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Application of inter-SINE amplified polymorphism (ISAP) markers for genotyping of *Cucumis melo* accessions and its transferability in *Coleus* spp.

SITI YULI MEILANDA SORMIN[1], AZIZ PURWANTORO[2], AGUS BUDI SETIAWAN[2,♥], CHEE HOW TEO[3]

[1]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia
[2]Laboratory of Genetics and Plant Breeding, Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia. Tel./fax. +62-274-551228, ♥email: setiawanab@ugm.ac.id
[3]Centre for Research in Biotechnology for Agriculture, Universiti Malaya. Kuala Lumpur 50603, Malaysia

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Abstract. *Sormin SYM, Purwantoro A, Setiawan AB, Teo CH. 2021. Application of inter-SINE amplified polymorphism (ISAP) markers for genotyping of* Cucumis melo *accessions and its transferability in* Coleus *spp. Biodiversitas 22: 2918-2929.* An unambiguous characterization of melon (*Cucumis melo* L.) accessions based on their morphological traits is often laborious and affected by environment when compared with molecular marker genotyping. Short interspersed nuclear elements (SINEs) are highly abundant non-autonomous and non-coding retrotransposons that are widely scattered over all chromosomes of eukaryotes. They can serve as a good molecular marker for routine genotyping in plant breeding and marker-assisted selection. This study aimed to apply inter-SINE amplified polymorphism (ISAP) markers for genotyping of *Cucumis melo* accessions and its transferability in *Coleus* spp. Twenty-one *C. melo* accessions, one *C. metuliferus* E. Mey. ex Naudin, and 15 accessions of *Coleus* spp. were used for ISAP marker development. A copy of cucumber-specific long interspersed nuclear element (LINE) and multiple copies of melon-specific SINE were identified and isolated. ISAP primers were designed from the highly conserved region of the SINEs and LINE. The melon and cucumber-specific ISAP markers showed a higher degree of polymorphism (87.5%-100%) than potato ISAP markers (60%-100%) in all the tested melon accessions. The unweighted pair group method with arithmetic average (UPGMA) dendrogram generated from polymorphic ISAP bands clearly distinguish the *Cucumis melo* accessions from their distantly related wild species *C. metuliferus*. The transferable nature of *Cucumis* ISAP marker system was demonstrated in *Coleus* species, where the marker differentiates the tested accessions.

Keywords: *Coleus*, *Cucumis melo*, ISAP marker, plant genotyping, SINE elements

INTRODUCTION

Melon (*Cucumis melo* L.) is an economically important horticultural crop in the genus *Cucumis*. It is a diploid species with 2n = 2× = 24 chromosomes (Setiawan et al. 2020a). Over the years, various melon varieties with distinct traits, stable yields, and uniform phenotypes have been developed by different melon breeding programs. In addition, numerous pre-breeding accessions exist, including the landraces and their wild accessions maintained by various gene banks around the world (Sebastian et al. 2010; Pitrat 2016). These melon accessions show a wide range of genetic diversity in their fruit size and shape, skin color, flesh color, seed characteristic, nutritional value, and floral diversity (Pandey et al. 2021). Various morphology and agronomic characters have been widely used for the selection of inbred LINEs and phenotyping of melon cultivars (Ali-Shtayeh et al. 2017; Merheb et al. 2020). However, the selection remains a laborious task because of the high number of melon cultivars (Pitrat 2016) and the phenotypic traits are often affected by environments. Molecular marker genotyping, which is less influenced by environmental factors, provides a reliable solution to phenotypic characterization.

The use of molecular markers in melon genotyping has been extensively studied. Random amplified polymorphism DNA (Karimi et al. 2016), sequence-related amplified polymorphism (Yildiz et al. 2011), chloroplast DNA marker (Rodríguez-Moreno et al. 2011), and internal transcribed spacer of ribosomal DNA (Renner et al. 2007; Endl et al. 2018) have been used to study the genetic diversity and domestication processes in melon. In addition, simple sequence repeat (SSR) markers have been applied in melon genetic diversity studies and genetic map development (Raghami et al. 2014; Zhu et al. 2016). However, RAPD is poorly reproducible, and the other markers are costly and require many combinations of SSR markers to obtain reliable results.

Retrotransposons are classified into two subclasses: long terminal repeat (LTR) retrotransposons, including Ty1/Copia and Ty3/Gypsy families, and non-LTR retrotransposons, including long interspersed nuclear element (LINE) and short interspersed nuclear element (SINE) families (Schulman et al. 2012; Elbarbary et al. 2016; Orozco-Arias et al. 2019). SINE elements are highly abundant non-LTR retrotransposons that are about 100 bp to 500 bp in length and show high sequence diversity (Wenke et al. 2011; Seibt et al. 2016). SINE has retrotransposition ability via an RNA intermediate, where new copies of the elements will transpose and integrate at

other genomic locations while maintaining their original location. Therefore, SINE is suitable for molecular marker development. Wenke et al. (2015) successfully developed inter-SINE amplified polymorphism (ISAP) marker for plant genotyping. The ISAP markers developed by various groups produced genotype-specific fingerprints at high resolutions, thus allowing cost-efficient, robust, and rapid plant genotyping (Seibt et al. 2012; Wenke et al. 2015; Diekmann et al. 2017; Pantchev et al. 2019).

The application of ISAP markers in melon genetic diversity and genotyping study is still limited. In addition, information on the transferability of the ISAP marker in other plant species is scarce. This study aimed to apply inter-SINE amplified polymorphism (ISAP) markers for genotyping of *Cucumis melo* accessions and its transferability in *Coleus* spp.

MATERIALS AND METHODS

Plant materials

Twenty-one *Cucumis melo* accessions, one *C. metuliferus* E. Mey. ex Naudin (Table 1), and 15 accessions of *Coleus* spp. were used in this study. The accessions of *Coleus* species (Col-K01, Col-K02, Col-K03, Col-L01 Col-L02, Col-L03, Col-M01 Col-M02, Col-M03, Col-N01 Col-N02, Col-N03, Col-O01 Col-O02, and Col-O03) were collected from the Yogyakarta region and maintained in the Genetic and Plant Breeding Laboratory, Faculty of Agriculture, Universitas Gadjah Mada. The seeds of *C. melo* accessions were germinated in moistened pot trays, grown, and maintained in the screen house of the Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia, from April until July 2019.

