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Development of Gene-Based Markers for The *Pun1* Pungency Gene in Pepper (*Capsicum* spp.) for Marker-Assisted Selection

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Abstract. Pungency in fruits of pepper (*Capsicum* spp.) is due to the accumulation of capsaicinoids. *Pun1* is an important gene responsible for pungency. To provide information for marker-assisted selection (MAS) of pungency, we developed and evaluated five codominant markers based on sequence of the *Pun1* gene. The usefulness of these gene-based markers for pungency in MAS was tested using 85 pungent and non-pungent lines and cultivars. Our results demonstrated that these markers can discriminate the pungent and non-pungent genotypes. Our results show that the five gene-based markers could be useful as a marker-assisted selection tool.

***Additional key words*:** capsaicinoid, C gene, MAS, molecular breeding

Introduction

Pepper (*Capsicum* spp.) is one of the most cultivated vegetable and spice crops over the world due to its high nutritional value, and plays an important role as a constituent in many of the food industries. One of the important quality attributes of pepper is pungency, which is defined as accumulation of alkaloid capsaicin and its analog, collectively known as capsaicinoids. Biosynthesis of this compound occurs only within genus of *Capsicum* (Walsh and Hoot, 2001). Although pungent peppers are widely used as spices or vegetables in cuisine, as a food additive, and as medicine, non-pungent or "sweet" peppers have also been selected for use as vegetable and as a spice known as paprika.

As in the other crops, the search for lines with the best general combining ability is an extremely important part of pepper hybrid breeding. In the genus *Capsicum*, wild species have been useful in breeding for disease resistance, high yield and adaptability to environmental stresses (Yoon et al., 2004). However, inter-specific incompatibility resulting in no viable hybrid has been one of the limiting factors in using wild and related species in breeding program. With a few notable exceptions such as introgression of resistance to tobacco mosaic virus from *C. chinense* and *C. chacoense* into *C. annuum* (Pickersgill, 1997), inter-specific hybridization has not commonly been used for breeding in *Capsicum*. Thus, to improve disease and pest resistances in sweet pepper, inter-specific hybridization breeding method is now beginning to be exploited by breeders.

Nowadays, breeders are using hot peppers as a donor in breeding sweet peppers in order to introgress resistant genes from pungent peppers into non-pungent peppers, because many resistant genes were found in *C. chinense* and *C. annuum* (Wang and Bosland, 2006). However, accurate selection of non-pungent lines is not easy to apply routinely. Current methods for phenotyping pungency such as high-performance liquid chromatography (HPLC) analysis (Collins et al., 1995) and panel tasting of fruits are time-consuming, labor intensive and require specialized facilities. In addition, pungency level is also significantly affected by the environment, for example, capsaicin concentration of genotypes is lower under lower temperature and light intensity (Murakami et al., 2006) and could be unstable across environments (Zewdie and Bosland, 2000). Availability of molecular genetic markers tightly linked to this gene may help in selection of genotypes within a breeding program through MAS. In application, MAS allows determination of pungency at very early stages of plant development. Therefore, distinguishing between pungent and non-pungent genotypes in large population will be easier and faster, helping to provide a low cost and efficient approach in breeding.

Early genetic studies have identified a single dominant gene, *C* (now known as *Pun1*), which is essential for capsaicinoid production. *Pun1* probably functions as an acyltransferase to complete the capsaicinoid synthesis (Stewart et al., 2005). The absence of pungency, known as a recessive allele *pun1*, apparently results from a 2.5-kb deletion spanning the first exon and part of the promoter region therefore preventing expression of Acyltransferase 3 (AT3) (Stewart et al., 2005). In addition, putative acyltransferases *Catf-1* and *Catf-2* have been identified and are involved in pungency of Capsicum (Lang et al. 2006). Based on these genetic studies, several molecular

