RESEARCH ARTICLE

Genetic diversity revealed by morphological traits and ISSR markers in 48 Okras (*Abelmoschus esculentus* L.)

Cong-Ying Yuan¹ · Ping Wang¹ · Pang-Pang Chen³ · Wen-Jun Xiao¹ · Cheng Zhang¹ · Shuai Hu¹ · Ping Zhou¹ · Hong-Ping Chang¹ · Zhuang He¹ · Rong Hu¹ · Xiu-Tao Lu¹ · Jia-Zhuo Ye² · Xin-Hong Guo¹

Received: 14 April 2015 / Revised: 8 June 2015 / Accepted: 8 June 2015 / Published online: 30 June 2015
© Prof. H.S. Srivastava Foundation for Science and Society 2015

Abstract Okra is a widely distributed crop in the tropics, subtropics, and warmer areas of the temperate zones. Its major potential uses as a vegetable, oil and protein source, and source of paper pulp and fuel, or biomass are compatible. It is expected to have high value of exploitation and application. Due to the limited number of molecular studies focused on okras, the methods of morphological and ISSR markers were used to analysis the genetic diversity of 48 okras in the present study. The 22 primers were picked for ISSR-PCR, and a total of 154 fragments were amplified with an overall average polymorphism of 54.55 %. We used the 154 markers to construct the dendrogram based on the unweighted pair group method with arithmetic means (UPGMA). A high level of genetic diversity was found among 48 individuals. The 48 Okras was divided into four clusters at Dice's coefficient of 0.19 with clustering analysis. Based on these data of the genetic diversity, it will be possible to exploit the available resources of okra in more valuable ways.

Cong-Ying Yuan and Ping Wang contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s12298-015-0303-5) contains supplementary material, which is available to authorized users.

✉ Jia-Zhuo Ye
yzj@hnu.edu.cn

✉ Xin-Hong Guo
gxm@hnu.edu.cn

¹ College of Biology, Hunan University, Changsha 410082, People's Republic of China

² College of Electrical and Information Engineering, Hunan University, Changsha 410082, People's Republic of China

³ Jiangxi Wugongshan Agriculture Development Limited Company, Luxi 337200, Jiangxi, People's Republic of China

Keywords Okra · ISSR · Polymorphism · Morphological markers

Introduction

Okra (*Abelmoschus esculentus* L.), known in many English-speaking countries as ladies' fingers, is a flowering plant in the mallow family. It is valued for its edible green seed pods. It is an allopolyploid of uncertain parentage (proposed parents include *Abelmoschus ficulneus*, *A. tuberculatus* and a reported "diploid" form of okra). Truly wild populations are not known with certainty and the species may be a cultigen. The geographical origin of okra is disputed, with supporters of West African, Ethiopian, and South Asian origins. The plant is cultivated in tropical, subtropical and warm temperate regions around the world (Babaei et al. 2012; Dhall et al. 2014). China, as a large agricultural nation, has a long history of cultivating okra. There is over 100-year history of planting in Pingxiang, Jiangxi Province. Okra is a popular health food due to its high fiber, vitamin C, and folate content. Okra is known for being high in antioxidants. It is a good source of calcium and potassium (Shamsul and Arifuzzaman 2007). Therefore it has extensive application value and industrialization development prospect. In ice cream it can be used as stabilizers to increase mix viscosity, promote smooth texture, and improve frozen stability (Yuennan et al. 2014). It not only tastes good and is nutrient rich, but also it can be used as medicines. For example, its leaves and seeds are considered as valuable traditional medicine. Sub-Saharan people use okra in the folk medicine to alleviate fever and pain, in the treatment of conjunctivitis, rheumatism, hemorrhoid, abscesses rheumatism, hemorrhoid (Gul et al. 2011).

