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Development of an ISSR based SCAR marker to identify small cardamom Malabar (prostrate panicle) variety (*Elettaria cardamomum* Maton.)

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Abstract

Small cardamom (*Elettaria cardamomum* Maton) a perennial monocot, exhibits an array of variation in nature, mainly due to cross pollination. Based on the nature of the panicle orientation, cardamom is broadly grouped into three main ‘cultivated types’ – Malabar, Mysore and Vazhuka having prostrate, erect and semi-erect panicles respectively. These morphologically discriminative markers manifest itself during panicle emergence as is only possible. Among the three varieties Malabar variety is relatively superior with respect to different qualitative and quantitative characteristics. The objective of the present study was to develop and characterize molecular markers for enabling differentiation of Malabar variety at juvenile stage. One accession specific ISSR marker generated by UBC 866 was selected which consistently amplified an intact, distinct, ≈1500bp band specifically in individuals of Malabar variety, which was therefore cloned, sequenced and characterized. Ten primers were designed from the sequences for converting them to SCAR markers. The developed SCAR markers were tested for variety specificity and one primer pair (SBBT4F/SBBT3R) was validated using small cardamom accessions belonging to Malabar variety from different geographic locations and varieties with erect panicles as well as hybrids. The findings suggest that the SCAR marker is promising in identifying cardamom varieties having prostrate panicle (Malabar) and therefore is expected to make significant contributions in selection of F1 hybrids during breeding programmes.

Introduction

Small cardamom (*Elettaria cardamomum* Maton) popularly known as ‘Queen’ among spices, is a rhizomatous perennial belonging to the family *Zingiberaceae*. It is considered as one of the most favoured spices in national and international cuisines by virtue of its versatility to impart the unique flavour - the sweetness together with pungency and aroma, to sweets and savouries. Because of its richness in essential oil and therapeutic components, small cardamom becomes the raw material for various pharmaceutical formulations. India has a rich wealth of genetic resources of cardamom as the crop originated in the tropical forests of Western Ghats (Mayne 1951; Abraham and Tulasidas 1958) and thus has a long tradition since centuries in the country. The cardamom varieties are highly location specific and therefore different varieties are adapted to different geographical regions along the Western Ghats (Anon 2009). Hence development of high yielding varieties specific to particular eco-geographic situation is of paramount importance.

The three main cultivated types or natural varieties of cardamom *viz.* Malabar, Mysore and Vazhukka were grouped mainly based on the nature of the panicle - prostrate, erect and semi erect respectively and is regarded as the major discriminative morphological markers of cardamom (Madhusoodanan et al. 2002). Among the three varieties of small cardamom, Malabar variety possesses several distinctive characters such as its relative tolerance to drought (Sudarsan et al. 1991), higher number of panicles per plant (Padmini et al. 1999), early maturing character (Madhusoodanan et al. 2002) which is suitable for high density planting (Madhusoodhanan et al. 2002) and the relatively higher flavonoid content of 0.159% (Govindarajan et al., 1982). It was also reported that most of the resistant genotypes reported in

cardamom are of Malabar type (Madhusoodhanan et al. 2002). However, all the three varieties and races of cardamom are inter-fertile and the observed variations are probably due to natural crossing (Korikanthimath 2003).

Cultivar selection during breeding programmes can be extremely difficult for a perennial like small cardamom as morphological discrimination of the three varieties is possible only after panicle emergence. Molecular markers including SCAR markers are an effective tool that can be used for varietal authentication and species identification at a very early developmental stage itself when key morphological features are indistinguishable (Das et al. 2008, Ghosh et al. 2011). SCAR markers have emerged as tools to distinguish between species that have similar morphology and phenotype, in several plants such as in spruce (Nkongolo et al. 2003), bamboo (Das et al. 2005), fenugreek (Surendra et al. 2012), gladiolus (Singh et al. 2017) and maple (Boyd et al. 2019).

Variety specific ISSR markers were previously identified in cardamom by the present authors (Sherin et al. 2013) but were not developed and validated as SCAR marker. In the present study we describe the conversion of ISSR derived specific marker to SCAR and its validation on small cardamom varieties. This is the first report on generation of SCAR markers in small cardamom.

