Electronic Journal of Biotechnology ISSN: 0717-3458
Vol.10 No.1, Issue of January 15, 2007

Received May 11, 2006 / Accepted October 5, 2006
DOI: 10.2225/vol10-issue1-fulltext-16
RESEARCH ARTICLE

Assessment of genetic diversity in sesame (*Sesamum indicum* L.) detected by Amplified Fragment Length Polymorphism markers

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Financial support: This work was performed under the fellowship for G.M. Ali. Authors are grateful to Japan Society for Promotion of Science and Natural Science and Engineering Research Council Canada for this award and financial support.

Keywords: AFLP, genetic diversity, geographical origin, morphology, sesame.

Abbreviations: AFLP: Amplified Fragment Length Polymorphism.

Sesame (*Sesamum indicum* L.) is one of the oldest oil crops and is widely cultivated in Asia and Africa. To determine the level of genetic diversity in relation to geographical origins and morphological characteristics, a total of 96 accessions have been collected from different parts of the world and were analyzed using AFLP techniques. Twenty-one primer pairs generated a total of 445 bands and among them 157 (35%) were polymorphic. Using UPGMA clustering analysis method based on the similarity coefficient, accessions were separated into two major groups. The first group mostly consists of Eastern Asian origin and another group consists of South Asian origin. Sub-clusters separated the accessions and form distinct diversity among groups. Considering the relatednessof accessions, geographical origin and their morphological characteristics are reflected to the similarity of AFLP pattern.

Sesame (*Sesamum indicum* L.) family Pedaliaceae, is one of the most ancient oilseeds crop known to mankind. It was cultivated and domesticated on the Indian subcontinent during Harappan and Anatolian eras (Bedigian et al. 1985; Bedigian et al. 2003) but now it is grown in many parts of the world. However, Asia is rich in diversity of cultivated sesame. It is an important source of edible oil and is widely used as a one of the ingredients in food products especially in bakery foods and animal feed. Sesame oil has medicinal and pharmaceutical value and is being used in many health cure products. Sesame seed contains 50-60% oil and 25% protein with antioxidants lignans such as sesamolin, sesamin and has been used as active ingredients in antiseptics, bactericides, viricides, disinfectants, moth repellants, anti-tubercular agents (Bedigian et al. 1985) and considerable source of calcium, tryptophan, methionine and many minerals (Johnson et al. 1979). These lignan contents have beneficial physiological effects in animal and human health (Ashakumary et al. 1999). Composition of fatty acid in sesame oil is variable between different cultivars

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(Yermanos et al. 1972; Brar, 1982). Effective antioxidant properties of sesame seed oils are characterized by the presence of lignans, sesamin and sesamolin (Fukuda et al. 1986). These highly important characteristics have lead researchers to develop interest in biochemical analysis and in identifying the accessions having rich beneficial oil contents, in order to make efforts for the improvement of this crop using advanced technologies.

Recently, the use of AFLP in genetic marker technologies has become the main tool due to its capability to disclose a high number of polymorphic markers by single reaction (Vos et al. 1995). It is a useful technique for breeders to accelerate plant improvement for a variety of criteria, by using molecular genetics maps to undertake marker-assisted selection and positional cloning for special characters. Molecular markers are more reliable for genetic studies than morphological characteristics because the environment does not affect them. In sesame, few reports have been published on the analysis of the diversity *viz*., RAPD (Bhat et al. 1999), isozymes (Isshiki and Umezaki, 1997), morphological and agronomic characters (Bedigian et al. 1986) but a little work has been done on sesame using AFLP molecular techniques for evaluating genetic diversity in relatedness with geographical origin.

AFLP markers have successfully been used for analyzing genetic diversity in some other plant species such as peanut (Herselman, 2003), soybean (Ude et al. 2003), and maize (Lübberstedt et al. 2000). These studies have indicated that the AFLP technique is highly applicable for molecular discrimination at the species level.

