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Identification of molecular markers linked to determinate growth habit in sesame

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Abstract This is the first report on molecular studies and tagging of the *dt* gene regulating determinate growth habit in sesame. The development of determinate cultivars has become an objective of high priority in sesame breeding programmes. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) techniques were investigated for the development of molecular markers for this induced mutant character. In order to identify molecular markers linked to determinate growth habit, a segregating F_2 population was developed from a cross between determinate mutant line, dt-1 and an indeterminate Turkish cultivar, Muganlı-57. Using the F_2 segregating population and bulked segregant approach, two ISSR marker loci originated from a $(CT)_8AGC$ primer were detected. The association was confirmed by analysing the ISSR profile from single plants originating from a F_2 segregating population for the trait. This marker is potentially useful for assisting sesame breeding programmes by marker assisted selection and can facilitate the integration of determinate growth habit into new genetic backgrounds.

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Introduction

Sesame (*Sesamum indicum* L.) is an annual crop native to tropic and sub-tropic regions. The seeds contain 50–60% oil which is highly resistant to oxidative deterioration even though oleic and linoleic acids are the predominant fatty acids of sesame oil, about 80% of its total (Arslan et al. 2007; Uzun et al. 2007). The high level of unsaturated fat increases the quality of sesame oil for human consumption. This important oilseed crop has been grown for centuries for its oil and high energy content.

In spite of being the first oilseed crop known to man and its long history, sesame is a typically neglected crop. It is not studied by any of the international agricultural research centres (Ashri 1998). Most of the harvested area in the world is in developing countries. As a natural consequence of this situation, the use of molecular techniques for the improvement of sesame is very limited. Only a few reports are available on the use of molecular markers such as isozyme (Isshiki and Umezaki 1997), RAPD (Bhat et al. 1999), ISSR (Kim et al. 2002), AFLP (Uzun et al. 2003) and SSR (Dixit et al. 2005). Of these studies, only one focused on linkage analysis

for the improvement of an agronomic trait, indehiscence (Uzun et al. 2003).

The cultivation of sesame has many advantages as it sets seeds and yields relatively well under high temperature and can be grown on residual soil moisture (Bhat et al. 1999). However, it is difficult to decide when to harvest a sesame crop to maximise yield, because plant growth is originally indeterminate and capsules dehisce when mature (Day 2000). One of the most important issues of sesame cultivation is to be able to accurately determine seed maturity since mature capsules near the base of the plant split and lose seed while flowering continues on the top of the plant. Determinate growth habit in sesame offers the solution to such difficulties and it is now one of the prerequisites for adapting sesame to modern farming systems. This induced mutant trait permits synchronized flowering and improved lodging resistance with shorter plant stature. On the other hand, determinate plant yield is low compared to indeterminate counterparts. It is, therefore, important to improve the determinate types using both conventional and molecular plant breeding strategies. In order to enhance efficiency of the sesame breeding programme, the use and inclusion of molecular techniques are needed in the frame of marker-assisted selection approach. The aim of this research was to identify molecular markers linked to determinate growth habit in sesame.

Materials and methods

Plant materials

The determinate growth habit mutant, dt-1 (♀) of sesame (Çağırgan 2001, 2006), *Sesamum indicum* L. was crossed with the indeterminate wild type cultivar, Muganli-57 (♂) in the 2003 growing season. The F_1 hybrids were selfed to generate a segregating population for the monogenic and recessive determinate mutant trait in 2004. An F_2 mapping population was developed from this cross and grown in 2005. The F_2 individuals were phenotypically scored either as determinate or indeterminate and were used for bulk segregant analysis (BSA; Michelmore et al. 1991) with the two bulks contrasting for the trait of interest. The properties and usefulness of the mutant are well documented in previous studies (Arslan et al. 2007; Çağırgan 2006; Uzun and Çağırgan 2006).

Bulked segregant analysis

Two contrasting DNA bulks were made by pooling equal amounts of DNA from ten randomly chosen F_2 determinate and ten F_2 indeterminate individuals, respectively. DNA samples of determinate and indeterminate parents along with determinate and indeterminate bulks were screened for polymorphism with RAPD, ISSR and some specific primers.

