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Development and application of genotype-specific SCAR markers in *Eruca sativa* and in its commercial oil

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Abstract

Eruca sativa (L.) Mill. (ver. Taramira, Arugula, and Rocket) has various therapeutic and industrial applications worldwide. Although the oil of taramira is non-edible in nature, it has many medicinal properties. The adulteration of its oil with any other edible oil could be harmful. The present study has provided a method for the development of genotype-specific SCAR (Sequence Characterized Amplified Region) markers in *E. sativa*, representing an effective way of identification. Through ISSR (Inter Simple Sequence Repeats) analysis, 16 polymorphic fragments were selected for their conversion into SCAR but finally only 4 SCAR markers (ScFa-ScRa, ScF3-ScR3, ScF5-ScR5 and ScFm-ScRm) each from different genotype were successfully developed. Further, examination of these markers was performed with the help of commercial samples of oil, collected from three different locations. Developed markers could be considered as useful to check the adulteration of *Eruca* oil in consumable products and also for authentication purposes in various breeding experiments.

Key words: *Authentication purpose, eruca sativa, genotype specific, ISSR, SCAR*

Introduction

Taramira (*Eruca sativa* L. Mill. syn. *E. vesicaria* L. cav., family Brassicaceae) is an important oilseed crop as well as a medicinal plant around the world. It has diverse therapeutic and industrial applications. Different plant parts of taramira have been accepted in traditional medicines for their extensive therapeutic properties (Garg and Sharma, 2014). Seeds of *Eruca* though non-edible in nature contain more than 38% oil and possess therapeutic properties in the treatment of indigestion, ulcers, bacterial and fungal infections, respiratory and urinary tract infection, scurvy, hair loss and hair lice treatment (Mennicke *et al*., 1988; Gulfraz *et al*., 2011; Garg and Sharma, 2014). However the seeds are neither used for edible purposes nor as an ingredient in food products. The adulterations of taramira oil with any other edible oil could be harmful, and hence is an important issue for consumer protection.

Demand for taramira oil is also tremendously increasing at various industries as a lubricant, illuminating agent, bio-fuel, for soap-making, in body massage and in paper industry etc. (Varga *et al*., 2009; Pignone and Gomez-Campo, 2011). Being a non-edible oil producing crop, *Eruca* has a distinct advantageous edge in the industrial sector. As the environmentalists are in opposition for the use of edible oil for industrial purposes due to the competition between food and fuel economies for the same oil resources (Ahmad *et al*., 2011). Identification of superior genotypes is essential for pharmaceutically and industrially importantance. Although chemical methods and morphological evaluations are often used for quality check, but there are limitations (Warude *et al*., 2006).

There are various kinds of molecular markers that can be applied for plant identification. ISSR can be effectively used for authentication of medicinal plants (Tripathi *et al*., 2012). ISSRs have also been widely used in the genotype identification, genetic mapping and marker-assisted selection due to their simplicity, low cost and convenience and that there is no necessity to know sequence information. Despite this, the use of ISSR has been restricted due to reproducibility problems and imperfect transferability among different laboratories. To overcome these drawbacks, the polymorphic bands generated from ISSR could be converted into more specific, reliable and more reproducible markers known as SCARs (Sequence Characterized Amplified Region). SCAR markers are developed with a pair of longer primers, usually the extended sequence that has a specific sequence of approximately 20 bases. These longer primer

sequences increase the specificity of the PCR reaction and generate results less sensitive to changes in reaction conditions and thus more reproducible (Hernandez *et al*., 1999). SCAR markers have many advantages including their specificity, low cost, ease and fast use. The ISSR derived SCAR marker has proved useful DNA markers for identification of Korean ginseng cultivars (Lee *et al.*, 2011). The SCAR marker has also been used to authenticate the plant material in its commercial samples and ayurvedic formulations (Warude *et al*., 2006; Chavan *et al*., 2008). In the present study, we attempted to develop reproducible SCAR marker (genotype-specific) derived from ISSR marker in *E. sativa*. Additionally, developed markers were authenticated with the DNA extracted from commercial samples of taramira oil (Jamba oil) collected from local markets of three different locations.

