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Development of New Molecular Markers for the Identification of Male Sterile Cytoplasm in Peppers (*Capsicum annuum* L.)

Woong-Ki Min[1†], Byung-Dong Kim[2†], Sunggil Kim[3], and Sanghyeob Lee[1,4*]

[1]*Center for Agriculture and Life Science Research, Dongbu Advanced Research Institute, Dongbu Hannong Co., Ltd., Daejeon 305-708, Korea*
[2]*Center for Plant Molecular Genetics and Breeding Research, Seoul National University, Seoul 151-921, Korea*
[3]*Department of Plant Biotechnology, Chonnam National University, Gwangju 500-757, Korea*
[4]*Plant Engineering Research Institute, Sejong University, Seoul 143-747, Korea*

Abstract. Cytoplasmic male sterility (CMS) induced by mutant mitochondria genome, has been used for commercial seed production of F_1 hybrid cultivars in diverse crops. In pepper (*Capsicum annuum* L.), two sterile cytoplasm specific gene organization, *atp6-2* and *coxII* were identified. An open reading frame, *orf456* nearby *coxII* gene has been speculated to induce male sterility (MS) by mutagenic analysis. Moreover, molecular markers for *atp6-2* and *coxII* of mitochondrial genotype (mitotype) were developed. However, the Cytoplasmic MS specific markers, atp6SCAR and coxIISCAR markers appeared in both N and S cytoplasms when polymerase chain reaction (PCR) cycles prolonged more than 40 cycles. Since the reported molecular markers were dominant markers, the presence of the faint sterile-specific band in normal cytoplasm may lead to the mis-classification of pepper breeding lines. To solve this problem, one common forward primer and two different reverse primers specific to normal *coxII* and sterile *orf456* genes were designed after analyzing their gene organizations. By using these three primers, N and S *coxII* specific bands were co-amplified in male-sterile lines, but only normal *coxII* specific band was amplified in maintainer lines. Since the reverse primer for sterile *coxII* was specifically designed 275 bp downstream of *orf456*, relatively stable PCR amplification patterns were observed regardless of the number of PCR cycles. These primer sets easily identified different mitotypes among the divergent breeding lines, commercial cultivars and diverse germplasms.

***Additional key words*:** CMS, coxII, mitotype

Introduction

In eukaryotic organisms, mitochondria are associated with various pathways of energy production. The genome size of the mitochondria varies with plant taxa from several hundred to thousands kb (Palmer and Herbon, 1987; Ward et al., 1981). In higher plants, particularly, in organelle contained complex feature of genome structure, such as master circular DNA, and various sub-genomes (Knoop, 2004). The mono-circular form of mitochondrial DNA was found only restricted species (Palmer and Herbon, 1987) and the master circular mitochondrial genomes from *Arabidopsis* (Unseld et al., 1997), rice (Notsu et al., 2002), sugar beet (Kubo et al., 2000), and rapeseed (Handa, 2003) have been sequenced. The omnipresence of repeat sequences in the mitochondrial genomes was the major factors contributing to the complexity of plant mitochondrial genomes. Repeat sequence-mediated recombination yields multi-partite structures and sub-stoichiometric mtDNA molecules (Albert et al., 1998; Palmer, 1988; Small et al., 1989). In maize, the scheme of repeat sequence-mediated duplication of mitochondrial DNA was suggested (Small et al., 1989). Albert et al. (1998) have simulated the models for repeat sequence-mediated duplication and deletion of mitochondrial subgenomes. Recently, plant-specific ssDNA

***Corresponding author:** sol6793@gmail.com

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binding protein OSB1 involved in a stoichiometric transmission of mitochondrial DNA was found in *Arabidopsis* (Zaegel et al., 2006). The stoichiometry of subgenomic mtDNAs varies between closely related taxa (Bellaoui et al., 1998; Kim et al., 2007b; Sakai and Imamura, 1993). In *Arabidopsis*, significant differences were reported in the cox3 region of ecotypes C24, Landsberg erecta (L*er*), and Col-0 (Forner et al., 2005).

Uneven mitochondrial subgenomes often lead to change of maternally inherited phenotypes due to variation of gene expression through increasing or decreasing the copy numbers of substoichiometric mtDNA. Cytoplasmic male sterility (CMS) is a well-known trait induced by chimeric mtDNA created thorough repeat sequence-mediated mtDNA rearrangement (Hanson, 1991; Hanson and Bentolila, 2004; Palmer and Shields, 1984; Small et al., 1989). The mechanisms of such chimeric gene induced MS were proposed in some plant species. Though most of them were still unclear, ultimate result might be programmed cell death of either the tapetal layer or sporogenous cells and processed via anterograde/retrograde signaling (Balk and Leaver, 2001; Bergman et al., 2000; Fujii and Toriyama, 2008; Hack et al., 1991; Rhoads et al., 1995).

