

*Full Length Research Paper*

# A co-dominant molecular marker linked to the monoecious gene *CmACS-7* derived from gene sequence in *Cucumis melo* L.

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**This study aimed to find a molecular marker linked to the sex determination gene *CmACS-7* based on the sequence publicly available in GenBank (accession number EU791279). Four pairs of primers were designed to clone the *CmACS-7* gene on the monoecious parental line 'RH107' and the andromonoecious parental line '76-09'. Analysis of the fragments' sequences and the publicly available sequences showed homology of 99% and 98%, respectively. According to the insertion/deletion loci between the two parental lines, a pair of specific primers TI-L/TI-R was designed to assay the polymorphism in the parents and F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub> populations and additional 20 accessions. The specific fragment amplified with primers TI-L/TI-R, was identified as a co-dominant molecular marker co-segregated with the sexual genotype in melon, named C1A/C1a, and could be used in marker assistant selection in melon breeding.**

**Key words:** melon, sexual, *CmACS-7*, co-dominant, molecular marker.

## INTRODUCTION

Melon (*Cucumis melo* L. 2n=2x=24) is an important commercial crop grown in temperate and tropical regions all over the world. Cucurbits are well known for their sexual diversity. Like other members of the *Cucurbitaceae*, melons produce bisexual and unisexual flowers, leading to a diversity of sex phenotypes due to the different flower combinations. Previous research showed that the genetic control of sex determination in melon depends on three genes, *a* (andromonoecious), *g* (gynomonoecious), and *M* (maleness), which combine to generate the different sexual types (Rosa, 1928; Poole and Grimball, 1939; Keningsbuch and Cohen, 1990). Among these, monoecious (*A-GG*) and andromonoecious (*aaGG*) types separate female flowers and hermaphrodites, respectively, except for male flowers that occur on each plant. The hermaphrodite type (*aagg*) is homozygous for *a* and *g* recessive alleles, which produce hermaphrodite flowers only, while gynoecy type (*Aagg*) generates only female flowers and seems to be stabilized

by mm (Keningsbuch and Cohen, 1990; Noguera et al., 2005).

Many traditional melon cultivars are andromonoecious, but are being replaced by new hybrid varieties that tend to be monoecious because of the agronomic advantages this provides, as parental lines do not require hand emasculation to produce F<sub>1</sub> hybrids and thus have gradually become an important goal for melon breeding. Being monoecious also provides control of pollination, seed production and fruit quality (Keningsbuch and Cohen, 1990; Pe'rin et al., 2002b; Noguera et al., 2005). It is easy to transfer the *A* gene from monoecy to andromonoecy with crossing and backcrossing, but it is difficult to get rid of linkage-drag, for example, the fruit shape or the size of bottom scar (Noguera et al., 2005), leading to new varieties differing from the breeding objective. Moreover, breeding is slow and time-consuming when selection is based on phenotypic traits. The use of molecular markers closely linked to the *A* gene make tracing the transferred gene in such recombinant individuals in earlier backcross generations more efficient (Wu et al., 2000; Noguera et al., 2005).

Several significant molecular markers have been

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**Table 1.** Sequences of primers designed and used in the present study.

Primers	Primer sequences 5'—3'	Amplified regions (bp)	Fragment size (bp)
1-L	AATAGAGGTGCCCATTTGTAAG	First exon –169 to 334	482
1-R	ATAATTCCCTTCTCCCTCACA		
2-L	TGGAAAGCGTATGATGAAGAT	Second exon 99–875	775
2-R	TTGGATTTTGAAGAATTTGGTT		
3-L	CATTACCAAGGATAATTGCAACT	Third exon 950–1640	690
3-R	CGACAACATGGACGCTAAAA		
4-L	GCAGCTACAAAAACGCCG	Third exon 1455–2264	809
4-R	GCTAAAAGCAAGAAAACAAGGAT		
TI-L	AACGGATGAAGAAGGAAAACG	3'UTR of the third exon 1978–2163	186
TI-R	ATATTGGGCAGTGTCCACACA		

Polymorphic sites were positioned relative to the first nucleotide of the start codon ATG in 'PI124112' reported by Boualem et al. (2008).