Materials and Methods

Plant material

Thirty-five genotypes of *Eruca sativa*, representing all ten agro-climatic zones (divided on the basis of climatic conditions and prevailing agricultural practices) of Rajasthan, India were obtained from Germplasm Storage Facility, SKN College of Agriculture (SKN AU), Jobner, Rajasthan, India. The list of genotypes and their agro-climatic zones along with their geographical coordinates are shown in Table 1. All genotypes were grown at the

Table 1. *Eruca sativa* genotypes, representing ten different agro-climatic zones in the state of Rajasthan, India

Genotypes	Name of district	Agro-climatic zones	Geographical coordinates
RTM-314	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-577	Udaipur	Humid Southern	24.58° N, 73.68° E
RTM-608	Hanumangarh	Irrigated North Western Plain	29.58° N, 74.32° E
RTM-613	Hanumangarh	Irrigated North Western Plain	29.58° N, 74.32° E
RTM-633	Hanumangarh	Irrigated North Western Plain	29.58° N, 74.32° E
RTM-650	Hanumangarh	Irrigated North Western Plain	29.58° N, 74.32° E
RTM-655	Hanumangarh	Irrigated North Western Plain	29.58° N, 74.32° E
RTM-686	Shriganganagar	Irrigated North Western Plain	29.92° N, 73.88° E
RTM-707	Tonk	Semi Arid Eastern Plain	26.16° N, 75.79° E
RTM-725	Pali	Transitional Plain	25.77° N, 73.33° E
RTM-733	Jodhpur	Arid Western	26.28° N, 73.02° E
RTM-738	Ajmer	Semi Arid Eastern Plain	26.45° N, 74.64° E
RTM-760	Bhilwara	Sub-Humid Southern Plain	25.35° N, 74.63° E
RTM-777	Bhilwara	Sub-Humid Southern Plain	25.35° N, 74.63° E
RTM-789	Rajsamand	Sub-Humid Southern Plain	25.07° N, 73.87° E
RTM-800	Nagaur	Internal Drainage Dry Zone	27.20° N, 73.73° E
RTM-1035	Kota	Humid Southern Eastern Plain	25.18° N, 75.83° E
RTM-1107	Tonk	Semi Arid Eastern Plain	26.16° N, 75.79° E
RTM-1212	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-1301	Ajmer	Semi Arid Eastern Plain	26.45° N, 74.64° E
RTM-1309	Ajmer	Semi Arid Eastern Plain	26.45° N, 74.64° E
RTM-1311	Nagaur	Internal Drainage Dry Zone	27.20° N, 73.73° E
RTM-1316	Bikaner	Hyper Arid Partial Irrigated	28.02° N, 73.31° E
RTM-1328	Bikaner	Hyper Arid Partial Irrigated	28.02° N, 73.31° E
RTM-1332	Sikar	Internal Drainage Dry Zone	27.62° N, 75.15° E
RTM-1351	Bikaner	Hyper Arid Partial Irrigated	28.02° N, 73.31° E
RTM-1354	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-1355	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-1359	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-1361	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-1397	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-1398	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-1413	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
RTM-2002	Jaipur	Semi Arid Eastern Plain	26.93° N, 75.82° E
TMLC2	Bharatpur	Flood Prone Eastern Plain	27.22° N, 77.48° E

green house of Banasthali University, Rajasthan, India.

Three commercial *E. sativa* oil samples from different regions of the state Rajasthan, India viz. Bharatpur, Jaipur and Tonk districts, were purchased from Ayurvedic product suppliers as well as from the different oil traders and stored at a cool and dry place.

DNA extraction from plant leaves

DNA was extracted from bulked fresh leaves (2 weeks old) collected from 5–10 randomly selected plants per genotype according to Doyle and Doyle (1990) with slight modification viz. chloroform and isoamylalcohol extraction step was repeated four times. DNA was qualitatively assessed on 0.8% agarose gel stained by ethidium bromide. Purity of extracted DNA was quantified spectrophotometrically (Nano Drop ND-1000 Version 3.1.1, Wilmington, DE, USA) at 260 nm and 280 nm. After quantification, each DNA sample was diluted with TE buffer to 25 ng /µl and stored at -20ºC.