The study of population and genetic diversity is a complex subject that involves the analysis of DNA sequences, gene

adaptability, inter-individual variation and speciation, and an understanding of the interactions among organisms that compose communities (Bertoni et al. 2010). The study of genetic diversity helps to further explore the history of biological evolution and adaptation potential. It contributes to conservation and utilization of biological resources. Furthering knowledge about genes, individuals, species and communities provides an ever greater understanding of biodiversity and, consequently, allows the development of the most adequate strategies for environmental preservation (Bertoni et al. 2010). In recent years, more and more markers are used to detect genetic diversity, such as Random Amplified Polymorphic DNA (RAPD), DNA amplification finger (DAF), Amplified Fragment Length Polymorphism (AFLP), and Simple Sequence Repeat (SSR). In 1994, a molecular marker technique called inter simple sequence repeat (ISSR) was proposed. This method is rapid and inexpensive with no requirements of probes or sequence information, and can detect far more polymorphism than other methods. It can provide the repeatable and stable experimental data. In the present study, the ISSR method was applied to detect genetic variation of 48 accessions of okra germplasm from China. Genetic diversity of 48 accessions of okra also was analysed by the morphological marker. If identified, these accessions could be utilized for breeding and develop okras cultivars. Moreover, we aimed to verify the classification of accessions and define the method for genotype identification. In each accession particular attention was paid to the genetic diversity revealed by ISSR and morphological markers.

Materials and methods

Plant materials

Plant materials were collected from various areas of China. PK-1~PK-6 come from Pingxiang, Jiangxi Province. LXQ-1~LXQ-2 come from Luxi, Jiangxi Province. WGS-1~WGS-2 come from Wugongshan, Jiangxi Province. CH-1~CH-4 come from Changsha, Hunan Province.

Morphological measurements

According to the essay, the 48 okras were chosen to observe the 23 morphological markers, such as plant shape, ramification, dasycaulon, stem color, margin, leaf shape, leaf color, leaf width, leaf length, and so on.

Total DNA extraction

DNA samples were extracted from 0.5 g of fresh leaf materials of 48 Okras using modified CTAB method. The quantity and quality of total genomic DNA were determined by agarose gel electrophoresis and spectrophotometer.

ISSR-PCR analysis

ISSR-PCR reactions were performed in 20 μ L volume of reaction mixture containing 10 ng template DNA, 2.0 μ L 10X PCR buffer, 1.8 mM $MgCl_2$, 0.1 mM dNTPs (TaKaRa, Dalian, China), 2 % formamide, 100nM of each primer, 1.5U *Taq* polymerase (TaKaRa, Dalian, China), and double-distilled water. And reactions were as followed: 5 min of initial denaturing at 94 $^{\circ}C$, 36 cycles of 94 $^{\circ}C$ for 45 s, 1 min at 47–51 $^{\circ}C$ (depending on the primer sequence) and 72 $^{\circ}C$ for 90s, followed by a final extension of 5 min at 72 $^{\circ}C$. The PCR products were analyzed by electrophoresis on 2.0 % agarose gel with 0.5X TAE buffer then ran at 90 V (constant) for 1 h A 100-bp DNA ladder was used as a size marker.

Data analysis

The information was analyzed by the software of DPS (V9.50). Only reliable, intensive bands of ISSR-PCR reaction across 48 accessions were processed in a binary system for

Fig. 1 Dendrogram constructed by cluster analysis for 48 okras resources based on morphological data

