Hort. Environ. Biotechnol. 52(2):204-210. 2011.
DOI 10.1007/s13580-011-0164-y

Research Report

Use of Cf-9 Gene-based Markers in Marker-assisted Selection to Screen Tomato Cultivars with Resistance to *Cladosporium fulvum*

Hai Thi Hong Truong[1,2], HakSoon Choi[1*], Myoung Cheoul Cho[1], Hye Eun Lee[1], and Jung Ho Kim[1]

[1]*Vegetable Research Division, National Institute of Horticultural & Herbal Science, Rural Development Administration, Suwon 440-706, Korea*
[2]*Department of Horticulture Science, Agronomy Faculty, Hue University of Agriculture and Forestry, 102 Phung Hung, Hue, Vietnam*

*Corresponding author: choichoy@korea.kr

Received August 6, 2010 / Accepted January 4, 2011

Abstract. Identification of the PCR markers tightly linked to genes that encode important agronomic traits is useful for marker-assisted selection (MAS). The *Cf*-9 locus confers resistance to most races of the leaf mold fungus *Cladosporium fulvum* and belongs to a large gene family. We developed three gene-based markers (8F/10R, 8F/12R, and 10F/10R) from the *Cf*-9 gene sequence. Of the 101 tomato genotypes tested, 9 commercial F_1 hybrids carried 8F/10R-, 8F/12R-, and 10F/10R-specific bands. Our results demonstrate the usefulness of these markers for MAS for *C. fulvum* resistance.

***Additional key words*:** leaf mold, MAS, *Solanum lycopersicum*

Introduction

Cladosporium fulvum is a semi-biotrophic pathogen which only infects tomato (*Solanum* spp.) where it causes leaf mold. It is a common and destructive disease on tomatoes worldwide grown under humid conditions. The traditional methods for controlling leaf mold consist of frequent applications of fungicides and use of resistant varieties. Moreover, reduction of chemical treatments limits risks for farmers and consumers. Therefore, in order to realize a sustainable agriculture and to get high quality products in terms of health safe, the use of resistant varieties becomes a principle tool to reduce damages caused by pathogen. In commercial tomato varieties, a number of genes that confer resistance to *C. fulvum* have been introgressed from wild tomato species. The *Cf-2*, *Cf-9*, and genes were introgressed from *S. pimpinellifolium*, *Cf- 4* was derived from *S. habrochaites* (Thomas et al.,1998), and *Cf-5* was derived from *S. lycopersicum* (Dickinson et al., 1993). However, races of *C. fulvum* can overcome the resistances of the *Cf* genes (Lindout et al., 1989). For example tomato varieties 'Lovely-Ai' and 'Momotaro-Natsumi' with the *Cf-9* gene are resistant to all Japanese *C. fulvum* races; however, symptoms of leaf mold were observed on these varieties in 2007 (Enya et al., 2009). Thus, new races seemed to have appeared, which make resistant varieties are of limited use. Successive backcrossing was used to generate a series of near isogenic lines (Tigchelar, 1984) containing single introgressed *Cf* genes (*Cf-2*, *Cf-4*, *Cf-5*, and *Cf-9*). However, pyramiding a number of resistant genes to a desirable cultivar is space- and time-consuming. Availability of molecular genetic markers for these genes may help in selection of the resistance genotypes in a breeding program without disease screening. Availability of co-dominant markers that distinguish between heterozygous and homozygous plants facilitates easy and fast selection. Among the *Cf* genes, the tomato resistance gene *Cf-9* confers resistance to most races of *C. fulvum*; however, there is no marker suitable for marker-assisted selection.Two AFLP markers tightly linked to the *Cf-9* gene have been reported (Thomas et al., 1995). However, those markers are dominant markers and not suitable for MAS, because heterozygous plants can not be distinguished from homozygotes. Since the *Cf-9* gene was sequenced (Parniske et al., 1997), there was no publicly available marker developed from the sequence of the *Cf-9* gene. The gene-based markers are simple, fast, and low-cost and more accurate, no chances for linkage break. We report here the development of *Cf-9* gene-based markers and demonstrate their usefulness for disease-resistance breeding.