The identification of genetic relationship among the cultivars based on biochemical and molecular analysis will be used in further genetic improvement. It will also provide support for selection of crossing combinations from bulk parental genotypes and for broadening the genetic basis of breeding programs. Therefore, it is necessary to study cultivars at the molecular level to distinguish them for their special characters and to differentiate varieties collected from different regions of the world.

In this context, the aims of the present study were to find out the relationships between sesame cultivars including breeding lines and, to analyze their genetic relationships for further genotypes identification. First, to determine varietals differences among varieties collected from different regions of the world, and second to describe the genetic similarity between accessions and confirm them by using morphological parameters.

MATERIALS AND METHODS

Plant material

Ninety-six accessions including breeding lines, experimental lines and local varieties collected from different regions of the world were analyzed (Table 1) for AFLP. This material was maintained at the National Institute of Crop Sciences Tsukuba, Japan. Each accession had homogeneous material therefore a single plant was used from each one.

AFLP methodology

All accessions were grown in a greenhouse and a total of 100 mg of fresh leaves were collected for DNA isolation using Plant DNA ZOL kit (Invitrogen life technology USA). AFLP analysis was performed according to Vos et al. (1995) method with little modifications. Initially, genomic DNA (120 ng) was digested using 1 µl of EcoR1/Mse1 (1.25) unit enzymes (Invitrogen AFLP Core reagent kit) at 37ºC for 12 hrs. Digested reactions were ligated following manufacture instructions. Diluted ligations (1:10) mixture were pre-amplified using the E00 (GACTGCGTACCAATTC) and M00 (GATGAGTCCTGAGTAA) primers. PCR reactions were performed in a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, USA) at 94ºC denature for 30 sec, annealing at 56ºC for 60 sec and extension at 72ºC for 60 sec for 20 cycles. Pre-selective PCR products (1:100 diluted) were stored at -20ºC.

For selective amplification, thermocycler was programmed to a touchdown temperature cycle at 94ºC for 30 sec, 65ºC for 30 sec and 72ºC for 60 sec for 13 cycles. The annealing temperature was decreased 0.7ºC each cycle and then, 23 cycles at 94ºC for 30 sec, 56ºC for 30 sec and 72ºC for 60 sec. Combinations of primers showed in Table 2 were used for the AFLP analysis.

PCR products were loaded on 0.8% Bis, 30% Acryl-amide, 1.5 M Tris-HCl (pH 8.8), 10% APS, TEMED gel. Marker VIII ladder (Roche diagnostic GmbH Germany) was used as molecular weight standard. Gels were stained using a Vistra green (Amersham) solution (60 µl in 200 ml sterilized H2O) for 40 min. Stained gels were washed with 25% ethanol for 1 hr. Bands were scored visually from gel pictures. An example is shown in Figure 1.

AFLP analysis

A total of 21 primer combinations were selected to carry out the analysis in the ninety-six varieties (Table 1). Total bands were scored visually and polymorphic bands were analyzed as presence (1) or absence (0). Phylogenetic relations were determined by the UPGMA method using the Jaccard's similarity coefficient (SPSS - 10 software).

RESULTS

For an initial screening, seven-hundred-four primers combinations were tested in eight varieties (data not shown). From this study, the twenty-one most effective primers were selected by scoring the amount of polymorphic bands. Results showed (Table 2) that E-ACT/M-GTT primer combination produced maximum polymorphic bands (65% of total detected bands) whereas the primers E-AAC/M-GGT, E-AGA/M-GTC, E-AGC/M-

GAG and E-ACT/M-TAT produced superior number of polymorphic bands. E-AGG was found to be the best performer primer, having more ability to produce polymorphic bands with other M primers. Among twenty-one selected combinations, eight combinations were composed by the E-AGG primer.

Results for AFLP data and phenotypic data are presented in Figure 2 and Table 1, respectively. Main clusters were related to geographic origin but the small clusters also present a phenotypic relatedness for four morphological traits viz., branching habit, number of flowers per axil, type of capsule and seed coat colour. Molecular data categorized the sesame accessions in two main groups (Figure 2). Group I and II, which discriminate varieties related with geographical origin. Countries were separated in the two main groups with some exceptions; both groups accumulated most of the accessions from countries of close origin. It is clear in cluster analysis that the accessions from Japan, India, Myanmar, and Pakistan showed a close phylogenetic relationship, based on their origin.