CMS has been widely used for the production of commercial F_1 hybrid plants because no self-pollination is occurred in maternal lines. For fruit or seed crops, however, CMS systems are useful only when nuclear restorer gene was introduced to suppress MS in the hybrid plants (Budar et al., 2003; Hanson and Bentolila, 2004; Schnabel and Wise, 1998).

At present, the mitochondrial gene inducing CMS has been reported in various cultivated plants (Hanson and Bentolilla, 2004; Jo, 2007; Kim et al., 2007a). After discovery of CMS in peppers (*Capsicum annuum* L.), it has been widely used for pepper breeding (Peaterson, 1958). There were two CMS cytoplasm specific genes, *ψ atp6-2* and *coxII* have been reported. The one open reading frame, *orf456* nearby *coxII* gene was proven to be responsible for MS in pepper (Kim et al., 2007a). Moreover, molecular markers for *ψ atp6-2* and *coxII* of mitochondrial genotype (mitotype) have been developed to apply for the selection of MS mitotypes in diverse germplasms. Recently, *orf507* rather than *orf456* was suggested that MS-related gene revealed by altered transcription (Gulyas et al., 2010).

In this study, sequence differences of *ψ atp6-2* and *coxII* genes between normal (S cytoplasm) and maintainer (N cytoplasm) breeding lines were surveyed using previously reported markers. Then, for precise selection of MS cytoplasm, improved markers were designed from *orf456* and normal *coxII* specific sequences.

Materials and Methods

Plant Materials

Four male-sterile and normal maintainer parental lines of commercial F_1 cultivars were used as control (Table 1). All parental lines were previously tested for their sterility in cytoplasm via testcrosses.

One hundred and ninety five breeding lines, their cytopalsms were already known from testcrosses, were used to examine the accuracy of improved marker. Moreover, total of 37 cultivars bred in Korea and 17 *C. annuum* germplasms were used for examining normal or male-sterile mitotypes (Tables 2 and 3).

Cytoplasm Specific Primer Sets

To detect N and S cytoplasm, the previously reported atp6SCAR, coxIISCAR and control markers were used (Kim and Kim, 2005). The forward primer, coxIITri-M1-F was designed from common sequences between N and S *coxII*, whereas coxIITri-M1-NR and coxIITri-M1-SR were designed from N *coxII* and 275 bp downstream of *orf456* sequences. The control marker for normal *atp6-2* gene was selected from specific primers (Kim and Kim, 2006) (Table 4, Figs. 1 and 2).

Amplification of Cytoplasmic Regions

Total genomic DNA was isolated using the protocol described by Kim and Kim (2005). Polymerase chain reactions (PCRs) of coxIISCAR markers were performed using the same conditions as in the previous reports, but Tm values of coxIITri-M1 primers specific to normal *coxII* and *orf456* gene were raised to 68℃. PCRs reactions were performed in a 10 μL reaction mixture containing 0.025 μg template, 1 μL 10× PCR buffer, 0.5 μL forward primer (10 μM), 0.5 μL reverse primer (10 μM), 1 μL dNTP (10 mM), and 0.1

Table 1. Pepper breeding lines used for the test of normal or male-sterile cytoplasm specific markers.

Male-sterile line	Cytoplasm	Nucleus	Maintainer line	Cytoplasm	Nucleus
D1201	Sterile	*rf/rf*	D1301	Normal	*rf/rf*
D1202	Sterile	*rf/rf*	D1302	Normal	*rf/rf*
D1203	Sterile	*rf/rf*	D1303	Normal	*rf/rf*
D1204	Sterile	*rf/rf*	D1304	Normal	*rf/rf*

Table 2. Commercial pepper cultivars used in this study.