DNA isolation and quantification

Total genomic DNA was extracted from the young leaves of *Cucumis* accessions and *Coleus* spp. By using the CTAB (hexadecyltrimethylammonium bromide) extraction method described by Setiawan et al. (2020a), DNA quantification was conducted with NanoDrop (2000c Spectrometer, Thermo Scientific). The DNA samples of each accession were diluted to a working solution (25 ng/µl) with nuclease-free water.

LINE and SINE sequences analysis

Cucumber LINE (CsLINE-1) was retrieved from the genome database of *Cucumis sativus* cv. 9930 by searching the corresponding database with the keywords "LINE" and/or "long interspersed nuclear element." SINE sequences were retrieved from melon and cucumber genomes by using the local blastN approach with CsLINE-1 as the search query. Sequence synteny analysis was performed by comparing the sequences of SINE elements (CsSINE-1, CmSINE-1, and CmSINE-2) in melon and cucumber genomes with CsLINE-1 in cucumber. Phylogenetic analysis of CsSINE-1 was conducted using MEGA 7 (Kumar et al. 2016) with the neighbor-joining (NJ) method with 1,000 bootstrap replicates. Insertion sites and copy numbers of CsSINEs inserted into cucumber genes were determined using blastN analysis against reference genome (*C. sativus* cv. 9930).

Table 1. Plant materials used in this study

Name/code	Accession number/status	Species	Origin
Golden Langkawi	Commercial variety	*Cucumis melo* L.	Malaysia
Silver Light	Commercial variety	*Cucumis melo* L.	Japan
Melani	Commercial variety	*Cucumis melo* L.	Indonesia
Japonica	Commercial variety	*Cucumis melo* L.	Indonesia
Rock Sonya	Commercial variety	*Cucumis melo* L.	Indonesia
Dainty	Commercial variety	*Cucumis melo* L.	Indonesia
Eksis	Commercial variety	*Cucumis melo* L.	Indonesia
Baladewa	Commercial variety	*Cucumis melo* L.	Indonesia
US340	PI 185111	*Cucumis melo* L.	Ghana
US143	PI 292190	*Cucumis metuliferus* E.Mey. ex Naudin	South Africa
Madesta F1	Commercial variety	*Cucumis melo* L.	Indonesia
Haru	PI 266944	*Cucumis melo* L.	England
NI19	940281	*Cucumis melo* L.	Chad
P34	Weedy melon	*Cucumis melo* L.	Korea
P107	Rio Gold	*Cucumis melo* L.	USA
US176	PI 614576	*Cucumis melo* L.	India (Center)
US128	PI 482398	*Cucumis melo* L.	Zimbabwe
US205	PI 182952	*Cucumis melo* L.	India (West)
US58	PI 116738	*Cucumis melo* L.	India (West)
US368	PI 505599-1	*Cucumis melo* L.	Zambia
US54	PI 614588	*Cucumis melo* L.	India (Center)
US171	PI 614542	*Cucumis melo* L.	India (Center)

Table 2. ISAP primers used in this study

Primer name	Sequence (5′-3′)	Tm (°C)	GC (%)	Reference
CmLINE_ISAP1	GAADRGTGTGAGGAGGAAGYTVTG	66.2	50.0	This study
CmLINE_ISAP2	CCAYTTKTCARMRTCACCCACC	68.7	52.3	This study
CsSINE1-01*	GTCCNGTGAGATTAGTHGAGGTG	63.9	51.4	This study
CsSINE1-02	GACAYBCAAATGTTGTAGGGTCAG	66.1	46.5	This study
CsSINE1-03	TTACCACTAGGNCAACCCANGATGG	70.9	52.0	This study
SolS-IIIaF	CCTATGTGGTTTGCGAGC	62.4	55.6	Seibt et al. (2012)
SolS-IVR	CCCTTTGGATCAATCACAGC	64.8	50.0	Seibt et al. (2012)

Note: *) The ISAP primer was tested in *Coleus* spp.

Table 3. LINE and SINE elements found in cucumber and melon genomes

Name	Accession	Position	Start-End	Length (bp)	Target site duplication
CsLINE-1	NC 026661.1	Chromosome 7	15506082-1528378	3153	Yes
CsSINE-1	NC 026658.1	Chromosome 4	1535820-1536085	260	Yes
CmSINE-1	NW 007546275.1	scaffold00008	72133-72388	241	Yes
CmSINE-2	NW 007546313.1	scaffold00046	1527032-1528378	1347	Yes

ISAP primer design and PCR assay

Multiple sequence alignment (MSA) of cucumber and melon SINE and LINE sequences were performed by using Bioedit (Hall 1999). Cucumber- and melon-specific ISAP primers were designed using FastPCR from a consensus sequence generated from highly conserved regions of cucumber-specific SINE and melon-specific LINE sequences (Table 2) (Kalendar et al. 2017). For PCR amplification, 50 ng of genomic DNA was added to a reaction mixture that consists of 1X GoTaq® Green Master Mix (Promega, USA), 0.2 mM dNTPs, 0.2 μM primer, 1.25 U/μL GoTaq® polymerase, and nuclease-free water in a 12.5 μL reaction volume. The amplification conditions are initial denaturation at 95 °C for 5 min; 30 cycles of 95°C for 1 min; annealing at the T_a specified in Table 3 for 1 min, 72°C for 1 min; and a final extension at 72°C for 7 min. The amplification was performed using T100™ thermal cycler (Bio-Rad, USA). PCR products were analyzed on 2.5% (w/v) MetaPhor™ (Lonza, USA) agarose gel.

Data analysis

The presence and absence of ISAP bands were scored as 1 and 0, respectively. Clustering analysis of ISAP bands was performed using NTSYS-PC software (Rohlf 2009). The SIMQUAL program was used to calculate Jaccard's similarity coefficient and a dendrogram was generated by the mean of the unweighted pair group method with arithmetic average (UPGMA) method.

RESULTS AND DISCUSSION

Analysis of truncated SINE elements originated from Cucumber LINE sequences

One copy of LINE (CsLINE-1) was identified from the cucumber genome (*C. sativus* cv. 9930). CsLINE-1 is a non-autonomous LINE as it contains only the reverse transcriptase (RVT_2) domain and lacks the GAG and AP domains. With the use of CsLINE-1 sequence as a homology search query for blastN analysis, multiple copies of SINEs (CmSINE-1, CmSINE-2, and CsSINE-1) were retrieved from the genomes of melon and cucumber. Sequence synteny analysis in this study allows clear identification of the SINE sequence features when comparing with CsLINE-1. SINE-1 sequences are highly conserved in both cucumber and melon genomes, while CsLINE-1 is found in the cucumber genome only. For CmSINE-2, most of the important retrotransposon domains such as GAG, AP, and RT domains were missing from the element (Figure 1). Detailed analysis of the melon and cucumber genomes found no evidence of a full-length autonomous copy of CsLINE-1 in these genomes. This finding indicates that CsLINE-1 was truncated and the autonomous copy was lost from the melon and cucumber genomes before the divergence of these two genomes.