Materials And Methods

Plant material

A total of 50 small cardamom accessions from the germplasm repository of Indian Cardamom Research Institute (ICRI), Spices Board, Kerala, India and two wild varieties from Regional Research Stations of ICRI were used to screen for variety specific markers (Table-1). Sources of genetic materials included 39 accessions and 11 released varieties. The accessions were selected based on the morphological analysis (IPGRI, 1994) and the panicle morphology was confirmed with the previous records available at ICRI. All the information concerning the collected samples was shown in Table 1. Secondly, the variety-specific marker was validated on 24 small cardamom accessions, which included 21 Malabar, 2 Mysore and 1 Vazhukka accessions.

Table 1
Details of small cardamom accessions included in the present study

Sl.No	Accession name	Panicle morphology	Location
1	ICRI 1	Prostrate	Chakupallam, Kerala
2	ICRI 2	Erect	Pampadumpara, Kerala
3	ICRI 3	Prostrate	Sakleshpur, Karnataka
4	ICRI 5	Semi-erect	ICRI, Kerala
5	MCC4	Semi-erect	Mudigeri, Karnataka
6	MCC6	Erect	Chemmannar, Kerala
7	MCC7	Semi-erect	Chemmannar, Kerala
8	MCC8	Semi-erect	Vandiperiyar, Kerala
9	MCC12	Semi-erect short	Vandiperiyar, Kerala
10	MCC34	Prostrate	Thondimala, Kerala
11	MCC35	Prostrate	Pampadumpara, Kerala
12	MCC36	Semi-erect	Mavady, Kerala
13	MCC37	Prostrate	Mavady, Kerala
14	MCC38	Semi-erect	Mavady, Kerala
15	MCC39	Semi-erect	Pampadumpara, Kerala
16	MCC40	Prostrate	Mavady, Kerala
17	MCC41	Prostrate	Bodimettu, TN
18	MCC42	Semi-erect	Bodimettu, TN
19	MCC43	Semi-erect	Bodimettu, TN
20	MCC44	Prostrate	Santhanpara, Kerala
21	MCC45	Prostrate	Santhanpara, Kerala
22	MCC46	Semi-erect	Vandiperiyar, Kerala
23	MCC47	Semi-erect	Kailasanadu, Kerala
24	MCC48	Semi-erect	Kattappana, Kerala
25	MCC50	Prostrate	Chakkupalam, Kerala
26	MCC51	Semi-erect	Chakkupalam, Kerala
27	MCC52	Semi-erect	Thandigudi, TN

Sl.No	Accession name	Panicle morphology	Location
28	MCC53	Prostrate	Thandigudi, TN
29	MCC55	Semi-erect	Thandigudi, TN
30	MCC58	Prostrate	Mudigeri, Karnataka
31	MCC60	Prostrate	Mudigeri, Karnataka
32	MCC64	Semi-erect	Pampadumpara, Kerala
33	MCC65	Erect	Pampadumpara, Kerala
34	MCC66	Semi-erect	Pampadumpara, Kerala
35	MCC67	Erect	Meghamalai, TN
36	MCC69	Prostrate	Meghamalai, TN
37	MCC70	Semi-erect	Chenkara, Kerala
38	MCC71	Semi-erect	Santhanpara, Kerala
39	MCC72	Semi-erect	Anavilasam, Kerala
40	MCC260	Semi-erect	Puliyanmala, Kerala
41	CRS Mudigere 1	Prostrate	IISR, CRS Appangala, Kerala
42	IISR Vijetha	Prostrate	Appangala, kodagu, Karnataka
43	IISR Avinash	Prostrate	Kandanakolli, Kodagu
44	MCC5	Prostrate	Maniyargudi
45	ICRI7	Prostrate	Wayanad, Kerala
46	ICRI4	Prostrate	Lower Pulney hills, TN
47	ICRI6	Prostrate	Attappadi, Kerala
48	Typical Mysore	Erect	Karnataka
49	ICRI8	Prostrate	Sakleshpur, Karnataka
50	MCC334	Erect	Anakkara, Kerala
51	*Amomum muricatum*	Terminal	Kandanakolli, Kodagu
52	*Amomum subulatum*	Terminal	Sikkim

