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RESEARCH ARTICLE

Genetic Diversity in Cultivated Sesame (*Sesamum indicum* L.) and Related Wild Species in East Africa

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Abstract

Genetic diversity of traditional sesame landraces and related wild species in East Africa remains largely unexplored. Knowing what fraction of the available genetic diversity is actually used by the farmers is of central importance for understanding how cultivation shapes the genetic structure of a crop and for the management of biodiversity preservation. Genetic diversity in cultivated sesame and related wild species in East Africa was determined using inter-simple sequence repeats (ISSR). Six reliable ISSR primers generated 51 amplification fragments of which 36 (70.6%) were polymorphic. The number of amplified fragments ranged from 7 to 12 with a mean of 8.5 fragments per primer. The overall gene diversity and Shannon's index were 0.28 and 0.34, respectively. Jaccard's similarity coefficient ranged from 0.26 to 0.96, with an average of 0.67. Forty-six accessions of sesame were divided into six clusters, although the clustering did not indicate any clear division among sesame accessions based on their origin. Each wild species was more distant from cultivated sesame than from other wild species, indicating that no cross-pollination with wild species occurred during sesame domestication. These results showed a relatively high genetic diversity in sesame and related wild species. Indian-1 and Indian-2 accessions showed a good amount of genetic divergence. The genetic diversity data uncovered in this study can be exploited to improve traditional landraces of sesame in East Africa.

Key words : genetic variation, ISSR, sesame breeding, sesame landraces, wild species

Introduction

Sesame (*Sesamum indicum* L.), is one of the oldest oil seed crops (Ashri 2010; Bedigian 2010a). Sesame seeds are important source of oil (44 - 58%), protein (18 - 25%), and carbohydrates (13.5%) (Bedigian et al. 1986). They are used as active ingredients in antiseptics, bactericides, viricides, disinfectants, moth repellants, and antitubercular agents because they contain natural antioxidants such as sesamin, sesamol, and sesamolin (Bedigian 2010b, c, 2011). Sesame oil has the highest antioxidant content (Anilakumar et al. 2010; Cheung et al. 2007; Elleuch et al. 2011) and contains several fatty acids such as oleic acid (43%), linoleic acid (35%), palmitic acid (11%), and stearic acid (7%) (Hiremath et al. 2007; Nzikou et al. 2009; Were et al. 2001, 2006). In addition, sesame oil is important in the food industry because of its distinct flavor (Elleuch et al. 2010). Sesame plays an important role in confectionary industries as well as being a source of vegetables in East Africa (Maundu et al. 1999). The crop is highly drought tolerant, grows well in most kinds of soils and regions, and is well suited to different crop rotations (Pham et al. 2010). Small-scale holders grow sesame under moisture stress with low management input (Cagirgan 2006).

Despite its nutritional value and medicinal importance, sesame yields in Kenya are very low, averaging 400 kg ha^{-1} compared to research seed yield of 2230 kg ha^{-1} (Ong'injo and Ayiecho 2009). Total area under sesame cultivation in Kenya has grown at a slow rate ranging from 20,000 ha in 1980 to 27, 000 ha in 2010 (FAO 2012) with concomitant

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low average seed yield/ha. One reason for low seed yield and reduced acreage under cultivation is lack of improved varieties for use by the farmers in East Africa (Were et al. 2001, 2006). This situation can be improved by selecting varieties of good quality and high adaptive potential to the diverse climatic conditions. Traditional sesame landraces as well as related wild species are an important source of genetic diversity for breeders and form the backbone of agricultural production (Ali et al. 2009; Bedigian 2011; Yol and Uzun 2012).