Materials and Methods

Plant Materials

Thirteen resistant commercial varieties and sixteen susceptible commercial varieties were provided by the National Institute of Horticultural & Herbal Science (NIHHS), Rural Development Administration (RDA). Among these, four commercial varieties for each of leaf mold resistance and susceptibility were used for genotyping analysis. Resistant genotypes included 'Kayachal', 'Cholchab205', 'Lycopin09', and 'Songalsongal'. Susceptible genotypes were 'Suuitu', 'Tichotu', 'Olenchikelol', and 'Minigelol'. In addition, 42 commercial F_1 hybrids and 59 lines/cultivars from NIHHS germplasm were also tested. All genotypes belong to *S. lycopersicum*.

DNA Extraction and Primer Design

Genomic DNA was extracted from young leaves of young seedlings following the protocol described by Murray et al. (1980) and slightly modified by Fulton et al. (1995). The genomic sequence of *S. pimpinellifolium Cf-9*resistance gene cluster (accession number: AJ002236) was obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/sites/entrez).

Primers were designed from the *Cf-9* gene sequence using the program Primer3 version 4.0 (Rozen and Skaletsky, 2000). Oligonucleotide primers were synthesized by Bioneer Corp. (Korea).

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), 1X PCR buffer and 0.5 U of DNA *Taq* polymerase (Roche, Korea), 0.25 μM of each primer. Reactions were performed on an Eppendorf Mastercycler Gradient. The amplification profile consisted of an initial denaturation for 5 min at 94°C followed by 35 cycles of PCR amplification under the following parameters: 1 min at 94°C, 1 min at the annealing temperature 45-60°C (depending on melting temperature of the primers), and 1 min of primer elongation at 72°C. A final incubation at 72°C for 5 min was programmed to allow completion of primer extension. For touchdown PCR reactions, samples were incubated for 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at the annealing temperature, 1 min at 72°C, followed by a final extension period for 5 min at 72°C. The annealing temperature was 45 or 60°C (depending on melting temperature of the primers) for the first cycle, and decreased by 1°C for each subsequent cycle. Amplified products were separated on a 2% ethidium bromide-agarose gels using 0.5 X TBE buffer for 1.5 hours at 120 V and photographed under UV light. A 100bp ladder was used as a molecular weight marker.

Southern Blot Analysis

A Southern blot analysis was performed using a standard protocol (Sambrook et al., 1989) using a *Cf-9* 1400-bp fragment which obtained from resistant genotype 'Gayachal' using primer 8F/10R. Briefly, the genomic DNAs (20-30 μg) of the resistant and susceptible inbred lines were digested with restriction enzyme *Eco*RI (200 unit) for 24 hours at 37℃ and separated by electrophoresis in 0.8% agarose gel at 30V for 16h. The procedure of the following steps such as electrophoresis, denaturation, neutralization, transblotting and hybridization with ^{32}P-labelled probes were carried out using the method of Sambrook et al. (1989). The hybridization was performed with Church buffer at 65℃ (Foster et al., 2002).

Results and Discussion

Marker Development

A total of twenty-eight primers were designed from the *Cf-9* gene sequence. Sixty-six primer combinations were screened on four resistant commercial varieties ('Gayachal', 'Jealjab205', 'Licopin-9', and 'Songalsongal') and four sus-

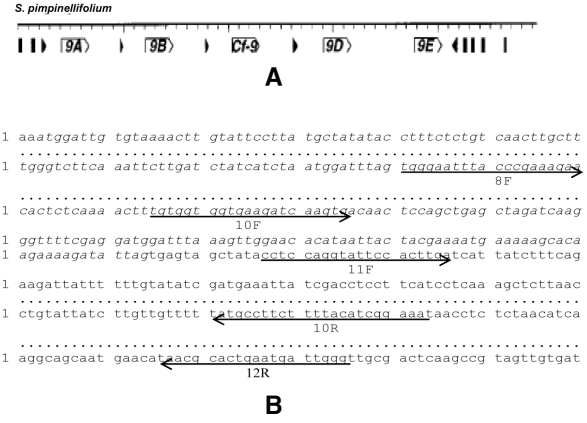

Fig. 1. A; Structure of *Cf-9* gene family (Parniske et al., 1997). 9A, 9B, 9D, 9E are homologs of *Cladosporium fulvum* resistance gene *Cf-9* (*Hcr9*). B; Selected *Cf-9* gene family sequence. Primers that revealed codominant polymorphism between resistant and susceptible tomato commercial varieties are indicated by arrows. Italic characters are *Cf-9* gene sequence. Non-italic characters are non coding region of *Cf-9* gene.