Table 1 ISSR primers with annealing temperature(Tm) 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'	TM(°C)	TB	PB	PPB (%)
809	AGAGAGAGAGAGAGAGG	47 °C	4	3	75.00 %
811	GAGAGAGAGAGAGAGAC	47 °C	7	5	71.43 %
817	CACACACACACACACAA	45 °C	8	6	75.00 %
818	CACACACACACACACAG	47 °C	7	5	71.43 %
823	TCTCTCTCTCTCTCC	47 °C	6	4	66.67 %
825	ACACACACACACACACT	45 °C	8	5	62.50 %
829	TGTGTGTGTGTGTGTC	47 °C	5	4	80.00 %
830	TGTGTGTGTGTGTGTGG	47 °C	7	3	42.86 %
834	AGAGAGAGAGAGAGAGCT	47 °C	6	3	50.00 %
835	AGAGAGAGAGAGAGAGCC	49 °C	7	3	42.86 %
840	GAGAGAGAGAGAGAGACT	49 °C	5	3	60.00 %
841	GAGAGAGAGAGAGAGACC	49 °C	6	3	50.00 %
842	GAGAGAGAGAGAGAGATG	49 °C	5	3	60.00 %
846	CACACACACACACACAAT	49 °C	9	5	55.56 %
847	CACACACACACACACAAC	51 °C	7	4	57.14 %
848	CACACACACACACACAAG	51 °C	4	1	25.00 %
855	ACACACACACACACACCT	49 °C	9	4	44.44 %
857	ACACACACACACACACCG	49 °C	5	1	20.00 %
884	ACTAGAGAGAGAGAGAG	47 °C	6	5	83.33 %
887	GCATCTCTCTCTCTCTC	49 °C	12	7	58.33 %
889	ACTACACACACACACAC	49 °C	10	3	30.00 %
891	ACTTGTGTGTGTGTGTG	49 °C	11	4	36.36 %
Total			154	84	54.55 %

band presence “1” or absence “0” for each primer. Each primer determined the number of monomorphic and polymorphic amplification products generated. The binary data were used to calculate levels of polymorphism by dividing the polymorphic bands from the total scored bands. Then we used the software to analysis the binary. The dendrograms were constructed through Nei-Li clustering program using unweighted pair group method with arithmetic means (UPGMA) and the genetic similarities were calculated using the numerical taxonomy multivariate analysis system NTSYS-pc version 2.10 (Rohlf 2000). The software of Structure v2 was used to estimate the number of genetic clusters and evaluate the degree of admixture among them (Pritchard et al. 2000).

Results

Cluster analysis of morphological index

We measured the morphological markers of 48 okras in 23 types. The data were showed in the tables (S1-S2). We used the statistics to calculate the similarity of samples. The software of DPS (V9.50) was applied for standardizing the statistics, then at the condition of euclidean distance and UPGMA. The results (Fig. 1) showed that at the threshold of 5.19, the 48 okras were divided into 4 groups: the Group I contained PK-6-4, PK-6-9 and PK-1. The Group II contained PK-3 which was collected from the mountain of Wugongshan and Luxi, the Group II covered samples coming from Changsha, PK-6 (except PK-6-4 and PK-6-9). The Group IV included PK-2 and PK-5.

Fig. 2 Agarose electrophoretic profiles of PCR products by using 887 ISSR primers for 48 okra varieties. Lane1-48=PK-1-1,PK-1-2,PK-1-3,PK-1-4,PK-1-5,PK-1-7,PK-1-8,PK-1-9,PK-1-10,PK-1-11,PK-1-12,PK-1-13,PK-2-1,PK-2-2,PK-2-3,PK-2-4,PK-3-1,PK-3-2,PK-3-3,PK-3-4,PK-3-4,PK-3-5,PK-3-6,PK-3-7,PK-3-8,PK-5-1,PK-5-2,PK-5-3,PK-5-4,PK-6-1,PK-6-2,PK-6-3,PK-6-4,PK-6-5,PK-6-6,PK-6-7,PK-6-8,PK-6-9,PK-6-10,CS-1,CS-2,CS-3,CS-4,WGS-1,WGS-2,WGS-3,LXQ-1,LXQ-2

Fig. 3 The map of clustering of 48 Okra germplasm based on ISSR data

ISSR polymorphism

The 48 okras accessions were screened using 22 primers, which yielded reproducible polymorphic banding patterns. A total of 154 bands were scored, of which 84 bands (54.55 %) were polymorphic. The electrophoretic bands ranged from 3 to 6 bands for each of the primers, with an average of 7 bands per primer. The approximate size of the amplified products ranged from 280 to 1500 bp. To characterize the capacity of each marker, the ISSR primer index (Table 1) was used to reveal polymorphic loci among the germplasm, which showed

that primers 884 were the most efficient for identifying genotype in okras. The results of ISSR amplification of primer 887 for 48 okras were showed in Fig. 2.