Genetic diversity in sesame, based on morphological, biochemical, metabolic, and molecular markers, has been reported by many researchers worldwide (Akbar et al. 2011; Bedigian 2010a; Cho et al. 2011; Furat and Uzun 2010; Isshiki and Umezaki 1997; Kim et al. 2002; Kumar et al. 2012; Laurentin et al. 2008; Nanthakumar et al. 2000; Parsaeian et al. 2011; Pham et al. 2010; Sharma et al. 2009; Tabatabaei et al. 2011; Uzun and Cagirgan 2009; Vinod and Sharma 2011; Wei et al. 2008; Were et al. 2001, 2006; Yol and Uzun 2012; Zhang et al. 2012). However, genetic diversity of traditional sesame landraces and related wild species in East Africa remains largely unexplored. In addition, most published work on genetic diversity in sesame has used accessions selected from germplasm banks. Knowing what fraction of the available genetic diversity is actually used by the farmers in East Africa is of central importance for understanding how cultivation shapes the genetic structure of a crop and for the management of biodiversity preservation. Inter-simple sequence repeats (ISSR) is a random amplified polymorphic DNA (RAPD)-like technique, that shares the simplicity of RAPD markers but uses longer polymerase chain reaction (PCR) primers (Kim et al. 2002). It is more reproducible and cost-effective for researchers in developing countries like Kenya. ISSR markers have been widely used for diversity analysis in a vast array of crops (Godwin et al. 1997).

The purpose of this study was to determine genetic diversity of traditional sesame landraces and related wild species in East Africa using ISSR and identify highly diverse accessions for the purposes of broadening the genetic base of the traditional landraces of sesame grown in East Africa.

Materials and Methods

Plant material

Forty-six accessions of sesame representing different regions in Kenya, Tanzania, and Uganda (Fig. 1; Table 1) were used in this study. These materials comprised thirty-five landraces, three commercial varieties, and eight accessions of three wild species. Ten seeds from each accession were planted in plastic pots and maintained in a greenhouse (28ºC/25ºC) day/night and 70% humidity.

Table 1. List of sesame accessions and place of collection

Serial Number	Accession	Seed source/ Locality
	S. indicum L.	
1.	105BU	Mumias, Kenya
2.	106BU	Busia, Kenya
3.	108BU	Busia, Kenya
4.	111BU	Luanda, Kenya
5.	113BU	Koyonzo, Kenya
6.	303BU	Kisii, Kenya
7.	304BU	Ahero, Kenya
8.	306BU	Kisumu, Kenya
9.	307KE-a	Ugenya, Kenya
10.	307KE-b	Ugenya, Kenya
11.	308KE	Ugenya, Kenya
12.	Lungalunga	Mombasa, Kenya
13.	Majengo	Mombasa, Kenya
14.	Msambweni	Mombasa, Kenya
15.	Sik114	Breeder
16.	Stewa	Shimo la Tewa, Kenya
17.	101UG	Kitale, Kenya
18.	102UG	Webuye, Kenya
19.	103UG	Kanduyi, Kenya
20.	107UG	Nambale, Kenya
21.	109UG	Adungosi, Kenya
22.	110UG	Bumala, Kenya
23.	Ug1	Uganda
24.	Ug2	Uganda
25.	Ug3	Uganda
26.	Ug4	Uganda
27.	Ug5	Uganda
28.	Ug7	Uganda
29.	301TZ	Tanzania
30.	302TZ	Tanzania
31.	Morogoro	Tanzania
32.	Mtwara-1	Mtwara, Tanzania
33	Mtwara-2	Mtwara, Tanzania
34.	Tan 3	Tanzania
35.	Tan6	Tanzania
36.	Tan7	Tanzania
37.	Indian-1	India
38.	Indian-2	India
Wild species	***S. angustifolium***	
39.	103w	Kitale- Kapenguria Rd.
	S. latifolium	
40.	104aw	Kambi ya Samaki Rd
41.	104w	Loboi (Marigat Rd.
	S. angolense	
42.	105w-a	Kapropita Rd
43.	105w	Kabarnet
44.	108w	Busia Air Strip
45.	109w	Eldama -Ravine Rd
46.	202w	Kabarnet

DNA extraction

Genomic DNA was isolated from leaves of 3-week-old seedlings following the protocol described by Uzun and Cagirgan (2009) and Uzun et al. (2003). DNA quality and quantity were determined by visual evaluation of band intensity in comparison with lambda DNA molecular standards of known concentrations as described by Sambrook and Russell (2001). DNA samples were stored at -20ºC, until further use.