Table 1. List of selected PCR primers used for screening polymorphism.

Primer	Sequence (5' - 3')	T_m^z(oC)
CF8	TGGGAATTTACCCGAAAGAA	54.4
CF10	TGTGGTGGTGAAGATCAAGTGA	54.0
CR10	TTTCCGATGTAAAAGAAGGCATA	54.4
CR12	CCCAATCATTCAGTGCGTTA	54.3

[z]Melting temperature.

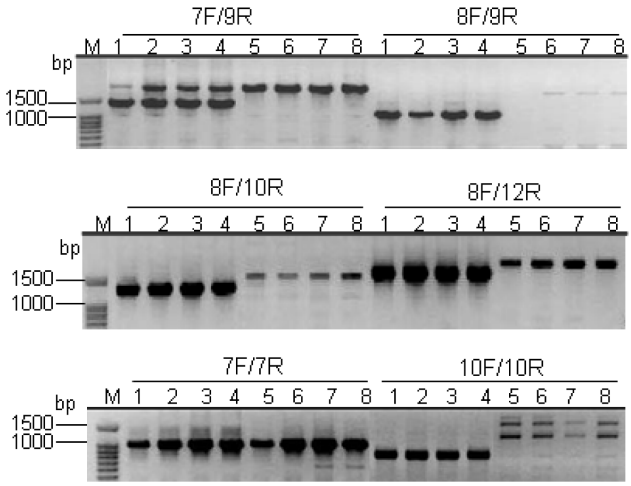

Fig. 2. Selected primer combinations were screened between resistant and susceptible genotypes. Lanes M, 100 bp molecular weight marker; 1-4, resistant commercial F_1 varieties 'Gayachal', 'Jealjab 205', 'Licopin-9', and 'Songalsongal', respectively; 5-8, susceptible commercial F_1 varieties 'Sweet', 'Dissert', 'Orangcaroll', and 'Minicaroll', respectively. Primer combinations are indicated at the top.

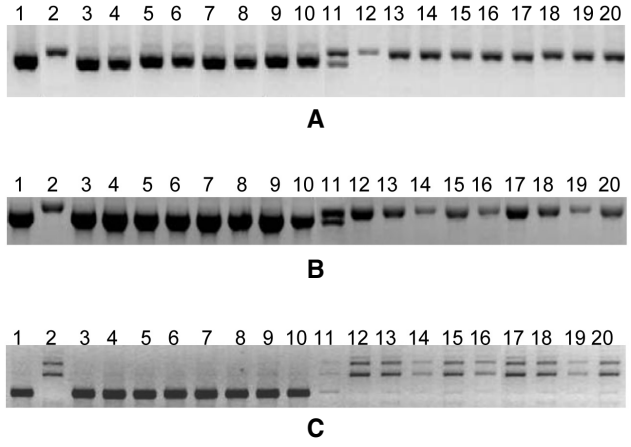

Fig. 3. Agarose gel elestrophoresis of 20 commercial F_1 hybrid varieties using the *Cf-9* gene-based markers: A; 8F/10R, B; 8F/12R, and C; 10F/10R. Lanes: 1, resistant commercial F_1 hybrid 'Gayachal' 2, susceptible commercial F1 hybrid 'Sweet' 3-20, selected commercial F_1 hybrids; 11, commercial variety 'Seagun' which showed heterozygous pattern.