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markers linked with *Pun1* have been identified. For example: Blum et al. (2002) have developed a cleaved amplified polymorphic sequence (CAPS) marker, which based on sequence of the *Capsicum* fibrillin gene, located 0.4 cM away from *Pun1*. Another CAPS marker was mapped, located 3.6 cM or 12.2 cM from *Pun1* depending on the population used in the study (Minamiyama et al., 2005). However, these markers are too far from the *Pun1* gene to be relevant for MAS. Five sequence-characterize amplified region (SCAR) markers were designed based on sequence of SB2-66, a cDNA clone, cosegregated exactly with *Pun1* locus (Lee et al., 2005). Most markers are dominant markers and not suitable to use in MAS, because heterozygous plants can not be distinguished from homozygotes. Recently, a single nucleotide polymorphism (SNP) associated with pungency was detected within an expressed sequence tag (EST) of 307 bp which was identified after expression analysis of the EST clone SB2-66 (Garces-Claver et al., 2007). We have tested these markers on our materials, but the results were not consistent over PCR reactions and the bands were very faint. They could not be useful for marker-assisted pepper breeding to remove pungency from sweet pepper. We report here the development of *Pun1* gene-based markers based on sequence of acyltransferase (*Pun1*) gene. The gene-based markers are simple, fast, low costing with no need for sequence information. The developed markers were tested for their usefulness with various pepper germplasms for non-pungent pepper breeding.

Materials and Methods

Plant materials

A total of eighty-five pepper genotypes were used in this study. Of these, six sweet pepper cultivars [62.2, 72.1, Tisana, 99G54, Early Calwonder (ECW), and 00G49] were provided by the Pepper & Breeding Institute (PBI), Korea; the other seventy-nine lines and cultivars were obtained from the National Institute of Horticulture & Herbal Science (NIHHS), Rural Development Administration (RDA), Korea. Most of the genotypes belong to *C. annuum*, two were *C. baccatum*, four were *C. chinense*, and one was *C. frutesens*.

DNA extraction and primer design

Genomic DNA of the genotypes was extracted from young leaves of field-grown plants following the protocol described by Murray et al. (1980) and slightly modified by Fulton et al. (1995).

The sequences of the acyltransferase (*Pun1*) gene, which were present in the three non-pungent genotypes, 'Maor', 'Jupiter' and 'Sweet 3575', and the four pungent genotypes, 'Thai Hot', 'Hot 1493', 'Habanero' and 'BG2814' (Stewart et al., 2005), the *Catf-1* and *Catf-2* genes (Lang et al., 2006), the clone SB2-66 (Kim et al., 2001) and the nonfunctional acyltransferase (*Pun1*) gene (Stewart et al., 2007) were obtained from the public databases National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/sites/entrez) and aligned using the programs BioEdit version 7 (Hall, 1999) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Primers were designed based on sequence of the *Pun1* gene presented in genotypes 'Sweet 3575' and 'Hot 1493' (Stewart et al., 2005). A total of twenty-one primers were designed including three primers specific to non-pungency with the program Primer3 version 04 (Rozen and Skaletsky, 2000). Oligonucleotide primers were synthesized by Bioneer (Korea). The primers were firstly screened for polymorphism on 'Habanero' and 'ECW' which are known to have the *Pun1* gene and the *pun1* gene, respectively. PCR conditions were optimized before genotyping was performed on the set of pepper lines and cultivars.

PCR amplification

Each PCR reaction was carried out in a total reaction volume of 25 μl containing 15-20 ng of genomic DNA, 200 μM deoxyribonucleotide triphosphate mix (Roche, Korea), 1 X PCR buffer and 1 U of *Taq* DNA polymerase (Roche), 0.25 μM of each primer. PCR reactions were performed on a Mastercycler epGradientS (Eppendorf). The amplification profile consisted of an initial denaturation for 5 min at 94℃ followed by 35 cycles of PCR amplification under the following parameters: 1 min at 94℃, 1 min at the annealing temperature 40-65℃ (depending on melting temperature of primers), 1 min of primer elongation at 72℃. A final incubation at 72℃ for 5 min was programmed to allow completion of primer extension. Amplified products were separated on 2% ethidium bromide-agarose gels using 1 X TBE buffer for 1.5 hours at 120 V and photographed under UV light. A 100 bp ladder was used as a molecular weight marker.