Phylogenetic analysis

The results were processed in a binary system for band presence “1” or absence “0” for each primer. The resulting data were obtained by DPS (V9.50), NTSYSpc 2.1 and Structure software. As compared with the morphology of cluster analysis, the software of DPS (V9.50) was also used to analysis the cluster dates. Cluster analysis based on the matrix of Nei and Li (Nei and Li 1979) genetic dissimilarity coefficient using group average method (UPGMA) grouped the 48 accessions in four clusters according to accession affiliation (Fig. 3). At the threshold of 0.19, the 48 okra were divided into 4 groups: the Group I contained PK-1, PK-2 and PK-3 (PK-3-7, PK-3-8 were not included). The Group II was PK-3-7. The Group III covered PK-5, PK-6, PK-3-8, and these samples were collected from the Wugongshan and Changsha. The Group IV was gathered from Luxi. The results were almost the same with the cluster analysis of pattern. They were both divided the 48 okras into 4 groups, as the molecular cluster results showed that samples from same accession were gathered in the same group, except PK-3-7 and PK-3-8. In the detail, the two methods were in difference. In the molecular dendrogram, the samples had more obvious distinguish than the cluster analysis of pattern.

The software of structure was used to analysis the array of 0 and 1. At the standard of run_1, $k=1$, the value of K is larger and the group is smaller. The bar chart and scatter of molecular dendrogram were showed in the Figs. 4 and 5. Each category contained individuals with DPS (V9.50) analysis derived from the same four categories of individuals included.

The arithmetic average (UPGMA) of NTSYSpc 2.1 statistical software was used to calculate the 48 genetic similarity coefficients between okra germplasm and constructed a two-dimensional map of its clustering (Fig. 6). By a two-dimensional map, we can find that the 48 okra points appeared clear cluster position, in accordance with the two dimensions, the samples will be divided into 4 groups. The genetic similarity coefficient of 48 okras was from 0.6558 to 0.9935, and the highest similarity is between PK-6-5 and PK-6-6, indicating that they have the germplasm affinity highest similarity.

Fig. 4 Genetic relationships among the 48 Okra populations estimated using the STRUCTURE program based on ISSR data. The model with $K=4$ showed the highest ΔK value

Fig. 5 The scatter of molecular clustering of 48 okras

The lowest were between LXQ-2 and PK-2-4, showing that they have the two lowest affinity germplasm.

Discussion

In okra breeding, the collection and use of diverse germplasms is indispensable. There are many ways to identify the germplasms. Domestic okra varieties were divided by phenotype. In this present study, we used the methods of morphological markers and ISSR markers to distinguish the accessions of 48 okras. The method of morphological marker was used to preliminarily classify the 48 samples. However, the sample of PK-6 and PK-3 was not the same with the accession. It

indicated that not all accession was alike with the previous distinction, and they may do evolve when the environment changes. However, the method of morphology is not very stable. With the technology improvement, microsatellite markers provide a new method of distinguishing the accession by a high degree of variability, thus making them powerful tools for analyzing of a large amount of genetic data (Sethy et al. 2006). In this study, we used the method of ISSR markers to analysis genetic diversity of 48 okras. The software of NTSYSpc 2.1 and Structure were used to analyze these data (Earl and vonHoldt 2012). The results indicated that the 48 okras were divided to 4 groups and the number of each accession was almost the same, proving that data were reliable and stable.

