 to 1500 bp. The number of bands by each primer ranged from 7 to 21, with an average of 13 per primer (Table 1). At the population level, the percentage of polymorphic bands ranged from 11 to 23%, with an average of 50%. The banding pattern of genotypes generated by ISSR primers 847 and 848 are shown in Figure 1.



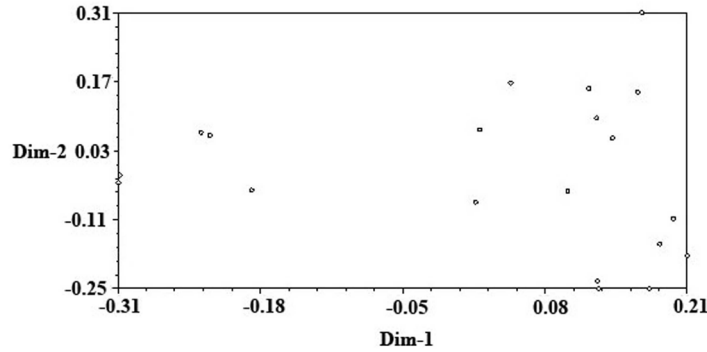
**Figure 1.** PAGE electrophoretic profiles of PCR products by using 847 and 848 ISSR primers for 24 okra varieties. Lanes 1-24 = WGS-1, WGS-2, WGS-5, WGS-3, WGS-4, PK-9, PK-10, WH-2, CS-1, CS-2, CS-4, CS-5, CS-3, WGS-7, WGS-6, PK-1, PK-2, WH-1, PK-4, PK-3, PK-7, PK-8, PK-6, and PK-5, respectively.

### Genetic differentiation and relationships

The neighbor-joining (NJ) dendrogram indicated that the 24 populations clustered into 4 geographically distinct groups (Figure 2). The populations (CS1-5) sampled from Hunan province formed Group I. Group II contained populations WH1 and WH2. Group III was comprised of populations from PK1 to PK10. The remaining populations (WGS1-7) formed Group IV. The above results were supported by correlation analysis between geographic and genetic distances for 24 populations of okra. The cluster results were entirely consistent with phenotype. Phenotype observations showed that PK-1, PK-2, PK-3, PK-4, PK-5, PK-6, PK-7, and PK-8 belonged to *Hibiscus coccineus* (Figure 3).



**Figure 2.** Neighbor-joining dendrogram based on genetic distances for the 24 populations of okra.



**Figure 3.** Two-dimensional matrix plot of the principal coordinate analysis showing associations among the cultivated bromeliad accessions.

We used the PowerMarker software to calculate the PIC value (i.e., 0.306659 to 0.694334; Table 2). The maximum and minimum values of allele frequency were 0.791666667 and 0.5, respectively. The value of genetic diversity ranged from 0.34375 to 0.713541667, and the highest value was found for primer 848. We found that the value of heterozygosity ranged from 0.416666667 to 1 (Table 2). Genetic distances were calculated via PowerMarker, and the highest distance (0.454545) was obtained between *WGS-3* and *Hibiscus coccineus* (Table 3). The genetic relationships among the investigated bromeliads were also investigated using PCoA. Two- and three-dimensional plots showing the dispersions of 24 okras are displayed in Figure 3; the sum of the eigenvalues was 4.538927. The STRUCTURE software was used in the ISSR admixture analysis (Figure 4). The highest likelihood of the data was obtained when samples were clustered into 4 groups ( $K = 4$ ). These groupings were entirely consistent with those of the NJ clustering results.

**Table 2.** Allele frequency, genetic diversity, heterozygosity, and polymorphism information content (PIC) for each primer calculated by the PowerMarker software.

Marker	Major allele frequency	Genotype No.	$N_A$	Gene diversity	Heterozygosity	PIC
809	0.520833333	3	3	0.565104167	0.958333333	0.474154
811	0.5	7	8	0.677083333	1	0.638317
817	0.541666667	5	5	0.638888889	0.916666667	0.596752
818	0.5	6	7	0.692708333	1	0.661782
823	0.5	3	4	0.660590278	1	0.611129
825	0.625	5	5	0.563368056	0.75	0.526246
829	0.5	2	3	0.611111111	1	0.535494
830	0.5625	4	4	0.608506944	0.875	0.558403
834	0.625	6	6	0.575520833	0.75	0.548282
835	0.770833333	3	3	0.361979167	0.458333333	0.309847
840	0.729166667	5	5	0.439236111	0.541666667	0.407969
841	0.791666667	3	3	0.34375	0.416666667	0.306659
842	0.5	5	6	0.686631944	1	0.65228
846	0.5	8	9	0.703125	1	0.678211
847	0.5	4	5	0.603298611	1	0.52822
848	0.5	10	11	0.713541667	1	0.694334
855	0.5625	4	4	0.596354167	0.875	0.539014
857	0.5	4	5	0.657986111	1	0.607522
884	0.583333333	3	3	0.551215278	0.833333333	0.475251
887	0.791666667	3	3	0.350694444	0.416666667	0.322187
889	0.520833333	4	4	0.605034722	0.958333333	0.53509
891	0.5	2	3	0.582465278	1	0.495298
Mean	0.573863636	4.5	4.954545	0.581281566	0.852272727	0.531929