DNA isolation

Leaf tissue samples were collected from the F_2 plants, immediately incubated with liquid nitrogen and stored at −80°C. Extraction of the DNA from the samples was carried out using a modification of the method of Dellaporta et al. (1985). The frozen samples were ground to fine powder in liquid nitrogen using a mortar and pestle and mixed with 7 ml extraction buffer (0.5 M EDTA, 500 mM NaCl, 100 mM Tris–HCl and 10 mM β-mercapthoethanol). The mixture was transferred to falcon tubes and 1 ml of 10% SDS was added. The sample was incubated at 65°C for 12 min. Then 2 ml of 5 M potassium acetate was added followed by vigorous mixing and incubation on ice for 5 min. The sample was then emulsified with 5 ml chloroform-octanol (24:1) and centrifuged at 15,000 rpm for 10 min. The aqueous phase was recovered and subjected to a second chloroform-octanol extraction. The aqueous phase was again removed and incubated at −20°C for 30 min. After incubation, the solution was mixed with an equal volume of isopropanol. The nucleic acid precipitate was recovered with a glass hook, washed twice with 500 µl of 80% ethanol and dried at room temperature. The pellet was resuspended in TE buffer (10 mM Tris–HCl and 1 mM EDTA).

DNA analyses

The protocols of Williams et al. (1990) for RAPD analysis and Zietkiewicz et al. (1994) for ISSR were adapted. DNA amplifications were performed in a 25 µl reaction volume containing approximately 50 ng template DNA, 30 pmol primer (MWG Biotech AG and Biosearch Technologies, Novato, CA), 0.2 mM deoxyribonucleotide triphosphates (dNTPs) (Larova Biochemie GmbH), 1 Unit (U) of Taq DNA polymerase, 1× PCR buffer and 2.5 mM $MgCl_2$

Table 1 The list of primers used in the study

No	Sequence (5′–3′)	Base pair	No	Sequence (5′–3′)	Base pair
1	CAGGCCCTTC	10	53	$(CT)_8AGC$	19
2	TGCCGAGCTG	10	54	$(AC)_8T$	17
3	AGTCAGCCAC	10	55	ACGAGAGGCC	10
4	AATCGGGCTG	10	56	CCGCCCAAAC	10
5	AGGGGTCTTG	10	57	AAGCGGCCTC	10
6	GTGACGTAGG	10	58	GAGAGCCAAC	10
7	GGGTAACGCC	10	59	GTGAGCGTCT	10
8	CAATCGCCGT	10	60	GGCATCGGCC	10
9	TCGGCGATAG	10	61	TGTAAGCTCG	10
10	CAGCACCCAC	10	62	TTGCTTGGCG	10
11	AGCCAGCGAA	10	63	CGGTGGCGAA	10
12	GACCGCTTGT	10	64	CGGCCCACGT	10
13	AGGTGACCGT	10	65	GCGGGAGACC	10
14	CAAACGTCGG	10	66	AGTCCTCGCC	10
15	GTTGCGATCC	10	67	GCTGGTACCC	10
16	GGTGATCAGG	10	68	GTCCTCGTAG	10
17	CCGAATTCCC	10	69	CGCATTTGCA	10
18	GGGAATTCGG	10	70	AGCGGCTAGG	10
19	GGGATATCGG	10	71	ACATCCTGCG	10
20	GGAAGCTTGG	10	72	GAGCCAGAAG	10
21	GGCTGCAGAA	10	73	GGTAACCGTA	10
22	TGCTGCAGGT	10	74	ACGGCGTCAC	10
23	CCAGTACTCC	10	75	ACGGCAGTGG	10
24	GGAGTACTGG	10	76	ACTTCCTCCA	10
25	GGTCTAGAGG	10	77	GGTCTCCTAG	10
26	AGTCGTCCCC	10	78	CCTCACCTGT	10
27	CACCGTATCC	10	79	CTAGGGGCTG	10
28	CTGGGCAACT	10	80	CGGAGAGCGA	10
29	AGGCGGGAAC	10	81	GTGGCCGCGC	10
30	GGTGGTCAAG	10	82	CCGGCATAGA	10
31	TGTTAGGCTC	10	83	ATCTAGGGAC	10
32	TCAGTCCGGG	10	84	GCCGCTACTA	10
33	AGGTCTTGGG	10	85	GACATCTCGC	10
34	TTGGCGGCCT	10	86	ACAGGGAACG	10
35	ACCTCGCCAC	10	87	TCTAAGCTCG	10
36	TGCTGGTTCC	10	88	CGGATCTCTA	10
37	CTGCGCTGGA	10	89	ATACGGCGTC	10
38	GAGGTCCACA	10	90	ATGGCCTTAC	10
39	GTCAGTGCGG	10	91	GCGAACCTCC	10
40	AGACCCAGAG	10	92	GGTGGTTTCC	10
41	CAATGCCATA	10	93	GCCTAGTCAC	10
42	ACTTCCCACC	10	94	AACGCGTAGA	10
43	ACAGGTTAAC	10	95	GAATGCGACG	10

