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Development and Application of Cleaved Amplified Polymorphic Sequence Markers for Classifying Ginger Using Reference Sequencing

Ji-Nam Kang[1)], So-Hee Yang[1)], and SIMYUNG LEE[1)*]

[1)] Genomics Division, National Institute of Agricultural Sciences, 370 Nongsaengmyeong-ro, Jeonju-si, Jeollabuk-do, 54874, Republic of Korea

[*](Corresponding author): E-mail: tataby@korea.kr Tel: +82-63-238-4560

Abstract

Background Medicinal plant ginger (*Zingiber officinale*) is mainly used as a spice and medicinal substance in East Asia region including Korea, Japan, and China. Ginger contains abundant secondary metabolites such as oleoresin and shogaol. However, because ginger mainly grows vegetatively by the infertility of flowers, research on ginger cultivar development has been limited. Furthermore, the available molecular markers for distinguishing ginger cultivars were unknown, which has become a vulnerability in protecting indigenous ginger cultivars in Korea. In this study, we developed cleaved amplified polymorphic sequence (CAPS) markers to protect domestic indigenous ginger and established a country of origin verification system for commercially available ginger samples. The developed markers can be applied as an efficient tool for protecting indigenous ginger in Korea.

Mathods and Results A total of 53 ginger resources were collected from farms located in Bongdong-eup, Wanju-gun, Jeollabuk-do, Republic of Korea. Genomic DNA was extracted from rhizome of 53 ginger samples and eight DNA samples were selected for Reference sequencing (Re-Seq). The Re-Seq data were mapped to the ginger reference genome, and CAPS positions were found through variant calling. As a result, *Bgl*II, *Dra*I, and *Cla*I-based CAPS markers were developed. These CAPS markers clearly distinguished between “Bongdong ginger (Bg)” and “Chinese imported ginger (Cg)”. Furthermore, we developed a *Cla*I-based TaqMan real-time PCR system that can distinguish ginger cultivars, which was effectively applied to verify the content and country of origin of commercially available gingers.

Conclusion The developed CAPS markers can distinguish between the Bg and Cg cultivars. Furthermore, we established a TaqMan real-time PCR system for *Cla*I-based CAPS marker, which was effectively applied to verify the content and country of origin for commercially available gingers. This work is the first to report the CAPS marker development in ginger and therefore provides important information for the breeding and conservation of ginger.

Materials and Methods

Plant Materials: Ginger samples were collected in Bongdong-eup, Wanju-gun, Jeollabuk-do, Republic of Korea.

Table 1. Collected information of ginger individuals.

Sample ID	Number	Cultivation region	Collection method
Bg_0	Bg_0_1-3	BONGDONG	Acquired from Wanju-Gun Aricultural Technology Center
Bg_1	Bg_1_1-5	BONGDONG	Direct collection
Bg_2	Bg_2_1-5	BONGDONG	Direct collection
Bg_3	Bg_3_1-5	BONGDONG	Direct collection
Bg_4	Bg_4_1-5	BONGDONG	Direct collection
Cg_6	Cg_6_1-5	Chinese imported	Direct collection
Cg_7	Cg_7_1-5	Chinese imported	Direct collection
Cg_8	Cg_8_1-5	Chinese imported	Direct collection
Cg_9	Cg_9_1-5	Chinese imported	Direct collection

Production of Sequencing Data: Illumina NovaSeq platform (Macrogen, Seoul, Korea).
Mapping: High-quality reads were mapped onto the ginger reference genome (ver. Zo_v1.1) using the mem option of the Burrows–Wheeler Aligner program (ver. 0.7.17).
Variant filtering and annotation: Variation calling was performed using the HaplotypeCaller module of the Genome Analysis Toolkit (GATK, ver. 4.2). The annotation of identified SNPs and InDels was performed using SnpEff software (ver. 5.2e).
CAPS primer design: For CAPS primer design, a 500-bp flanking sequence from the variant site was extracted from the reference genome sequence. PCR primer sets were designed using CAPS-finder.
TaqMan probe design: TaqMan real-time PCR primer sets and probes were specifically designed for SNP validation of *Cla*I-based CAPS using Primer Express (Thermo Fisher Scientific).

Results

Table 2. Polymorphism prediction of amplicons by CAPS digestion.