DNA Extraction And Quantification

Fresh, young leaves of the small cardamom varieties were collected for DNA extraction. The method of genomic DNA isolation followed the procedure developed by the authors in a previous study (Mathew et al. 2013). The integrity and quality of DNA were evaluated using 1.0% agarose gels electrophoresis, and the concentration of genomic DNA was determined using a UV spectrophotometer (Hitachi) by measuring absorbance at A260 and A280 nm and also using Nanodrop UV/Vis spectrophotometer. Based on the quantification data, DNA dilutions were made in TE buffer to a final concentration of 25ng/µl and stored at -20°C for further use.

Amplification Of ISSR Markers

PCR amplifications were performed using UBC 866 in 25µl reaction mixtures containing 25ng genomic DNA, 2.5mM $MgCl_2$, 1X PCR buffer, 10µM primer, 200µM of each dNTP (Genei, Bangalore) and 1 unit of Taq DNA polymerase (Genei, Bangalore). Amplifications were carried out using a thermal cycler (Biorad) with an initial denaturation/activation step of 4min at 95°C followed by 40 cycles of 30s at 94°C, 60sec at annealing temperature and 2min extension at 72°C. A final extension for 10min at 72°C was included. Optimal conditions were determined based on the resolvable PCR products generated by each primer. A negative control which contained all the PCR components except DNA (replaced by water) was included in every experiment to test for DNA contamination of the reagents. PCR products were subjected to electrophoresis on 2% agarose gels, stained with ethidium bromide (0.5µg/ml) and documented in a gel documentation imaging (Biorad, USA). The fragment sizes were estimated by comparing to 250bp and 100bp DNA ladders (Genei, Bangalore).

ISSR Analysis And Marker Selection

Each of ISSR amplification was performed at least twice to verify the reproducibility of the results and only repeatable amplicons were selected. The amplified products were recorded as ‘1’ for presence or ‘0’ for absence of the bands. The presence of an ISSR band in a particular variety, and its absence in all the other varieties, was considered to be a variety-specific marker.

Identification and gel elution of variety specific - ISSR markers

Among the generated ISSR fingerprints, the ~ 1500 bp fragment that amplified specifically in Malabar variety (prostrate panicle) was eluted using GeneJET Gel Extraction kit (Thermoscientific) following manufacturer’s instructions.

Cloning And Sequencing Of ISSR UBC 866 Fragment

The purified DNA fragment was ligated into pTZ57R (Fig-2) using InsTAclone PCR cloning kit (Thermoscientific) according to the manufacturer’s instructions and transformed into ultra-competent *Escherichia coli* strain JM109 and then plated onto LB/ampicillin/IPTG/X-Gal plates. Positive colonies were determined by blue/white screening. The presence of the insert and its orientation in the purified plasmids was confirmed by colony PCR using M13 Universal primers. The reactions were performed in an appropriate thermocycler (Biorad, USA) adopting the following programme, 94°C for 2 minutes for initial

denaturation, followed by 30 cycles of 94°C for 30s, 45°C for 30s and 72° C for 1min. This was followed by 72° C for five minutes for final extension. PCR products were separated and the colonies with desired size of insert were sequenced, using M13 universal primers in both forward and reverse directions, by Scigenome (Kochi, Kerala) using an automated ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA) method.

Sequence Data Analysis, Scar Primer Design And Validation

With the obtained nucleotide sequence, the similarity of the sequence was determined using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Based on the sequences of the cloned ISSR product, ten pairs of SCAR primer was designed using the Primer3 software. Care was taken to avoid possible secondary structure or primer dimer generation, false priming to match melting temperatures and to achieve appropriate internal stability while generating SCAR primers. The SCAR primers were synthesized through Sigma Aldrich Inc. Bangalore, India. To conduct primer validation, twenty-four selected small cardamom accessions were amplified with the SCAR marker. SCAR-PCR was performed in a final volume of 20 µl consisted of 25ng of DNA from each sample, 1x reaction buffer, 2.5mM of $MgCl_2$, 200µM each of dNTPs, 10µM of each SCAR primer and 1 unit of Taq DNA polymerase. Annealing temperatures was optimized for SCAR primers. The reactions were performed in Eppendorf Master Cycler with the program- 94˚c for 4min, followed by 35 cycles at 94˚C for 45s, 53–57˚C for 1min and 72˚C for 2min and a final extension at 72˚C for 10min.

