

Original Research Article

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Microsatellite in Coriander: A Cross Species Amplification within Apiaceae Family

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ABSTRACT

Coriander is a spice crop mainly used for seasoning in cooking. For the improvement of this crop very limited literature is available for molecular studies. Hence, it's a need to develop molecular markers for this crop. Development of SSRs (Simple Sequence Repeats) for new crop like coriander is an expensive and time consuming process. This can be easily achieved by transferring the microsatellite loci from the same family/genus/species which is a cost-effective approach for development of SSRs in lesser studied crops like coriander. Presently in carrot and celery SSRs are available on the public domain for cross-species investigation, both crops belongs to the same family. We examined the transferability of hundred carrot SSR loci to coriander. Thirty nine percent primer (39/100) amplified SSRs for coriander. Out of 39 SSR loci, 35 primers were monomorphic and specific to carrot primers, showing approximately the same size of SSR loci as reported for carrot. Four SSRs were polymorphic, seven primer pairs were non-specific which were deviating for band size from the source of SSRs. Primer GSSR-113 was non-specific, but showing polymorphism for the microsatellite loci. These SSR markers should provide a powerful tool for coriander breeding and genetics.

Keywords

Apiaceae,
Coriandrum sativum, Cross-Transferability, Microsatellite, SSRs

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Introduction

Coriander (*Coriandrum sativum* L) is one of the important seed spice crops after cumin. The crop belongs to the family Apiaceae and have diploid chromosome number $2n=22$. The coriander seeds and foliage possess a unique fragrance and taste, which are used all over the world as culinary spice, flavouring agent. In addition to its use as seed spice in our routine cooking, recent studies have shown its pharmaceutical and medicinal importance (Aruna and Baskaran, 2010). Coriander is native to the Mediterranean and commercially produced in India, Russia, Romania, Ukraine,

Morocco, Canada, France, Central America, Mexico, and USA (Qureshi *et al.*, 2009). India is the largest producer and accounting for 5.5 lakh tonnes per annum of seeds from 5.8 lakh ha area. The quantity of foliage production and consumption remains unaccounted. India is also the highest consumer and exporter of coriander. In India, Rajasthan, Gujarat, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Himachal Pradesh are the major coriander growing states in India. Very limited molecular and genomic studies on it, hinders the molecular research.

In addition, a lack of coriander molecular markers limits the process of molecular genetics breeding. Till date very limited literature is available for molecular studies in coriander. Molecular genetic breeding is also very difficult in coriander as lack of molecular markers limits the process. Problems associated with conventional breeding can be overcome by the use of molecular marker assisted selection. However, only a few molecular markers have been reported in coriander like RAPD (Al-Kordy *et al.*, 2013; Singh *et al.*, 2013). A very popular and informative marker SSR is still lacking for coriander crop. Development of new SSRs for new species like coriander is very time consuming, expensive and requires isolation, cloning, sequencing and characterization of microsatellite loci. Available collections of ESTs (Expressed Sequence Tags) on public domain can be explored for microsatellite development (Kantety *et al.*, 2002). This approach is, only applicable to well-studied species where abundant molecular data is available. Another very economic and reliable approach for the genome with very limited DNA sequence information is screening of the conservation of microsatellite flanking regions in closely related Species (Bushar *et al.*, 2001; Schnell *et al.*, 2005). Primer screening from different relevant sources are the most potential chance for development of SSR marker for species without prior sequence information (Barbara *et al.*, 2007). Transferability of microsatellite markers within genus, across the species (Ciprian *et al.*, 1999; Butcher *et al.*, 2000) and in leguminaceae across genera transferability, belongs to same family has been reported (Peakall *et al.*, 1998), Fagaceae (Al-drich *et al.*, 2003) and Casuarinaceae (Ramasamy *et al.*, 2005). In the present study, we analyzed the transferability of 100 microsatellite loci originally belongs to carrot (*daucus carota*) as both the crops coriander and carrot belongs to the same family.