PCR amplification and electrophoresis

A few individuals of each sesame accession were initially screened by testing with 20 ISSR primers, designed at the Biotechnology Laboratory, University of British Columbia (UBC) and synthesized by Sigma-Aldrich Company. The six

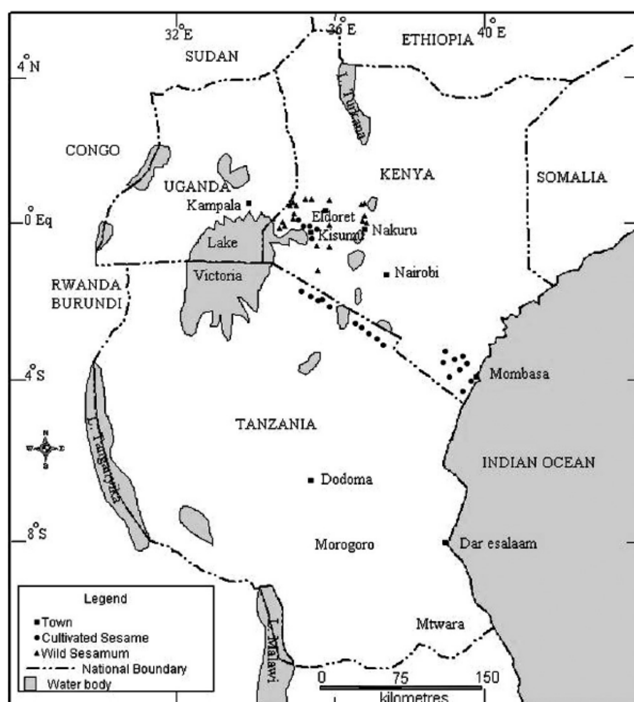

Fig. 1. Map of East Africa showing the collection sites for sesame landraces and wild species studied.

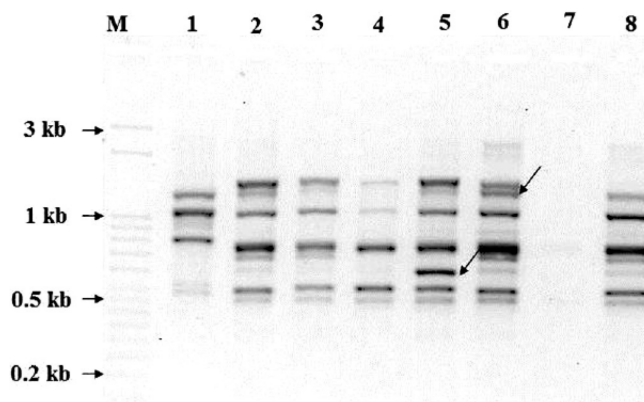

Fig. 2. PCR products from eight sesame genotypes (Lane 1 - 8) amplified with primer UBC868. Arrows show polymorphic fragment. Molecular weight marker (50 bp) is shown in the left lane (M).

primers (Figure 2; Table 2) that produced clear-cut DNA fragments and consistent polymorphisms were selected to amplify the DNA of each accession. The reproducibility of PCR banding patterns was tested on eight samples randomly selected from various accessions. Amplification reaction was carried out as described by Kim et al. (2002) and Uzun and Cagirgan (2009) with modification in annealing temperature and reaction volume. PCR reaction was performed in a volume of 15 µL containing 1×PCR buffer (200 mM Tris-HCl, pH 8.4, 50 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM primers, 1.0 unit of Taq DNA polymerase (Invitrogen®), and approximately 10 - 20 ng of genomic DNA. PCR amplification was carried out in a Crocodile III™thermocycler (Appligene Oncor) according to the following program: 4 min at 94ºC for initial denaturation, followed by 35 cycles of 45 s at 94ºC, 1 min for annealing temperature (Table 2) and 2 min at 72ºC for extension step, followed by 5 min at 72°C. ISSR fragments were separated on 1.8% agarose gels and run in 1×TAE buffer (40 mM Tris acetate, pH 7.5, 1 mM EDTA) for 2 h at 70 v. The gels were stained with ethidium bromide (0.5 µg $µL^{-1}$) as described by Sambrook and Russell (2001). DNA fragments were detected using UV trans-illumination and photographed using Bio Doc-It™ gel documentation system (UVP, Cambridge, UK).