Results

ISSR analysis and variety specific ISSR marker identification

Small cardamom accessions from three botanical varieties (Table 1, Figure-1) were analyzed using ISSR primers. A total of 18 primers that generated clear, reproducible polymorphic bands (Table 2) were selected from the initial 35 primers analysed for the study. The amplified fragments were within a range of 180 to 2000bp. Six ISSR primers had given a high percentage (100 %) of polymorphic bands. The average number of loci per primer was 11.17 and the number of polymorphic loci generated per primer was 10.05. Among the analysed genotypes, 191 (93.63%) of the ISSR fragments were found to be polymorphic.

Table 2

Details of ISSR primers analyzed, number of polymorphic loci and percentage of polymorphism obtained from fifty cardamom accessions and two out group varieties.

Sl No	Primer (UBC)	Primer Sequence	Annealing Temperature (°C)	Fragment size range (bp)	Number of fragments	Number of polymorphic loci	Polymorphism (%)
1	807	$(AG)_8T$	49	350–1250	17	16	94.12
2	808	$(AG)_8C$	52	270–1560	13	12	92.31
3	810	$(GA)_8T$	46	300–1750	10	9	90
4	812	$(GA)_8A$	52	200–1000	10	10	100
5	828	$(TG)_8A$	53	220–1450	15	15	100
6	835	$(AG)_8YC$	57	180–2000	21	21	100
7	857	$(AC)_8YG$	51	220–1750	20	19	95
8	868	$(GAA)_6$	52	290–1550	11	10	90.61
9	834	$(AG)_8YT$	52	250–1300	9	9	100
10	841	$(GA)_8YC$	52	200–1270	13	12	92.31
11	850	$(GT)_8YC$	51	200–1250	6	6	100
12	866	$(CTC)_6GT$	52	300–2000	14	14	100
13	880	$(GGAGA)_3$	51	300–1750	7	5	71.43
14	881	$(GGGGT)_3G$	57	400–1250	6	6	100
15	860	$(TG)_8RA$	53	270–1800	8	6	75
16	816	$(CA)_8T$	47	300–1500	7	4	57.14
17	873	$(GACA)_4$	52	400–900	7	7	100

Sl No	Primer (UBC)	Primer Sequence	Annealing Temperature (°C)	Fragment size range (bp)	Number of fragments	Number of polymorphic loci	Polymorphism (%)
18	840	$(GA)_8TT$	49	250–1220	10	5	71.43

Several ISSR fragments showed fixed frequencies in individual accessions. Accession specific polymorphisms were obtained for 20 of the 50 small cardamom accessions tested (Table 3). The reproducibility of the amplification pattern was checked by repeating each reaction twice under the identical conditions. Of the 20 specific polymorphisms, 14 accessions were characterized by the presence of bands and the rest by the absence of bands. Unique bands were obtained for most of the released varieties under study. Primers UBC 857 and UBC 807 have given specific fragments for the released varieties. The accession MCC7 a vazhukka (semi-erect) type, exhibited the maximum number of unique bands (Table 3).

Table 3

Accession - specific bands revealed by ISSR fingerprinting for analyzed small cardamom accessions

Cultivar	Characterised by presence of ISSR markers[a]	Characterised by absence of ISSR markers
ICRI 1	*UBC860- 500*	
ICRI 5	*UBC860-520*	
ICRI 4	UBC807-1000	
ICRI 7	UBC807-1400	
MCC 47	UBC807-720	
ICRI6		UBC812-900
ICRI 8	UBC812-650	UBC866-450
MCC70	*UBC841-800*	
Typical Mysore		UBC812-600
MCC 38	UBC828-550	
MCC 48	UBC828-700	
MCC 65	UBC857-900	
MCC6	UBC857-690	
MCC 7	UBC828-420, UBC873-600, UBC857-520, 600 UBC866-450	
IISR Avinash	UBC850-950, UBC866	
IISR Vijetha	UBC828-900	
CRS Mudigere I		UBC828-750
MCC4		UBC866-750
MCC 36		UBC873-300
MCC 53		*UBC841-450*

The primer UBC866 produced a clear fragment (approximately 1500bp) unique to Malabar variety (prostrate panicle), but was absent in all DNA samples of Mysore and Vazhukka varieties (Fig. 2). This fragment produced by UBC-866 was selected as a putative variety-specific marker.