**Table 3.** Genetic distance calculated for 24 okra populations by the PowerMarker software.

OTU	PK-6	PK-5	CS-2	CS-1	WH-2	WGS-7	WGS-6	PK-1
PK-6	0	0	0.363636	0.386364	0.386364	0.363636	0.363636	0.227273
PK-5	0	0	0.363636	0.386364	0.386364	0.363636	0.363636	0.227273
CS-2	0.363636	0.363636	0	0.045455	0.318182	0.318182	0.318182	0.318182
CS-1	0.386364	0.386364	0.045455	0	0.340909	0.363636	0.363636	0.318182
WH-2	0.386364	0.386364	0.318182	0.340909	0	0.318182	0.318182	0.363636
WGS-7	0.363636	0.363636	0.318182	0.363636	0.318182	0	0	0.340909
WGS-6	0.363636	0.363636	0.318182	0.363636	0.318182	0	0	0.340909
PK-1	0.227273	0.227273	0.318182	0.318182	0.363636	0.340909	0.340909	0
PK-2	0.227273	0.227273	0.318182	0.318182	0.363636	0.340909	0.340909	0
WH-1	0.454545	0.454545	0.340909	0.363636	0.272727	0.227273	0.227273	0.318182
PK-4	0.204545	0.204545	0.409091	0.409091	0.363636	0.340909	0.340909	0.204545
PK-3	0.204545	0.204545	0.409091	0.409091	0.363636	0.340909	0.340909	0.204545
PK-7	0.045455	0.045455	0.363636	0.386364	0.363636	0.363636	0.363636	0.181818
PK-8	0.045455	0.045455	0.363636	0.386364	0.363636	0.363636	0.363636	0.181818
CS-4	0.386364	0.386364	0.204545	0.204545	0.363636	0.295455	0.295455	0.340909
CS-5	0.386364	0.386364	0.204545	0.204545	0.363636	0.295455	0.295455	0.340909
CS-3	0.386364	0.386364	0.204545	0.204545	0.340909	0.272727	0.272727	0.340909
WGS-1	0.363636	0.363636	0.386364	0.409091	0.386364	0.272727	0.272727	0.363636
WGS-2	0.386364	0.386364	0.409091	0.431818	0.386364	0.318182	0.318182	0.431818
WGS-5	0.386364	0.386364	0.409091	0.431818	0.386364	0.318182	0.318182	0.431818
WGS-3	0.454545	0.454545	0.386364	0.363636	0.363636	0.318182	0.318182	0.431818
WGS-4	0.431818	0.431818	0.295455	0.318182	0.363636	0.295455	0.295455	0.409091
PK-9	0.295455	0.295455	0.318182	0.340909	0.318182	0.25	0.25	0.272727
PK-10	0.363636	0.363636	0.318182	0.363636	0.340909	0.25	0.25	0.340909
OTU	PK-2	WH-1	PK-4	PK-3	PK-7	PK-8	CS-4	CS-5
PK-6	0.227273	0.454545	0.204545	0.204545	0.045455	0.045455	0.386364	0.386364
PK-5	0.227273	0.454545	0.204545	0.204545	0.045455	0.045455	0.386364	0.386364
CS-2	0.318182	0.340909	0.409091	0.409091	0.363636	0.363636	0.204545	0.204545
CS-1	0.318182	0.363636	0.409091	0.409091	0.386364	0.386364	0.204545	0.204545
WH-2	0.363636	0.272727	0.363636	0.363636	0.363636	0.363636	0.363636	0.363636
WGS-7	0.340909	0.227273	0.340909	0.340909	0.363636	0.363636	0.295455	0.295455
WGS-6	0.340909	0.227273	0.340909	0.340909	0.363636	0.363636	0.295455	0.295455
PK-1	0	0.318182	0.204545	0.204545	0.181818	0.181818	0.340909	0.340909
PK-2	0	0.318182	0.204545	0.204545	0.181818	0.181818	0.340909	0.340909
WH-1	0.318182	0	0.409091	0.409091	0.409091	0.409091	0.295455	0.295455
PK-4	0.204545	0.409091	0	0	0.181818	0.181818	0.431818	0.431818
PK-3	0.204545	0.409091	0	0	0.181818	0.181818	0.431818	0.431818
PK-7	0.181818	0.409091	0.181818	0.181818	0	0	0.409091	0.409091
PK-8	0.181818	0.409091	0.181818	0.181818	0	0	0.409091	0.409091
CS-4	0.340909	0.295455	0.431818	0.431818	0.409091	0.409091	0	0
CS-5	0.340909	0.295455	0.431818	0.431818	0.409091	0.409091	0	0
CS-3	0.340909	0.272727	0.431818	0.431818	0.409091	0.409091	0.022727	0.022727
WGS-1	0.363636	0.363636	0.340909	0.340909	0.363636	0.363636	0.363636	0.363636
WGS-2	0.431818	0.386364	0.431818	0.431818	0.409091	0.409091	0.386364	0.386364
WGS-5	0.431818	0.386364	0.431818	0.431818	0.409091	0.409091	0.386364	0.386364
WGS-3	0.431818	0.318182	0.454545	0.454545	0.454545	0.454545	0.318182	0.318182
WGS-4	0.409091	0.318182	0.454545	0.454545	0.431818	0.431818	0.295455	0.295455
PK-9	0.272727	0.25	0.318182	0.318182	0.272727	0.272727	0.295455	0.295455
PK-10	0.340909	0.295455	0.386364	0.386364	0.340909	0.340909	0.318182	0.318182