Microsatellites (SSRs) have been widely recognized as the powerful marker of choice for plants and animals which is considered as the very informative genetic marker. Microsatellites normally occur in non-coding regions of the genome but they can also occur coding regions or in either 3' or 5' UTRs of ESTs. Microsatellites (SSRs) are composed of short (2-5 base pairs), tandemly repetitive tracts of DNA in which certain DNA motifs are repeated, typically 5-50 times (Caporale *et al.*, 2003). For example, the sequence TATATATATA is a dinucleotide SSR, and GTCGTCGTCGTCGTC is a trinucleotide SSR (with A being Adenine, T Thymine, G Guanine, and C Cytosine). Repeat DNA motifs of four and five nucleotides are referred to as tetra- and pentanucleotide SSRs, respectively. This marker is abundant, co-dominant, hypervariable and transportable between species and are distributed throughout the animals and plants genome (Roder *et al.*, 1995; Gupta and Varshney, 2000; Jarne and Lagoda, 1996). Due to their high polymorphism, and easy use with the PCR, microsatellite loci are considered as ideal markers (Kuleung *et al.*, 2004, Peakall *et al.*, 1998). In plants, SSRs are useful for cultivar identification, genetic linkage mapping and marker assisted selection in breeding and population genetic studies (Barreneche *et al.*, 1998; Butcher *et al.*, 2000; Steinkellner *et al.*, 1997).

Materials and Methods

Plant material and DNA extraction

To test the cross-species transferability of microsatellite loci, we obtained five samples of coriander Variety ACr-1 from Gene Bank, ICAR-National Research Centre on Seed Spices, Tabiji, Ajmer (Rajasthan), India. DNA was extracted from frozen leaves of 20 days old seedlings using cetyltrimethyl ammonium bromide (CTAB) method

described by Doyle and Doyle (1990). The quality and quantity of DNA was determined by electrophoresis on 0.8% agarose gel. DNA samples were diluted to 20ng/μl for polymerase chain reaction (PCR) amplification. A total of hundred primers screened for cross-species amplification were synthesized by Xcelris genomics (India).

PCR amplification with microsatellites and data analysis

Hundred microsatellite loci previously developed for carrot by Cavagnaro *et al.*, (2011) were tested for cross amplification in coriander species. PCR reactions (20 μl) were conducted in C1000™ Thermal cycler (BIO-RAD) containing 12.8 μl water, 2 μl 10X DNA polymerase buffer, 2 μl dNTPs (2 mM each), 0.5 μl of each primer at 100 μM, 0.2 μl Taq DNA Polymerase at 5 u/μl (SRL, biolit™) and 2 μl genomic DNA. Thermal cycling conditions were as follows: initial denaturation at 94°C (4 min), followed by 40 cycle of appropriate primer annealing temperature (1.0 min), elongation at 72°C (1.0 min), and denaturation at 94°C (20 sec). A final elongation step was allowed for 10.0 min at 72°C.

Electrophoresis was carried out for 3 hours at 150 V on 1.5% high-resolution agarose. For each 100 ml of TAE, TAE gels were supplemented with 5 ul (5.0 mg/ml) of ethidium bromide. For each microsatellite locus, sizes of the allele were estimated by comparison with standard DNA marker (100bp, SRL Biolit™) and were scored across all the samples manually. Amplification of products was scored for sharp and reproducible bands only.

Results and Discussion

To evaluate the potential utilization of SSRs, two different types of microsatellites were used for transferring carrot SSR markers to

coriander viz; genomic microsatellites (GSSRs) and BAC end sequences (BSSRs) based SSRs (Cavagnaro *et al.*, 2011) were tested in five sample of coriander. Specific PCR amplification were considered for those amplifications which were of approximate length expected for that particular SSR in carrot and those with different size were considered as non specific amplification.