No.	Cultivar	Seed Co.	No.	Cultivar	Seed Co.
1	Geosan	Monsanto Korea	20	DBTS-06-25[z]	DongBu Hannong
2	Geumdang	Syngenta Korea	21	DBTS-06-02	DongBu Hannong
3	Daejangbu	Syngenta Korea	22	DBTS-06-55	DongBu Hannong
4	Daecheong	DongBu Hannong	23	DBTS-06-59	DongBu Hannong
5	Daechon	Nongwoo-bio	24	DBTS-06-70	DongBu Hannong
6	Manitta	Nongwoo-bio	25	DBTS-06-71	DongBu Hannong
7	Ppallitta	DongBu Hannong	26	DBTS-06-72	DongBu Hannong
8	Buhong	DongBu Hannong	27	DBTS-06-73	DongBu Hannong
9	Singsinghong	DongBu Hannong	28	Jomyeong	DongBu Hannong
10	Supermanitta	Nongwoo-bio	29	Jota	DongBu Hannong
11	Sinnaneun	Monsanto Korea	30	DBTS-06-12	DongBu Hannong
12	Yeokgang	Koregon	31	DBTS-06-14	DongBu Hannong
13	Wangdaebak	Monsanto Korea	32	Keumsang	DongBu Hannong
14	Teobowang	Nunheme	33	DBTS-06-03	DongBu Hannong
15	Taesan	Nongwoo-bio	34	DBTS-06-04	DongBu Hannong
16	Cheonhajanggun	DongBu Hannong	35	DBTS-06-05	DongBu Hannong
17	Cheonhatongil	Monsanto Korea	36	DBTS-06-33	DongBu Hannong
18	Hanbando	Nongwoo-bio	37	DBTS-06-01	DongBu Hannong
19	Heungina	DongBu Hannong			

[z]DBTS, trial cultivars from Dongbu Hitek, Korea.

Table 3. Pepper germplasms used in this study.

No.	Accession No.[z]	Cultivar	No.	Accession No.	Cultivar
1	PI167061	Sivri Aci Biber	9	5G14	C00784
2	PI262903	Pimento del Pico	10	5G16	C00877
3	PI593491	10243	11	5G26	C01243
4	PI640705	Szeged 40017	12	5G29	C01281
5	PI640557	Anaheim F-6	13	5G38	C01393
6	PI640719	Fecske	14	5G72	C03165
7	PI640796	Anaheim TMR	15	5G77	C03375
8	5G12	C00719	16	5G82	C03657

[z]Grif and PI, accessions from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS); 5G and K, accessions from the National Horticultural Research Institute (NHRI), Korea.

Table 4. N and S cytoplasm specific markers used in this study.

Marker		Sequence	Description	Reference
atp6SCAR	F[z]	5'- AGTCCACTTGAACAATTTGAAATAATC -3'	ψ *atp6-2*	Kim and Kim
	R	5'- GTTCCGTACTTTACTTACGAGC -3'	specific	(2005)
Normal atp6-2	F	5'- GAGCACTATGACTTTGCCCTAAAC -3'	Normal *atp6-2*	Kim and Kim
	R	5'- GACTCGGAATGCTCCACGACCTAA -3'	specific	(2006)
Sterile atp6-2	F	5'- GAGCACTATGACTTTGCCCTAAAC -3'	ψ *atp6-2*	Kim and Kim
	R	5'- GATCTGGCTATTAACCACTTTTC -3'	specific	(2006)
coxIISCAR	F	5'- AGTCCACTTGAACAATTTGAAATAATC -3'	Sterile *coxII*	Kim and Kim
	R	5'- GTTCCGTACTTTACTTACGAGC -3'	specific	(2005)
Normal coxII	F	5'- AGTCCACTTGAACAATTTGAAATAATC -3'	Normal *coxII*	Kim and Kim
	R	5'- TAGACAAGGCTCGTTCCGTACT -3'	specific	(2005)
coxIICTR	F	5'- GATGCAGCGGAACCATGGCAATTA -3'	*coxII* control	Kim and Kim
	R	5'- ACTGCACTGACCATAGTAAACTCC -3'		(2005)
coxIITri-M1	F	5'- CTAAGAGTAGGCGTGGAGAGCTTTTTGC -3'	Common *coxII*	Kim and Kim
	NR	5'- AGAGCTAGAAGCTTCGCCAGAAGCAAG -3'	Normal *coxII*	(2005)[y]
	SR	5'- GAAATTGCAGTTCTGCTTCCGTTTTCA -3'	*orf456*	

[z]F, forward; R, reverse primer.
[y]Newly designed primers from the sequences in the reference.