Two different types of SINE derived from CsLINE-1 were detected in the melon genome (CmSINE-1 and CmSINE-2), whereas only one type (CsSINE-1) was found in the cucumber genome (Figure 1). CsLINE-1, CsSINE-1, CmSINE-1, and CmSINE-2 are 3153, 260, 241, and 1347 bp in length, respectively. All elements contain target site duplication at the 5′ and 3′-end of their sequences. The details of these sequences, including the accession numbers where these elements are located and their positions, are listed in Table 3. An NJ dendrogram was constructed from 131 copies of CsSINE-1 sequences retrieved from *C. sativus* cv. 9930. The NJ dendrogram divided CsSINE-1 sequences into 8 clades, i.e., clades A, A1, A2, B, C, D, D1, and E (Figure 2). The largest clade of CsSINE-1 consists of 30 members (D1), while clades A, A1, A2, B, C, D and E consist of 10, 10, 24, 14, 13, 13 and 11 members, respectively.

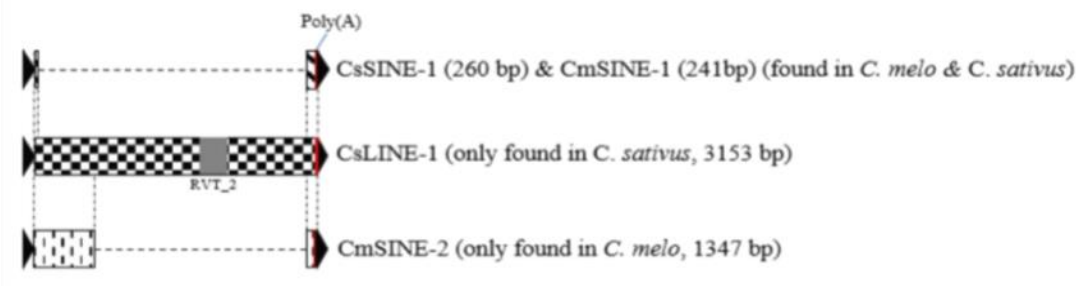

Figure 1. Sequence synteny analysis of cucumber-specific-LINE element (CsLINE-1) and its corresponding SINE elements (CsSINE-1, CmSINE-1, and CmSINE2) retrieved from cucumber and melon genomes. The CsLINE-1 contains target site duplication (arrowheads) at upstream and downstream, 3′ poly A-tails, and the reverse transcriptase domain but lacks other retrotransposon domains.

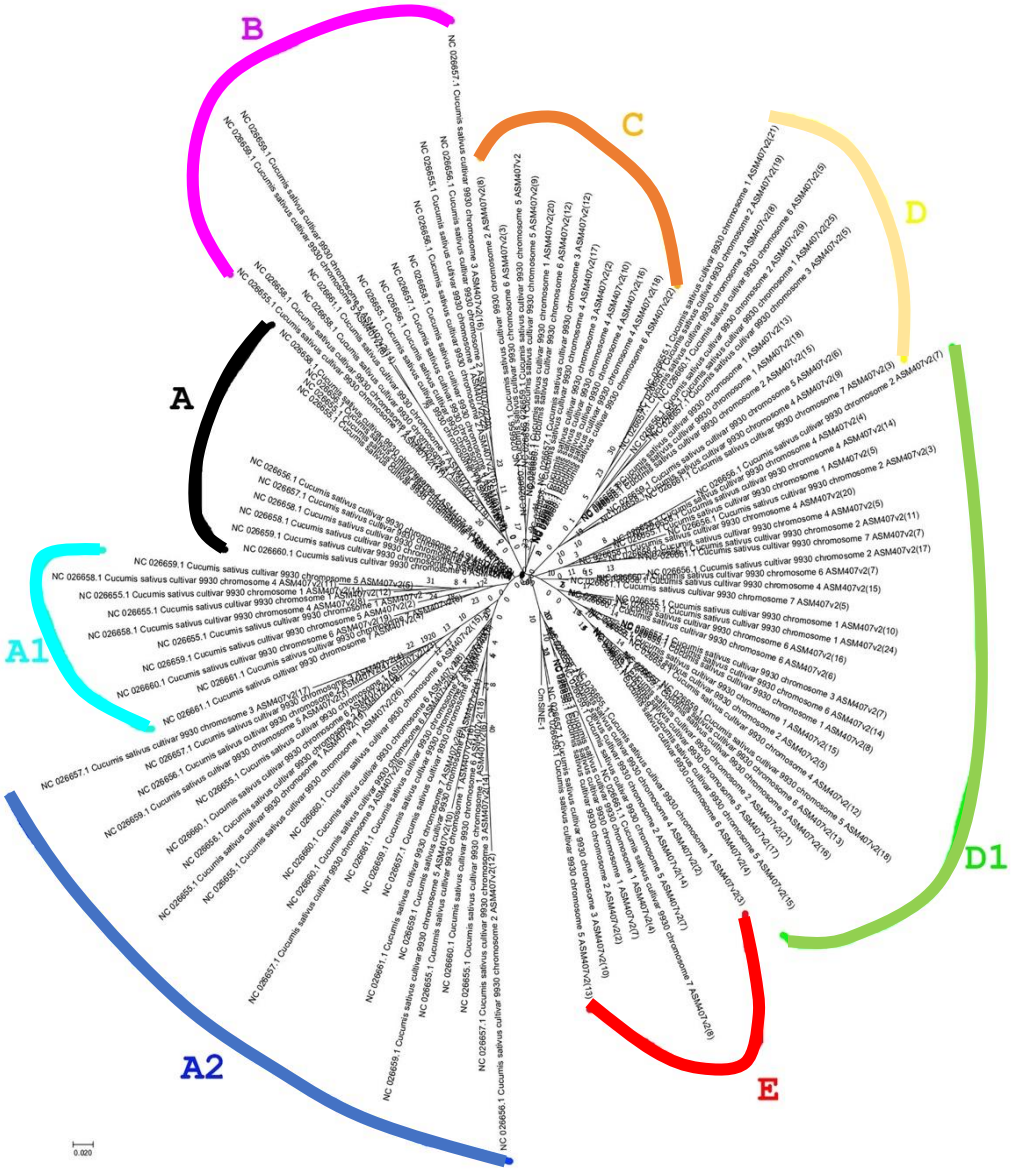

Figure 2. NJ tree of CsSINE-1 elements. The optimal tree with the sum of branch length = 5.38336563 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. The analysis involved 126 nucleotide sequences. All positions containing gaps and missing data were eliminated. The final dataset contained a total of 23 positions. Evolutionary analyses were conducted in MEGA7.