DNA extraction from oil samples

Extraction of DNA from oil samples was performed according to the protocol given by Busconi et al. (2003) with some modification, i.e. 12% CTAB and by repetition of extraction step two more times for better yield. DNA was qualitatively and quantitatively assessed as described above.

ISSR analysis

All ISSR primers used in this study have GC contents ranging from 50 to 80%. Initially, 135 ISSR primers were chosen for preliminary amplification of 35 selected genotypes. Protocol for PCR was optimized and the reaction mixture of 25 µl containing 25 ng of template DNA, 200 µM dNTPs, 1X *Taq* buffer, 1U *Taq* DNA polymerase and 6 pmol of each ISSR primer was prepared. PCR amplification was performed in thermo cycler (PQlab-Primus 96) using amplification program: 4 min at 94ºC for initial activation step followed by 44 amplification cycles of 1 min at 94ºC; 1 min at 40ºC; 2 min extension at 72ºC and final extension step on 72ºC for 7 min. The amplification products along with low range DNA ruler (for determination of the size of amplified products) were loaded on 1.5% agarose gel in 1X TBE buffer and were electrophoresed at 100 V for 2 hrs. The gels were stained with ethidium bromide and images were captured by the gel documentation system (Bio-rad). Reproducibility of each experiment was confirmed at least twice.

Data analysis and identification of polymorphic ISSR bands

Here, only sharp and consistent reproducible bands were chosen for further analysis. ISSR data was analyzed using NTSYS-PC software version 2.1 (Rohlf, 2002). The discrimination power (D) was calculated to find out the efficiency of the random primers to distinguish between individuals (Tessier *et al*., 1999). Few ISSR putative bands were identified for development of SCAR markers among all genotypes.

Cloning and sequencing of ISSR bands

The targeted ISSR fragments were extracted from agarose gel using the Qiagen Gel Extraction kit (Qiagen Pvt. Ltd.). The fragments were ligated into the pGEM-T easy vector (Promega Co. USA) by following supplier's instructions. *E. coli* DH5á competent cells were transformed with the recombinant vector according to the protocol of Sambrook *et al*. (2001). Positive white colonies were picked up from the LB-ampicillin plate. Recombinant DNA from randomly selected white colonies was extracted using the QIA mini preparation kit (Qiagen). Restriction digestion with *Eco*RI enzyme and amplification of DNA by SP6 and T7 promoter primers was performed to confirm the size of the inserted fragment. The plasmids containing the fragments of the correct size were sequenced with ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystem) in ABI PRISM® 3730 automated sequencer using SP6 and T7 promoter primers.

SCAR primer designing, amplification of the genomic region and validation

Based on the sequence of cloned ISSR markers, specific primer pairs were designed using Primer 3 software and synthesized by Xcelris genomics. PCR amplification was carried out with all 35 genotypes of *E. sativa* using 5 pmol of each SCAR primer (forward and reverse), 1X PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1U *Taq* DNA polymerase and 25 ng template DNA in 10 µl reaction volume. Thermal cycling conditions for amplification using SCAR primers were optimized as: 94°C for 4 min followed by 35 cycles of 1 min at 94°C, 1 min at according to Tm of each primer, and 2 min at 72°C, and finally terminated with an extension of 5 min at 72°C. PCR amplification for authentication of commercial samples was done by using the SCAR primer pairs with the above-mentioned thermal cycling conditions.

Analysis of sequence data

Homology searches were performed within GenBank's database using the BLAST (Basic Local Alignment Search Tool) algorithm at http://www.ncbi.nlm.nih.gov/BLAST/ of the National Center for Biotechnology Information (NCBI), with the program BLAST-N.

The sequences of ISSR fragments EsI7 (967 bp), EsI19 (825 bp), EsI21 (740bp) and EsI22 (787 bp) have been published online at NCBI, DDBJ, and EMBL Nucleotide Sequence Databases under the accession numbers KF976388, KF976389, KF976390 and KF976391 respectively.

Results and Discussion

Selection of polymorphic ISSR bands

In the present study, high molecular weight genomic DNA was extracted from all selected genotypes of *E. sativa*, but some degree of fragmentation was observed in the DNA extracted from commercial oil samples. A total of 135 primers were used in the ISSR analysis, 25 primers produced a distinct amplification profile and expressed well among all genotypes (Table 2).