reported in early exploration in this field. Early on, a partial linkage map of melon was constructed from a cross between 'PI414723' and 'Dulce', and a map spanning 14 linkage groups was produced in which the *A/a* locus was located to molecular linkage group 4 (Danin-Poleg et al., 2002). Later, a genetic map placed the gene on a 25.2-cM linkage region containing two quantitative trait loci for ovary and fruit shape (Périn et al., 2002); and another 7-cM RFLP (restriction fragment length polymorphism) marker was obtained (Silberstein et al., 2003). More recently, an AFLP (amplified fragment length polymorphism) marker linked to the *A* gene at 3.3 cM was found with a BSA (Bulked Segregant Analysis) approach performed in a set of 38 double-haploid lines, then it was transformed into a co-dominant SCAR (sequence characterized amplified region) marker at 5.5 cM distance (Noguera et al., 2005); this was the nearest molecular marker to the *A/a* locus before the gene found by Boualem and collaborators in August 2008 (Boualem et al., 2008).

In Boualem's research, a 14-kbp region encoding for aminocyclopropane-1-carboxylic acid synthase (ACS) and containing the *A* gene was identified in the melon genome, and designated *CmACS-7* on the basis of homology to the *Arabidopsis thaliana ACS-7* gene (AT4G26200). The role of *CmACS-7* in sex determination was confirmed by TILLING (a targeting induced local lesion in genomes) approach. One of the two missense mutations, the G19E in a highly conserved amino-acid position affects the function of the protein. The change of amino-acid A57V reduces ACS enzymatic activity which is required for stamen growth in bisexual flowers on andromonoecious lines.

In this paper, we exploited a co-dominant marker linked to sexual traits, based on *A* gene sequence reported in GenBank (accession number EU791279), using some melon varieties, to provide a technique for molecular marker assistant selection in melon breeding.

## MATERIALS AND METHODS

### Plant materials

Two homozygous breeding lines of melon (*C. melo* L.) were used in this study. A monoecious line 'RH107' and an andromonoecious line '76-09', an F<sub>2</sub> population and a BC<sub>1</sub> (RH107-76-09). 76-09] population consisting of 157 and 87 individuals were used in PCR analysis. Furthermore, 20 accessions including one monoecious accession from Russia, and one and 18 andromonoecious accessions from Japan and China, respectively, were used to assay the marker. All plant materials were grown in a greenhouse in Shenyang Agricultural University in April 2008. Their sexual phenotypes were surveyed from 5 - 10 bisexual or female flowers on axillary branches of each plant.

### Primers

Four pairs of primers were designed based on the *CmACS-7* gene sequence (accession number EU791279) with Primer 5.0 software used to clone the *CmACS-7* gene of 'RH107' and '76-09' in the following study (1-L to 4-R; Table 1). Polymorphic sites according to the sequence and BLAST results were considered, and a pair of specific PCR primers T1-L/T1-R (Table 1) was designed to evaluate the polymorphism among the parents; F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub> populations; and other accessions. All primers were synthesized by Beijing SBS Genetech Co. Ltd.

### DNA isolation

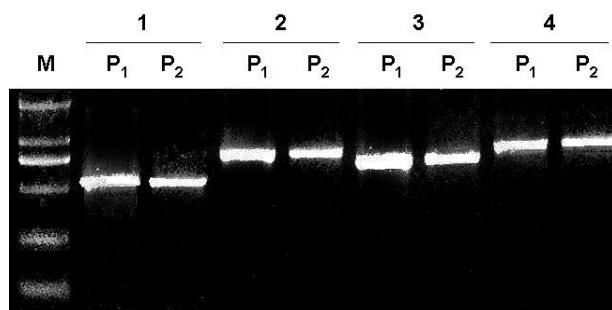
Young leaves either from parents or individual F<sub>1</sub>, F<sub>2</sub>, or BC<sub>1</sub> plants were harvested, quickly frozen in liquid nitrogen, and stored at -80°C. The total DNA was isolated using CTAB method (Liu, 2007) dissolved in water, diluted to 10 ng/μl, and kept frozen at -20°C until used in PCR reactions.