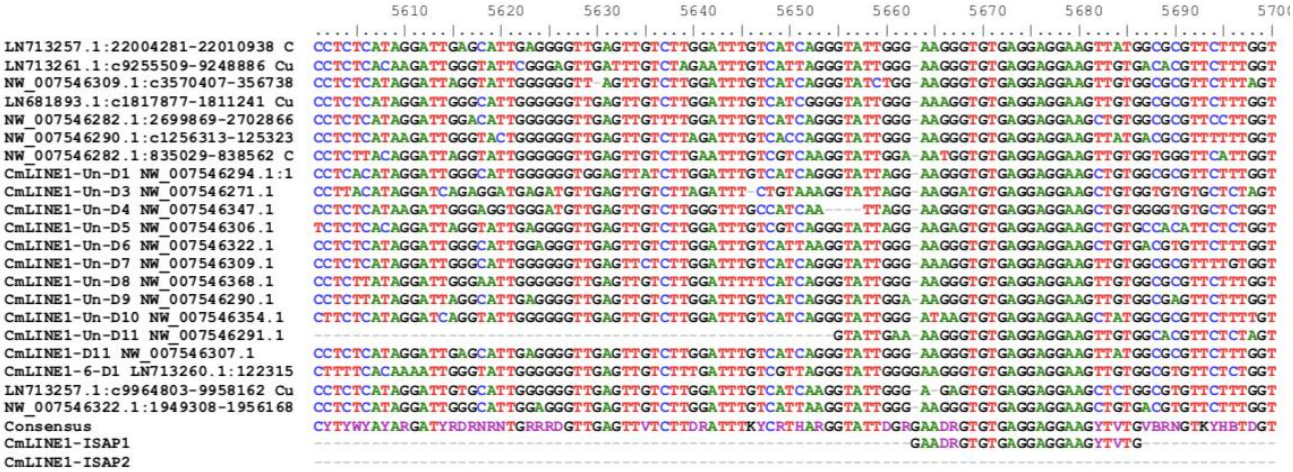

Figure 3. MSA of LINE elements of *C. melo*. CmLINE-1 primers used for ISAP markers were designed from the conserved regions. Only one part of the MSA with one primer site (starts-ends: 5663-5685) is shown here due to the length of MSA, which reaches up to 6245 bp.

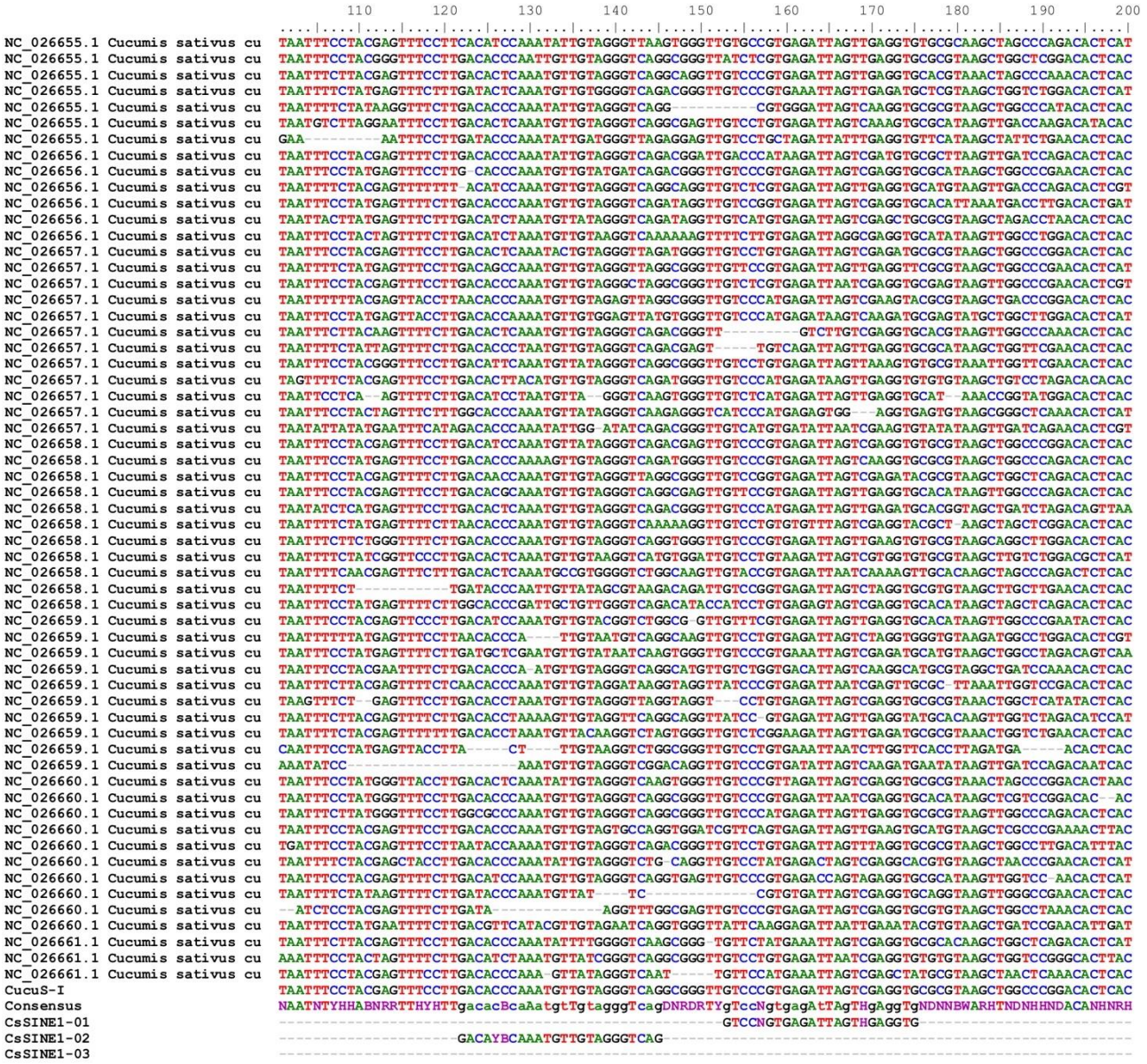

Figure 4. MSA of SINE elements of *C. sativus*. CsSINE-1 primers used for ISAP markers were designed from the conserved regions. The primer site starts from 153 up to 175.