Reference genome position	Bg-CAPS	Cg-CAPS	Restriction enzyme		Predicted PCR product size (bp)	Amplicon cutting size			
						Bg-CAPS		Cg-CAPS	
			Bg-CAPS	Cg-CAPS		1	2	1	2
NC_055986.1 _153787420	C	T	*Bgl*II		470	137	333		
NC_055987.1 _85483445	AC	A		*Dra*I	570			277	293
NC_055989.1 _143739477	G	A	*Cla*I		579	418	161		

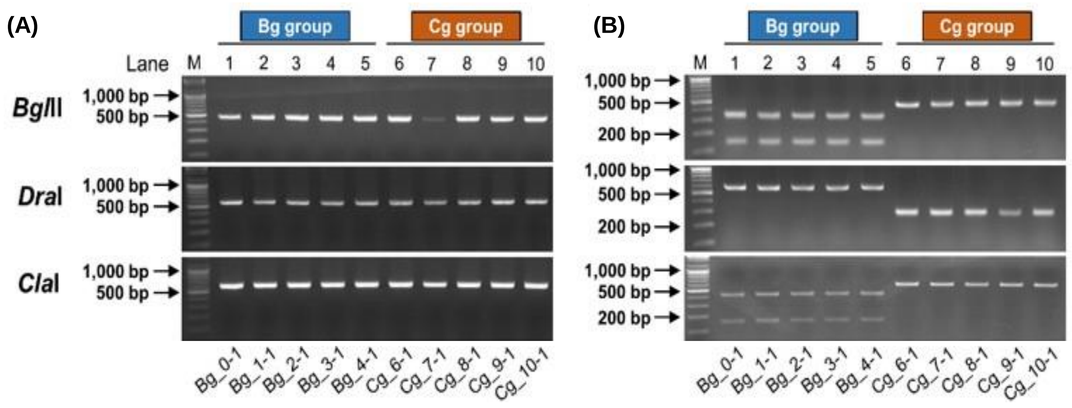

Figure 1. Polymorphism verification of CAPS candidates. (A) Confirmation of amplicon. **(B)** CAPS cleavage confirmation of each amplicon by restriction enzymes. Lanes 1 to 5 are Bg cultivar groups and lanes 6 to 10 are Cg cultivar groups.

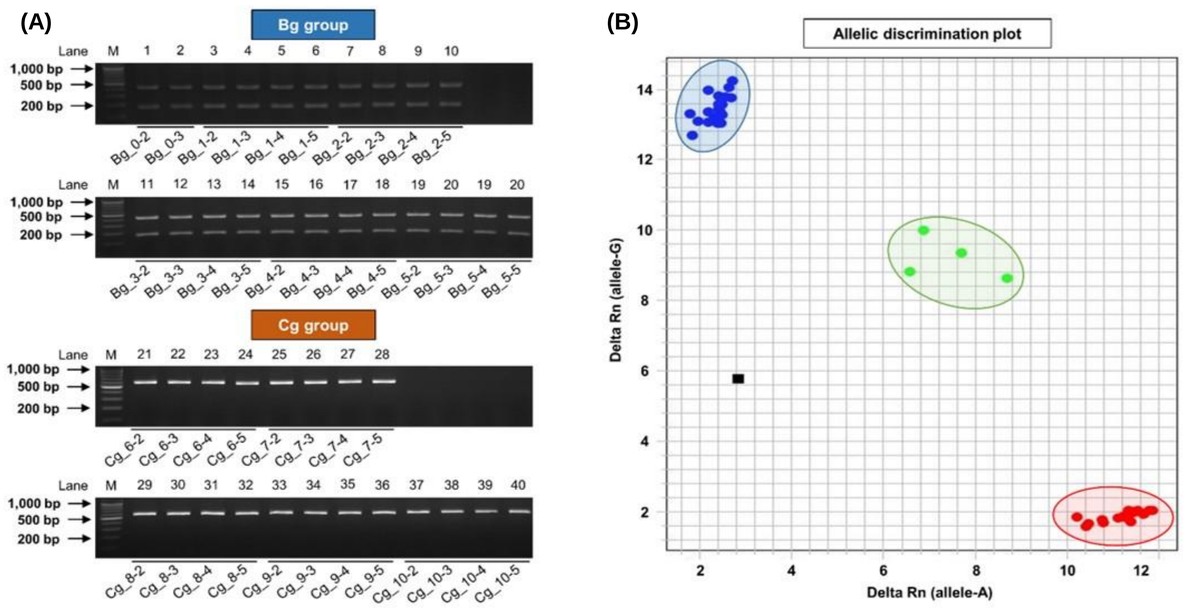

Figure 2. TaqMan real-time PCR application of *Cla*I-based CAPS markers. (A) Electrophoresis results of *Cla*I-based CAPS markers for Bg and Cg cultivars collected in the “Bongdong” region. Lanes 1 to 20 are Bg cultivars and lanes 21 to 40 are Cg cultivars. **(B)** Allelic discrimination plot. A total of 44 ginger DNA samples were used for TaqMan real-time PCR. The allele-G of 20 Bg cultivars was detected by FAM fluorescent dye linked-probes (grouped in blue). The allele-A of 20 Cg cultivars was detected by VIC fluorescent dye linked-probes (grouped as red). Green group represents DNA samples mixed with Bg and Cg cultivars, which were artificially prepared for heterozygous type detection. Black square indicates the negative control. Allelic discrimination plots were generated based on Delta-Rn values.