### PCR reaction

The PCR reaction mixture contained 14.2 μl ddH<sub>2</sub>O, 10 ng of genomic DNA, 1U Taq DNA polymerase (Tiangen), 10 × reaction buffer (containing Mg<sup>2+</sup>), 0.2 mmol/L dNTP, and 0.5 μmol/L each of primers in a 20 μl reaction volume. PCR reactions were run with a

	160	170	180	190	200															
Vedrantaïis :	GTTATTCAAATGGGCTTAGCTTGAAAATCAAGTAAGAATATATAACTTTTTTTGTTTTGT																			
76-09 :	GTTATTCAAATGGGCTTAGCTTGAAAATCAAGTAAGAATATATAACTTTTTTTGTTTTGT																			
PI124112 :	GTTATTCAAATGGGCTTAGCTTGAAAATCAAGTAAGAATATATAACTTTTTTTGTTTTGT																			
RH107 :	GTTATTCAAATGGGCTTAGCTTGAAAATCAAGTAAGAATATATAACTTTTTTTGTTTTGT																			
amino acid	V	I	Q	M	G	L	V/A	E	N	Q	V	R	I	Y	N	F	F	L	F	C

**Figure 2.** Comparison between the nucleotide sequences of 'RH107' and '76-09' and *CmACS-7* of 'PI124112' and 'Vedrantaïis'. The gray bar indicates the mutated locus 170<sup>th</sup> bp.



**Figure 1.** Electrophoretogram of amplified fragments of *CmACS-7* on 'RH107' and '76-09'. M: DL2000 DNA ladder; P<sub>1</sub>:RH107; P<sub>2</sub>:76-09; 1: 1-L/1-R; 2: 2-L/2-R; 3: 3-L/3-R; 4: 4-L/4-R.

program of 35 cycles at 95°C for 45 s, 56°C for 45 s and 72°C for 30 s with an initial denaturing at 95°C for 5 min and a final extension of 72°C for 7 min with BIO-RAD iCycler. PCR products were loaded onto 1.5% agarose gel in 0.5 × TBE (Tris-borate-EDTA) buffer containing EB (ethidium bromide), quantified by comparison with DNA standards (D2000, Tiagen), and run at 2.8 V/cm for 60 min for electrophoresis. The agarose gel were observed under UVP 3UVTM Trans-illuminator, and photographed with a Canon A610 digital camera.

The PCR reactions with specific primers T1-L/T1-R were as above, except the annealing temperature was 58°C. PCR products were examined in 6% denaturing polyacrylamide gels electrophoresis for 1 h in 1 × TBE, stained with 0.1% AgNO<sub>3</sub>.

#### Extraction, cloning, sequencing and analysis of the PCR products

The PCR products were excised from the gel and purified with DNA Extraction Kit (TaKaRa) according to the recommendations of the supplier. Briefly, the fragments were inserted into pBS-T vectors and transformed into competent cell, *Escherichia coli* strain TOP10 (Tiagen). The recombinants were screened with ampicillin and X-gal; and positive clones identified with PCR, using the bacteria as template, were sent for sequencing at the Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. The sequences were analyzed with DNAstar and BLAST in NCBI (National Center for Biotechnology Information).

## RESULTS

Both F<sub>2</sub> and BC<sub>1</sub> populations fitted segregation ratios of 3:1 and 1:1, confirming the monogenic inheritance of the monoecy/andromonoecy as described previously

(Kenigsbuch and Cohen, 1990; Noguera et al., 2005).

#### Cloning of the *CmACS-7* gene

Fragments of about 500 bp, 750 bp, 700 bp and 800 bp were amplified with primers 1-L/1-R, 2-L/2-R, 3-L/3-R, and 4-L/4-R on the parents 'RH107' and '76-09', respectively (Figure 1.) Among these, the fragment amplified with primers 1-L/1-R and 2-L/2-R covered the first and the second exons of the *CmACS-7* gene, and the joined fragments amplified with 3-L/3-R and 4-L/4-R covered the third exon. Thus, all three exons of *CmACS-7* were cloned with the four primer pairs.