Table 4. Cucumber genes with inserted CsSINE-1

Chr.	Gene	Copy no.
1	Uncharacterized protein LOC101215194	1
	DNA damage-binding protein 1	1
	GPI ethanolamine phosphate transferase 3	1
	Mediator of RNA polymerase II transcription subunit 13	2
	Probable ATP-dependent RNA helicase DHX35	1
	ER membrane protein complex subunit 6	1
	Histone deacetylase 15	1
	Protein PIR	1
	MAG2-interacting protein 2	1
	Translation machinery-associated protein 22	1
	Transcription initiation factor TFIID subunit 2	1
	Cellulose synthase A catalytic subunit 8 [UDP-forming]	1
	E3 SUMO-protein ligase SIZ1	1
	Endoribonuclease Dicer homolog 2	1
2	Nuclear pore complex protein NUP160	1
	Cleavage and polyadenylation specificity factor subunit 1	2
	Uncharacterized protein LOC101217421	1
	Translational activator GCN1	1
	Superkiller viralicidic activity 2-like 2	1
	DEAD-box ATP-dependent RNA helicase 17	1
	WD repeat-containing protein 48	1
	Translation initiation factor eif-2B subunit epsilon	1
	Adagio protein 1	1
	UPF0400 protein C337.03	1
	Gamma carbonic anhydrase 1, mitochondrial	1
	Calcium-transporting atpase 3, endoplasmic reticulum-type	2
	Probable inactive leucine-rich repeat receptor-like protein kinase At3g03770	1
	Polyadenylate-binding protein RBP47	1
3	Importin-11	1
	Methyltransferase-like protein 10	1
	Inactive poly [ADP-ribose] polymerase RCD1-like	1
	Putative ATP-dependent RNA helicase DHX33	1
	Uncharacterized protein LOC101216506	1
	Protein disulfide-isomerase 5-4-like	1
	Diacylglycerol O-acyltransferase 2	1
	Dual-specificity protein phosphatase 12-like	1
	Elongation factor Ts, mitochondrial	1
	Protein VASCULAR ASSOCIATED DEATH 1, chloroplastic	1
	Spastin	1
	Uncharacterized protein At1g04910	1
	Uncharacterized protein LOC101209700	1
	Uncharacterized protein LOC101220986	1
	TOM1-like protein 2	1
4	Small G protein signaling modulator 1	1
	Uncharacterized protein LOC101218523	1
	Sm-like protein LSM4	1
	Putative callose synthase 8	1
	Protein NLP9	1
	Probable sphingolipid transporter spinster homolog 2	1
	AP-4 complex subunit epsilon	1
	Golgin subfamily B member 1	1
	Restin homolog	1
	Phosphoglycerate mutase-like protein 1	1
	Probable cytosolic oligopeptidase A	1
	UDP-N-acetylglucosamine-dolichyl phosphate N acetylglucosaminephosphotransferase	1
	MADS-box transcription factor 23	1
	FH protein interacting protein FIP2	1
	Replication factor C subunit 3	1
	Uncharacterized protein LOC101208689	1
	Phosphoglucan, water dikinase, chloroplastic	1
5	Transcription factor GTE1	1
	Anaphase-promoting complex subunit 1	1
	DNA repair protein complementing XP-C cells homolog	1
	Uncharacterized protein LOC101212003	1
	Structural maintenance of chromosomes protein 4	1
	Protein decapping 5	1
	Dolichyl phosphate beta-glucosyltransferase-like	1
	Putative nuclear matrix constituent protein 1-like protein	1
	Signal peptide peptidase-like 2	1
	Uncharacterized protein LOC101212864	1
	DNA-directed RNA polymerase V subunit 1	1
	GDP-Man:Man(3)glcnac(2)-PP-Dol alpha-1,2-mannosyltransferase	1
6	50S ribosomal protein L20, chloroplastic	1
	DNA-directed RNA polymerase I subunit RPA2	1
	COP9 signalosome complex subunit 3	1
	DNA polymerase epsilon catalytic subunit A-like	1
	Signal peptide peptidase-like 5	2
	Uncharacterized protein LOC101209603	1
	Ankyrin repeat domain-containing protein 2	1
	Uncharacterized protein LOC101204078	1
	Myosin-6-like	1
	Probable plastidic glucose transporter 1	1
	Mediator of RNA polymerase II transcription subunit 33A	1
	Nardilysin	1
	Uncharacterized protein LOC101216170	1
	DNA gyrase subunit A, chloroplastic/mitochondrial	1
7	GPI transamidase component PIG-T	1
	Glyoxylate/hydroxypyruvate reductase HPR3	2
	DEAD-box ATP-dependent RNA helicase 39	1
	Protein FAM188A	1
	Exosome complex exonuclease RRP44	1
	Phosphatidylserine decarboxylase proenzyme 2	1
	HUA2-like protein 3	3
	Brefeldin A-inhibited guanine nucleotide-exchange protein 1	1
	Ubiquitin thioesterase OTU1	1
	E3 ubiquitin-protein ligase PRT1	1
	Nuclear pore complex protein NUP107	1
	Methylcrotonoyl-coa carboxylase subunit alpha, mitochondrial	1
	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	1
	Probable galacturonosyltransferase 6	1
	TBC1 domain family member 15-like	1
	Pyrophosphate-energized membrane proton pump 2	1
	Nuclear pore anchor	1
	Protein-tyrosine-phosphatase PTP1	1
	Dol-P-Man:Man(5)glcnac(2)-PP-Dol alpha-1,3-mannosyltransferase	1