Cloning of ISSR UBC 866_{1500} fragment

The characteristic DNA fragment amplified by ISSR UBC 866 was eluted and ligated to pTZ57R vector by InsTAclone PCR cloning kit. Blue and white colonies were obtained and white colonies were cultured (Supplementary figure S1). The positive clones were then confirmed by PCR amplification using M13 universal primers (Supplementary figure S2). Blue colony (without insert) was used as a control for comparing the results. All clones from recombinant colonies showed fragments similar to the inserted fragment except the blue colony, which produced fragment of ~ 750bp in size. The recombinant clones were selected for sequencing.

The sequence of the prostrate panicle-specific fragment of 1534 bp (Fig. 3) was deposited in GenBank (Accession number: MN276062). The sequence analysis of cloned product revealed that the 1534 bp ISSR fragment was bordered by the original bases of the microsatellite repeats and no repeats were found within the sequence. BLAST searches of the nucleotide sequences with the NCBI nucleotide database using BlastN tool did not reveal any significant match with any known nucleotide sequence, which confirmed the first report of this SCAR marker.

To generate a stable Malabar-specific diagnostic SCAR marker, ten pairs of primers were designed and synthesized based on cloned sequences and were named SBBT 1–10 (Supplementary table-1). The efficacy of each SCAR primer set for amplification of specific PCR product size in particular variety was assessed using DNA from each of the three varieties. Of the 10 SCAR primers designed only a single primer pair SBBT4F and SBBT3R (Table 4) produced specific fragment in Malabar variety and this was used for further validation.

Table 4
Characteristics of SCAR developed for distinguishing Malabar variety

ISSR primer	Variety specific locus	Cloned sequence length (bp)	SCAR primer pair	Primer sequence	Tm (°C)	Amplicon length (bp)
UBC 866	UBC 866–1500	1534	SBBT4F	GGAGGTCGGCTATGATCTGC	57	1350
			SBBT3R	CATCTGGATGAAAGCCCACT		

Validation of the designed SCAR marker

The SCAR marker primer pair (SBBT4F and SBBT3R) was used to amplify 24 samples to test variety-specificity at an annealing temperature of 57°C (Fig. 4). The 24 tested accessions include 21 Malabar

accessions (prostrate panicles), 2 erect panicles and 1 semi-erect panicle types. A clear specific band was detected in 95% of prostrate panicle type but not in samples of erect and semi-erect accessions (Fig. 4). The result revealed that the SCAR primer SBBT 4 F and SBBT 3 R that produced specific fragment (~ 1350 bp) in prostrate panicle is indicative of its Malabar-specificity. Moreover, the initial UBC866 primer was also analysed in the same gel in Malabar and Mysore accession to confirm the product size and specificity. This validated the relevance of designing Malabar- variety specific SCAR marker.

Discussion

The present study was to identify suitable ISSR polymorphisms among cardamom varieties and to transform them into more-specific SCAR markers in an efficient and reliable manner. ISSR reported first by Zietkiewicz (1994) is a popular marker system, owing to their reproducibility, ability to detect polymorphisms, and cost-effectiveness. The reliability of the ISSR marker system has been supported by different investigators in a wide range of crop species including wheat (Nagaoka & Ogihara 1997) and rice (Joshi et al. 2000). In the present study, a specific ISSR fragment of high intensity and reproducibility was identified for Malabar varieties (prostrate panicle) and was used for development of SCAR primers for consistent and repeatable amplification. Subsequently a Malabar variety-specific SCAR marker was developed from the sequence derived from ISSR primer UBC-866. The developed SCAR marker (SBBT4F/SBBT3R) was specific to DNA from all Malabar varieties, as they showed no amplification of DNA from Mysore and Vazhukka varieties. Nucleotide BLAST analyses of ISSR sequence did not reveal any homology to documented GenBank sequences, thereby proving that sequence of the marker under discussion does not belong to any identified portion of the small cardamom genome.