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Table 3. Continued.

OTU	CS-3	WGS-1	WGS-2	WGS-5	WGS-3	WGS-4	PK-9	PK-10
PK-6	0.386364	0.363636	0.386364	0.386364	0.454545	0.431818	0.295455	0.363636
PK-5	0.386364	0.363636	0.386364	0.386364	0.454545	0.431818	0.295455	0.363636
CS-2	0.204545	0.386364	0.409091	0.409091	0.386364	0.295455	0.318182	0.318182
CS-1	0.204545	0.409091	0.431818	0.431818	0.363636	0.318182	0.340909	0.363636
WH-2	0.340909	0.386364	0.386364	0.386364	0.363636	0.363636	0.318182	0.340909
WGS-7	0.272727	0.272727	0.318182	0.318182	0.318182	0.295455	0.25	0.25
WGS-6	0.272727	0.272727	0.318182	0.318182	0.318182	0.295455	0.25	0.25
PK-1	0.340909	0.363636	0.431818	0.431818	0.431818	0.409091	0.272727	0.340909
PK-2	0.340909	0.363636	0.431818	0.431818	0.431818	0.409091	0.272727	0.340909
WH-1	0.272727	0.363636	0.386364	0.386364	0.318182	0.318182	0.25	0.295455
PK-4	0.431818	0.340909	0.431818	0.431818	0.454545	0.454545	0.318182	0.386364
PK-3	0.431818	0.340909	0.431818	0.431818	0.454545	0.454545	0.318182	0.386364
PK-7	0.409091	0.363636	0.409091	0.409091	0.454545	0.431818	0.272727	0.340909
PK-8	0.409091	0.363636	0.409091	0.409091	0.454545	0.431818	0.272727	0.340909
CS-4	0.022727	0.363636	0.386364	0.386364	0.318182	0.295455	0.295455	0.318182
CS-5	0.022727	0.363636	0.386364	0.386364	0.318182	0.295455	0.295455	0.318182
CS-3	0	0.363636	0.386364	0.386364	0.318182	0.295455	0.272727	0.295455
WGS-1	0.363636	0	0.295455	0.295455	0.340909	0.272727	0.272727	0.340909
WGS-2	0.386364	0.295455	0	0	0.295455	0.295455	0.363636	0.363636
WGS-5	0.386364	0.295455	0	0	0.295455	0.295455	0.363636	0.363636
WGS-3	0.318182	0.340909	0.295455	0.295455	0	0.204545	0.340909	0.363636
WGS-4	0.295455	0.272727	0.295455	0.295455	0.204545	0	0.318182	0.363636
PK-9	0.272727	0.272727	0.363636	0.363636	0.340909	0.318182	0	0.113636
PK-10	0.295455	0.340909	0.363636	0.363636	0.363636	0.363636	0.113636	0