Protein transport protein SFT2-like	1
Piezo-type mechanosensitive ion channel homolog	1
V-type proton atpase subunit a3-like	1
Calcium-transporting atpase 9, plasma membrane-type	2
Protein transport protein SFT2-like	1
Uncharacterized protein LOC101216857	1
Inositol 1,3,4-trisphosphate 5/6-kinase 4	1
Uncharacterized protein LOC101209189	1
Nitrogen regulatory protein P-II homolog	1
NAD-dependent protein deacetylase SRT1	1
Uncharacterized protein LOC101216108	1
Serine/threonine-protein kinase TOR	1
Pentatricopeptide repeat-containing protein At2g31400, chloroplastic	1
Pre-mrna-processing protein 40C	1
Probable protein phosphatase 2C 60	1
Uncharacterized protein LOC101208216	2
Epidermal growth factor receptor substrate 15-like 1	1
Uncharacterized protein LOC101215677	1
Alpha-1,3-mannosyl-glycoprotein 2-beta-n-acetylglucosaminyltransferase	1
Transcription initiation factor TFIID subunit 1	2
Cycloartenol synthase	1
DNA polymerase kappa	1
LOW QUALITY PROTEIN: phospholipase A-2-activating protein	1
Exocyst complex component SEC5A	1
30S ribosomal protein 2, chloroplastic	1
Dynamin-like protein ARC5	1
Phosphatidylserine decarboxylase proenzyme 2	1
BTB/POZ domain-containing protein At4g08455	2
Uncharacterized protein LOC101205573	2
Ceramide-1-phosphate transfer protein	1
Probable indole-3-pyruvate monooxygenase YUCCA10	1
Sacsin	1
N-alpha-acetyltransferase 15, nata auxiliary subunit	1
Indole-3-glycerol phosphate synthase, chloroplastic	1
Calmodulin-binding transcription activator 2	1

Note: Chr.: Chromosome

Table 5. Summary of ISAP marker analysis

Primer name	Ta (°C)	Total amplified bands	Band size (bp)	Total polymorphic bands	Degree of polymorphism (%)
CmLINE_ISAP1	59.4	4	300-2050	4	100
CsSINE1-01	53.8	19	270-1600	19	100
CsSINE1-02	56.1	8	250-1200	7	87.5
CsSINE1-03	60.4	10	260-1300	14	100
SolS-IIIaF	49.5	5	300-1600	3	60.0
SolS-IVR	53.0	8	400-2500	8	100
CsSINE1-01/CsSINE1-02	60.0	15	216-1900	14	93.0
CsSINE1-01/CsSINE1-03	45.0	18	240-1400	18	100
CsSINE1-02/CsSINE1-03	45.0	9	220-620	9	100

CsSINE-1 was found to insert into many gene loci in the cucumber genome (Table 4). This result indicates that SINE elements are dispersed all over 7 pairs of cucumber chromosomes. Sixty and 21 copies of CsSINE-1 and CmLINE-1 were retrieved from the cucumber and melon genomes, respectively, for MSA analysis. For ISAP marker development, only highly conserved DNA sequences were used for MSA (Figures 3 and 4).

Amplification of genomic DNA with ISAP marker

The ISAP primers were designed from the conserved regions of CsSINE-1 and CmLINE-1 (Figure 3 and Figure 4). Six out of seven ISAP primers (single-primer PCR) and three combinations of CsSINE-1 primers generated multiple PCR band patterns when applied on different *Cucumis* accessions. Single-primer PCR of ISAP primers generally produced 4 to 19 PCR bands, while the combination of CsSINE-1 primers produced 9 to 18 PCR bands. The highest number of PCR bands (19) was generated by a single CsSINE1-01 primer, while the highest number of amplified bands from the ISAP primer combination was achieved by CsSINE1-01/CsSINE1-03 (Figure 5a). All ISAP primers designed from *Cucumis* species showed higher degrees of polymorphism (87.5%-100%) than potato ISAP primers (60%-100%) (Table 5). This finding indicates that *Cucumis* ISAP primers designed in this study have better discrimination power than ISAP primers from Seibt et al. (2012).

The transferability of *Cucumis* ISAP markers was demonstrated in *Coleus* species, an ornamental plant that is known for its attractive leaf feature. High degree of polymorphisms was observed among the *Coleus* accessions, and the amplicon sizes of *Cucumis* ISAP range from 150 bp to 2000 bp (Figure 6a). Clustering analysis using the UPGMA method showed that the *Coleus* accessions are clustered into four clusters. Cluster 1 consists of both pink and green leaf accessions (Col-K01, Col-N03, Col-O01, Col-M02, Col-N02, Col-N01, Col-L01, and Col-L02). The second cluster consists of species with pink leaf color (Col-K03 and Col-O02). The third and fourth clusters consist of only one member, namely, Col-M01 and Col-K02, respectively (Figure 6b).

Clustering analysis of ISAP marker

Clustering analysis of ISAP marker was performed on 21 accessions of *C. melo* to investigate their genetic diversity. Pitrat (2008) divided *Cucumis melo* into two subspecies, i.e. *melo* and *agrestis*. The melon accessions

and the distantly related wild species *Cucumis metuliferus* were also included in the analysis. The UPGMA dendrogram of ISAP marker separated *C. metuliferus* (US143) from all *C. melo* accessions and provided good discrimination power, with the similarity coefficient ranging from 0.09 to 1.00 (Figure 5b). All 22 melon accessions were grouped into three major clusters. The largest cluster consists of the subcluster of the *C. melo* group (i.e., Golden Langkawi, Melani, Japonica, NI19, P107, Dainty, Madesta, Haru, Silver Light, Rock Sonya, US176) and the subcluster of the *C. agrestis* group (i.e., US128, US205, and US 368). However, two accessions of the *C. agrestis* group (US 340 and P34) were clustered together with members of the *C. melo* subcluster. The second cluster consists of Eksis, US58, Baladewa, and US54 originated from the *C. melo* group. The third cluster consists of only *C. metuliferus* (US143). These results suggest that ISAP is a fairly good marker in discriminating *Cucumis* accessions.