Table 1 continued

No	Sequence (5′–3′)	Base pair	No	Sequence (5′–3′)	Base pair
44	CATGGTGTTG	10	96	ATGGCAAAGC	10
45	GTCATTGTTC	10	97	TGGACCACCC	10
46	AGAGTTGGTA	10	98	GCCACGGAGA	10
47	GCTCTTGATC	10	99	TCCCGAACCG	10
48	TAGATCTGAT	10	100	CTGTGGCGGT	10
49	ATCTGAATTC	10	101	CTCACTTGGG	10
50	$(AGC)_6$	18	102	Fwd-ATTGGAGGATTTTTGGTTGG Rev-TGACGATGAGTCCTGAGTAAGC	42
51	$(TG)_8$AGA	19	103	Fwd-AATTCAAGTAAGATATTG Rev-TAACATGACGATGAGTCCT	37
52	$(CA)_8$AGT	19	104	Fwd-TACCAATTCAAGAAAATGGT Rev-TCCCGGCCGCCTGGCGGCCGCG	42

provided by the manufacturers of the enzyme (Bioron GmbH, Ludwigshafen) for ISSR and RAPD analyses. PCR amplifications of the genomic DNA with different primers were carried out using an Eppendorf Mastercycler Gradient. The PCR schedules were adapted as follows: 94°C for 2 min followed by 35 cycles of 94°C for 1 min., 50.5°C for 1 min., 72°C for 1 min., and a final extension of 5 min at 72°C. The annealing temperature was changed based on *Tm* values of each primer both for ISSR and RAPD.

The PCR products were run with gel electrophoresis system (Biorad, CA, USA) on a 1.5% agarose gel (Serva Electrophoresis GmbH) at 70 V constant powers in 0.5 × Tris-borate-EDTA (TBE) buffer and stained with ethidium bromide for 20 min. A 100 bp standard molecular weight ladder (Amresco, Solon, OH, USA) was used in the electrophoretic runs. The UV transilluminated gels were photographed with a gel documentation and image analysis system (Vilber Lourmat, France).

Results

One hundred and four RAPD, ISSR and other primers were used to find molecular markers linked to the determinate growth habit in sesame. The primers were selected from the previous studies of Bhat et al. (1999); Kim et al. (2002) and Uzun et al. (2005, 2006). The list of the primers used in the study is shown in Table 1.

Each of the tested primers produced approximately 8–12 bands and thus about 1,040 loci in sesame were scanned in a segregating population of the trait. The primers were screened in PCR reactions with DNA extracted from the parental lines, determinate and indeterminate bulks in the frame of BSA. All markers were scored for the presence or absence of the amplification products. Of 104 primers, only two gave polymorphic bands in the contrasting bulks and parents. One polymorphic band was obtained from the RAPD primer with a sequence of AGTCGTCC CC. However, this polymorphism was not reproducible due to the nature of RAPD technique. This RAPD primer showed also an association with closed capsule mutant trait in a previous study (Uzun et al. 2005). The specific primers previously developed for the closed capsule mutant trait (Uzun et al. 2006) were also tested for identifying molecular markers linked to determinate growth habit. However no polymorphism was detected.