Scoring and data analysis

Each amplification product was assigned numbers in order of decreasing molecular weight. The amplified fragments in each of the 46 accessions were scored manually for their presence (1) or absence (0) for each primer combination. The

Table 2. Selected ISSR primers, annealing temperature, number of total fragments (TF), polymorphic fragments (PF), percent polymorphism (%P), gene diversity (H_t), and Shannon's index (S) observed in 46 sesame accessions

Primer Code	*Ta (°C)	Sequence (5'-3')	TF	PF	%P	Ht	S	Size (bp)
UBC 811	47	$(GA)_8C$	12	9	75	0.24	0.40	350 - 2000
UBC 825	47	$(AC)_8T$	7	5	71	0.28	0.44	300 - 2000
UBC 845	60	$(CT)_8R$**G	8	7	88	0.29	0.44	300 - 3000
UBC 868	47	$(GAA)_6$	7	5	71	0.27	0.42	450 - 2000
UBC 873	47	$(GACA)_4$	9	5	56	0.35	0.53	200 - 3000
UBC 900	60	-[5]	8	5	63	0.34	0.51	350 - 3000
Total			51	36	70.6	0.28 ± 0.14	0.44 ± 0.19	
Range			7 - 12	5 - 9	56 -88			200 - 3000

*Ta = annealing temperature, R** = (A, G), -[5] = ACTTCCCCACAGGTTAACACA

molecular size of the DNA fragments was estimated using 50 bp DNA ladder (Fermentas). For each primer, the number of total fragments, polymorphic fragments, and polymorphism percentage were calculated (Table 2). Nei's (Nei, 1978) genetic diversity (H_t) and Shannon diversity index (S) of phenotypic diversity for the ISSR binary data was derived using Popgene® software version 1.32 (Yeh et al. 1999). The binary data (1/0) was used to generate a similarity coefficient. Genetic similarity, S, between genotype *i* and *j* was estimated by using Jaccard's coefficient as described by Sneath and Sokal (Sneath and Sokal 1973) as follows:

$$S_{ij} = \frac{a}{a+b+c}$$

Where S_{ij} = similarity between two individuals, i and j, a = bands shared by both individuals.
b = bands present in i but not j, and c = bands present in j but not i.

The resulting similarity coefficient was employed to generate a dendrogram using the un-weighted pair-group method with arithmetic average method (UPGMA). These analyses were carried out using NTSYS-pc, Version 2.1 package (Rohlf 2000). Bootstrap analysis using WINBOOT software package developed at the International Rice Research Institute (Yap and Nelson 1996) with 1,000 replicates was performed to obtain the confidence of the branches of the

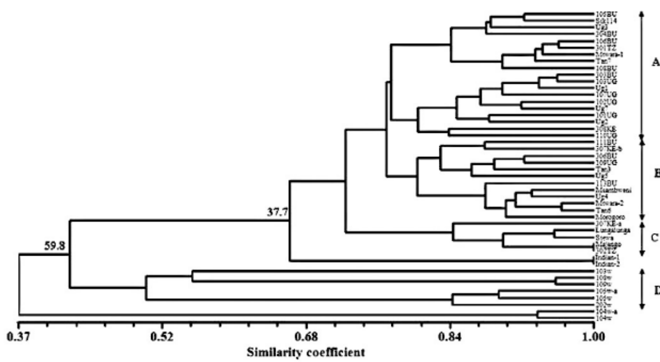

Fig. 3. UPGMA dendrogram showing relationships among accessions of sesame and related wild species. The values at the fork show the percentage contribution of the group bracketed by the fork by bootstrapping.

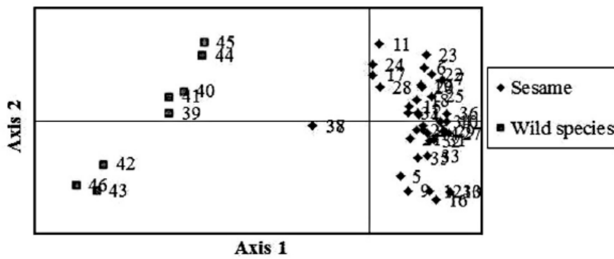

Fig. 4. Principal coordinate analysis showing relationships among accessions of cultivated sesame and related wild species.

tree. The principle coordinate analysis (PCA) via distance matrix with data standardization for the 46 accessions was derived using GenAlEx 6.2 software (Peakall and Smouse 2006).