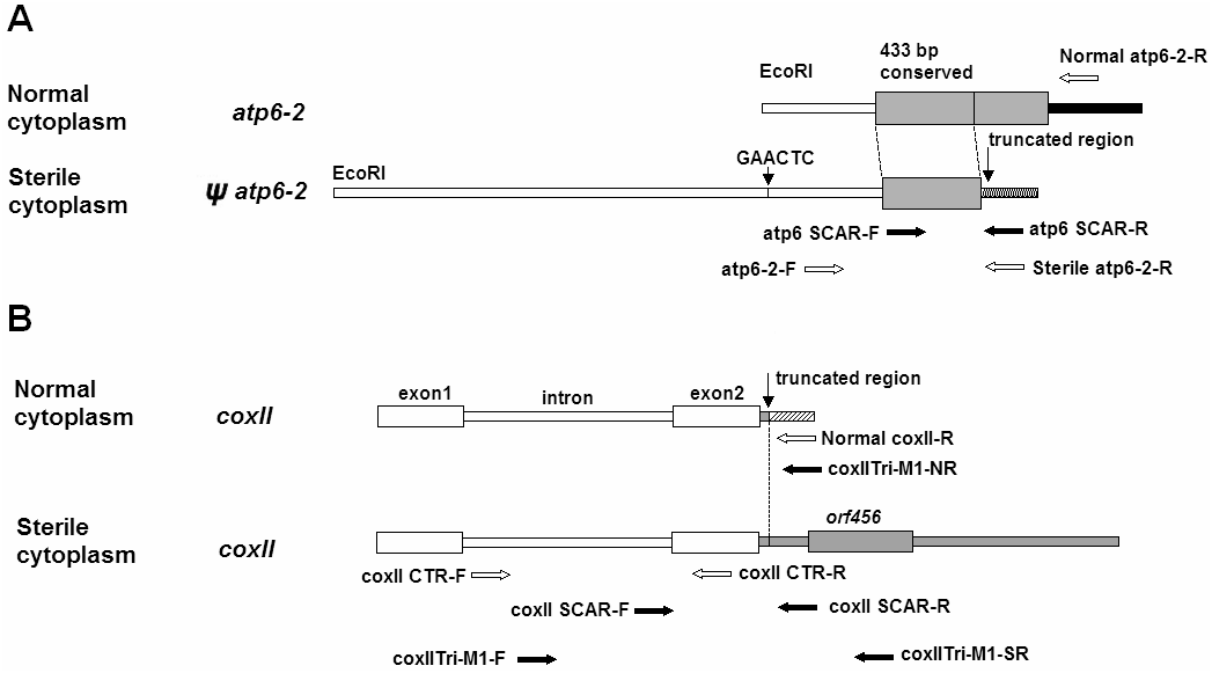

Fig. 1. Structural comparison of two mitochondrial genes, *atp6-2* (A) and *coxII* (B) from N and S cytoplasm in pepper (modified from Kim and Kim, 2005).

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          coxIISCAR-F                                  coxIITri-M1-F
NcoxII  GTCGGGAGAACTACCTAACTAAAGAAAAATAGTGTTCTTTCTAAGAGTAGGCGTGGAGAGCTTTTTGCGGGGAAACTTGCAAGTACAGTTTGGGGGGAGGCGGGCGTCGACCCTACCTTA 120
ScoxII  GTCGGGAGAACTACCTAACTAAAGAAAAATAGTGTTCTTTCTAAGAGTAGGCGTGGAGAGCTTTTTGCGGGGAAACTTGCAAGTACAGTTTGGGGGGAGGCGGGCGTCGACCCTACCTTA 120
        ************************************************************************************************************************

NcoxII  TGAGTATTCGGACTATAACAGTTCCGATGAACAGTCACTCACTTTTGACAGTTATACGATTCCAGAAGATGATCTAGAATTGGGTCAATCACGATTATTAGAAGTCGACAATAGAGTGGT 240
ScoxII  TGAGTATTCGGACTATAACAGTTCCGATGAACAGTCACTCACTTTTGACAGTTATACGATTCCAGAAGATGATCTAGAATTGGGTCAATCACGATTATTAGAAGTCGACAATAGAGTGGT 240
        ************************************************************************************************************************

NcoxII  TCTACCAGCCAAGAGCCATATACGTTTTATTGTAACATCTGCTGATGTACCTGATAGTTGGGCTGTACCTTCCTTAGGTGTCAAATGTGATGCTGTACCTGGTCGTTTAAATCAGACCTC 360
ScoxII  TCTACCAGCCAAGAGCCATATACGTTTTATTGTAACATCTGCTGATGTACCTGATAGTTGGGCTGTACCTTCCTTAGGTGTCAAATGTGATGCTGTACCTGGTCGTTTAAATCAGACCTC 360
        ************************************************************************************************************************