The development and characterization of SCAR markers is a valuable molecular technique for the genetic identification of any species. This method is mainly derived from the molecular cloning of the amplified DNA fragments achieved from the dominant markers. Since, SCAR markers are more straightforward than RAPD, ISSR and AFLP, they can identify a single or few bands instead of a complex pattern (Fu et al. 2015). So identification of any organism becomes more authentic and well-verified if molecular marker analysis is combined to SCAR marker technology (Surendra et al. 2012). Recently, SCAR markers have been used widely for discriminating plant species/varieties for different conditions and traits. Surendra et al. (2012) used SCAR marker for varietal identification in fenugreek, whereas and Rajesh et al (2013) developed RAPD-SCAR marker for identifying tall-type palm trait in coconut. Semsang et al. (2013) utilized SCAR markers for identifying Thai fragrant rice mutants. Singh et al. (2017) used SCAR marker for differentiating *Gladiolus* germplasm and Hao et al. (2018) used SCOT (Start Codon Targeted polymorphism) based SCAR for authentication of *Taxus* media. Boyd et al. (2019) distinguished red maple from silver maple using species-specific SCAR markers.

Madhusoodanan et al. (1994) reported that phenotypic variants exist naturally due to the cross-pollinating nature of cardamom. Among this morphological variability, the panicle type such as prostrate, erect and semi-erect panicles were considered as the major discriminative morphological varietal markers of cardamom. In the present study, 95 % of the prostrate panicle type (Malabar group) expressed the variety-

specific band however it was absent in erect and semi-erect types. This might be due to the presence of distinct variability within and between the cardamom varieties as reported by Padmini et al (1999) based on assessment of morphological characteristics. Varietal identification is important for breeding processes and to protect breeder's rights for newly developed varieties. Presently, the traditional methods for characterization and assessment of genetic variability in many plant species including cardamom, based on morphological, physiological and biochemical studies, *per se* are time consuming and affected by the environment.

Designing SCAR markers for varietal identification would be of immense use for crops like cardamom, where accurate identification of taxonomic status persists as a long-term problem. Due to many remarkable characteristics of Malabar variety, the present results with SCAR markers would be very useful for identifying Malabar varieties and thereby reducing the time and labour during breeding programmes. This is the first study on ISSR-SCAR in *Elettaria* for varietal identification.

Conclusion

The variety-specific SCAR marker developed during the course of the present investigation proved to be reliable and efficient for discriminating small cardamom varieties based on panicle morphology. The specific primer pair (SBBT4F/SBBT3R) clearly demonstrated this unique band only in Malabar varieties and not in Mysore and Vazhukka varieties of cardamom. The SCAR marker reported here could be used for effective and rapid identification of this variety, for utilizing in plant breeding activities.

Declarations

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Conflict of Interest

The authors declare that they have no conflict of interest to disclose.

Availability of data and material

All data are included in the manuscript.

Code availability

Not applicable

Authors' contributions

SJ conceived, designed and executed the experiments, collected the plant material and analyzed the experimental data. SS and ACS provided experimental help in the laboratory. MMK and RYS conceived the overall research project and are the PI's for the initiative. SJ wrote the manuscript and MMK provided editorial advice. All authors revised and approved the final version of the manuscript.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate