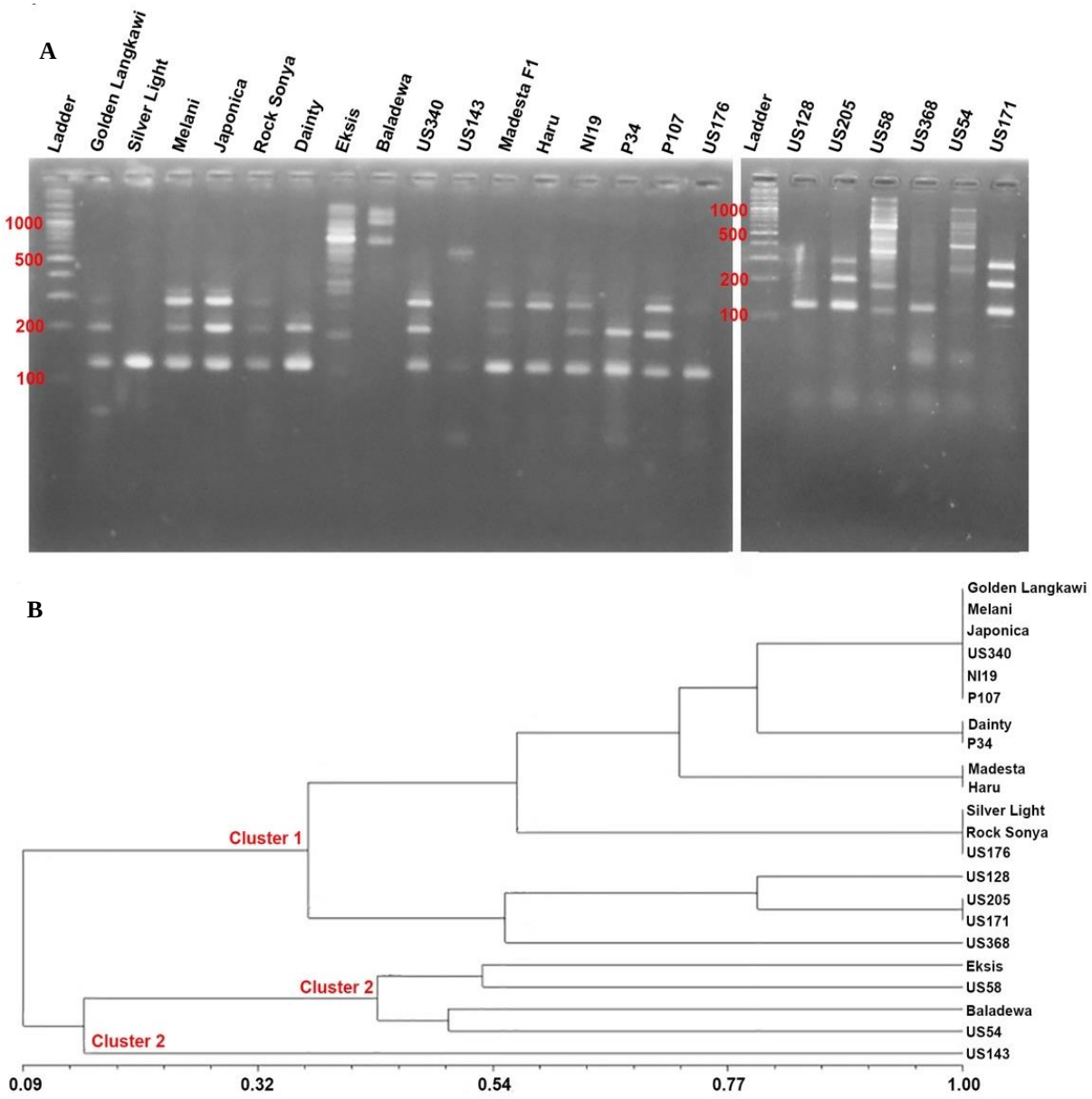

Figure 5. Genomic DNA amplification and cluster analysis of 22 melon accessions. A. ISAP banding patterns generated by the primer combination CsSINE1-01/CsSINE1-03. B. Dendrogram was constructed with the UPGMA clustering method. The scale beneath the dendrogram depicts the genetic similarity coefficient.

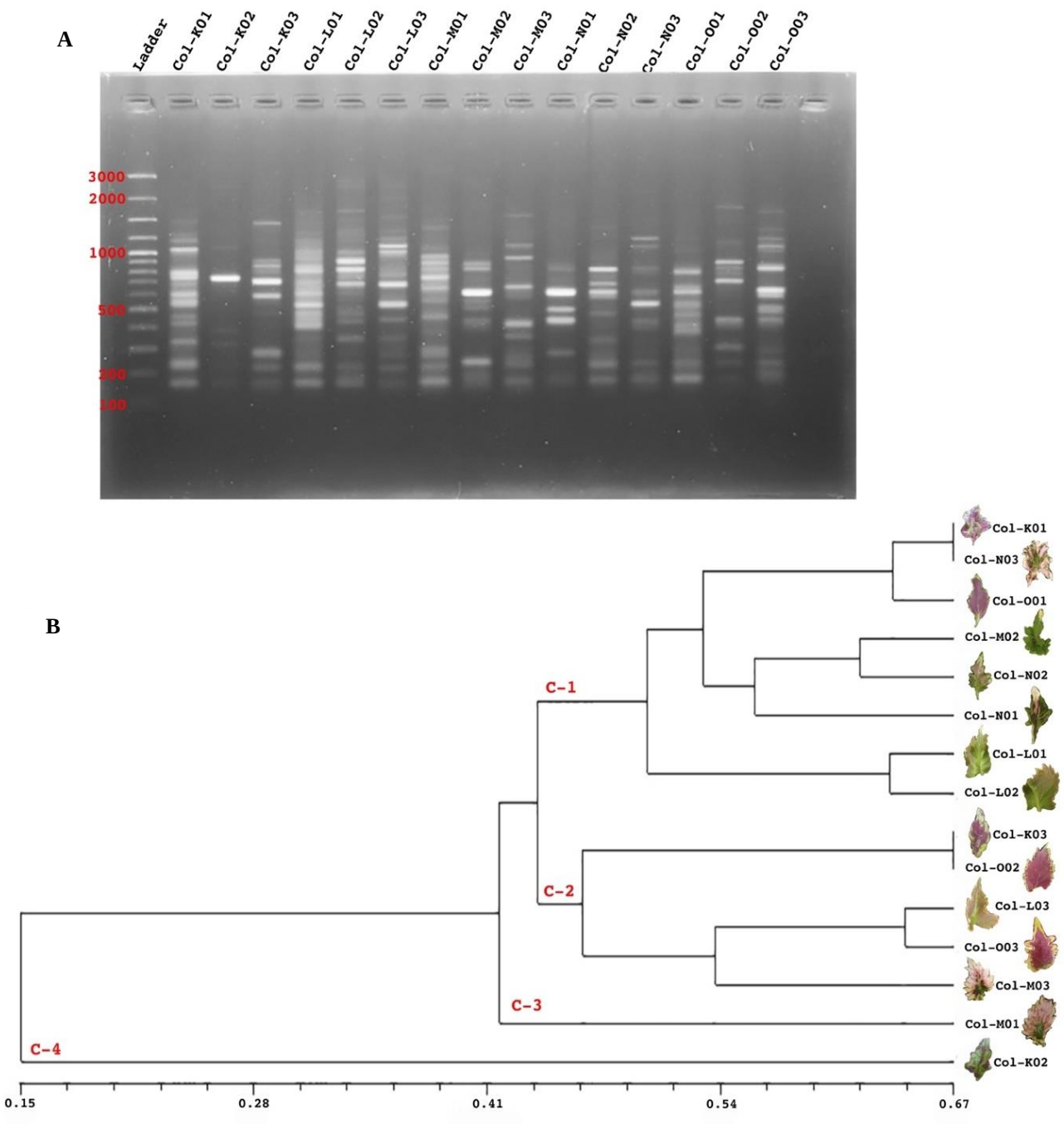

Figure 6. Genomic DNA amplification with ISAP marker and cluster analysis of *Coleus* accessions. A. ISAP banding patterns of *Coleus* accessions generated by CsSINE1-01 primer. B. Dendrogram constructed with the UPGMA clustering method among 15 *Coleus* accessions. The scale beneath the dendrogram depicts the genetic similarity coefficient. C-1, C-2, C-3, C-4 depict cluster 1, 2, 3, 4, respectively.