NcoxII  TATTTCGGTACAACGAGAAGGAGTTTACTATGGTCAGTGCAGTGAGATTTGTGGAACTAATCATGCCTTTATGCCGATCGTCGTAGAAGCTGTTCCTAGGAAAGATTATGGGTCTCGGGT 480
ScoxII  TATTTCGGTACAACGAGAAGGAGTTTACTATGGTCAGTGCAGTGAGATTTGTGGAACTAATCATGCCTTTATGCCGATCGTCGTAGAAGCTGTTCCTAGGAAAGATTATGGGTCTCGGGT 480
        ************************************************************************************************************************

NcoxII  ATCCAATCAATTAATCCCATAATAAAGCGCGGAAGCTTAAGCGGAAATGAAAGAGGAGGTTGAGG----GAAGCCACTAAATTG----------------AGGGCTTCGCT-------- 571
ScoxII  ATCCAATCAATTAATCCCATAATAAAGCGCGGAAGCTTAAGCGGAAATGAAAGAGGAGGTTGAGGTTATGAAGTCACTTAGCCGTATACTATACAAAGGGAAAGGCGTCGGTACGGAGTC 600
        ************************************************************** **** **** * * * *** *** *

NcoxII  -CGCTCGCTCTAACGCTCGTTTAGTAGACAGCGAGTGGAGTGCATAAGCCCCTTTAGAGATAGGGGTG-----AGTACTACACGAGCTCGTAAGTAAAGTACGGAACGAGCCTTGTCTA- 684
ScoxII  ACGTCAGCTGTGGATATAGACTAGGCTATAAGGAACGGAGTCTTAAACTATGGACCGAGACAGATATATAGAAAGTGTGCAGTGAGGGTGCTTGTAAATCACTAGGT-AGCCTAGCTCGA 719
         **   *** *    * *  ***   * *  **  *****    **        **** **   *     ***       ***   *   *****  **      ***** *
                                                                                                  coxIISCAR-R
                                    coxIITri-M1-NR
NcoxII  -CGAAGCAGAGCGACCTCATCTTGCTTGCTTCTGGCGAAGCTT-CTAGCTCTAAATAATAGGGAATTC---------------------------------------------------- 750
ScoxII  CCCAAGCAATGCCCAAAAGTCCCATGTATTTCTGGTTAAACAAACCAGCAATTTCCGACAAGTCTTTCTTCATTGGAAGAGCAAGAAGCGGAACTACAACATTTACATGCAATTTCACCA 839
         * *****  **      **    *  ******  ** *   * ***  *    * * *   ***
              orf456 start

NcoxII  ----------------------------------------------------------------------------------------------------------------------------
ScoxII  TGAATTTTATTGATTATGGCACATTGTTTACTTTTTCTTTTTATCTCGGTATTTCAATCGGCATTTTTGCGGGCCGGTTTTTTGAGCGAAGTGAAGTTTTACAGGAATTGGAGAACTTCC 959

NcoxII  ----------------------------------------------------------------------------------------------------------------------------
ScoxII  AGCTAGAAAAAATAAAACTGAAAACGGAAGCAGAACTGCAATTTCTTTGTAGAGAGCACTTGAGAATGAATGAAGAATTACAATTACCTGTTCCAGATGGAACGAGTATGCACATCTCCG 1079
                                  coxIITri-M1-SR

NcoxII  ----------------------------------------------------------------------------------------
ScoxII  ACTTTTTAGGGAAAGCCTTTTTGGTCGACGAGACTGTGAGGGAACGAATATTAGGGCTGACTCAAATTTATATGGATCTAAAAAACAATGGAGCAACCGAGTAA 1183
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Fig. 2. Comparison of the DNA sequences of N and S *coxII* in pepper mitochondria including primer positions used in this study.

μL polymerase mix (Advantage 2 Polymerase Mix, Clontech, Palo Alto, CA, USA). The PCR products were visualized on a 1.5% agarose gel.

Results

The atp6SCAR and coxIISCAR Markers Were Not Distinguished between N and S Cytoplasm

Total eight male-sterile and maintainer lines for commercial F_1 cultivars were analyzed using the previously reported N or S cytoplasm specific markers. When amplification cycles were raised from 30 to 40 cycles, both sterile cytoplasm specific atp6SCAR and coxIISCAR markers were amplified in maintainer lines having normal cytoplasm. Furthermore, sterile atp6-2 marker specific to 3' truncated region of *ψ atp6-2* also amplified in all maintainer lines, normal cytoplasm specific markers (Normal atp6-2 and Normal coxII) were amplified in all male-sterile lines (Fig. 3A). These results indicated that previously reported markers can not be used for identification of mitotype.