Based on ISSR screening, 16 fragments were selected for their conversion into SCAR marker, but due to failure in obtaining repeatability of bands and also insignificant response in cloning experiment, finally 4 bands were selected. As a result, IS-7, IS-19, IS-21 and IS-22 consistently amplified a single and unique band in some genotypes (Figure 1). Interestingly, primer IS-7 and IS-19, each amplified an intense band of 970bp (EsI7) in RTM-1212 and 830bp (EsI19) in RTM-1107 respectively. Similarly, primer IS-21 and IS-22, each amplified a polymorphic band of 742bp (EsI21) in TmlC2 and 790bp (EsI22) in RTM-1361 respectively. These bands were selected as putative *E. sativa* genotype-specific markers and were further used for cloning.

Table 2: Total no. of ampliûed fragments, no. of polymorphic fragments and discrimination power generated by using ISSR primers in 35 genotypes of *E. sativa*

Primers code	Primer sequence	Total bands	No. of monomorphic bands	No. of polymorphic bands	% polymorphism	D
IS- 5	$(AG)_8T$	13	0	13	100.0	0.82
IS- 7	$(GA)_8T$	14	0	14	100.0	0.91
IS- 9	$(GA)_8A$	10	3	7	70.0	0.13
IS- 10	$(CT)_8T$	6	0	6	100.0	0.30
IS- 17	$(TC)_8C$	10	0	10	100.0	0.92
IS- 19	$(AC)_8T$	14	1	13	92.8	0.88
IS- 20	$(AC)_8C$	15	0	15	100.0	0.92
IS- 21	$(AC)_8G$	17	2	15	88.2	0.98
IS-22	$(TG)_8A$	16	0	16	100.0	0.89
IS- 23	$(TG)_8C$	11	0	11	100.0	0.69
IS- 24	$(ACC)_6$	15	0	15	100.0	0.91
IS- 29	$(CT)_8C$	2	0	2	100.0	0.52
IS- 35	$(CT)_8GC$	10	0	10	100.0	0.51
IS- 40	(GA)8CC	6	0	6	100.0	0.88
IS- 50	(CA)8GT	18	1	17	94.4	0.93
ISS-05	$(TGC)_5$	14	0	14	100.0	0.98
ISS-06	$(GCC)_5$	15	3	12	80.0	0.98
ISS-10	$(CTAG)_4$	8	3	5	62.5	0.45
ISS-11	$(TGCA)_4$	10	0	10	100.0	0.94
ISS-14	$(GGAGA)_3$	20	3	17	85.0	0.92
ISS-17	$(GTG)_3GC$	12	0	12	100.0	0.93
ISS-21	(CAC)3GC	9	1	8	88.8	0.84
ISS-26	$(AGC)_6G$	5	0	5	100.0	0.88
ISS-29	$(GAC)_3GC$	14	1	13	92.8	0.91
ISS-30	$(AC)_{16}TG$	11	1	10	90.9	0.96
Total		295	19	276		
Average		11.8	0.76	11.04	93.8	0.79