Table 3. Information of collected commercial ginger powder in the market.

Sample No.	Manufacturer	Product type	Country of origin	Content
2	A	Powder	Domestic (Korea)	Ginger 100%
3	B	Powder	Domestic (Korea)	Ginger 100%
4	C	Powder	Domestic (Korea)	Ginger 100%
5	D	Powder	Domestic (Korea)	Ginger 100%
6	E	Powder	Domestic (Korea)	Ginger 100%
8	F	Powder	Domestic (Korea)	Ginger 100%
9	G	Powder	Domestic (Korea)	Ginger 100%
14	H	Powder	Domestic (Korea)	Ginger 100%
15	I	Powder	Domestic (Korea)	Ginger 100%

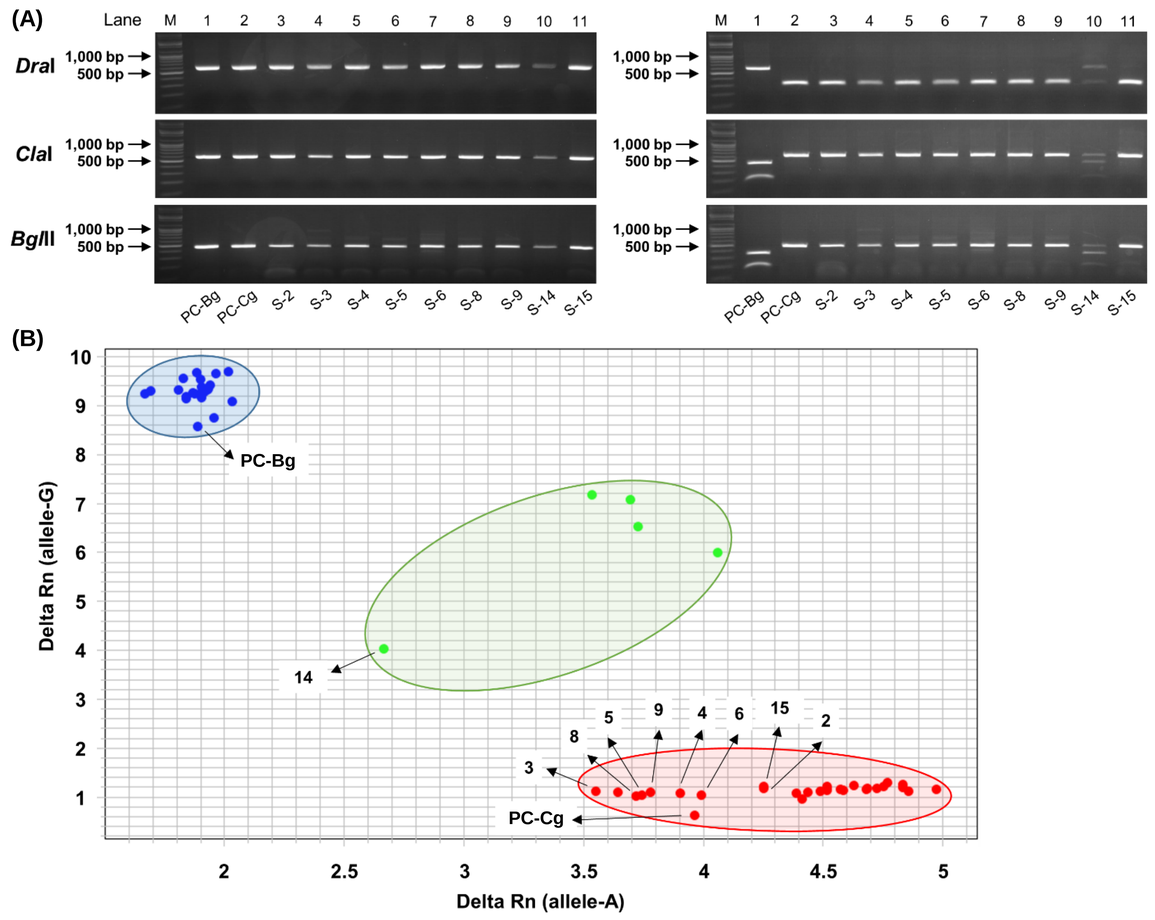

Figure 3. Application of CAPS markers to commercial ginger samples. (A) Electrophoresis results of CAPS markers for commercial ginger samples. Lanes 1 to 2 are positive control of Bg and Cg cultivars. Lanes 3 to 11 are commercial ginger samples. **(B)** Allelic discrimination plot. A total of 9 commercial ginger samples were used in *Cla*I-based TaqMan real-time PCR verification along with positive Bg and Cg samples. The allele-G of Bg cultivars was detected by FAM fluorescent dye linked-probes (grouped in blue). The allele-A of Cg cultivars was detected by VIC fluorescent dye linked-probes (grouped as red). Green group represents DNA samples mixed with Bg and Cg cultivars, which were artificially prepared for heterozygous type detection. Allelic discrimination plots were generated based on Delta-Rn values.