Results

Marker development

A total of twenty-one primers were designed from the full-length nucleotide sequence of the *Pun1* gene. Seventy-eight primer combinations were screened *C. annuum* 'Habanero' and *C. annuum* 'ECW' but only twelve showed polymorphism. The amplicons, which were obtained by the twelve combinations, are shown in Fig. 1B. The *Pun1* gene structure and primer positions are shown in Fig. 1A. Primers 5NF, 5NR and 11NR were designed to target non-pungent genotypes; however, both primer combinations 5NF/11NR and 5NF/5NR generated a fragment larger than 3000 bp from pungent genotype, whereas 800 and 700 bp fragments were produced in non-pungent genotype, respectively. Primers 2F/2R and 3F/3R generated 500 and 1400 bp products from pungent peppers, respectively, while no fragment was generated from non-pungent peppers. These primers were combined with non-

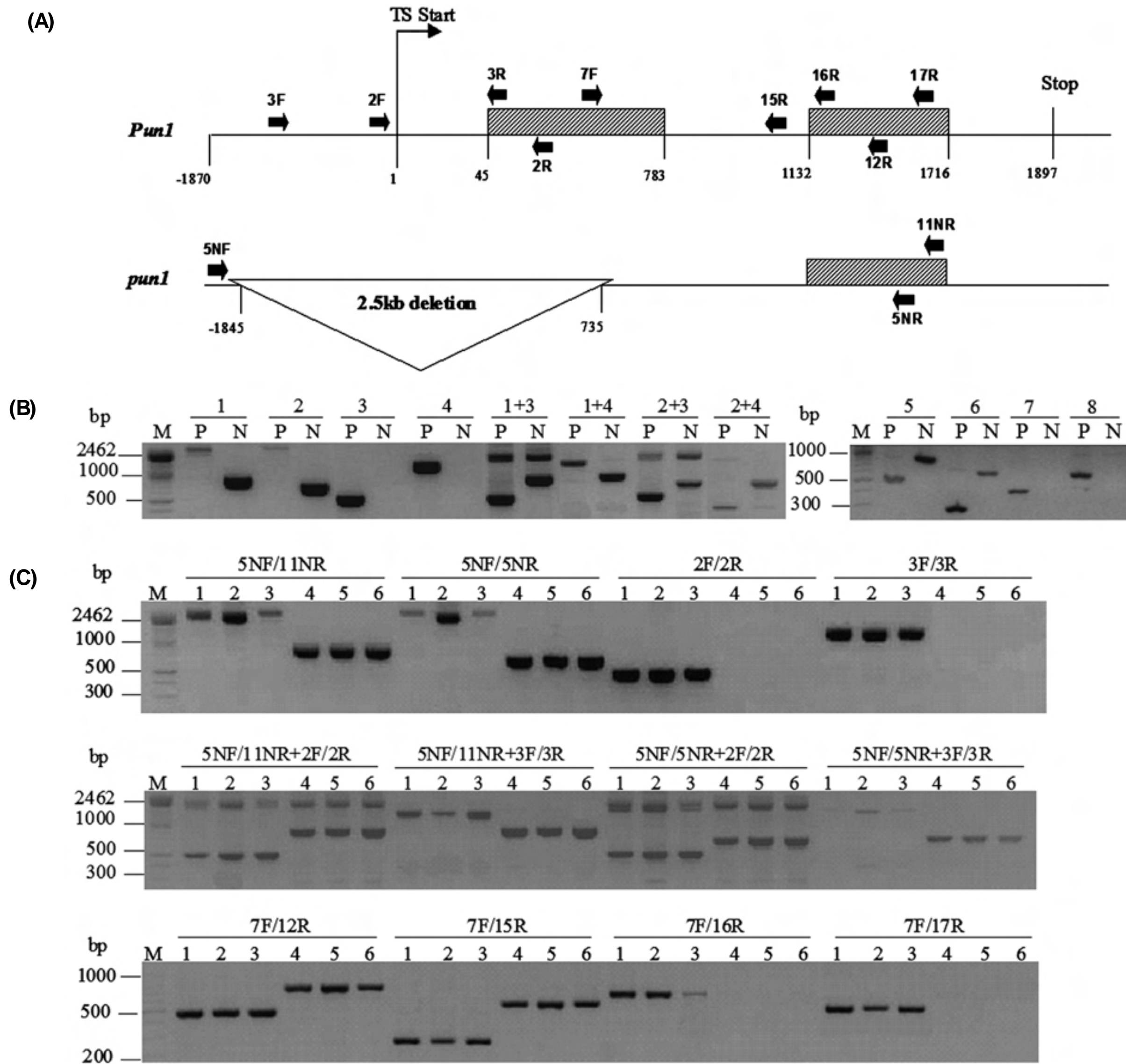

Fig. 1. Comparison of gene-based markers. (A): Structure of *Pun1* loci from pungent and non-pungen genotypes (Stewart et al., 2005). Primers, which were combined and showed polymorphism between pungent and non-pungent peppers, are indicated by arrows. Exons are presented with closed boxes and the deletion in the *pun1* allele is presented with an inverted triangle. (B): Polymorphic primers screening between pungent and non-pungent genotypes. M, 100 bp molecular weight marker; 1, primer combination 5NF/11NR; 2, 5NF/5NR; 3, 2F/2R; 4, 3F/3R; 5, 7F/12R; 6, 7F/15R; 7, 7F/16R; 8, 7F/17R; P, pungent genotype *C. annuum* 'Habanero'; N, non-pungent genotype *C. annuum* 'ECW' (C): Selected polymorphic primers screening between pungent and non-pungent genotypes. M, 100 bp molecular weight marker; 1-3, pungent genotypes *C. annuum* 'RNaky', *C. baccatum* var. pendulum 'C-130', *C. annuum* '8NH1', respectively; 4-6, non-pungent genotypes *C. annuum* 'Goal', *C. annuum* '62.2', *C. annuum* 'Tisana', respectively. Primer combinations are indicated at the top.