Cloning, sequencing and confirmation of selected ISSR bands

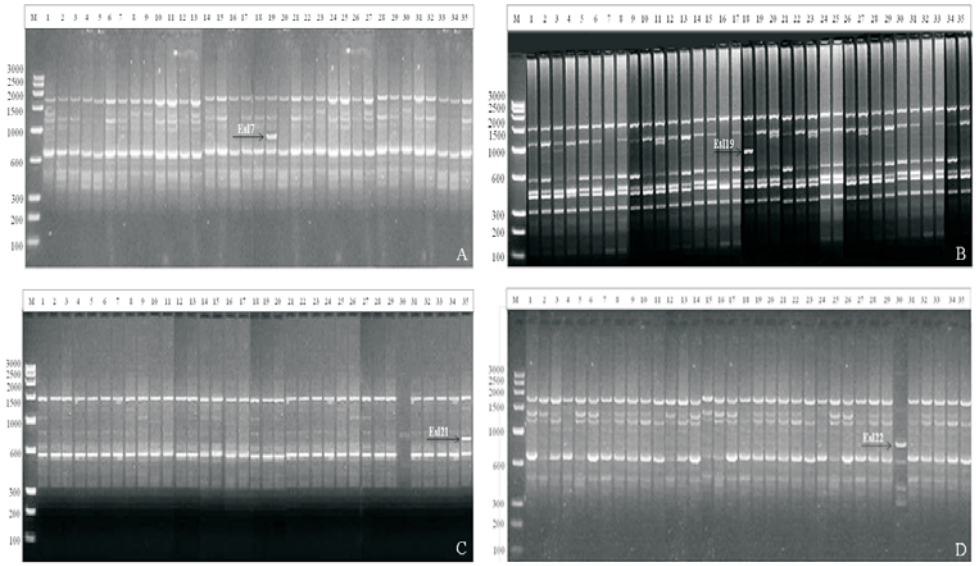

Fig 1. Screening 35 genotypes of *E. sativa* with ISSR primers (A): IS-7 (B): IS-19 (C): IS-21 (D): IS-22. Lane M- Low range DNA ruler, Lane 1-35- *E. sativa* genotypes. EsI7 (970bp), EsI19 (830bp), EsI21 (742bp) and EsI22 (790bp) represents genotype specific ISSR amplicons.

EsI7, EsI19, EsI21 and EsI22 were cloned and recombinant plasmids were then sequenced. Confirmation of the desirable insert in the vector was done by restriction digestion analysis. *Eco*RI restriction enzyme revealed the desirable bands on 1.5% w/v agarose gel (Figure 2). Sequencing of recombinants was performed by using SP6 and T7 promoter primers. In all the clones, initial nucleotides of the sequence were completely matched with the corresponding ISSR primers used.

Sequence data analysis

All sequences were analyzed with the help of BioEdit software and sequences from different plasmids were used to form final contig of each fragment. After analyzing the sequence data, the length of EsI7, EsI19, EsI21 and EsI22 were similar as measured in eluted products (Figure 3, 4, 5, and 6). BLAST results showed that all sequences have partial homology with known plant nucleotide sequences at different sequence similarity levels. Considerable

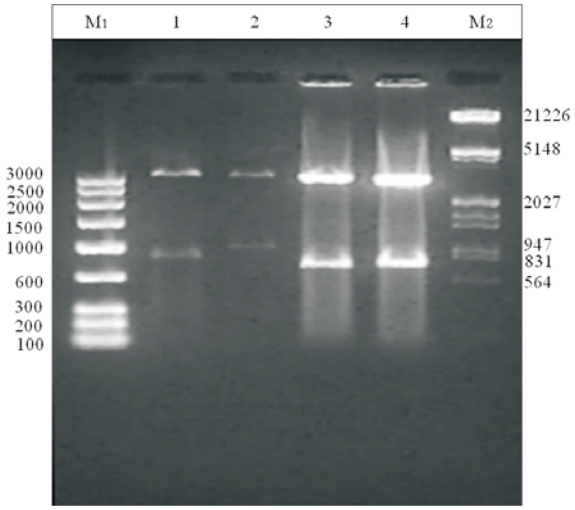

Fig 2. Restriction digestion of plasmid. M_1- Low range DNA ruler, M_2-Lamda DNA *Eco*R1/ *Hind III* double digested marker, Lane 1-4- Restriction digestions of plasmid.

Fig 3. Nucleotide sequence of ISSR amplicon EsI7.

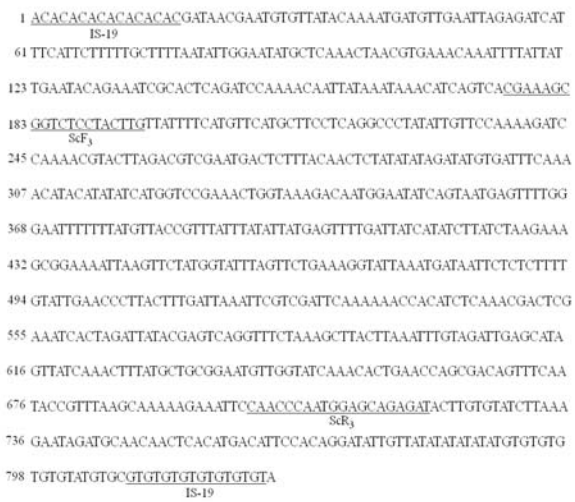

Fig 4. Nucleotide sequence of ISSR amplicon EsI19.