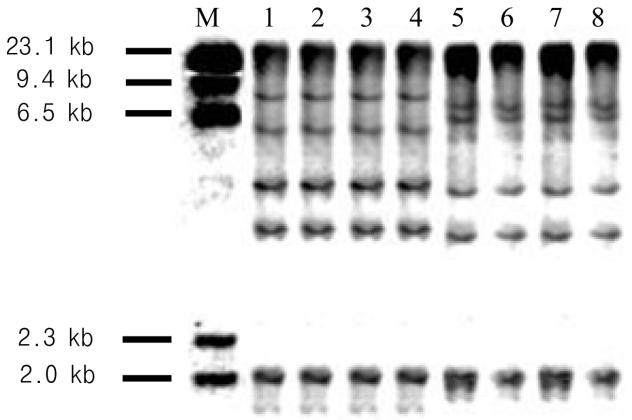

Fig. 4. Homologs of the *Cf-9* gene in DNA gel blots of the resistant and susceptible varieties. Genomic DNAs were digested with restriction enzyme *Eco*RI cut then probed with the *Cf-9* 1400-bp PCR product which obtained from variety 'Gayachal' using primer 8F/10R. Lanes M, 23-kb lambda *Hind*III marker; 1-4, resistant commercial F_1 varieties 'Gayachal', 'Jealjab205', 'Licopin-9', and 'Songalsongal', respectively; 5-8, susceptible commercial F_1 varieties 'Sweet', 'Dissert', 'Orangcaroll', and 'Minicaroll'.

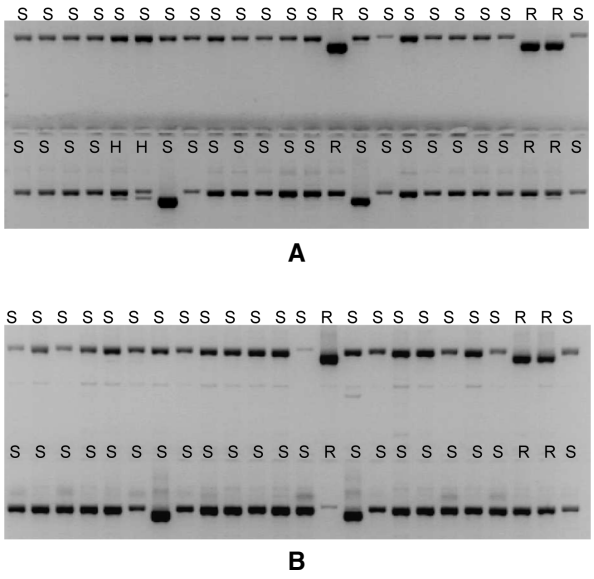

Fig. 5. Agarose gel elestrophoresis of 48 tomato germplasm using the *Cf-9* gene-based markers: A; 8F/10R, B; 8F/12R. Lanes R, resistance; S, susceptibility; H, heterozygosity.

ceptible commercial varieties ('Sweet', 'Dissert', 'Orangcaroll', and 'Minicaroll'). Of these, three primer combinations detected codominant polymorphisms between resistant and susceptible varieties, while seven showed dominant bands, 56 were monomorphic (Fig. 1). Primer sequences are listed in Table 1. Structure of the *Cf-9* gene family is shown in Fig. 1a, and the selected primer positions are showed in Fig. 1b. To develop co-dominant markers for MAS, all dominant markers were discarded. Primers 8F/10R and 8F/12R generated about 1400 bp and 1500 bp products from resistant genotypes, respectively, while about 1500 bp and 1600 bp fragments were generated from susceptible genotypes. Combination 10F/10R produced an 850 bp fragment from resistant genotypes and double bands, which were 1200 bp and 1500 bp fragments, from susceptible genotypes (Fig. 2). This could be the homologous sequences located in the other regions of the gene cluster.

Before these markers were used in MAS, another nine resistant commercial varieties and 12 susceptible commercial varieties were tested using the above polymorphic primers (Table 2). PCR amplifications of the genotypes are shown in Fig. 3. All the resistant and susceptible genotypes gave the same results as 'Gayachal' and 'Sweet', respectively, except resistant commercial F_1 hybrid 'Kingcaroll' and 'Macarena',

Table 2. Genotyping results of the tomato germplasm tested.