pungent specific primers in a multiplex PCR. Combinations 5NF/11NR+2F/2R and 5NF/5NR+2F/2R produced a 500 bp fragment from the pungent genotype and 800 and 700 bp fragments from the non-pungent genotype, respectively. Additionally, these combinations amplified 2.5 and 2.0 kb fragments to pungent and non-pungent genotypes, respectively. Primer combination 5NF/11NR+3F/3R produced an 800 bp fragment; 5NF/5NR+3F/3R generated a 700 bp fragment in the non-pungent genotype. Both primer combinations amplified a 1300 bp product in the pungent genotype.

When forward primer 7F was combined with the four reverse primers, 12R, 15R, 16R, and 17R, combination 7F/12NR generated about 450 bp fragment from the pungent pepper and 800 bp fragment from the non-pungent genotype, whereas 7F/15NR produced 600 and 800 bp products from the pungent and non-pungent genotypes, respectively. Fragments of 400 bp and 600 bp were generated from the pungent genotype by primer combinations 7F/16NR and 7F/17NR, respectively, whereas no fragments were detected in the non-pungent genotype.

Before these markers used in MAS, another three pungent genotypes such as *C. annuum* 'RNaky', *C. baccatum* var. *pen-*

Table 1. List of primers showing polymorphism between pungent and non pungent genotypes.

Primer code	Primer sequence (5' → 3')	Tm (C°)	Source of sequence
2F[z]	GGGGTTGGGTAGAGGTTGTT	54.0	Hot 1493
2R[y]	CTGCAAGAGATGCATGAGGA	53.3	
3F	CCTCGGGTCTACGAACAGAA	53.9	
3R	AGAGGGGGTGAGAGAGGAAG	53.5	
7F	GGTTCTCTCATTACGCCACAAATATT	57.7	
12R	ACAACTTCAGCCCTTGTTGG	53.8	
15R	ACACCAATAAGTGGAGTGCT	48.2	
16R	TTCATTTCGGAAAAACTACAA	49.3	
17R	TTTGATGGTAGCATTGATGA	48.4	
5NF	ATTAGAAGGTCATACCGCTCCA	54.3	Sweet 3575
5NR	GGTCTTTCTTGTATGCCACCTC	54.1	
11NR	AGGTCTTCCCCATCCAAAAT	53.6	

[z] F: forward.
[y] R: reverse.

Table 2. Genotyping and phenotyping results of the *Capsicum* accessions tested.