Fig 5. Nucleotide sequence of ISSR amplicon EsI21.

Fig 6. Nucleotide sequence of ISSR amplicon EsI22.

similarity was found with three different species of *Brassica* genus and with *Oryza sativa*.

SCAR marker design

On the basis of sequences of cloned fragments, SCAR primers were designed using primer 3 software. Table 3 shows the sequences of SCAR primer designed in our study.

Amplification using SCAR primers

Further testing of designed SCAR primer pairs was done by amplifying genomic DNA from all 35 genotypes of *E. sativa*. Here, different SCAR primer combinations supposed to amplify bands ranging from 505 to 681bp in EsI7, 499 to 544bp in EsI19, 246 to 472bp in EsI21 and 274 to 480bp in EsI22 were used (Table 3). SCAR primers clearly amplified the desired length of band in their related genotype of *E. sativa*. SCAR primer pairs ScF_a-ScR_a, ScF_3-ScR_3, ScF_5-ScR_5 and ScF_m-ScR_m each amplified a single and unique 681bp (B1), 544bp (B2), 472bp (B3) and 480bp (B4) bands correspondingly in respective genotypes from which they were eluted, but not amplified in the rest of the genotypes (fig. 7), proving its genotype specificity in *E. sativa*. Among the remaining SCAR primer pairs, some did not show any amplification and some primer pairs showed multiple bands. On the other hand, these primers have low product size and hence, were not further considered in this study.

Applicability or validation of SCAR primers

The SCAR primers were further used to test the applicability of ScF_a-ScR_a, ScF_3-ScR_3, ScF_5-ScR_5 and ScF_m-ScR_m markers as tools for authentication of commercial samples of oil of *E. sativa*. For this, DNA extracted from commercial oil samples were amplified with ScF_a-ScR_a, ScF_3-ScR_3, ScF_5-ScR_5 and ScF_m-ScR_m markers. Significant results were found with less intensity but the reproducible band in two oil samples i.e. from Bharatpur district and Jaipur district (fig. 8). However no bands were obtained in oil collected from the Tonk district.

Worldwide, the oil of taramira is used in various types of remedies and also in industrial sector but its traces with any other edible oil could be dangerous due to presence of antinutritional compound. Therefore, the identification of genotypes with reliable and specific DNA markers is urgently required. The objective of the present work was to develop a genotype-specific SCAR marker from ISSR markers. SCAR markers have been proved their utility in different identification purposes (Vidal *et al*., 2000; Busconi *et al*., 2006; Yuskianti and Shiraishi, 2010). SCAR marker has been applied various time for the purpose of authentication of traditional medicines such as Echinacea