For the accurate selection of both sterile and normal cytoplasm, two different reverse primers and one common forward primer were newly designed from the sequences of *coxII* and *orf456*. Forward primer was presented in intron between exon1 and exon2 of *coxII*, N cytoplasm specific reverse primer was designed in the 3' truncated region besides of exon2, and S cytoplasm specific primer was tagged to *orf456* sequence (Figs. 1 and 2). When amplified using three primers, sterile *coxII* specific band was not amplified in the maintainer lines in all cycles (Fig. 3B).

Evaluation of the coxIITri-M1 Marker for Use in Breeding Programs

To test the usefulness of the new primer sets, total 195 breeding lines including MS, maintainer and restorer lines for commercial cultivars were screened and compared with

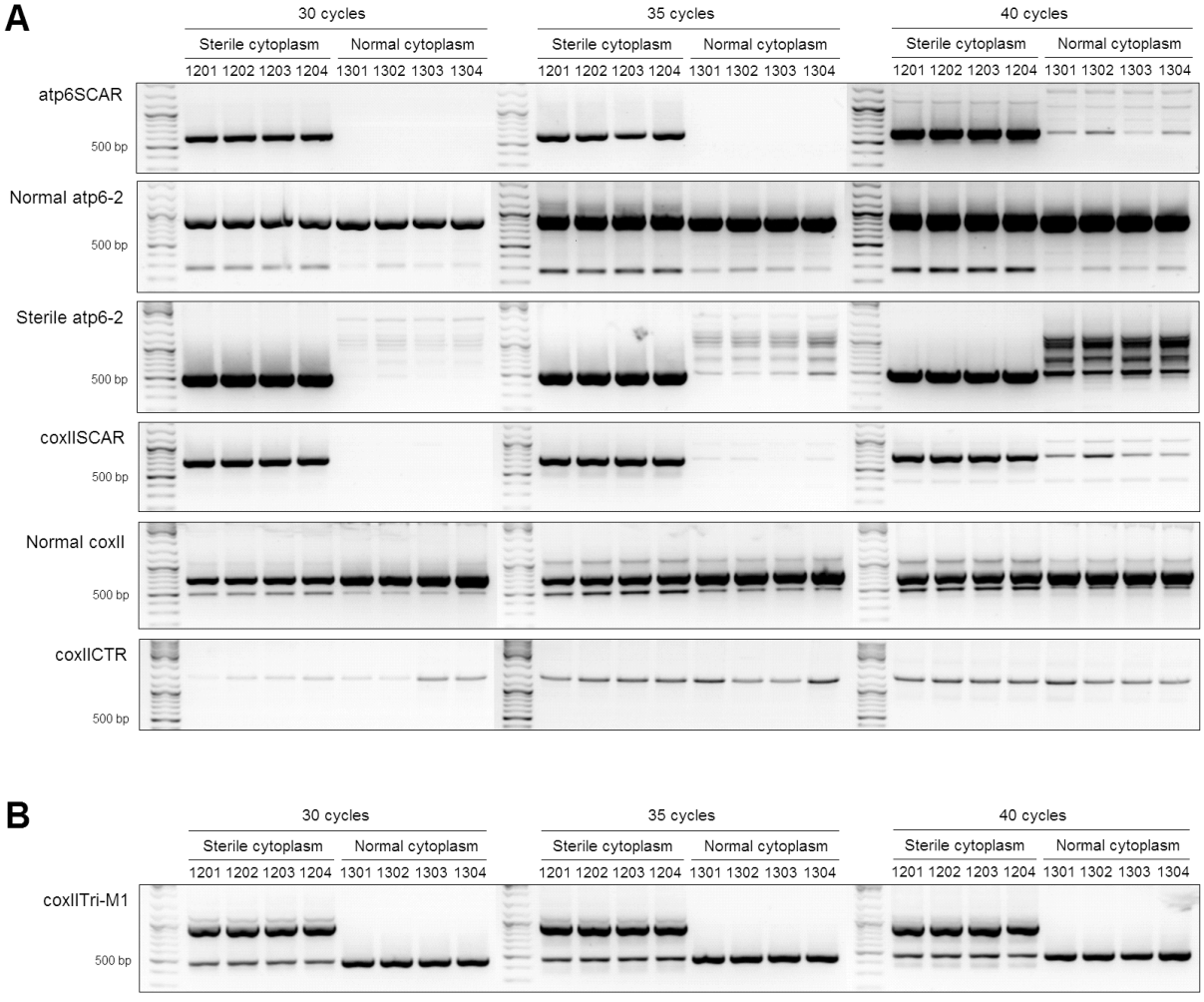

Fig. 3. PCR results of N and S cytoplasm specific markers with different amplification cycles in pepper breeding lines. A. atpSCAR, coxIISCAR, and Sterile atp6-2 markers, male-sterile cytoplasm markers; Normal atp6-2 and Normal coxII, normal cytoplasm markers, respectively. B. Tri-primer PCR results for specific detection of *orf456* in sterile cytoplasm.