Pattern of cross species amplification in plant across genera is beginning to emerge as a source for developing microsatellites in lesser-studied or orphan crops. Very few studies are available for transferability of microsatellites to related species.

In present study cross-species amplification was scored as positive only when sharp band/bands were reproduced in at least two out of three PCR replicates. Using this criterion Under standard, high-stringency PCR amplification conditions, out of the hundred microsatellite loci assayed, 39% were amplified in at least one of the used coriander samples (Table: 1). The remainder could not be consistently scored or failed to amplify.

When hundred microsatellite loci of carrot were amplified in PCR for cross-species transferability at annealing temperature 54°C or below, very feint and stutter bands were produced and therefore the annealing temperature was increased to 55°C and up to 65°C for few primer pairs (Table: 1). Such modification in the PCR amplification protocol specially primer annealing temperature are recommended for the successful transfer of microsatellite loci between species (Rosotto *et al.*, 2001).

Out of tested 100 carrot SSRs only 39 were showing PCR amplification with coriander, the putative SSR loci of 35 primers were monomorphic.

Table.1 Characteristics of 39 carrot microsatellites showing amplification in coriander

S.N.	Microsatellite	SSR Motif	Sequence 5'-3'	Annealing temp °C	Amplicon size (bp)	NCBI number	GC content	Allel Size (bp)	Spec
1	GSSR-4	(TCTA)21 (p4)	F: CAATCTTGCCACTAAAAGAGCA R: CAGATACAATAGACAGGAAACATCG	55/55	314	FJ816114	40.9	350	+
2	GSSR-5	(AC)9 (AC)6 (p2)	F: ATAATAAACCCAACCAGACCCC R: ATCAGGCAAATCCCATACTGAC	54/58	120	FJ816115	45.5	120	+
3	GSSR-10	(GACA)7 (p4)	F: CTTAGTAGTAGCACACACCAGACG R: GAGCTGAACGAGTCAGAAAGG	55/55	255	FJ816120	50	250	+
4	GSSR-16	(TG)9tacgc(ATGT)3 (c)	F: ATGCAAAACGACAATATCCACAG R: GCCCAGCCACTTCCTAGAT	57/57	212	FJ816126	40.9	220	+
5	GSSR-35	(GA)13 (p2)	F: AATTCACAATCACCGACTCTCC R: ACGTCAAAGTCCCTGTTCATTT	55/57	173	FJ816145	45.5	175	+
6	GSSR-37	(TATG)7 (TTGC)3 (p4)	F: CGAGGGAGAATGACGAAAATTA R: TCTGTGACGAGTAGGATCAGGA	55/55	197	FJ816147	40.9	200	+
7	GSSR-40	(CAT)7 (p3)	F: TAGAAGCTCCAACAAATCACCC R: CAAGGAACCCTAGATCACAAATG	55/55	171	FJ816150	45.5	900	-
8	GSSR-43	(TG)12 (p2)	F: TTCTTCACCTATGTTGGGGC R: CGTTCATATGCACAACACTCA	58/55	198	FJ816152	50	200 700	P
9	GSSR-81	(CT)15 (CT)8 (p2)	F: TGGGTCTCTGGCCAATTCTA R: CAATTTGCACATAACTCATCAAG	58/58	228	FJ816190	50	900	-
10	GSSR-87	(TCT)15 (TCT)4 (p3)	F: CCAACAACCATCCAACAACTA R: AGTCGTCCGATAAGCGAATCTA	58/58	391	FJ816196	40.9	400	+
11	GSSR-92	(ACAT)11 (p4)	F: AGAAGGCATCGTGTTCATAA R: CAACGGTGATTAAGTGGGTTCT	55/55	276	FJ816201	40.9	250	+
12	GSSR-96	(CAT)5 (p3)	F: AGCGTCGTTTTTCGCGAGT R: CGCGGTTAAAGCAAAGCTAAT	55/60	334	FJ816205	55.6	350, 400, 600, 700	P
13	GSSR-97	(GA)8 (GA)7 (AG)7aagtattcca(AG)6 (c)	F: GGCAAAGAAACAGATTTGGAGA R: CTGCCCTAGCATCAAAACAAAC	55/55	265	FJ816206	40.9	300	+
14	GSSR-100	(ACA)4 (p3)	F: TTCCCCTGTGCTGAACTCCA R: CTGCAACTCTGGATTTGAGGT	54/55	362	FJ816209	50	350	+
15	GSSR-101	(GA)6 (GA)10 (p2)	F: TGGGGTGGTTTTGTCTGATT R: TGGGGTGGTTTTGTCTGATT	54/58	328	FJ816210	45	350	+
16	GSSR-104	(GTT)5(GAG)4 (c)	F: TGGTCTGAGGAAGATGTTTGA R: GCCTGCACTAAATTGATGACAC	54/60	330	FJ816213	40.9	350	+
17	GSSR-107	(ATAC)8 (ACAT)4 (p4)	F: TTCTGGTCTTTTGACATGAAGG R: CGGATTTGAGGTGAGTTGAATA	54/55	265	FJ816216	40.9	300	+
18	GSSR-111	(ATAC)3atccatc(CATA)9tat(CA)20 (c)	F: GAGGAAGGGTAGATCCAGTCA	55//55	355	FJ816220	52.4	350	+