Spicies	Accession	Origine	Phenotype	Genotype				
				M1	M2	M3	M4	M5
C. annuum	3_25_27	AVRDC	NP	-	-	-	-	-
C. annuum	8avs1	AVRDC	NP	-	-	-	-	-
C. annuum	8avs2	AVRDC	NP	-	-	-	-	-
C. annuum	8avs3*	AVRDC	NP	+	+	+	+	+
C. annuum	8avs4	AVRDC	NP	-	-	-	-	-
C. annuum	8avs5	AVRDC	NP	-	-	-	-	-
C. annuum	De Cheiro	Brazil	NP	na	+	na	+	+
C. annuum	Valentina	Commercial var, Isreal	NP	-	-	-	-	-
C. annuum	Baltasar	Commercial var, Isreal	NP	-	-	-	-	-
C. annuum	Amadeo	Commercial var, Isreal	NP	na	na	na	na	na
C. annuum	PBY-16000	Commercial var, Isreal	NP	-	na	-	na	na
C. annuum	PL-4007	Commercial var, Isreal	NP	-	-	-	-	-
C. annuum	Goal	Commercial var, Korea	NP	-	-	-	-	-
C. annuum	Score	Commercial var, Korea	NP	-	-	-	-	-
C. annuum	ECW	PBI, Korea	NP	-	-	-	-	-
C. annuum	62.2	PBI, Korea	NP	-	-	-	-	-
C. annuum	72.1	PBI, Korea	NP	-	-	-	-	-
C. annuum	Tisana	PBI, Korea	NP	-	-	-	-	-
C. annuum	99G54	PBI, Korea	NP	-	-	-	-	-
C. annuum	00G49	PBI, Korea	NP	-	-	-	-	-
C. annuum	8N2	NIHHS, Korea	NP	-	-	-	-	-
C. annuum	LV2319	AVRDC	P	na	+	na	+	+
C. annuum	AC2258	AVRDC	P	na	+	na	+	+
C. annuum	PI201234	AVRDC	P	+	+	+	+	+
C. annuum	LF	AVRDC	P	+	+	na	+	+
C. annuum	Blue Dragon	Local var, Korea	P	na	+	na	+	+
C. annuum	Emsung	Local var, Korea	P	na	na	na	+	+
C. annuum	Bungeo	Local var, Korea	P	+	+	+	+	+
C. annuum	Kangwong Kalcho	Local var, Korea	P	na	+	na	+	+
C. annuum	Kalmi	Local var, Korea	P	na	+	na	+	+
C. annuum	Mac min	Local var, Vietnam	P	na	+	na	+	na
C. annuum	Ot Thoc	Local var, Vietnam	P	na	+	+	na	na
C. annuum	YCM334	NIHHS, Korea	P	+	+	+	+	+
C. annuum	Tean	NIHHS, Korea	P	+	+	+	+	+
C. annuum	7HR3	NIHHS, Korea	P	na	+	na	+	+
C. annuum	7HR5	NIHHS, Korea	P	na	+	na	+	+
C. annuum	7HR6	NIHHS, Korea	P	na	+	+	+	+
C. annuum	7HR27	NIHHS, Korea	P	na	+	+	+	+
C. annuum	LE5	NIHHS, Korea	P	-	na	na	na	na
C. annuum	8NH1	NIHHS, Korea	P	+	+	+	+	+

Table 2. Genotyping and phenotyping results of the *Capsicum* accessions tested.(Continue)