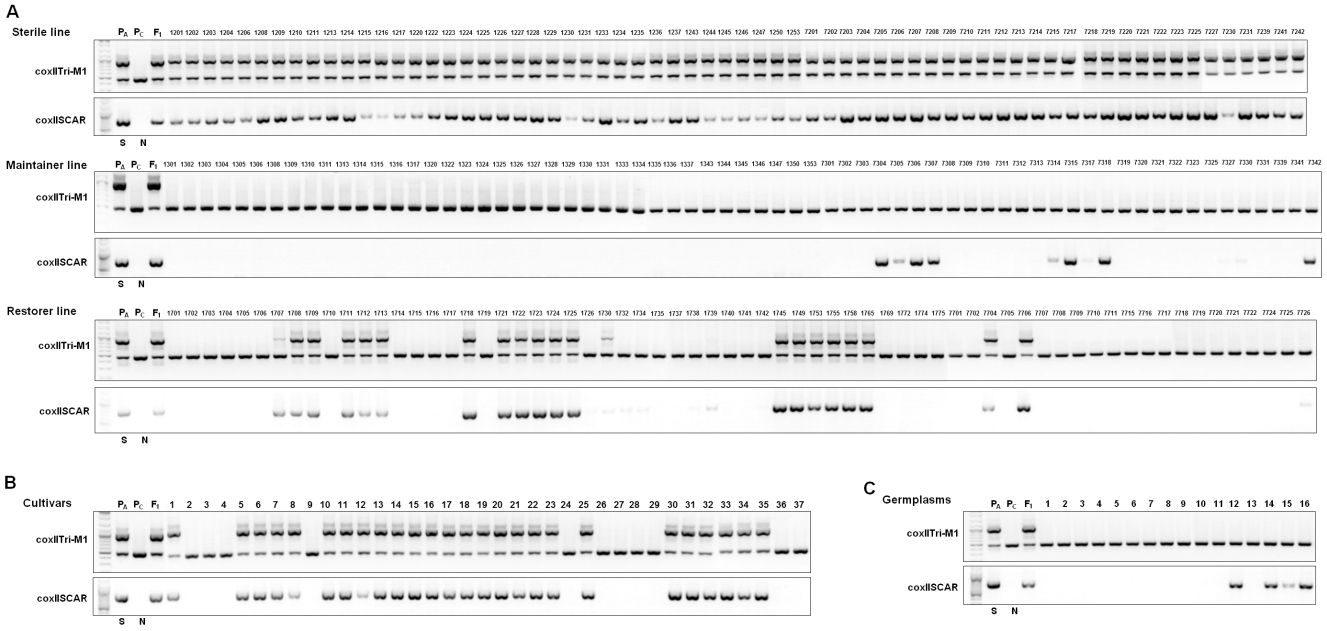

Fig. 4. Screening of mitotypes in diverse pepper breeding lines, commercial cultivars and germplasms using coxIITri-M1 marker. A, CGMS breeding lines; B, Commercial cultivars bred in Korea; C, Germplasms from the USDA-ARS and the NHRI. The numbers of analyzed plants were the same as in Tables 2 and 3, respectively.

the results of coxIISCAR marker. In the case of coxIITri-M1, all MS lines showed *orf456* specific band and all maintainer lines amplified only normal *coxII* specific bands (Fig. 4A). However, some of the maintainer lines showed S mitotype when using coxIISCAR marker. These results indicated that genotyping using coxIITri-M1 marker for breeding lines matched with phenotyping.

Application of the coxIITri-M1 Marker for Selection of MS Cytoplasms in Commercial Cultivars and Germplasms

Diverse commercial cultivars bred in Korea and germplasms were screened using coxIITri-M1 and coxIISCAR markers. Among analyzed 37 cultivars, 11 cultivars showed normal cytoplasmic band pattern. This result indicated that they were bred by using genic male sterility (GMS) lines (Fig. 4B). When 16 germplasms accessed from foreign countries were analyzed, all of them showed normal *coxII* specific bands when using coxIITri-M1, but 4 plants were typed as S cytoplasm by coxIISCAR marker (Fig. 4C). Regardless of these inconsistent results, the frequency of MS mitotypes was relatively high in the commercial cultivars, probably due to CMS F_1 hybrid breeding programs.