It was noticed that due to genetic difference, major genotype clusters were related to main geographic origin. However, small clusters were also formed based on some known characteristics, pedigree relations or belonging to close area of cultivation within main group. Group 1 was divided into nine (a to i) sub groups. Results displayed that both S79 and S80 were sister breeding lines with high lignin contents as could be confirmed by their close distance. AFLP markers produced identical fingerprints between these lines and, one of their parents (S81) was also neighboured within small distance.

Three accessions viz., S4, S5, and S6 gathered in cluster "c" were originated in USA, especially S4 and S6 carried indehiscent character with short molecular distance from another indehiscent accession S90. Whereas three accessions from China were grouped in cluster "d". Cluster "e" bunched four Japanese accessions collected from western region of Japan. Varieties S22, S24 and S83 from Korea as well as others accessions from central Japan were in the cluster "g". Cluster "h" was composed by 18 accessions; one from Korea, two from China and the rest were from central Japan. It was noticeable that most of the accessions from clusters "g" and "h" were from Korea and western region (Shimane prefecture). Some other varieties in these clusters belong to central region that is neighbouring to western Japan, reflecting that geographical association being close position in clusters.

Group II consisted of two main clusters "a" and "b". Cluster "a" was mainly composed by accessions collected from Myanmar, three from India, one from Bangladesh and, one from Sri Lanka r. Cluster "b" was formed by most of the accessions from India, Pakistan, Bangladesh, Sri Lanka, Thailand and Nepal. Dendrogram (Figure 2) confirms that the accessions collected from same countries were closely associated. Pakistan, Thailand and India dominantly showed their association in small units considering molecular similarities. Overall consideration could be that the entire South Asian region was a place of origin from the studied accessions.

Similarities in morphological characters, such as basal branching, one flower, bicarpels and white seed coat colour were also showed in the accessions accumulated in Group-1 a, b, f and g. Basal branching, three flowers and bicarpels were gathered in cluster "c" and "d". Cluster "e" was characterized by accessions with basal branching, no branching, one flower, tetracarpels and white seed colour.

Some of the accessions in cluster "h" had similarity having basal or no branching and bicarples. Group II was divided into two main clusters "a" and "b"; majority of the accessions in cluster "a" produced basal branching habit, one flower bicarples and reddish brown. In cluster "b" most of the accessions produced white seed colour whereas some accessions had few exceptional morphological characters in each sub group, which may differentiate the clusters.

From above results, it has been observed that different geographical regions could be characterized by the presence of AFLP fragments, and a possible correlation between some morphological characters and geographic origin was also evident.

DISCUSSION

Genetic diversity of different materials can be studied together by morphological traits, the geographical origin and by using molecular marker techniques like RFLPs, RAPDs or AFLPs. Work on the subject has already been described in many other species, especially in cereals (Cho et al. 1998), horticultural crops (Aranzana et al. 2003), medicinal plants, ornamental plants and, oilseed plants (Hansen et al. 2003). Microsatellites and SSRs are also considered a powerful tool to investigate plant variability (Donini et al. 1998; Huang et al. 2002; Khlestkina et al. 2004). Recently, it has been assumed that in plant breeding, diversity can be reduced using biochemical molecular techniques. Present study was carried out on diversity of ninety-six sesame accessions collected from different parts of the world, mainly from the Asian region.

In our work, close genetic relations between the accessions were determined by geographical origin using AFLP markers. The accessions were clustered in two main groups; mainly corresponding to their geographical origin as well morphological characteristics. All accessions from Japan were clustered in Group I and, none of Japanese accessions were outside this group. It is remarkable to mention that accessions from neighboring countries of Japan (Korea and China) were also pooled in this group and showed low diversity.