The second polymorphic primer was an ISSR primer with a sequence of CTCTCTCTCTCTCTC TAGC. This primer produced an amplification pattern that was different in the determinate bulk and indeterminate bulk samples of the cross. When ISSR amplification reactions were carried out with DNA from single individuals originating from a F_2 segregating population for the trait, the pattern was consistent in that fragments of 800 and 1,100 bp bands were present in DNA from determinates and was absent in amplifications with DNA from

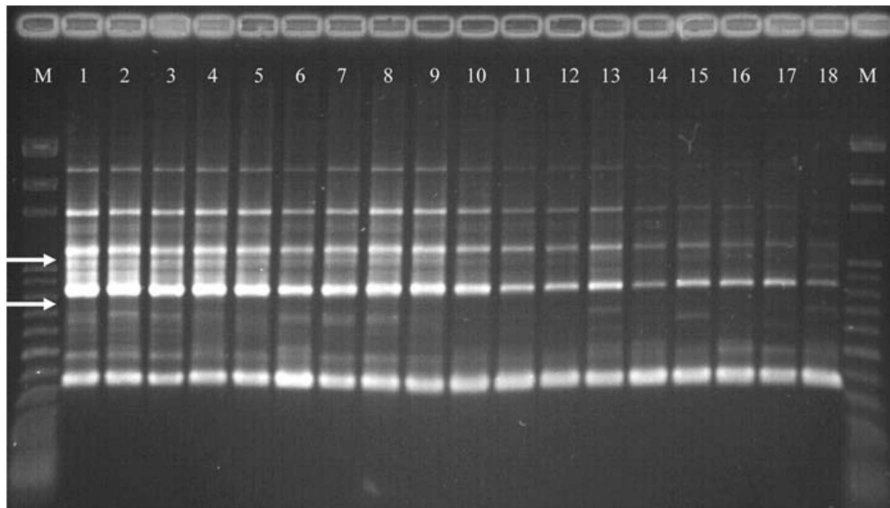

Fig. 1 A representative gel photograph showing the markers segregation in individuals of F_2 population. Lane M: A 100 bp standard molecular weight ladder (Amresco, Solon, OH, USA). Lane 1–9: determinate individuals. Lane 10–18: indeterminate individuals. *Arrows* indicate the polymorphic marker bands

indeterminates (Fig. 1). It is concluded that the bands obtained from this ISSR primer are associated in coupling phase linkage with the gene *dt* that control determinate growth habit in sesame and can be considered to be a reliable molecular marker for the determinate character in sesame.

Discussion

RAPD and ISSR analyses were successfully and reliably applied to sesame molecular analysis. The ISSR technique was previously performed in sesame to study the genetic relationship of a sesame germplasm by Kim et al. (2002). The present study was the first report on the use of ISSR for linkage analysis in sesame. The technique does not need any prior information about DNA sequence and overcomes many of the technical limitations of RAPD and AFLP because of its high reproducibility and simplicity (Danilova and Karlov 2006; Ratnaparkhe et al. 1998). Since there is no sequence information and molecular studies are limited in sesame, ISSR-PCR offers great applicability to identify molecular markers linked to determinate growth habit in sesame. Although a limited number of ISSR primers were tested, a marker linked to the gene of interest was identified. The result is consistent with that of Danilova and Karlov (2006) who found that testing 22 ISSR primers was enough to find two male specific markers in hops. For RAPD, hundreds of primers were tested for the same target (Polley et al. 1997). In fact, combination of RAPD and ISSR should, in theory, give better coverage of genome because molecular markers generated by RAPD and ISSR randomly target different regions of the genome (Archak et al. 2003).

In addition to RAPD and ISSR primers, three specific primers originally developed for the closed capsule mutant trait in a different study were also used for finding markers linked to determinate growth habit. The RAPD primer, AGTCGTCCCC, showed an association to both mutant traits. These specific primers might have been associated with determinate growth habit because both mutants were the result of the same mutation event following gamma irradiation (Çağırgan 2001). The specific primers were tested but no polymorphism was obtained. One primer showed a unique band while the others produced non-specific band patterns.

The objective of the present study was to develop markers for tagging the determinate growth habit so that the markers could be effectively used in marker assisted selection (MAS) to select high yielding determinate genotypes. The development of determinate cultivars has become an objective of high priority in sesame breeding programmes. MAS can facilitate a PCR-based test for the rapid identification of

determinate types without costly and extensive field trials. PCR analysis can greatly reduce the amount of labour needed for evaluating phenotypes by pre-screening with MAS (Fuentes et al. 2007). Thus, cost-effective ISSR markers linked to *dt* gene, and suitable for agarose gel electrophoresis can facilitate the integration of determinate growth habit into new genetic backgrounds. This marker is potentially useful for assisting sesame breeding programmes. However, the practical usefulness of the identified markers in assisting an applied sesame-breeding programme should be tested in segregating populations with different genetic backgrounds.

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