In the ISSR amplification fragments of the 48 samples, 54.55 % of polymorphism bands were screened. Their similarity is between 0.6558 and 0.9935. It indicated that the high genetic diversity existed between them. By using ISSR-PCR method to study genetic diversity of okra germplasm resources, this will lay a good foundation for the follow-up study. The 54.55 % of polymorphism bands is quite high. Although we collected the plant samples from close geographical distance, the polymorphic bands is rich, indicating that these okra germplasm had a relatively high level of genetic diversity.

Okra was domesticated in West and Central Africa, but is now widely cultivated throughout the tropics primarily for local consumption around the world. In Nigeria, it ranks third in terms of consumption and production area following tomato and pepper (Ibeawuchi 2007). The immature pods are used as boiled vegetable while in dried form it is used as soup thickener. The green pods are rich sources of vitamins, calcium, potassium, and other minerals (Shamsul and Arifuzzaman 2007). The leaves and seeds of okra are considered a valuable

Fig. 6 Two-dimensional matrix plot of the principal coordinate analysis showing associations among the 48 okras

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. Therefore, okras have important edible, medicinal and ornamental value. These 48 okras can be further measured specific protein, pectin, flavonoids and other ingredients contents. This will lay the foundation for the purpose of breeding fine varieties. It is called “green ginseng” in South Korea. It is called “Plant Viagra” in the United States. Such a multi-function (anti-fatigue, anti-cancer, enhance immunity, etc.), nutrient-rich plants, its function mechanism is worth further study.

Acknowledgments This research was supported by grants from the National Natural Science Foundation of China (31071076), Changsha Science and Technology Funds (2014 to X. Guo), International S&T Cooperation Program of Hunan Province (2014WK3012 to J. Ye; 2015WK to X. Guo), the interdisciplinary research project of Hunan University (2015 to X. Guo), the Ph.D. Programs Foundation of Ministry of Education of China (20130161110005), Jiangxi Province Science and Technology Support Project (20133BBF60080), and the SIT Project of Hunan University, 2013 and 2014.

References

- Babaei N, Abdullah NA, Saleh G, Abdullah TL (2012) Isolation and characterization of microsatellite markers and analysis of genetic variability in *Curculigo latifolia* Dryand. *Mol Biol Rep* 39(11): 9869–9877
- Bertoni BW, de C Telles MP, Malosso MG, Torres SC, Pereira JO, Lourenço MV, de C França S, Pereira AM (2010) Genetic diversity in natural populations of *Jacaranda decurrens* Cham. determined using RAPD and AFLP markers. *Genet Mol Biol* 33(3): 532–538
- Dhall RK, Shamma SR, Mahajan BV (2014) Development of post-harvest protocol of okra for export marketing. *J Food Sci Technol* 51(8): 1622–1625
- Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4:359–361
- Gul MZ, Bhakshu LM, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA (2011) Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using in vitro assays. *BMC Complement Altern Med* 11:64
- Ibeawuchi IK (2007) Intercropping a food production strategy for resource poor farmers. *Nat Sci* 5(1):46–49
- Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci U S A* 76: 5269–5273
- Oyelade OJ, Ade-Omowaye BIO, Adeomi VF (2003) Influence of variety on protein, fat contents and some physical characteristics of okra seeds. *J Food Eng* 57:111–114
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Rohlf FG (2000) NTSYS-pc: numerical taxonomy and multivariate analysis system Version 2.1. Exeter Publications, New York
- Sethy NK, Shokeen B, Edwards KJ, Bhatia S (2006) Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L.). *Theor Appl Genet* 112:1416–1428
- Shamsul A, Arifuzzaman K (2007) Chemical analysis of okra bast fiber (*Abelmoschus esculentus*) and its physico-chemical properties. *J Text Appar Technol Manag* 5(4):1–9
- Yuennan P, Sajjaanantakul T, Goff HD (2014) Effect of okra cell wall and polysaccharide on physical properties and stability of ice cream. *J Food Sci* 42:1750–1781