Results

Six ISSR primers generated a total of 51 well-resolved fragments of which 36 (70.6%) were polymorphic. The highest number of polymorphisms was observed with primer UBC 845, while UBC 873 detected the lowest number of polymorphisms (Table 2). The number of amplified fragments ranged from 7 to 12 per primer. Average number of fragments and polymorphic fragments per primer were 8.5 and 6, respectively. The fragment size ranged from 200 to 3000 bp. A representation of the ISSR fragment profile obtained with primer UBC 868 is shown in Fig. 1. The evaluation of the ISSR by six primers resulted in overall species diversity of 0.34 and gene diversity of 0.28, repectively. A wide range (0.26 - 0.96) in the value of Jaccard's similarity coefficent was observed among the accessions (data not shown). Forty-six accessions of sesame and related wild species were divided into six clusters that were dermarcated at a cut-off similarity coefficient level of 0.74 (Fig. 2). Accessions of cultivated sesame and related wild species formed separate clusters. The most distinct sesame accessions were Indian-1 and Indian-2. Genetic distances among three wild species were larger than distances among landraces and cultivars. Sesame landraces from different geographical regions grouped together in the same cluster with the exception of two commercial varieties, Indian-1 and Indian-2. The PCA (Fig. 3), indicated that the first two principal coordinates, PCA1 and PCA2, explained 41.4 and 16.7% of the variation, respectively.

Discussion

The level of polymorphism obtained in this study (70.6%), was high compared with previous studies in which a low level of polymorphism was detected among Korean sesame genotypes (33%) (Kim et al. 2002), and Indian sesame varieties (57%) (Vinod and Sharma 2011). However, our results were comparable with the 76.47% of polymorphism reported in analysis of genetic diversity in Iranian sesame genotypes by Parsaein et al. (2011). The difference in polymorphism may be due to the genotypes used, nature of ISSR primers and annealing temperatures used. Low annealing temperature may increase non-specific amplification, leading to artefact bands (Sanchez et al. 1996). The modification of annealing temperature by Bornet and Branchard (2001) has a great impact on the richness and legibility of fingerprints. Minimum and maximum fragment size, number of polymorphic fragments, and percentage of polymorphic loci reported in this study indicate high genetic diversity among accessions of sesame and related wild species. In addition, the overall species and gene diversity indices were relatively high, indicating a high level of genetic diversity in the accessions of sesame and related wild species. The gene diversity observed in this study (0.28) was comparable to 0.29 reported by Sharma et al. (2009) using ISSR markers. Although sesame is self-pollinated, some authors have reported levels of out-crossing ranging between 5 and 60% (Pathirana 1994; Yermanos 1980). Out-crossing plant species tend to present between 10 to 20% of the genetic variation between populations (Hamrick and Godt 1989). Hence, out-crossing among neighbouring fields could explain the high genetic variation observed. Our results support the previous studies where a high level of genetic variation was observed in East African sesame germplasm based on morphological traits (Pham et al. 2010; Were et al. 2001, 2006). Although a relatively high genetic diversity exists among accessions of cultivated sesame and related wild species from various geographical locations, some accessions situated geographically far apart grouped together in the same cluster. These results are in agreement with earlier studies which showed that geographical separation did not generally result in greater genetic distance (Kumar et al. 2012; Parsaeian et al. 2011; Wei et al. 2008; Zhang et al. 2012). This could be a consequence of exchange of genetic materials among the neighbouring farmers as well as traders in the region. The movement of traders in East Africa region could be a possible explanation for the spreading of sesame landraces. The human factor has been

previously shown to be responsible for the lack of correlation between genetic and geographical distance (Stankiewicz et al. 2001). The accessions of sesame and related wild species formed separate clusters indicating a relatively distant relationship. This distinctness could be attributed to different modes of evolution coupled with sexual incompatibility barriers that exist in some *Sesamum* species (Kobayashi 1981). A high number of chromosome (2n = 32) observed in wild species in this study (data not shown) may also explain the genetic divergence between sesame and related wild species in East Africa. Genetic divergence between wild and cultivated plant species has been reported in a number of crops including *Lens* species (Duràn and Pérez de la Vega 2004), mulberry accessions (Zhao et al. 2004), jute (Bandyopadhyay et al. 2006), and Pigeon pea (Panguluri et al. 2006).

In conclusion, cultivated sesame and related wild species growing in East Africa showed a wide genetic diversity. Indian-1 and Indian-2 accessions showed a good amount of genetic divergence and would therefore be useful in broadening genetic base of traditional landraces of sesame in East Africa. Wild species were more distant from cultivated sesame than from other wild species, indicating that no cross-pollination with wild species occurred during sesame domestication.

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