It is important to stand out that in the collected materials from Japan, most of the accessions from the same or neighbour regions were closely grouped, *i.e.* accessions

S49, S28, S29, S27, S51, S53 and S91 from central region pooled together (g and h clusters). Accessions S38, S39 and S36, S40 and S82 from western region and some Korean accessions (Figure 2) were also grouped together, probably due to a very close place of origin. It has been concluded that sesame cultivated in these countries had a very narrow genetic base. Present results support the evidences of previous studies from Isshiki and Umezaki (1997*)* and Bhat et al. (1999). Similarly, majority of the accessions from Myanmar were grouped in cluster "a" of group II, while all other south Asian neighboring countries were pooled together in cluster "b". Figure 2 showed a very consistent relationship between these accessions. Our results are in accordance with the conclusion on distribution of genetic diversity for soybean observed by Cui et al. (2000) and Ude et al. (2003).

However, there are some exceptions, accession S63 from Myanmar showed a drastically distinct position (Figure 2) being clustered in "I a" indicating the highest diversity. Bhat et al. (1999) found comparable results using RAPDs for accessions collected in India. As the accessions representing different regions were grouped in different clusters, further strategies could be following for both breeding management and usage.

Considering morphological data (branching habit, number of flowers per axel, capsule type and seed coat colour), some sesame genotypes were closely grouped in sub-clusters. Group I clusters "c" and "d" included basal branching, three flowers and bi-carpals, while accessions from cluster "e" produced one flower with tetra-carpel trait. Clusters I f, g and II b mostly accumulated accessions with basal branching, one flower, white seed coat colour, but f and g might be separated because of tetra carpel character. Whereas "II a" showed similarities on the basal branching, one flower but with different seed coat colour, altogether point toward their relatedness (Kobayashi, 1981; Bisht et al. 1998). Similar results indicating relationship between molecular data with morphological traits have been reporter by Furini and Wunder (2004) for complex *Solanum* genus and, by Sharma et al. (2000) in *Morus* genus.

In coincidence with Kobayashi (1981) results, tetra-carpals characters appears mostly in accessions belonging to Japan and far east countries, whereas those belonging to other Asian countries produced bi-carpals. Results of cluster pattern showed a relationship when comparing molecular and morphological data for most of the phenotypic characters. Federici et al. (2001) observed this kind of relationship in rice. In this case, about 90% of the samples having straw hull and short awns were clustered together and, about 75% with black hull and long awns were accumulated separately by AFLP data. Furini and Wunder (2004) also reported consistency between molecular and morphological data in eggplant. Additionally, this relationship has been studied in different crops, *i.e.* rice (Federici et al. 2001*)*, common vetch (Sharma et al. 2000), *Morus* (Potokina et al. 2002; Baranger et al. 2004).

Two lines (S79 and S80) with high lignin contents showed strong relation on the basis of biochemical analysis (Sirato-Yasumoto et al. 2001) as was revealed in the dendrogram. Both lines were breed for high lignin contents; which showed feasibility of AFLP technique as a tool for identification of parental genotypes (Marsan et al. 1998). In addition, it was remarkable that accessions S4, S6 and S90 (with indehiscent trait) were closely grouped. Linkage for indehiscent characters in sesame has also been reported by Uzun et al. (2003).

Summarizing, we demonstrated that for genetic relatedness studies in sesame AFLP was a reliable tool. AFLP patterns will be useful to identify the different sesame accessions and to make relatedness by biochemical analysis. Morphological traits, geographical origins, and observations on genotype-specific amplified bands of AFLP will also be useful for their economic value and explore the different genotypes for further classification.

ACKNOWLEDGMENTS

We would like to thank Dr. Ryoji Takahashi for all his technical and moral support for this work and also like to thanks Evangelos D. Leonardos and Javaid Iqbal, Department of Plant Agriculture, University of Guelph, Guelph, Canada for critical reading of the manuscript.