Table 3: SCAR primer pairs designed in our study

ISSR Primers	SCAR Primer ID	Sequence (5'-3')	Tm	% GC	Productsize (bp)
	ScF_a	CCCAACAGCTCTTTCCTCAG	59.98	55	681
	ScR_a	AGCCCAAGAAGAGCAACAAA	59.99	45	
	ScF_b	ACATCAGTCACGAAAGCGGT	59.97	50	505
IS-7	ScR_b	ACTGTCGCTGGTTCAGTGTT	59.82	50	
	ScF_c	CACGAAAGCGGTCTCCTACT	59.47	55	543
	ScR_c	TCTGCTCCATTGGGTTGGAA	59.22	50	
	ScF_d	TCCCACACACGAGATTACCA	59.96	50	632
	ScR_d	AGCCCAAGAAGAGCAACAAA	59.99	45	
	ScF_1	CGAAAGCGGTCTCCTACTTG	60.01	55	499
	ScR_1	TGAAACTGTCGCTGGTTCAG	60.02	50	
	ScF_2	CATGCTTCCTCAGGCCCTAT	59.23	55	511
IS-19	ScR_2	ATCTCTGCTCCATTGGGTTGG	60.06	52	
	ScF_3	CGAAAGCGGTCTCCTACTTG	60.10	55	544
	ScR_3	ATCTCTGCTCCATTGGGTTG	60.70	50	
	ScF_4	ATCAGTCACGAAAGCGGTCT	59.87	50	507
	ScR_4	TGAAACTGTCGCTGGTTCAG	60.02	50	
	ScF_5	AACACACAACCCAACGCAAC	60.11	50	472
	ScR_5	GATACTCCATGGACCAGCCG	59.97	60	
	ScF_6	GCAACCCAACATCTCAACCT	59.67	50	436
IS-21	ScR_6	CACGACCTGTGCTCTGAAAA	60.02	50	
	ScF_7	CATGTTGAACTCGCCACAGC	60.11	55	285
	ScR_7	AAAGCTTCCCCCATGTCGTT	59.89	50	
	ScF_8	CATGGCTTCCAAGCTTCTTC	59.96	50	246
	ScR_8	CACGACCTGTGCTCTGAAAA	60.02	50	
	ScF_m	ACCTGGCGACTGAAACTTTG	60.29	50	480
	ScR_m	GGGGAATTCGATTTGTGTGT	59.65	45	
	ScF_n	TTTCCCGGCTGCAAATCGTA	60.32	50	374
IS-22	ScR_n	AAATGGGGGCGAGTAGTGTC	59.75	55	
	ScF_o	ACCTGGCGACTGAAACTTTG	60.29	50	431
	ScR_o	GCCTTGCACAACTTTTACCC	59.61	50	
	ScF_p	GGAATGAAGTCCATCCATCG	60.28	50	274
	ScR_p	GGGGAATTCGATTTGTGTGT	59.65	45	

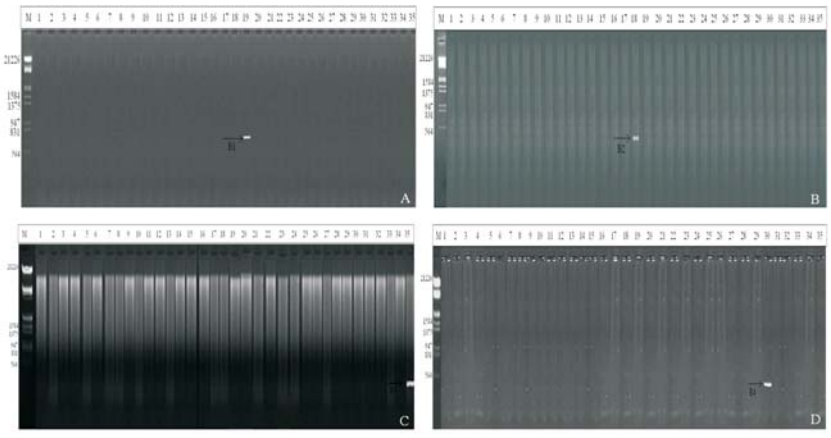

Fig 7. PCR amplification of all 35 genotypes of *E. sativa* using SCAR primers (A): with ScF_a-ScR_a, (B): with ScF_3-ScR_3, (C): with ScF_5-ScR_5 and (D): with ScF_m-ScR_m. Lane M- Low range DNA ruler, B1, B2, B3 and B4 showing amplification with SCAR primers in respective genotypes.

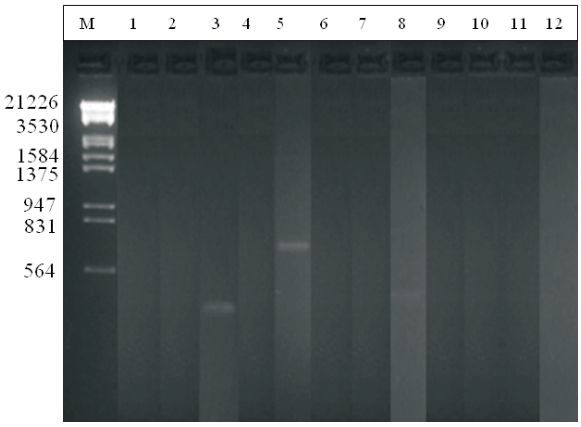

Fig 8. Testing of all SCAR primer pairs in commercial oil samples. Lane M- Low range DNA ruler. Lane 1-4- amplification of four SCAR primers with the oil collected from Bharatpur district, Lane 5-8- amplification of four SCAR primers with the oil collected from Jaipur district, Lane 9-12- amplification of four SCAR primers with the oil collected from Tonk district.