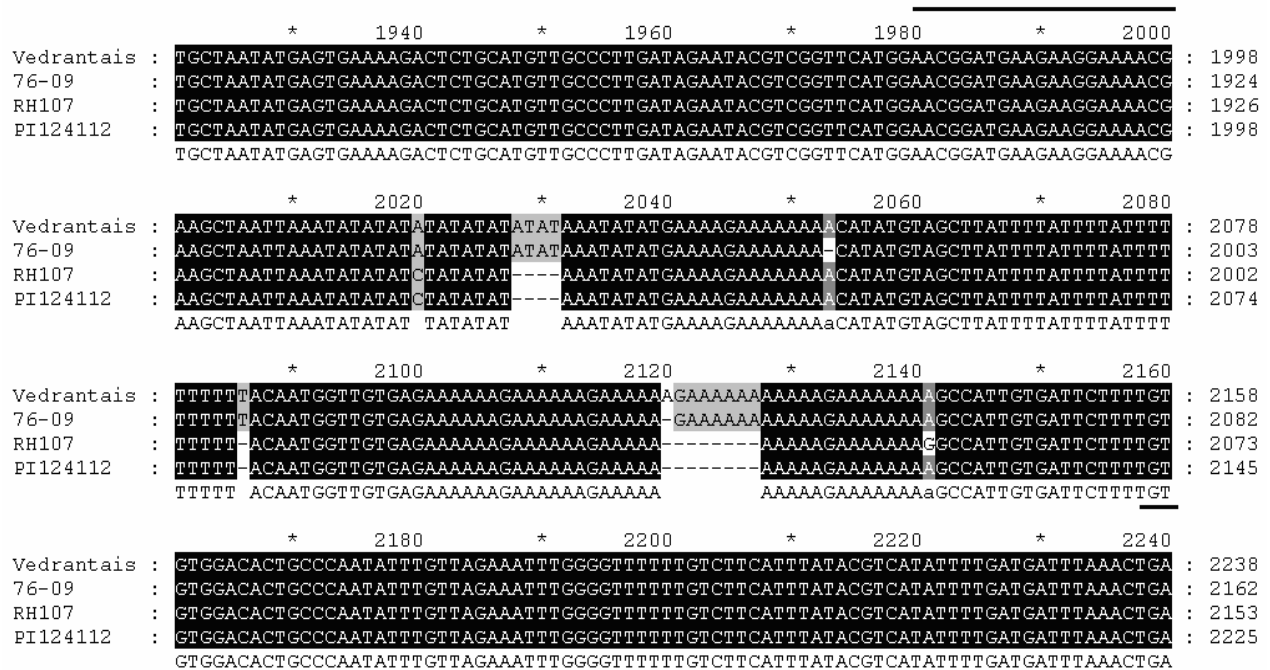
#### Sequence analysis

The sequence analysis and BLAST search in the database between the amplified fragments of 'RH107' and '76-09' from the gel and the publicly available sequences of 'PI124112' and 'Vedrantaïis' revealed that the homology was 99 and 98%, respectively. Accordingly, the fragments cloned by the four primer pairs were identified as the *CmACS-7* gene.

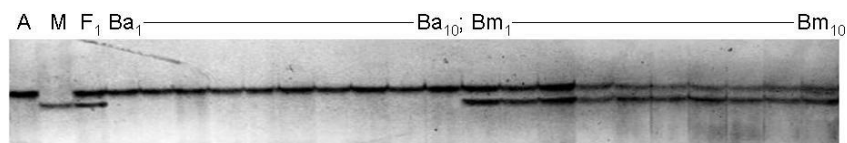
The 170<sup>th</sup> nucleotide of 'RH107' was "C", consistent with that of 'PI124112', while the 170<sup>th</sup> nucleotide of '76-09' was "T", just as the 'Vedrantaïis', which leading to the change of the 57<sup>th</sup> amino acid from alanine to valine (Figure 2). In addition, 17 insertion/deletion loci and 13 transition/transverse loci were detected along the whole sequence, except for a small fragment in the second intron region. Analysis showed that most were located in non-coding regions or did not affect the amino acid translation. Only two loci affected the amino acid translation but seemed not to be associated closely with the sexual phenotype (data not shown). A pair of primers was designed according to the insertion/deletion loci (Figure 3) to examine the polymorphism of populations and accessions in the following studies.

#### Development of a co-dominant marker linked to sexual gene

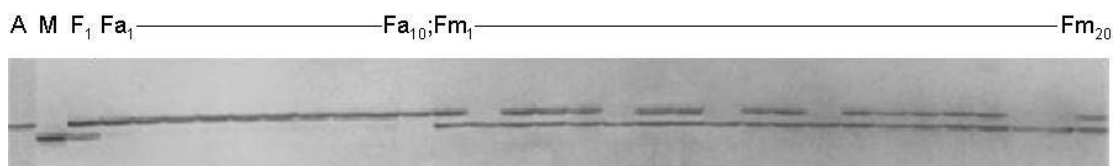
The primer combinations T1-L/T1-R were used in the parents and F<sub>1</sub> to analyze polymorphism. As expected, the banding pattern of fragments exhibited a polymor



**Figure 3.** Comparison between the nucleotide sequences of 'RH107' and '76-09' and that of *CmACS-7* of 'PI124112' and 'Vedrantaïs'. The black threads show the locations of the specific primers T1-L/T1-R.