19	GSSR-112	(CT)12(CA)6a(AC)6 (c)	R: ATGGGATGTCTTTCCCTCTAT F: TCTTGTYYAAGAAMACCACA R: GTCCACCAAGTATGCTC	55/55	197	FJ816221	37.5	200	+
20	GSSR-113	(TTG)4 (TGT)4 (p3)	F: AGTGGTTGTGAGGTTGATTGTG R: TAATGTCGGAAAGGTTCAATGCT	55/55	336	FJ816222	45.5	600, 900	-P
21	GSSR-131	(GAT)11 (p3)	F: AAATTACTGGAGATGGAGCGAG R: GTTTGTTGATTCGGACTTTGTG	55/55	165	FJ816240	45.5	150	+
22	GSSR-132	(TGT)4 (p3)	F: CAGGTAGTGGTTGWGAGYTTGA R: TCCCCTTGTCGTAACCTCCATAC	54/56	332	FJ816241	47.7	350	+
23	GSSR-136	(TG)5 (p2)	F: GAAGAAAGGAGCTAAAGTGGGA R: CCCATTCCATTTTCACTTGC	58/58	426	FJ816244	45.5	450	+
24	GSSR-138	(GT)5ata(GT)7 (AG)21 (p2)	F: CGCTCGAGTTTCGTAGAGT R: CCTCCCCAACTCAATCCAAT	56/56	519	FJ816246	52.6	500	+
25	GSSR-139	(TGTC)4 (p4)	F: GCAAGTGTTCGTGACATGC R: AACATGAGTTAATCGAAGGGGA	56/56	365	FJ816247	50	350	+
26	GSSR-140	(TC)10 (p2)	F: GGATACGAAGGAAAGACTCCAC R: AGGAGAGTAAAAGATTGAGGACTTG	56/58	158	FJ816248	50	700	-
27	GSSR-141	(CT)8cct(CCTC)3 (c)	F: CTACAATACCAACATACCAAAGG R: GGCTACAATGAACCAAGAAATG	55/55	184	FJ816249	40	700	-
28	GSSR-142	(AAAC)5 (p4)	F: GTAGAAAACCTTTTGGCAGTAACG R: CCAAGACCATGAAGAAATCACTC	56/56	138	FJ816250	43.5	150	+
29	GSSR-143	(TATC)4(TA)7 (c)	F: GGAAGAATAAAAACCAACACA R: TCAGTAAATCAGGAGTGCAGAA	55/55	346	FJ816251	40.9	350	+
30	GSSR-149	(GAGAGG)3(GA)8 (c) (GA)6 (AG)8 (p2)	F: TGAAGCAACTCGTGATACAGAGA R: TTCTCTTGTCCTGGTTAGCTC	57/57	342	FJ816257	43.5	350	+
31	GSSR-153	(TC)15 (p2) (TAGTTG)2 (p6)	F: GTCACCCACCAAGAAAAA R: CCAAACACAAGACAGTCAAATG	56/56	255	FJ816261	47.4	500	-
32	GSSR-154	(TC)11 (p2)	F: CTTATATGTGATGGCGTCGAAA R: GACTGCACCGCTCCTAACTC	56/56	328	FJ816262	40.9	350	+
33	GSSR-18	(TG)7tt(TG)4 (c) (TG)7 (P2)	F: TTGGGGAACAAGCAACTCAG R: CTCTCTTGAATGTTGGCATAACAC	54/55	130	FJ816126	50	150	+
34	GSSR-58	(TAAAA)2 (CAAAA)2 (p5)	F: CAAGAACTACGCACATAACTCA R: TGCTCTAGCCTTTTTACCGC	54, 56/55	301	FJ816167	40.91	300	+
35	GSSR-61	(CTCA)4 (p4)	F: TGTATTAAGGCACAACCTGGA R: CCTTGTACCTAGAGTGG	54/65	402	FJ816170	38.1	400	+
36	BSSR-1	(TCT)4 (p3)	F: TTCTTGGTCTGTTGATGTCAGTGTAG R: TGGATATAGAAGCCATCAGACTTGAG	56/56	180	FJ147759	42.3	150	+
37	BSSR-6	(TAAT)3 (p4)	F: GTCGCGGAAGTGAATCTGA R: TGTTCTGTAACCTTGCTTGATCG	54/60	223	FJ147807	52.6	600	-
38	BSSR-8	(AT)6 (p2)	F: TGAAGCTAATATCCAACAAAGGAAA R: AGGAGCATGTTATGCTATTACCAACA	52/60	159	FJ147825	32	150- 900	P
39	BSSR-14	(TTA)4 (p3)	F: TACCATAAECTCAAGTTGGATAATTC R: AATGTCTAAACCCACTGATTTAAAAG	52/58	240	FJ816268	30.8	250	+