Spicies	Accession	Origine	Phenotype	Genotype				
				M1	M2	M3	M4	M5
C. annuum	8NH2	NIHHS, Korea	P	+	+	+	+	+
C. annuum	8NH3	NIHHS, Korea	P	+	+	+	+	+
C. annuum	8NH4	NIHHS, Korea	P	na	+	+	+	+
C. annuum	8N1	NIHHS, Korea	P	+	+	+	+	+
C. annuum	8N3	NIHHS, Korea	P	-	h	-	+	+
C. annuum	8N4	NIHHS, Korea	P	na	+	+	+	+
C. annuum	8N5	NIHHS, Korea	P	+	+	+	+	+
C. annuum	K134203	Turkey	P	na	+	na	+	+
C. annuum	K134214	Turkey	P	+	+	+	+	+
C. annuum	K134237	Turkey	P	na	+	na	+	+
C. annuum	Konchowang	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Gold Medal	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Golden Light	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Dangcho	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Dangcho Welbeeing	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Dogbul King	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Dogyachungchung	Commercial var, Korea	P	-	h	-	+	+
C. annuum	Manita	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Mansahyungtong	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Bukang	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Bukang Q	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Buja	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Buchon	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Palita	Commercial var, Korea	P	na	+	+	+	+
C. annuum	Gumtap	Commercial var, Korea	P	na	+	+	+	+
C. annuum	Super Manita	Commercial var, Korea	P	na	+	+	+	+
C. annuum	Super Bigarim	Commercial var, Korea	P	na	na	na	+	na
C. annuum	Shinhong	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Omchungna	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Yuggkang Red King	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Wanggun	Commercial var, Korea	P	+	+	+	+	+
C. annuum	Wangdaebag	Commercial var, Korea	P	+	+	+	+	+
C. annuum	Urigun	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Chammani	Commercial var, Korea	P	na	+	+	+	+
C. annuum	Chunhajeil	Commercial var, Korea	P	na	+	na	+	+
C. annuum	Tesan	Commercial var, Korea	P	na	na	na	+	+
C. annuum	PR Mujug	Commercial var, Korea	P	+	+	na	+	+
C. annuum	PR Chohong	Commercial var, Korea	P	na	+	na	+	+
C.baccatum	GA	NIHHS, Korea	P	na	+	na	+	+
C.baccatum pendulum	C-130	NIHHS, Korea	P	+	+	+	+	+
C.chinense	Bode Rosinha	Brazil	P	na	na	na	na	na
C. chinense	Biquinho	Brazil	P	na	na	na	+	+
C. chinense	Murupi	Brazil	P	+	+	na	+	+
C. chinense	Habanero	NIHHS, Korea	P	+	+	na	+	+
C. frutesens	Malagueta	Brazil	P	na	+	na	+	na

* Genotype was phenotyped as non-pungency but carried *Pun1* allele with five markers tested. NP and '-': non-pungency, P and '+': pungency, M1: marker 5NF/5NR, M2: marker 5NF/5NR+2F/2R, M3: marker 5NF/11NR+3F/3R, M4: marker 7F/12R, M5: marker 7F/15R, 'h': heterozygosity, na: not amplified.

dulum 'C-130', and inbred line '8NH1', and three non-pungent genotypes, *C. annuum* 'Goal', *C. annuum* '62.2', and *C. annuum* 'Tisana', were tested using the above polymorphic primers. PCR amplifications of the genotypes are shown in Fig. 1C. All the three pungent genotypes and three non-pungent genotypes gave the same results as *C. annuum* 'Habanero' and *C. annuum* 'ECW', respectively.

Marker-assisted selection

Out of the twelve markers developed, five markers, 5NF/5NR, 5F/5NR+2F/2R, 5F/11NR+3F/3R, 7F/15R and 7F/12R, were more informative and selected to assay 85 pepper genotypes representing our germplasm including 'Habanero' and 'ECW' which were used as control. Of the eighty-five genotypes evaluated (Table 2), we found 33 produced PCR products for the five markers, and no fragments were amplified