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APPENDIX

Figures

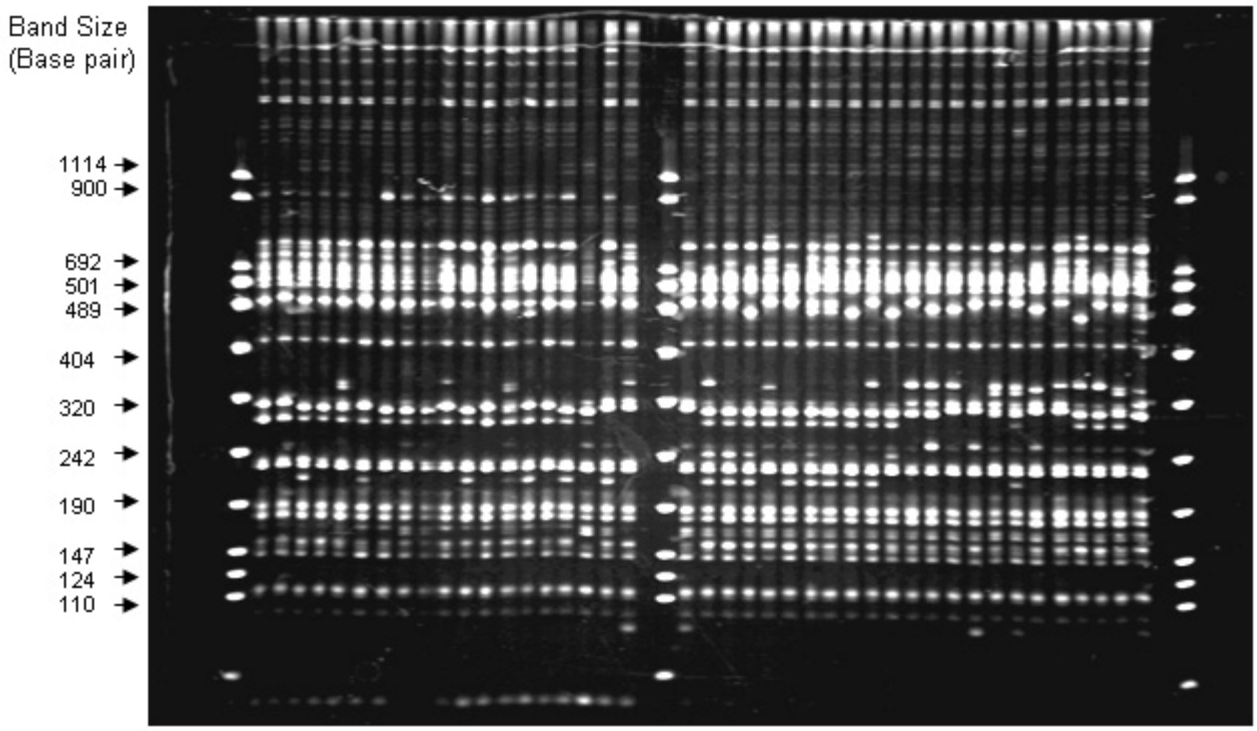

Figure 1. Example of an AFLP profile for some selective sesame accessions with the primer pair of *Eco*R1-AGG and *Mse*1-GGA.