Discussion

Both LINE and SINE elements were found in the cucumber (*C. sativus*) genome (CsLINE-1 and CsSINE-1), whereas only SINE element (CmSINE-1 and CmSINE-2) was found in the melon (*C. melo*) genome. Multiple copies of these elements were detected in both genomes through homology search analysis. This finding indicates that both SINE and LINE elements are abundant in the melon and cucumber genomes. Non-LTR retrotransposons, particularly LINEs and SINEs, are highly abundant in plant genome, and they have been identified in *C. melo* (Rodríguez-Moreno et al. 2011; Garcia-Mas et al. 2012; Setiawan et al. 2020b); *Brassica rapa, B. napus, B. nigra, B. juncea, B. oleracea, B. carinata* (Nouroz et al. 2017, 2018); *Solananum tuberosum, Brachypodium distachyon, Populus trichocarpa, Vitis vinifera, Nuphar advena, Medicago truncatula* (Wenke et al. 2011), and *Lotus japonicus* (Gadzalski and Sakowicz 2011). Sequence

synteny analysis revealed that CmSINE-1, CmSINE-2, and CsSINE-1 originated from CsLINE-1 (Figure 1). This result indicates that SINE elements derived from CsLINE-1 are highly conserved in *C. melo* and *C. sativus*. Weiner (2002) reported that SINEs are similar to LINEs but have a shorter sequence length (100-500 bp), are almost certainly dependent on the activity of LINE RT/EN for their retrotranspositions, and contain polymerase III as their internal promoter instead of polymerase II. SINE is a type of non-autonomous retrotransposon and can increase its copy number through retrotransposition with the help of LINE-encoded proteins where the proteins recognize the 3′ tail of SINE sequences, which is similar to LINE (Liu et al. 2020).

The genome distribution of SINEs in grass plants, such as *Oryza sativa*, *Triticum aestivum*, *Triticum urartu*, *Aegilops tauschii*, *Phyllostachys edulis*, *Setaria italica*, *Setaria viridis*, *Panicum virgatum*, *Dichanthelium oligosanthes*, *Sorghum bicolor*, and *Zea mays* showed high-level enrichment in gene-rich regions and at the end of chromosome arms (Mao and Wang 2017). In the potato genome, the chromosomal distributions of SINE elements are scattered in all potato chromosomes (Seibt et al. 2012, 2016). In melon, SINE elements are abundant in copy number (Zhang et al. 2019; Castanera et al. 2020; Yang et al. 2020) and are mostly located in the heterochromatic region in the chromosomes (Setiawan et al. 2020b). In addition, SINEs together with other transposable elements are the major component of *Citrullus lanatus*, *Cucurbita argyrosperma*, *C. pepo*, *C. maxima*, and *C. moschata* genomes (Guo et al. 2013; Sun et al. 2017; Montero-Pau et al. 2018; Barrera-Redondo et al. 2019). These findings suggest that SINE is highly abundant in plants and is a suitable source for the development of molecular markers in plants.

Our results showed that CsSINE-1 elements are inserted into multiple gene loci in the cucumber genome (Table 4). Non-LTR retrotransposons have been reported to insert into gene loci and are involved in the genome evolution of *Cucumis* species (Setiawan et al. 2020b) and other plant species such as sunflower (*Helianthus annuus*) (Nagaki et al. 2015) and banana (*Musa* spp.) (Čížková et al. 2013). Castanera et al. (2020) reported that transposable elements can have a potential impact on melon genes and affect their phenotype. This result suggests that SINE elements may play an important role in cucumber cell development, genome evolution, and chromosome organization. Further studies are required to investigate the genome evolution and the chromosomal distribution of SINE elements in cucumber accessions together with their closely and/or distantly related species.

The ISAP marker system is designed based on the principle that PCR amplification of sequence regions between two copies of SINE elements using SINE specific primers will generate a multiple banding pattern with a high level of polymorphism (Wenke et al. 2015). High levels of polymorphism were observed when *Cucumis* ISAP markers were used for genetic diversity study in 21 accessions of *C. melo* (Figure 5 and Table 5). The clustering analysis showed that melon accessions are separated into three clusters (Figure 5b). The *Cucumis* ISAP marker also allows clear discrimination among *Cucumis* accessions, particularly in distinguishing the cultivated melon accessions from the wild accessions. Similar findings were observed when ISAP markers were used for genotyping in potato (Seibt et al. 2012; Wenke et al. 2015). The transferability of *Cucumis* ISAP marker to other plant species was demonstrated in this study when one of the ISAP markers was used in *Coleus* species (Figure 6). *Coleus* spp. are ornamental plants and show high variation in their leaf colors and shapes (Shoaib et al. 2020). In addition, the transposable element has been reported as the major driver for different color expressions in ornamental plants (Hsu et al. 2019). The highly polymorphic ISAP banding patterns observed in different *Coleus* accessions indicate that *Cucumis* ISAP marker can be applied for genetic diversity study in *Coleus* species. In addition, ISAP primers described in Seibt et al. (2012) from potatoes were used in this study and showed a high level of polymorphism among *Cucumis* accessions (Table 5). This finding further supported the transferable nature of ISAP markers in genotyping of plant species. This result is also consistent with the transferable nature of barley IRAP markers in *Musa* species (Teo et al. 2005). Thus, our studies indicate that the SINE-based markers can be applied directly across different plant species. Further analysis is required to determine the sequence of SINE and/or LINE in *Coleus* species to confirm the *Cucumis* ISAP specificity in this species.

In conclusion, the SINE family identified from cucumber and melon genomes can be designed as ISAP markers and can be used for genotyping of *Cucumis* accessions. This marker can also be applied for genotyping of *Coleus* accessions. This study demonstrated a simple PCR-based molecular marker system by using a transposable element, namely, the SINE elements.

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