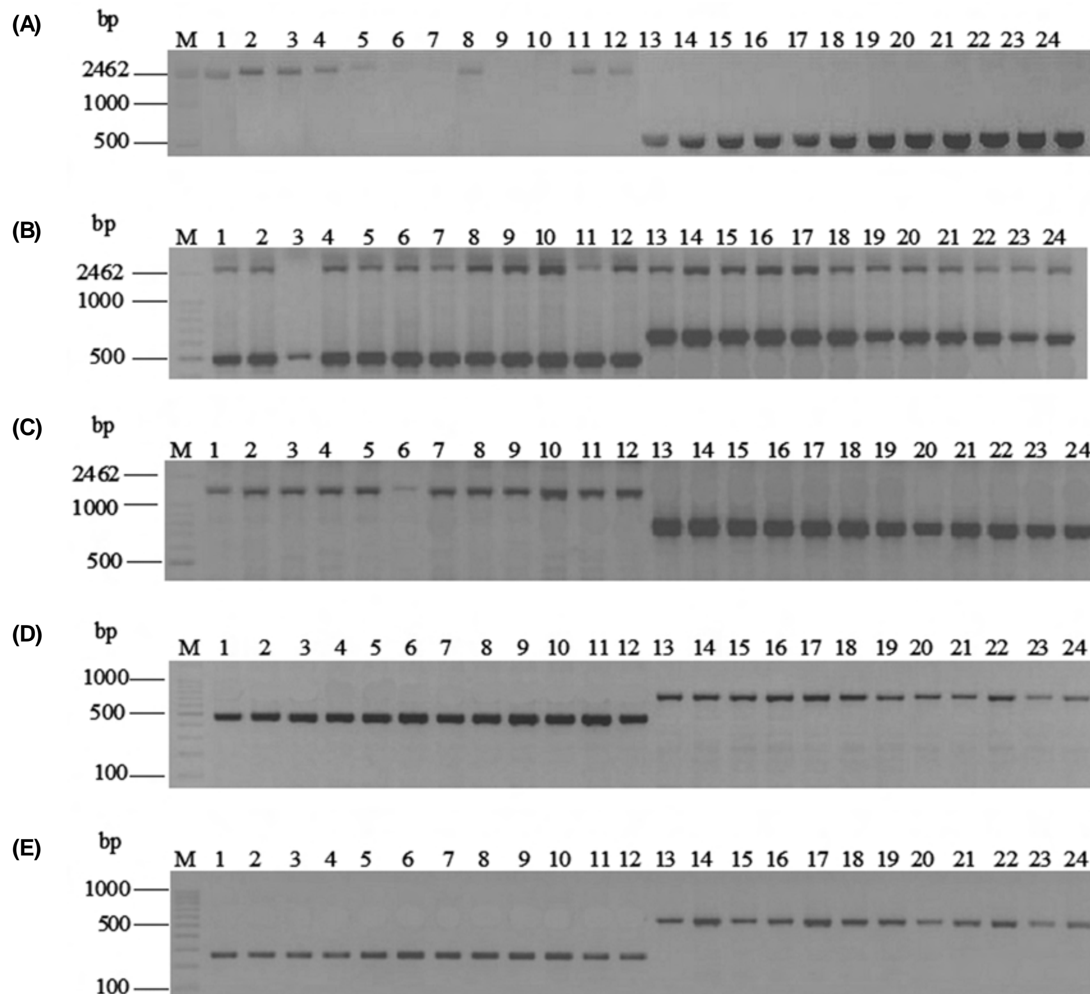

Fig. 2. Agarose gel electrophoresis of gene-based markers. M, 100 bp molecular weight marker; 1, *C. annuum* 'Habanero'; 13, *C. annuum* 'ECW'. Numbers from 2 to 24 are selected genotypes from germplasm. Markers used were (A) 5NF/5NR, (B) 5NF/5NR+2F/2R, (C) 5NF/11NR+3F/3R, (D) 7F/12R, and (E) 7F/15R.

in *C. chinense* 'Bode Rosinha' and *C. annuum* cv. 'Amadeo'. Sixty-two lines and cultivars were genotyped as pungency and nineteen were non-pungency. 'Dogyachungchung' and '8N3' were genotyped as non-pungency by primer combinations 5NF/5NR and 5F/11NR+3F/3R, as pungency by 7F/15R and 7F/12R, and as heterozygous by 5F/5NR+2F/2R (Table 2). In the case of the combinations 5NF/5NR and 5F/11NR+3F/3R, there were no amplification in a large of number of genotypes, 54.1% and 49.4%, respectively, while 9.4%, 5.9% and 9.4% of total genotypes were not amplified by the combinations 5F/5NR+2F/2R, 7F/15R, and 7F/12R, respectively. The sizes of the PCR products generated by these primers were similar among the pungent genotypes as well as among the non-pungent genotypes. The results of genotyping from 22 lines and cultivars by these markers are shown in Fig. 2.

In order to determine whether the pungent and non-pungent peppers associated with that response, a tasting panel was performed for all genotypes (Dr. Myoung-Cheol Cho, personal communication). The genotyping results of the lines and cultivars were consistent with the phenotypic data, except for '8avs3', 'LE5', 'Dogyachungchung' and '8N3' (Table 2). '8avs3' was identified as a pungent pepper using all five markers, while a tasting panel identified this line as a non-pungent pepper. On the contrary, 'LE5' was phenotyped as a pungent pepper, while the marker 5NF/5NR identified this line as a non-pungent pepper. The two pungent genotypes 'Dogyachungchung' and '8N3' were identified as non-pungent peppers using markers 5NF/5NR and 5F/11NR+3F/3R.