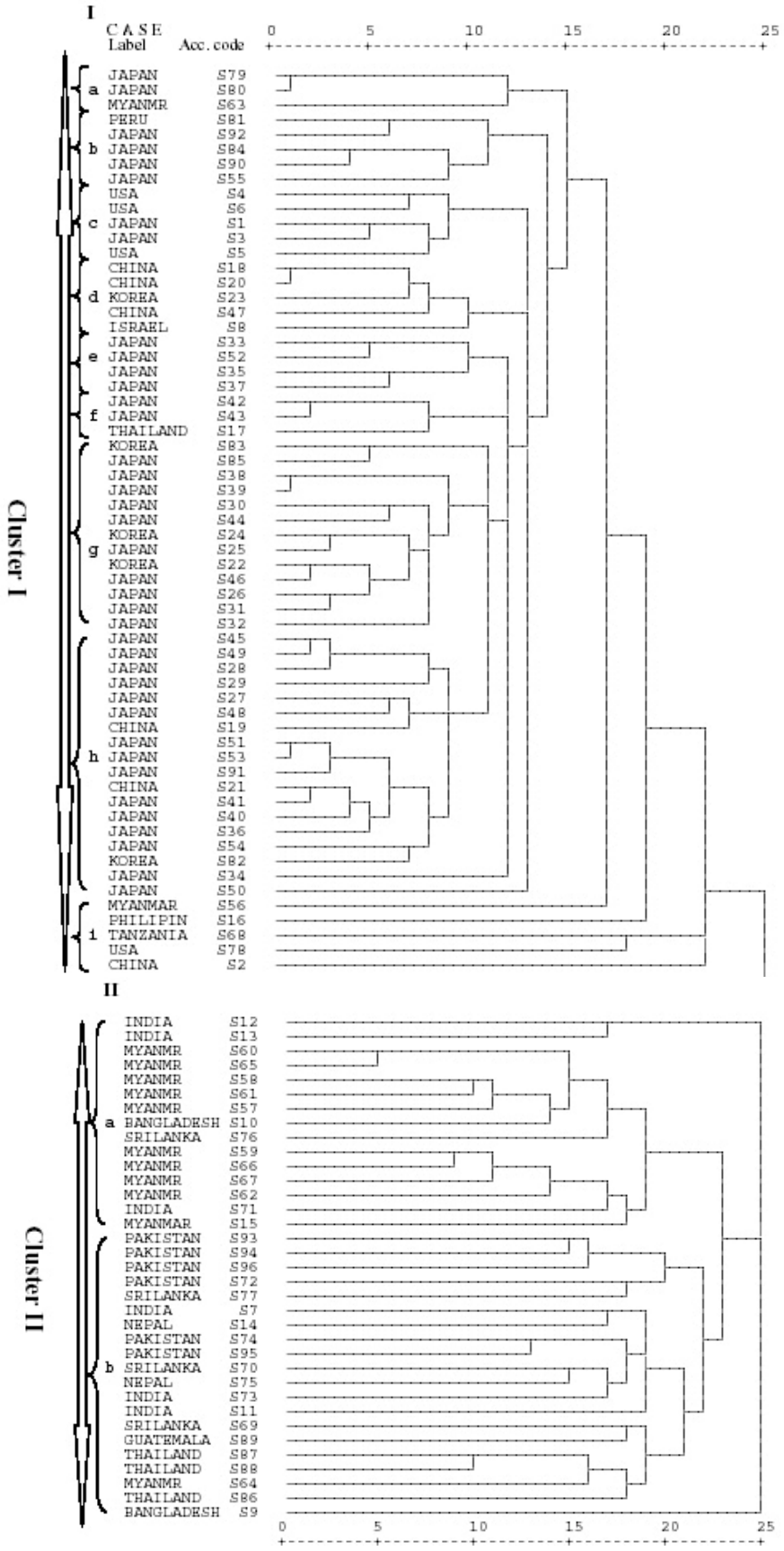

Figure 2. Dendrogram based on AFLP data of 96 sesame lines, using Jaccards coefficient of similarities and UPGMA clustering method.

Tables

Table 1. Morphological data and country of origin for the sesame accessions used in AFLP analysis.