Not applicable

Consent for Publication

All authors consented for publication in this journal

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Figures

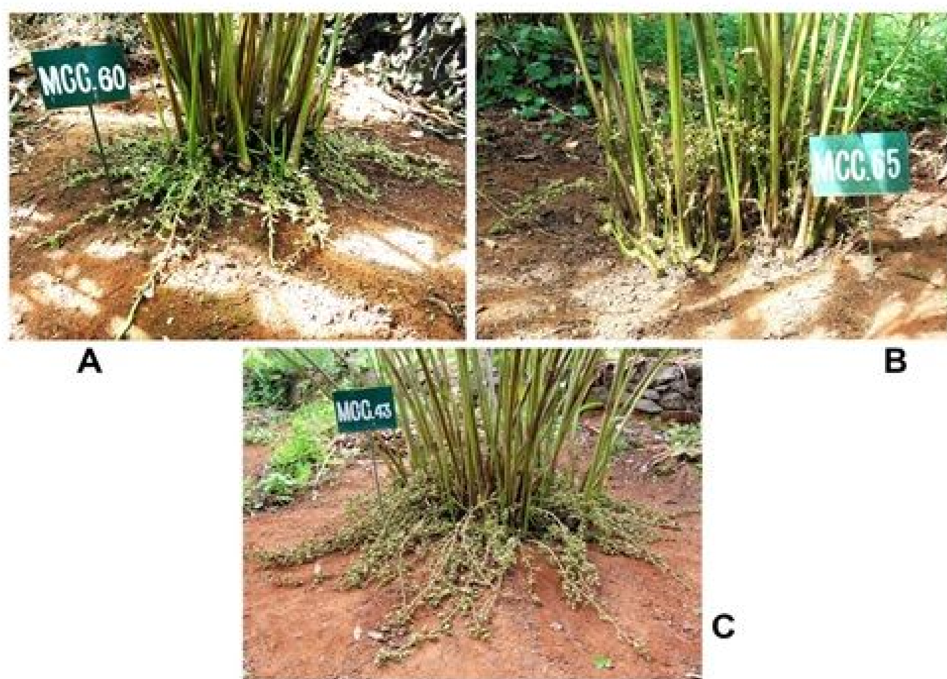
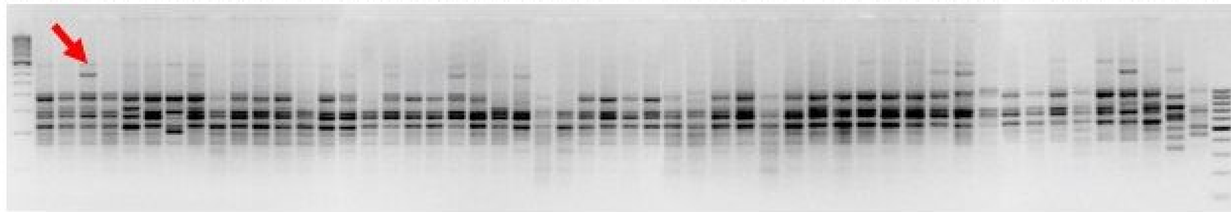


Figure 1

Botanical varieties of cardamom. A) Malabar; B) Mysore and C) Vazhukka



M- 250bpladder; 1-ICRI1, 2-ICRI2, 3-ICRI3, 4-ICRI5, 5-MCC4, 6-MCC6, 7-MCC7, 8-MCC8, 9-MCC12, 10-MCC34, 11-PV1, 12-MCC36,13- MCC37, 14-MCC38, 15- MCC39, 16-MCC40, 17-MCC41, 18-MCC42, 19-MCC43, 20-MCC44, 21-MCC45, 22-MCC46, 23-MCC47, 24-MCC48, 25-MCC50, 26-MCC51, 27-MCC52, 28-MCC53, 29-MCC55, 30-MCC58, 31-MCC60, 32-MCC64, 33-MCC65, 34-MCC66, 35-MCC67, 36-MCC69, 37-MCC70, 38-MCC71, 39-MCC72, 40-MCC260, 41-CRS Mudigere1, 42-IISR Vijetha, 43-IISR Avinash, 44-MCC5, 45-ICRI7, 46-ICRI4, 47-ICRI6, 48-Typical Mysore, 49-ICRI8, 50-MCC334, 51-*A.muricatum*, 52-*A.subulatum;* M-100bp ladder