(Wolf *et al*., 1999), Ginseng (Um *et al*., 2001), and Atractylodes (Chen *et al*., 2001).

The first step for the present work required is obtaining the purified DNA from oil that could be used in authentication purpose for SCAR analysis. We were able to successfully isolate good quality DNA from oil samples. The oil samples were collected in the form of cold-pressed oil that does not require refining and hence preserves the essential fatty acids and genetic material. Then on the basis of the pattern of ISSR markers, we were able to identify some genotypes which contained a few unique bands. In ISSR analysis, significant genetic polymorphism was observed among selected genotypes of *E. sativa* due to its cross-pollinated nature. Here, many ISSR primers showed high discriminatory power (D) and were recognized as the best primers.

Thus, reproducible results were identified through the ISSR marker system. However for better reproducibility and specificity, polymorphic ISSR bands were chosen for the development of SCAR marker. According to Lee *et al.* (2011), there might be an intricacy during the conversion of randomly amplified polymorphisms to SCAR marker. The heterogeneous nature of polymorphic bands does not allow the desirable cloning of bands. During conversion some non-targeted sequences could frequently be generated and further leading to misinterpretation of the results. If in case, the targeted sequence could be obtained, it is not necessary that SCAR marker designed from the sequence could frequently be amplified the expected polymorphisms. There are several cases, where SCAR markers amplify the dominant type of product. The reason behind is that ISSR technique generally amplify dominant alleles, as their polymorphisms were caused by mismatches in primer binding sites (Ardiel *et al*., 2002). The SCAR marker is a genomic fragment, generally designed from internal region and is known to be defined locus. If no variation exist, specific primers produce expected polymorphisms that frequently appear in the form of unique band those are more stable and reproducible (He *et al*., 2007; Su *et al*., 2008). This study represents the successful development of four SCAR markers from four different genotypes (Table 4).

Table 4: Genotype-specific SCAR markers from different genotypes

Genotype	Eluted band	Size	SCAR primer pair	Amplified band	Size
RTM-1212	EsI7	967bp	ScF_a-ScR_a	B1	681bp
RTM-1107	EsI19	825bp	ScF_3-ScR_3	B2	544bp
TMLC-2	EsI21	740bp	ScF_5-ScR_5	B3	472bp
RTM-1361	EsI22	787bp	ScF_m-ScR_m	B4	480bp

Moreover, among these four genotypes RTM-1107 and RTM-1212 are also frequently used by plant breeders as a check variety (Keer and Jakhar, 2012).

BLAST results revealed that three sequences (EsI7, EsI19 and EsI21) have partial homology with *Brassica* genus (*B. oleracea*, *B. rapa* and *B. oleracea*, respectively) and one sequence (EsI21) has partial homology with *Oryza sativa* genome. Our results are in conformity with Mizushima (1950) and Sastry (2003) who showed that the genome of *E. sativa* has partial homology with the genome of *Brassica* genus. The study is a successful attempt in authentication of developed SCAR markers. It has been done with commercial samples of oil collected from local markets of three different places. Such an experiment helped us to find out the application of developed markers. Selected SCAR markers (ScF_a-ScR_a, ScF_3-ScR_3, ScF_5-ScR_5 and ScF_m-ScR_m) established as a promising tool for identification of taramira oil as ScF_5-ScR_5 (B3) was amplified with the oil collected from the Bharatpur district and ScF_a-ScR_a (B1) was amplified with the oil collected from the Jaipur district. Here, ScF_m-ScR_m (B4) was also

slightly amplified with the same but it produced a very faint band in the amplification. This confirmed the genotype-specific behavior since the seeds were collected from the same place from which the commercial sample of oil was collected. No amplification was found with the oil collected from the Tonk district, showing that oil might be processed from any other genotype of *E. sativa*. Further screening with a larger number of oil samples collected from more different places is being further required. The study confirmed that the developed SCAR markers are positively related to *E. sativa*. It will serve as a useful DNA marker in traceability of taramira oil for adulteration of edible oils. In the future, these genotype-specific markers would allow fast and accurate genotype identification in *E. sativa* which is especially important for industries as it is a prospective biofuel crop.

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