Genotype	Origin	Phenotype	Genotyping/Marker		
			8F/10R	8F/12R	10F/10R
Gayachal	Commercial var, Korea	R	R	R	R
Jealjab205	Commercial var, Korea	R	R	R	R
Kingcaroll	Commercial var, Korea	R	S	S	S
Licopin-9	Commercial var, Korea	R	R	R	R
Songalsongal	Commercial var, Korea	R	R	R	R
Macarena	Commercial var, Korea	R	S	S	S
Dotaerang gold	Commercial var, Korea	R	R	R	R
Supertop	Commercial var, Korea	R	R	R	R
Yoyocaptin	Commercial var, Korea	R	R	R	R
Supersunrod	Commercial var, Korea	R	R	R	R
Bigdena	Commercial var, Korea	R	R	R	R
Rokusanmaro	Commercial var, Korea	R	R	R	R
Rapsodi	Commercial var, Korea	R	R	R	R
Seagun	Commercial var, Korea	S	H	H	H
Sweet	Commercial var, Korea	S	S	S	S
Dissert	Commercial var, Korea	S	S	S	S
Orangcaroll	Commercial var, Korea	S	S	S	S
Minicaroll	Commercial var, Korea	S	S	S	S
Sysilianroze	Commercial var, Korea	S	S	S	S
Housechelange	Commercial var, Korea	S	S	S	S
Housedotaerang	Commercial var, Korea	S	S	S	S
Solution	Commercial var, Korea	S	S	S	S
Legend	Commercial var, Korea	S	S	S	S
Special	Commercial var, Korea	S	S	S	S
Liliangs	Commercial var, Korea	S	S	S	S
Madison	Commercial var, Korea	S	S	S	S
Tenten	Commercial var, Korea	S	S	S	S
B-Blocking	Commercial var, Korea	S	S	S	S
Naver	Commercial var, Korea	S	S	S	S
IT142186	NAC	nt	S	S	S
IT142188	NAC	nt	S	S	S
IT142190	NAC	nt	S	S	S
IT142191	NAC	nt	S	S	S
IT142195	NAC	nt	S	S	S
IT156673	NAC	nt	S	S	S
IT156677	NAC	nt	S	S	S
IT160384	NAC	nt	S	S	S
IT180572	NAC	nt	S	S	S
IT189947	NAC	nt	S	S	S
IT200624	NAC	nt	S	S	S
IT201639	NAC	nt	S	S	S
IT201642	NAC	nt	S	S	S
IT201657	NAC	nt	S	S	S
IT201665	NAC	nt	S	S	S

Table 2. Continued.

Genotype	Origin	Phenotype	Genotyping/Marker		
			8F/10R	8F/12R	10F/10R
IT203278	NAC	nt	S	S	S
IT203280	NAC	nt	S	S	S
IT203287	NAC	nt	S	S	S
IT203291	NAC	nt	S	S	S
IT203294	NAC	nt	S	S	S
IT203296	NAC	nt	S	S	S
IT203449	NAC	nt	S	S	S
IT203452	NAC	nt	S	S	S
IT203455	NAC	nt	S	S	S
IT203466	NAC	nt	S	S	S
IT203468	NAC	nt	S	S	S
IT207036	NAC	nt	S	S	S
IT207222	NAC	nt	S	S	S
IT207244	NAC	nt	S	S	S
K044840	NAC	nt	S	S	S
K052128	NAC	nt	S	S	S
K060617	NAC	nt	S	S	S
K060625	NAC	nt	S	S	S
K060627	NAC	nt	S	S	S
K060650	NAC	nt	S	S	S
K060707	NAC	nt	S	S	S
K100254	NAC	nt	S	S	S
K100255	NAC	nt	S	S	S
K100256	NAC	nt	S	S	S
K100259	NAC	nt	S	S	S
K100262	NAC	nt	S	S	S
K100263	NAC	nt	S	S	S
K100265	NAC	nt	S	S	S
K100266	NAC	nt	S	S	S
K100267	NAC	nt	S	S	S
K101301	NAC	nt	S	S	S
K121155	NAC	nt	S	S	S
K124968	NAC	nt	S	S	S
K125501	NAC	nt	S	S	S
K125502	NAC	nt	S	S	S
K126944	NAC	nt	S	S	S
K130763	NAC	nt	S	S	S
K133673	NAC	nt	H	S	H
K146467	NAC	nt	S	S	S
Wonmeonghwageoja	Commercial var, China	nt	S	S	S
Yachesee	Commercial var, China	nt	S	S	S
Sinkwanwoo	Commercial var, China	nt	R	R	R
Damangolyo	Commercial var, China	nt	S	S	S
Wonmeong (rkn-108)	Commercial var, China	nt	R	R	R

Table 2. Continued.