**Figure 4.** Amplification result with T1-L/T1-R on two parents and individuals of the BC<sub>1</sub> population. A: andromonoecious parent '76-09'; M: monoecious parent 'RH107'; Ba<sub>1</sub>–Ba<sub>10</sub>: andromonoecious BC<sub>1</sub> individuals; Bm<sub>11</sub>–Bm<sub>20</sub>: monoecious BC<sub>1</sub> individuals.



**Figure 5.** Amplification result with T1-L/T1-R on parents and individuals of F<sub>2</sub> population. A: andromonoecious parent '76-09'; M: monoecious parent 'RH107'; Fa<sub>1</sub>–Fa<sub>10</sub>: andromonoecious individuals of F<sub>2</sub>; Fm<sub>1</sub>–Fm<sub>20</sub>: monoecious individuals of F<sub>2</sub>.

phism between parents, with an approximately 200-bp band in the andromonoecious '76 - 09', but a 180-bp band in the monoecious 'RH107'. Interestingly, both bands were displayed in F<sub>1</sub> plants. The banding pattern was also maintained when the primers were used to estimate the two separated populations of BC<sub>1</sub> and F<sub>2</sub>. In the BC<sub>1</sub> population all of the 46 andromonoecious

individuals each had one 200-bp band, while all 41 monoecious individuals had both bands (partially shown in Figure 4). In the F<sub>2</sub> population, all the 42 andromonoecious individuals also had one 200-bp band; however, the 115 monoecious individuals were more complex (Table 2): 34 of them had only one 180-bp band, but 81 had two bands (partially shown in Figure 5).

**Table 2.** Pattern of bands amplified with T1-L/T1-R in the BC<sub>1</sub> and F<sub>2</sub> populations.

Population	Phenotype	Total number	Number of individuals with a band of 180 bp	Number of individuals with two bands	Number of individuals with a band of 200 bp
BC <sub>1</sub>	mono	41	0	41	0
	andro	46	0	0	46
F <sub>2</sub>	mono	115	34	81	0
	andro	42	0	0	42

Mono: Monoecious; andro: andromonoecious.

**Table 3.** The result of amplification with specific primer T1-L/T1-R on 20 accessions.

Accession Name	Origin	Sexual phenotype	Amplified with T1-L/T1-R
No. 112	Russia	mono	180 bp
Tianbao	Japan	andro	200 bp
No. 111	Liaoning, China	andro	200 bp
No. 114	Heilongjiang, China	andro	200 bp
No. 116	Heilongjiang, China	andro	200 bp
Chaozaomeiyu	Heilongjiang, China	andro	200 bp
Jingtian No. 208	Heilongjiang, China	andro	200 bp
Tianguan No. 2	Heilongjiang, China	andro	200 bp
Tianguan No. 4	Heilongjiang, China	andro	200 bp
Hongcheng No.16	Inner Mongolia, China	andro	200 bp
A-2	Henan, China	andro	200 bp
Y-2	Henan, China	andro	200 bp
ML-19-4	Henan, China	andro	200 bp
Elizabeth	Henan, China	andro	200 bp
Huapiniujiaomi	Henan, China	andro	200 bp
No.108	Henan, China	andro	200 bp
Tiandiaoya	Hebei, China	andro	200 bp
68-3-3	Gansu, China	andro	200 bp
Yingua	Shandong, China	andro	200 bp
91-4	Shandong, China	andro	200 bp

Mono: Monoecious; andro: andromonoecious.

This result indicated that the fragments of 180 bp and 200 bp amplified with the primer combination T1-L/T1-R putatively co-separated with the *A/a* gene, behaving as co-dominant molecular markers. To confirm this hypothesis, 20 additional accessions were tested (Table 3).

In summary, the 200-bp and 180-bp bands indicated the homozygous recessive genotype *aa* and homozygous dominant genotype *AA*, respectively. Furthermore, both bands were given by heterozygous *Aa*. The 180-bp and 200-bp fragments behaved as a co-dominant molecular marker with two alleles, co-segregating to the *A* (monoecious) and *a* (andromonoecious) alleles, named C1A/C1a, and could be used to identify the sexual genotype of melon.