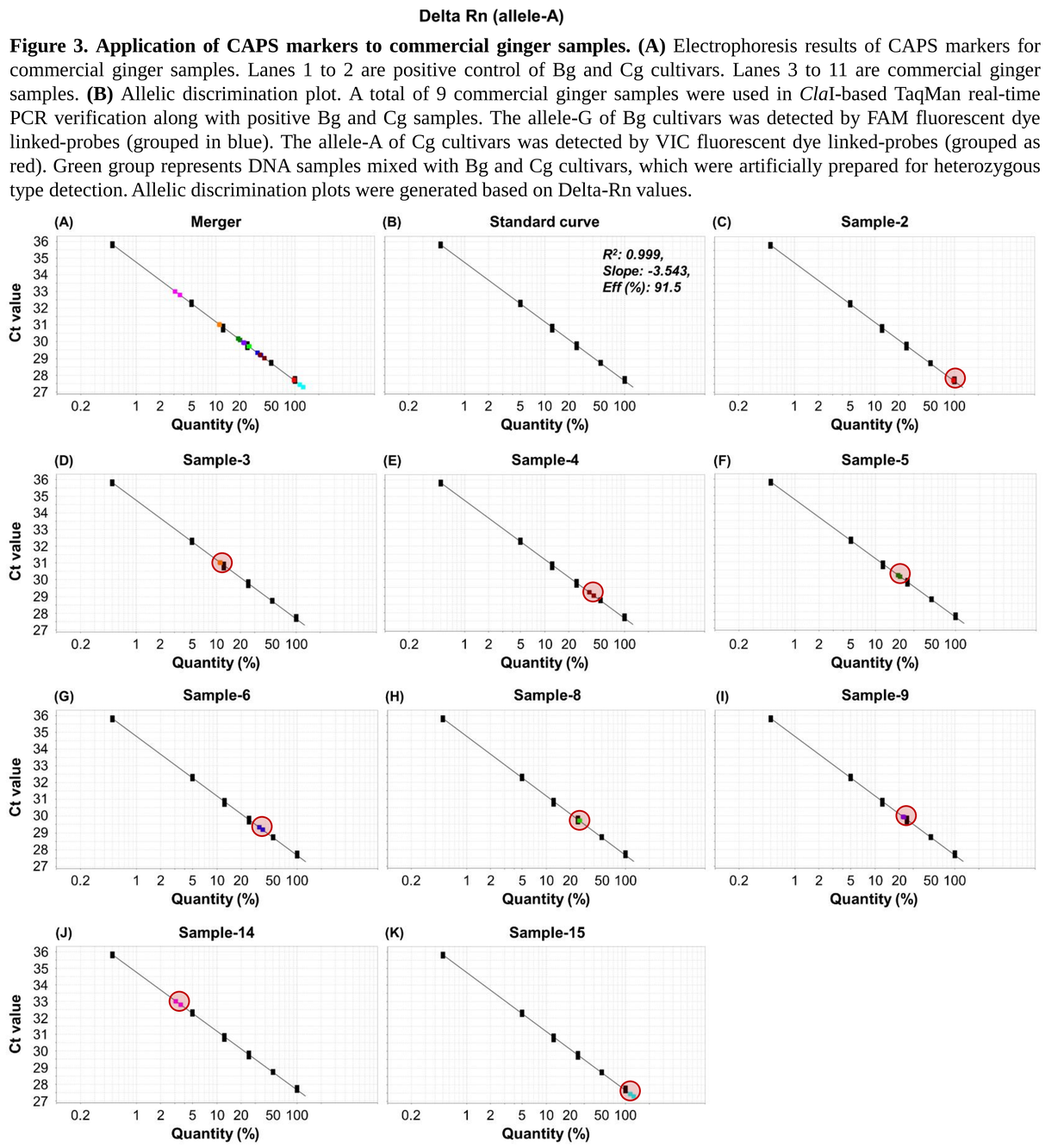

Figure 4. Measurement of Cg content for nine commercially available ginger samples. The Cg content of nine commercial ginger samples were caculated using *Cla*I-based TaqMan real-time PCR system, which was detected allele-A in Cg cultivars by VIC fluorescent dye linked-probes. Stepwise diluted Cg DNA sample was used to create a standard curve **(B)**, and PCR results of nine commercially available ginger samples are substituted for this **(A-K)**. The red circle represents the Cg content measured in commercially available ginger samples.

References

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