Discussion

Detection of N and S Cytoplasm in Peppers

Repeat sequence-mediated recombination yields chimeric genes in mitochondria (Cui et al., 1996) and causing CMS in some plant. In maize, the scheme of repeat sequence-mediated duplication of mitochondrial DNA was suggested (Small et al., 1989). Infrequent homologous recombination between short direct repeats in two independent ancestral master circles gave rise to two pairs of subgenomic circles. One of each subgenomic circles can be recombined by shared repeat sequences to generate a new master circle. Therefore, the new master circle contains a duplicated cluster and lacks other clusters presented in the ancestral master circle. Albert et al. (1998) have simulated the models for repeat sequence-mediated duplication and deletion of mitochondrial subgenomes. The master circle 1 generated circles 2 to 14 through recombination of different repeat sequences including 4 of duplicated-deleted circles. Recently, similar way of repeat sequence-mediated recombination in mitochondrial genome between coxII intergenic region of *orf456* and *ψ atp6-2* was reported in pepper (Jo, 2007).

To date, various mitochondrial gene constructed from CMS cytoplasm were reported from various crop species (Hanson and Bentolilla, 2004). In radish, *orf138* gene was known for Ogura MS was detected in normal cultivars as low-copy-number substoichiometric molecules (Kim et al., 2007b). Similar amplifying patterns were observed in pepper, *ψ atp6-2* and *coxII* genes responsible for MS were detected in maintainer lines. Conversely, markers for normal cytoplasm tagged to the truncated regions in *atp6-2* and *coxII* also amplified in sterile lines. The low-copy-number substoichiometric molecules of *ψ atp6-2* and sterile *coxII* in normal cytoplasm might be affected from multipartite mitochondrial genes. The reverse primer for coxIISCAR marker was designed

in the intergenic region between exon2 and *orf456* (17 bp upstream from 5' end of *orf456*), it was supposed to be the fused region via homologous recombination in the previous report (Jo, 2007).

Since the previously reported atp6SCAR and coxIISCAR were dominant markers, the presence of the faint sterile-specific band in N cytoplasm may lead to the mis-classification of pepper breeding lines. To solve this problem, one common forward primer and two different reverse primers specific to N *coxII* and *orf456* genes were designed after aligned their sequences (Figs. 1 and 2). By using three primers (coxIITri-M1 marker), N and S *coxII* specific bands were co-amplified in MS lines, but only N *coxII* specific band was amplified in maintainer lines (Figs. 2 and 3A). Moreover, reverse primer for S *coxII* was specifically designed 275 bp downstream of *orf456*, relatively stable amplifying patterns were observed in all cycle conditions and breeding lines. The newly designed primer sets showed co-dominant patterns which are very useful tool for pepper breeding and germplasm evaluation.

Total nine maintainer lines and four germplasms showed different results when using coxIISCAR and coxIITri-M1 markers. Maintainer lines analyzed in this study were used for the production of commercial F_1 hybrid seeds, their mitotypes have been confirmed by repeated testcrosses by breeders. Recently, *orf507* in S cytoplasm was reported as putative causal gene of MS phenotypes in pepper (Gulyas et al., 2010). The *orf507* specific primers were applied to the same breeding lines, commercial cultivars and germplasms, the results were same as when using coxIISCAR marker (data not shown). Because *orf507* region overlaps *orf456* and more precise amino acid termination codon was defined 49 nt downstream from the original site, these two SCAR markers showed similar patterns when amplified with genomic DNAs.

At present, coxII region was regarded as the possible causal gene of MS phenotype in pepper, partial results of this study implied controversial hypothesis. For example, 'Daecheong' and 'Singsinghong' were bred using CMS lines, 'Cheonhajanggun' was bred using GMS lines. But their mitotypes analyzed using coxIISCAR and coxIITri-M1 markers were different from actual cytoplasms. Though possible inconsistency of mitotypings using present markers, the frequency of male-sterile cytoplasm contained *orf456* was relatively high in the commercial cultivars bred in Korea, but nearly absent in the germplasm originated from foreign countries. Although large potion of commercial F_1 hybrid cultivars were bred using CMS except for some of disease resistant cultivars or non-pungent green fruit lines. However, we analyzed limited numbers of plants in this study, more diverse germplasms should be tested to confirm the reliability of coxIITri-M1 marker.

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