## DISUSSION

Molecular marker technology and its applied research developed quickly since RFLP was first proposed as a genetic marker used to build genetic linkage maps (Botstein et al., 1980). More than 20 kinds of DNA molecular marker techniques have been created, represented by three generations namely RFLP, SSR (simple sequence repeat) (Tautz, 1989) and SNP (single nucleotide polymorphism) (Lander, 1996). All of these are defined random DNA markers (RDMs) in that they are derived at random from polymorphic sites in the genome. By contrast, gene targeted markers (GTMs) are derived from polymorphisms

within genes (Andersen and Lübberstedt, 2003). Millions of gene sequences, a vast amount of informative data are deposited at GenBank and facilitate the assessment of genetic diversity. To design or exploit new markers it is essential to rely on this available data.

In fact, little research on GTM has been reported. A PCR-based marker for *VRN-A1*, based on the gene sequence information obtained from previous studies, was designed to amplify an 810-bp segment of the *VRN-A1* gene, the common wheat (*Triticum aestivum* L.) growth habit gene. Three nucleotide changes were identified that differentiated the dominant *Vrn-A1* and recessive *vrn-A1* alleles, one of which was developed into a CAPS marker that can be used to determine the allelic state at *VRN-A1* in germplasm collections (Sherman et al., 2004). A co-dominant PCR-based marker for allelic selection of the pink trait in onion (*Allium cepa*), based on an insertion mutation in the promoter of the anthocyanidin synthesis gene promoter sequences of both alleles were developed (Kim et al., 2005). In another case, a PCR primer combination I-2/5F and I-2/5R was designed according to the sequence of tomato fusarium wilt resistance gene *I-2* (accession number AF118127), and as a functional co-dominant marker could distinguish homozygous resistant, heterozygous and homozygous susceptible materials (Yu and Zou, 2008). As a type of molecular marker, GTM originates from the DNA sequence and it is unnecessary to know the function of the polymorphic site, it has lower development costs, and is a higher quality marker than RDMs (Andersen and Lübberstedt, 2003). Use of GTMs is a promising and practical technique based on known gene sequences.

Long ago, it was noticed that sex expression in cucumber and muskmelon was correlated with endogenous ethylene production. Gynoecious (all female) plants of these species produced more ethylene than monoecious plants. Hypobaric ventilation treatment of melon reduced ethylene concentrations and increased maleness, which was reversible by addition of exogenous ethylene (Byers et al., 1972). In cucumber, the inheritance of sex expression has been elucidated (reviewed by Perl-Treves, 1999), and three major genes, *M*, *F*, and *A*, account for most phenotypes. *F* controls femaleness; this partially dominant gene causes the female phase to start earlier. Gynoecious lines (*FFM*-) bear exclusively female flowers, while *ffMM* is the ordinary monoecious genotype, displaying a long male phase. The *M* locus affects the presence of stamens in pistillate flowers. Recent research reveals that it is through ethylene biosynthesis enzyme that the ethylene controls the sex expression. For instance, a fragment homologous to the ACC-synthase 1 gene (*CS-ACS1*) is only observed in gynoecious cucumber plants, not in monoecious ones, and was named *CS-ACS1G* (Trebitsh et al., 1997). These results not only suggested a functional role of ethylene in sex determination but also that *CS-ACS1G*, a gene involved in ethylene biosynthesis, could correspond to the *F* (female) gene of cucumber. However, other research sug-

gested only *CS-ACS2* mRNA was detected in the apices of gynoecious cucumber in which female flowers were developing (Kamachi et al., 1997). These results demonstrate that *CS-ACS2*-mediated biosynthesis of ethylene in individual flower buds is associated with the differentiation and development of female flowers (Saito et al., 2007). After the promoter regions of the genes *CsACS1G* and *CsACS1* were amplified and sequenced, the new sequence *CsACS1G* isolated in gynoecious (*MMFF*) and sub-gynoecious (*MMFf*) genotypes was confirmed as the dominant *F* allele (Mibus and Tatlioglu, 2004).