S. No.	Accession name	Acc. code	Acc. type	Branching habit	Number of flowers per axel/Capsule type	Seed coat colour	Country of origin
1	Ses-146	S9	L	3	1B	4	Bangladesh
2	Ses-191	S10	L	3	1B	4	Bangladesh
3	H-65	S2	L	1	1B	1	China
4	22M1439	S18	L	3	3B	1	China
5	Toyama-802	S19	L	3	3B	6	China
6	Toyama-803	S20	L	3	3Q	1	China
7	China Kanan	S47	L	3	3B	1	China
8	Danbaeckae	S21	L	3	3B	1	China
9	Introduction line	S89	L	3	1B	1	Guatemala
10	EC-244632	S7	L	3	1B	1	India
11	IC-23279-1	S11	L	3	1B	6	India
12	IC-43110	S12	L	3	1B	6	India
13	IC-96175	S13	L	3	1B	1	India
14	Toyama 585	S71	L	1	1B	4	India
15	NIC-16365	S73	L	3	1B	1	India
16	1991-2003	S8	L	3	3B	2	Israel
17	T.006 Sesamin less	S84	E. L	1	1B	5	Japan
18	T.4292 Sesamin less	S85	E. L	4	3-1Q	1	Japan
19	Toyama 40221 Indehiscent	S90	E. L	3	3B	1	Japan
20	Toyama 308 Indehiscent	S91	E. L	3	1B	4	Japan
21	Iwatekuro	S1	L	3	1B	7	Japan
22	Masekin	S3	L	4	3B	3	Japan
23	Toyama-925 (Aomori)	S25	L	3	1B	5	Japan
24	Col/fukushima/1990/9001	S26	L	4	1Q	5	Japan
25	Azuma Gunma local	S27	L	4	3B	3	Japan
26	Chichibu local (Saitama)	S28	L	4	3Q	3	Japan
27	Col/Chichibu/Maruteru2/1995/Saitama	S29	L	3	3B	6	Japan
28	Nagatoro Zairai (Saitama)	S30	L	4	3B	3	Japan
29	Birodo (Saitama)	S31	L	3	1B	5	Japan
30	Boushu Shiro (Chiba)	S32	L	3	1B	1	Japan
31	Aichi Shiro	S33	L	4	1Q	1	Japan
32	Col/Okayama/Takahashi/ 092005	S34	L	4	3B	3	Japan
33	Col/Mie/NIAR/1998/029	S35	L	3	1B	2	Japan
34	Col/Iimi,Huse/Kawakami/(Shimane)	S36	L	4	1Q	1	Japan
35	Col/Imadu/Saigou/Sakaki (Shimane)	S37	L	4	1Q	1	Japan
36	Col/Matugaura, Saigou/Mori/Shimane	S38	L	4	1Q	1	Japan
37	Col/Minamikata/Goka/Matuyama	S39	L	4	1Q	1	Japan
38	Col/Uehama,Saigou/Tomita/(Shimane)	S40	L	3	3B	1	Japan
39	Toyama-959 (Fakuka)	S41	L	3	1B	1	Japan
40	00037803 (Kagoshima)	S42	L	3	1Q	2	Japan
41	Shiro Goma	S43	L	4	3Q	1	Japan
42	Col/Nagasaki/NIAR/1994/111	S44	L	3	1B	7	Japan