Discussion

Since cDNA clone SB2-66, which corresponded to biosynthesis of capsaicinoid (Kim et al., 2001), was isolated, several research groups have used its sequence to study genetic control of pungency. Lee et al., (2005) have found an EST clone, SB2-66, tightly linked to the *Pun1* and co-localized with capsaicinoid syntheses (CS) gene, and demonstrated that the cause of lacking capsaicinoid synthesis in non-pungent

pepper fruits was due to a 2.5 kb deletion presented in non-pungent peppers. Several SCAR markers have been developed; however, most of them are dominant markers, which would not be able to distinguish heterozygous lines. Stewart et al. (2005) have identified SB2-66 as a candidate gene for *Pun1* which was referred as Acyltransferase 3 (AT3). Genomic sequence of *Pun1* from *C. chinense* and *C. annuum* were determined and a large 2.5-kb deletion was observed in the non-pungent genotypes. The approach adopted was to design primers specific to non-pungent and pungent genotypes and combine them in a multiplex PCR to achieve more efficiency in MAS. From the results shown in Fig. 1B, primer combinations 2F/2R and 3F/3R only generated fragments in pungent peppers while 5NF/5NR and 5NF/11NR produced a large fragment from pungent peppers and smaller fragments in non-pungent peppers. This could be due to the sequence of primer 5NF that is matched with the left border of the deletion in the non-pungent genotypes, indicating presence of the deletion in the non-pungent peppers as described previously (Lee et al. 2005; Stewart et al. 2005).

Garces-Claver et al. (2007) have recently developed a SNP marker using the tetra-primer ARMS-PCR method (Ye et al., 2001); however, this marker was not consistent over PCR tests with our germplasm. The aim of this study was to develop gene-based markers to be able to distinguish pungent and non -pungent genotypes as well as homozygous and heterozygous plants at seedling stage for breeding program. Because of the absence of fragments in the non-pungent genotypes by dominant markers, all dominant markers were discarded. Among the eight codominant markers developed in this study, five markers, 5NF/5NR, 5NF/5NR+2F/2R, 5NF/11NR+3F/3R, 7F/12R, and 7F/15R, were much more informative and visible (Fig. 1C).

In the past, researchers needed both a good breeding strategy and a good sense for selecting desirable phenotypes. However, evaluation of traits often requires specialized techniques. Molecular marker technology which is especially based on PCR analysis can greatly reduce the amount of labor needed for evaluating phenotypes by prescreening with MAS. The usefulness of MAS can increase by creating markers tightly linked to a target gene. In this study, we developed five codominant markers based on the sequence of the *Pun1* gene (Stewart et al., 2005) and were able to amplify 33 out of the 85 selected pepper genotypes representing our germplasm. Expected bands not amplified from remaining genotypes could be because these markers are not well-conserved in these genotypes. Among thirty-three pepper genotypes identified through our pre-screening process, thirty-one manifested phenotype that perfectly matched PCR-genotype. Markers 7F/15R and 7F/12R identified two pungent peppers ‘Dogyachungchung’ and ‘8N3’ as pungency, while markers 5NF/5NR and 5F/11NR +3F/3R identified these lines as non-pungency and similar with ‘LE5’. Only marker 5F/5NR+2F/2R can identify heterozygous plants. This could be due to a genetic recombination which occurred between the marker and the gene during evolution or during plant breeding process (Park and Crosby, 2007, Yang et al 2008). ‘8avs3’ was genotyped as pungency by the five markers, while phenotyping resulted as a non-pungent pepper. This could be because the tasting panel was conducted under lower temperature and light intensity, leading to very low capsaicin concentration (Murakami et al., 2006). These results demonstrate efficiency and preciseness of MAS in that target genes can be identified at any plant growth stage with the use of tightly linked DNA molecular markers. The new codominant markers developed in this study make it possible to detect pungency in early stage in *Capsicum* for non-pungent pepper breeding.

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