Since the ACS gene has a close relationship with the *F* gene of cucumber, the issue of whether it is associated with the monoecious gene *A* in melon puzzles geneticists. Yet none of the few ethylene-related genes mapped to date are located in the same linkage group as the *A* gene (Pe'rin et al., 2002a; Silberstein et al., 2003). Noguera et al. (2005) compared the sequence between the parental lines of the doubled-haploid population, regarding three ACC-oxidase genes and five ACC-synthase genes, but found no molecular polymorphism in the coding regions of these regulatory genes that could be associated with sex type. Constitutive expression of ACS in transgenic melon resulted in increased femaleness as measured by earlier and increased number of carpel-bearing buds (Papadopoulou et al., 2005), but this is only an indirect proof. The discovery and identification of the melon sexual gene *CmACS-7*, as a milestone, would be valuable not only for melon breeding but for elucidating sex-determining mechanisms (Boualem et al., 2008).

A CAPS marker, which was developed according to the C-T mutation on the position of 170 bp (Boualem et al., 2008), was considered as a reliable tool to distinguish the sexual genotype of *Cucumis melo* L. But the application of this marker in the marker-assisted selection was limited by its complicated procedures of identification. On this point, the markers which were developed in the present study are more applicable.

Being a part of the gene itself, the markers C1A/C1a had complete linkage with trait locus alleles without genetic recombination in the present study, which make identity more specific and sensitive than any previous markers although GTM had a very small separation probability with target gene (Andersen and Lübberstedt, 2003). However, as a co-dominant marker, C1A/C1a will enable us to distinguish between homozygous and heterozygous monoecious individuals, and allow faster and more reliable selection of melon breeding lines. In contrast with the CAPS and SNP markers, the low cost and convenient operation make it suitable for high-throughput screening. In future, it could provide a powerful tool in multi-gene identification and marker-assisted selection for melon germplasm innovation and polymerization of many genes.

Only two monoecious and 20 andromonoecious accessions involved, and the populations were smaller than those previously reported. Different germplasm and expand-

ed populations should be used to identify the universality and practicalities of C1A/C1a to take this research past the level of gene mapping and molecular marker identification, and into the breeding process.

It is well known that sexual expression of cucurbits can also be influenced by environment and hormonal applications (Calun et al., 1965; Roy and Saran, 1990; Rudich, 1990; Wien, 2004; Dennis, 2008). Phenomena such as andromonoecious individuals bearing female flowers, monoecious ones bearing bisexual flowers, a few stamens being produced in female flowers, or bisexual flowers with insufficient stamens were occasionally observed in the present study; with a higher frequency at higher temperatures. However, this occurred at most on 1–3 single flowers per plant and so had no impact on identification of sexual phenotype. Some of these flowers were manually self-crossed and seed collected; phenotypes of the offspring showed their genotypes were not changed. Therefore it was speculated that C1A/C1a was effective to identify materials impacted by high temperatures, but should be further tested.

## Conclusion

Four pairs of primers were designed according to the sequence publicly available in GenBank to clone the *CmACS-7* gene on the monoecious parental line 'RH107' and the andromonoecious parental line '76-09'. According to the insertion/deletion loci between the two parental lines, a pair of specific primers TI-L/TI-R was designed to assay the polymorphism in the parents and F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub> populations and additional 20 accessions. The fragments amplified with the specific primers TI-L/TI-R, named C1A/C1a, were a co-dominant molecular markers Linked to monoecy in melon and could be used in marker assistant selection in melon breeding.