43	Col/Okinawa/NIAR/1991/060	S45	L	4	3B	3	Japan
44	KANTO-1 Breeding line Ibaraki	S46	B.L	3	1B	1	Japan
45	Int/Iwate/NICS/ 2001/011	S48	L	3	1B	7	Japan
46	Int/Ibaraki/NICS/ 2001/003	S49	L	3	3B	3	Japan
47	Int/Ibaraki/NICS/ 2001/004	S50	L	3	3B	1	Japan
48	Int/Ibaraki/NICS/ 2001/006	S51	L	3	1B	7	Japan
49	Int/Kagoshima/NICS/2001/001	S52	L	3	1Q	1	Japan
50	Col/Nagano/NICS/ 2001/1333	S53	L	3	1-3BQ	5	Japan
51	Col/Nagano/NICS/ 2001/1356	S54	L	4	3B	1	Japan
52	Nagasaki	S55	L	3	1Q	1	Japan
53	0731	S79	B.L	3	1B	5	Japan
54	0732	S80	B.L	3	1B	4	Japan
55	Kanto	S92	L	3	1B	1	Japan
56	Korea-58	S82	L	3	1B	1	Korea
57	Korea-39	S83	L	3	1B	1	Korea
58	Boteni	S15	L	3	1B	4	Myanmar
59	9A	S56	L	1	1B	1	Myanmar
60	Al	S57	L	3	1B	4	Myanmar
61	Ashri-118	S58	L	1	1B	4	Myanmar
62	Hnan Ni	S59	L	3	1B	4	Myanmar
63	Khway Lay Ni	S60	L	3	1B	4	Myanmar
64	Magway7/9	S61	L	3	1B	4	Myanmar
65	Me Thi La	S62	L	3	1B	2	Myanmar
66	MMT-995-501	S63	L	2	3B	1	Myanmar
67	Shwe Tasoke	S64	L	3	1B	1	Myanmar
68	Thee Kone (local) variety)	S65	L	3	1B	4	Myanmar
69	Water LoggedResistant/Kachin	S66	L	3	1B	4	Myanmar
70	Yoe Sein	S67	L	3	1B	4	Myanmar
71	Col/Nepal/1984/1325	S14	L	3	1B	1	Nepal
72	Col/Nepal/1984/2412	S75	L	1	1B	7	Nepal
18	Col/Pak/1989/IBPGR/ 2541-(2)	S72	L	1	1B	1	Pakistan
73	Toyama 9463	S74	L	1	1B	4	Pakistan
74	86014	S93	L	3	1B	1	Pakistan
75	K-S95	S94	L	3	1B	4	Pakistan
76	S-105	S95	L	3	1B	1	Pakistan
77	SGP-31	S96	L	3	1B	1	Pakistan
78	Toyama-016	S81	L	3	1B	1	Peru
79	HSI-173	S16	L	3	1B	1	Philippines
81	Korea-44	S22	L	3	3B	1	Rep. Korea
82	Korea-61	S23	L	3	3B	1	Rep. Korea
83	Korea-68	S24	L	3	3B	1	Rep. Korea
84	Kalu Tala	S69	L	1	1B	7	Sri Lanka
85	Sudu Tala	S70	L	3	3B	1	Sri Lanka
86	Toyama 5261	S76	L	1	1B	7	Sri Lanka
87	Toyama5262+E26	S77	L	1	1B	4	Sri Lanka
88	T-6	S68	L	1	1B	2	Tanzania
89	Boder Racet	S17	L	3	1B	2,4,5	Thailand
90	Chaiphum white seed	S86	L	1	1B	1	Thailand
91	Loei white seed	S87	L	1	1B	1	Thailand

92	Nakorn Sawan Black Seed	S88	L	3	1Q	7	Thailand
93	9310	S5	B.L	3	1B	5	USA
94	Shiro Goma	S78	B.L	1	1B	1	USA
95	Toyama-062321 (Indehiscent)	S4	B.L	3	3B	1	USA
96	Toyama-3201 (Indehiscent)	S6	B.L	3	3Q	1	USA

Accession type: Breeding line: B.L; Experimental lines: E.L; Local: L.
Branching habit: More branching: 1; Top branching: 2; Basal branching: 3; No branching: 4.
Number of flowers per axel: One flower: 1; Three flowers: 3.
Capsule shape: one capsule with bi-carpals: 1B; three capsules with bi-carpals: 3B; one capsule with tetra-carpals: 1Q, three capsule with tetra-carpals: 3Q.
Seed coat colour: White: 1; Yellow brown: 2; Yellow: 3; Reddish brown: 4; Blackish gray: 5; Violet: 6; Black: 7.

Table 2. Selected primer combinations and polymorphic percentage for AFLP analysis in sesame.

No.	*Eco*R1 site sequence	*Mse*1 site sequence	Total bands	polymorphic bands	Polymorphic bands %
1	AGG	ATA	20	8	40
2	AGG	CCC	15	6	40
3	AGG	GGA	33	9	27
4	AGC	GAG	22	10	45
5	ACT	TAT	23	10	43
6	AAC	GGT	39	13	33
7	ACC	ATT	17	7	41
8	ACT	GTT	26	17	65
9	ACT	TCC	13	7	54
10	AGA	GTC	27	11	41
11	AGC	AGC	11	4	36
12	AGG	TTC	15	2	13
13	AGA	TTG	24	9	38
14	AAA	GTC	23	4	17
15	AAC	TTA	19	3	16
16	AAG	ACG	14	5	36
17	ACC	TGA	33	6	18
18	AGG	AGC	20	9	45
19	AGG	CTT	15	8	53
20	AGG	GGC	20	4	20
21	AGG	CAT	16	5	31
Total			445	157	35

Percentage for polymorphic bands of total = 35.