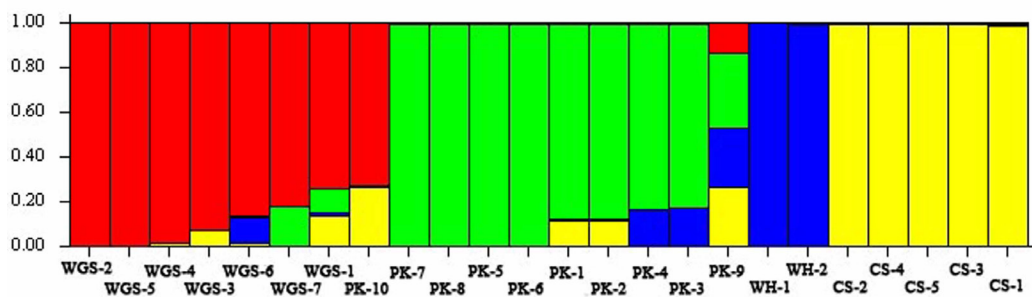


Figure 4. Genetic relationships among the 24 okra populations estimated using the STRUCTURE program based on ISSR data. The model with  $K = 4$  showed the highest  $\Delta K$  value.

## DISCUSSION

Microsatellite markers are distinguished by a high degree of variability, thus making them powerful tools for population genetic analyses (Sethy et al., 2006). The common protocols used for the isolation of microsatellites are costly, time consuming, have low efficiency, and are laborious (Zane et al., 2002). To reduce the time invested in microsatellite isolation and significantly increase yield, ISSR-PCR can be used an alternative strategy. Furthermore, ISSR-PCR targets only those regions of the genome that are rich in microsatellite motifs. In plants, the construction of microsatellite markers has been proven to be effective with the 3'



and 5' anchored ISSR-PCR strategy in producing polymorphic loci for different species. This technique was highly successful, as over 86% of unique clones obtained contained internally located microsatellite motifs (in addition to those at the ends). The familiar protocols were used for agarose gels to separate the PCR products. It can certainly save both time and cost, however, a low percentage of polymorphic bands may be obtained. Nondenaturing polyacrylamide gels can produce clear bands and detect more polymorphic bands. In this study, the ISSR-PCR products were first visualized on a 1.5 or 2.0% agarose gel with ethidium bromide staining. Then, the PCR products were electrophoretically separated using 8.0% nondenaturing polyacrylamide gels, and the satisfactory experimental results were obtained.

As represented earlier, the okra, although widely distributed in Africa and all countries throughout the Caribbean, Europe, China, and Southeast Asia, has not been characterized with regard to genomic sequencing and microsatellite development. Therefore, these developed microsatellite markers for okra could promote future population genetic studies and breeding programs for this plant and related species. In the present study, the NJ dendrogram indicated that the 24 populations clustered into 4 geographically distinct groups (Figure 2). Phenotypic observations showed that PK-1, PK-2, PK-3, PK-4, PK-5, PK-6, PK-7, PK-8, PK-9, and PK-10 belonged to *Hibiscus coccineus*. The results showed that PK-9 and PK-10 had a close genetic relationship. WGS-1, WGS-2, WGS-3, WGS-4, WGS-5, WGS-6, and WGS-7 belonged to *Abelmoschus esculentus* L., and, therefore, they clustered together to form Group IV.

Information on genetic diversity and population structure will be essential for providing further insight into the breeding history and genetic relationship of crop germplasm. In this study, 145 (~50%) of the 289 bands generated by the 22 ISSR primers were polymorphic. The results obtained in our study showed that the ISSR markers were successfully used for okra. The values of allele frequency, genetic diversity, and heterozygosity were high, which showed that the ISSR primers can produce some measurable fragments. The PIC provides an estimate of the discriminatory power of a marker to differentiate genotypes based on both the  $N_A$  expressed and the relative frequencies of alleles (Nagl et al., 2011). PIC was used to measure the genetic diversity in okra. High, medium, and low polymorphism is represented as  $PIC > 0.5$ ,  $0.5 > PIC > 0.25$ , and  $PIC < 0.25$ , respectively (Vaiman et al., 1994; Xie et al., 2010). The PIC value ranged from 0.306659 to 0.694334, and the mean value in this study was 0.531929, demonstrating that the primers could develop high polymorphism, which is useful for genetic variation of genotypes in this research. Genetic distances were examined for all pairwise comparisons between subpopulations. The mean distance ranged from 0.045455 to 0.454545. The test showed high differences in the population of the 24 okras. The two- and three-dimensional graphs of accession differentiation were revealed by principal component analysis. This type of graphical illustration enables an assessment of the population structure and geometric distances among all of the accessions in the study (de Sousa et al., 2011). These groupings were entirely consistent with those of the clustering results. It can be concluded that there is a wide genetic base of okra in China and ISSR markers are effective in determining the genetic diversity and relationships among them.

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