p2: Poly-dinucleotide, p3: Poly-trinucleotide, p4: Poly -tetranucleotide, c: Compound SSR, P: polymorphic, +: Specific, -: Non specific, Ca/Co: carrot/coriander.

Hille *et al.*, (2002) reported that monomorphic loci observed in cross amplification could be due to null heterozygous condition. Four (GSSR-43, GSSR-96, GSSR-113 and BSSR-8) out of thirty nine SSR markers revealed polymorphic loci. Moreover non-specific amplifications were observed in seven primers, namely, GSSR-40, GSSR-81, GSSR-113, GSSR-140, GSSR-141, GSSR-153 and BSSR-6, and one primer GSSR-113 was showing non-specific but polymorphic banding pattern (Table.1). Our data provide an undoubted evidence for the potential transferability of SSRs in plants more closely related to carrot like coriander, as earlier reported that closely related species share similar SSR priming sites that results in easier transferability of SSRs (Rai *et al.*, 2013). The impact of evolutionary relationship between source and target species on SSR transfer success have been widely observed in many plant families (Rossetto *et al.*, 2001; Arnold *et al.*, 2002). Transferability of carrot SSR markers across coriander is achieved in this study which proves that screening of SSR markers from different species can lead to the development of SSR loci in coriander. It may further be possible to increase the success rate of cross-species SSR amplification by searching expressed sequence tags (ESTs) and heterogeneous nuclear RNA (hnRNA) libraries or database for SSRs that can be anchored in exons. Therefore, these SSR loci should be a powerful tool for coriander breeding programs and conservation genetic studies.

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