## REFERENCES

- Andersen JR, Lübberstedt T (2003). Functional markers in plants. *Trends Plant Sci.* 8:554-560.
- Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32:314-331.
- Boualem A, Fergany M, Fernandez R, Troadec C, Martin A, Morin H, Sari MA, Collin F, Flowers JM, Pitrat M, Purugganan MD, Dogimont C, Bendahmane A (2008). A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. *Science* 321:836-838.
- Byers RE, Baker LR, Sell HM, Herrner RC, Dilley DR (1972). Ethylene: A natural regulator of sex expression of *Cucumis melo* L. *Proc. Natl. Acad. Sci.* 69:717-720.
- Calun E, Izhar S, Atsmon D (1965). Determination of relative auxin content in hermaphrodite and andromonoecious *Cucumis sativus* L. *Plant Physiol.* 40:321-326.
- Danin-Poleg Y, Tadmor Y, Tzuri G, Reis N, Hirschberg J, Katzir N (2002). Construction of a genetic map of melon with molecular markers and horticultural traits, and localization of genes associated with ZYMV resistance. *Euphytica.* 125: 373-384.
- Kenigsbuch D, Cohen Y (1990). The inheritance of gynoccy in muskmelon. *Genome* 33:317-320.
- Kim S, Yoo KS, Pike LM (2005). Development of a codominant PCR-based marker for allelic selection of the pink trait in onions (*Allium cepa*), based on the insertion mutation in the promoter of the anthocyanidin synthesis gene. *Theor. Appl. Genet.* 110:573-578.
- Lander ES (1996). The new genomics: global views of biology. *Science.* 274:536-539.
- Liu ZY (2007). Analysis of polyamine metabolism and differentially expressed genes in tomato (*Solanum numlyeopersicum*) under heat stress. Chinese Academy of Agricultural Sciences. Doctoral Dissertation, 2007.
- Mibus H, Tatlioglu T (2004). Molecular characterization and isolation of the F/f gene for femaleness in cucumber (*Cucumis sativus* L.). *Theor. Appl. Genet.* 109:1669-1676.
- Noguera FJ, Capel J, Alvarez JI, Lozano R (2005). Development and mapping of a codominant SCAR marker linked to the andromonoecious gene of melon. *Theor. Appl. Genet.* 110:714-720.
- Papadopoulou E, Little HA, Hammar SA, Grumet R (2005). Effect of modified endogenous ethylene production sex expression, bisexual flower development and fruit set in melon (*Cucumis melo* L.). *Sex. Plant Reprod.* 18:131-142.
- Périn C, Hagen LS, Giovinazzo N, Besombes D, Dogimont C, Pitrat M (2002b). Genetic control of fruit shape acts prior to anthesis in melon (*Cucumis melo* L.). *Mol. Genet. Genomics.* 266:933-941.
- Périn C, Hagen S, De Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002a). A reference map of *Cucumis melo* based on two recombinant inbred line populations. *Theor. Appl. Genet.* 104:1017-1034.
- Poole CF, Grimboll PC (1939). Inheritance of new sex forms in *Cucumis melo*. *J. Hered.* 30:21-25.
- Rosa JT (1928). The inheritance of flower types in *Cucumis melo* and *Citrullus*. *Hilgardia.* 3:233-250.
- Roy RP, Saran S (1990). Sex expression in the *Cucurbitaceae*. In Cornell University Press. pp: 251-268.
- Rudich J (1990). Biochemical aspects of hormonal regulation of sex expression in Cucurbits. Cornell University Press. 269-280.
- Saito S, Fujii N, Miyazawa Y, Yamasaki S, Matsuura S, Mizusawa H, Fujita Y, Takahashi H (2007). Correlation between development of female flower buds and expression of the *CS-ACS2* gene in cucumber plants. *J. Exp. Bot.* 58: 2897-2907.
- Sherman JD, Yan L, Talbert L, Dubcovsky J (2004). A PCR marker for growth habit in common wheat based on allelic variation at the *VRN-A1* gene. *Crop Sci.* 44:1832-1838.
- Silberstein L, Kovalski I, Brotman Y, Pe'rin C, Dogimont C, Pitrat M, Klingler J, Thompson G, Portnoy V, Katzir N, Perl-Treves R (2003). Linkage map of *Cucumis melo* including phenotypic traits and sequence-characterized genes. *Genome* 46:761-773.
- Tautz D (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17: 6463-6471.
- Trebitsh T, Staub JE, Neill SD (1997). Identification of a 1-aminocuclop ropene-1-carboxylic acid synthesis gene linked to the female (F) locus that enhances female sex expression in cucumber. *Plant physiol.* 113:987-995.
- Wien HC, Stapleton SC, Maynard, DN, McClurg C, Riggs, D (2004). Flowering, sex expression and fruiting of Pumpkin (*Cucurbita* sp.) cultivars under various temperatures in greenhouse and distant field trials. *HortScience* 39: 239-242.
- Wu MZ, Yi HP, Feng JX, Erken, Zhang YB (2000). Ecobreeding of hami melon and soil less cultivation of organic ecotype. *Engineering science.* 2:47-52.
- Yu SC, Zou YM (2008). A co-dominant molecular marker of fusarium wilt resistance gene *I-2* derived from gene sequence in tomato. *Hereditas.* 30: 926-932.