Figure 2

Variety specific bands produced by ISSR marker UBC 866

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GCCGACTAGACTCGCGATGCATCTAGATTCTCCTCCTCCTCCTCCTCGTATTCGCTTCCGGATTCATTCGGTTGCCCC
TTCGTGACCGGCGACGGACACCGCTCCTTCAGGCGTCGGCCAGCCCACTGCACCATTTCCCACGCACCACCCAATT
TTTATTCCCAATTCAAGCACTTACTGGAACTCTGCCTGCCATGCAACTCTGTGACCGCACCACGTCCTCCACCTCCAC
CCACGTATTCTCCTCCCAGTTGCCATCTGGATGAAAGCCGCACGACTATAATTATAGCTACTACCACCGTCTTACAT
ATGCCATCGGATCATCGCTCTCCAAATTAACCTCTATTCTTGGCATCCTTGCTTTGGTACGTAGGAGAATCATCTACC
TTTAATTTCGTTTTCCTAGCGAGTAGAACCATAACTAATTGTTGAACTTGTGCTTTACGTGCATGCGTGAGCTTAGA
TTACTGGTGAATGGCATCGCGGGAAGCAATAAGCAAAGTGAGAATAGGGAAGACGACGACGTACCGGAAGGGC
ACCGATACGGAACTGCTTGCCTTCGGAAGCGAAGCGGCGATGGCAGCCAAGCTGCTGGTTTTGGGAGGCGACGG
CGGCGGCAGGCAGGTCTTCCGCGCTGGACGGCTTTCCGTACACATGATCCGGGTGGCGTCCTCCCCCGTGTGCTT
GGTCAGTTGCATGGTCGGGGATCACCTCTGGACGATGGCAAGGGACGCGGTCGTCCTCCGGGCAGCTCCGCGCA
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CCCTTTCCGATTTGCGCGCGCCGAGATCAATTTTTTGCTTTGATTTCTTGCGCGCCAAGATCAATTTATTATTTGATT
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GCGGCGGCGACGCCCAGTGGGCTCTGGTGCATCAACrGCAGAGGGGTTGAAGCTGCTGGTGTTGGTACTTTCGGC
GTGCAGCACCGCGGCGAAGAGAAAAAGGCAGCCGCTAGAGACGCTGAACGAGGAGGAGGAGGAGGAGAA
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Figure 3

Nucleotide sequence of ISSR UBC- 866 specific to the Malabar variety (prostrate panicle) flanked by the SSR sequences (underlined)

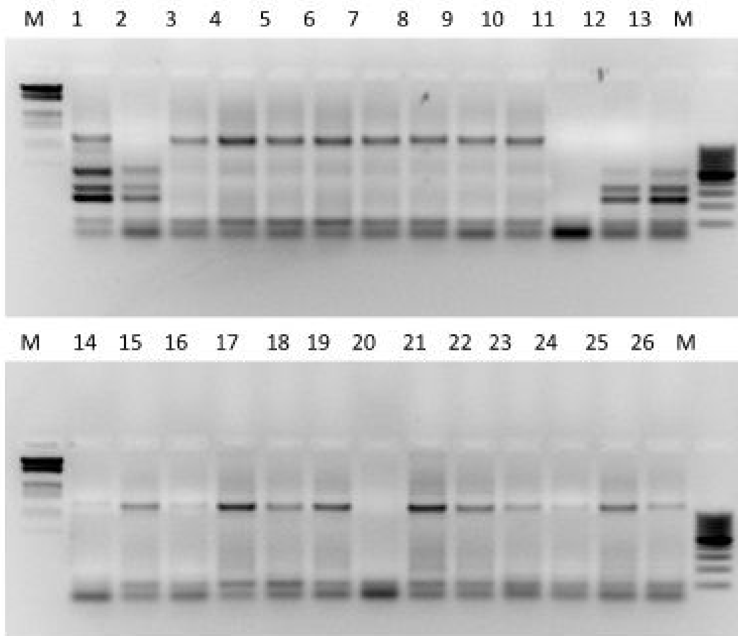

Figure 4

Amplification profile of SCAR marker SBBT4F/SBBT3R in small cardamom accessions. Lane M- 250bp ladder; lane (1&2) Malabar and Mysore variety resp using ISSR UBC 866 primer; lane (3-10) Malabar accessions (prostrate panicle); lane (11) Vazhukka (semi-erect panicle); lane (12&13) Mysore (erect panicle); and lane (14-26) Malabar accessions (prostrate panicle) last lane (M) 100bp ladder.

Supplementary Files

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