Genotype	Origin	Phenotype	Genotyping/Marker		
			8F/10R	8F/12R	10F/10R
Hapjak206	Commercial var, China	nt	R	R	R
Wonmeongokyeohong	Commercial var, China	nt	S	S	S
Sinkwan106	Commercial var, China	nt	R	R	R
Taegong1	Commercial var, China	nt	R	R	R
Shinbon-N88	Commercial var, China	nt	H	S	H
Wonmeong-909	Commercial var, China	nt	S	S	S
Wonmeongjinjuhwang	Commercial var, China	nt	S	S	S
Daehongangdo	Commercial var, China	nt	S	S	S
Alt516	Commercial var, China	nt	S	S	S
Bizuhongka	Commercial var, China	nt	S	S	S
Hapjak903	Commercial var, China	nt	S	S	S
Hapjak906	Commercial var, China	nt	H	S	H
Sinkwan1ho	Commercial var, China	nt	R	R	R
Hapjak908	Commercial var, China	nt	S	S	S
Hongjinjubunga	Commercial var, China	nt	S	S	S
Shinbon-158	Commercial var, China	nt	R	R	R
Hapjak919daehongbunga	Commercial var, China	nt	R	R	R
Hapjak928bunga	Commercial var, China	nt	R	R	R
Woonaehwangsija	Commercial var, China	nt	S	S	S
Daejosija	Commercial var, China	nt	S	S	S
Jukbooyou	Commercial var, China	nt	S	S	S
Daehwangsija	Commercial var, China	nt	S	S	S
Angdobunga	Commercial var, China	nt	S	S	S
Chumjuengbunga	Commercial var, China	nt	S	S	S
Bukdoseoungsea	Commercial var, China	nt	S	S	S
Americadaehong	Commercial var, China	nt	S	S	S
T1-Mobir	Local var, Uzbekistan	nt	S	S	S
T2-Riogranidu	Local var, Uzbekistan	nt	S	S	S
T3-Bargagradski	Local var, Uzbekistan	nt	S	S	S
T4-Timkaiyuorit22	Local var, Uzbekistan	nt	S	S	S
T5-Uzbekistan	Local var, Uzbekistan	nt	S	S	S
T6-Yousoofarboski	Local var, Uzbekistan	nt	S	S	S
Galkandeu	Local var, Uzbekistan	nt	S	S	S
Avemaria	Local var, Uzbekistan	nt	S	S	S
Seyihum	Local var, Uzbekistan	nt	S	S	S
Bohol	Local var, Uzbekistan	nt	S	S	S
Arisenna	Local var, Uzbekistan	nt	S	S	S
Hawaii7996	AVRDC	nt	S	S	S
Hawaii7998	AVRDC	nt	S	S	S
Gc9	University of Wisconsin	nt	S	S	S
Gc171	University of Wisconsin	nt	S	S	S
Gc173	University of Wisconsin	nt	S	S	S

NAC; National Agrobiodiversity Center, AVRDC; Asian Vegetable Research and Development Center, S; susceptibility, R; resistance, H; heterozygosity, nt; not tested.

which did not produced *Cf-9* specific bands. 'Seagun' produced heterozygous pattern by using the three primer combinations (Fig. 3). This could be due to criteria of disease classification. For example: 'Kingcaroll' and 'Macarena'displayed moderate resistance to *C. fulvum* in the field trial evaluation, while 'Seagun'displayed a lower susceptibility. This demonstrates efficiency and preciseness of MAS that target genes that can be identified at any plant growth stage with the use of tightly linked DNA molecular markers.

Cf-9 belongs to a large gene family consisting of clustered Hcr9 genes (Hcr9s; Homologues of *Cladosporium fulvum* resistance gene *Cf-9*) (Parniske et al., 1997). In order to confirm the copy number of the *Cf-9*gene, a Southern blot analysis was performed using *Cf-9* 1400-bp fragment, which obtained from variety 'Gayachal'using primer 8F/10R, as a probe. The result showed that multiple copies of the 1400-bp fragment were generated (Fig. 4). Thus, our result was consistent with result of Parniske et al. (1997).

Using *Cf-9* Markers in Marker-assisted Selection

In the past, to select desirable phenotypes, researchers needed both a good breeding strategy and a good sense. However, the evaluation of disease resistance or other traits often requires specialized techniques. Recent molecular-marker technology especially that based on PCR analysis can greatly reduce the amount of labor needed for evaluating phenotypes by prescreening with MAS. The usefulness of MAS can be increased by creating markers tightly linked to a target gene. In this study, we developed three *Cf-9* gene-based markers, 8F/10R, 8F/12R, and 10F/10R, and used to assay 101 tomato genotypes (Fig. 5). The results showed that these markers identified nine commercial varieties possessing the *Cf-9* gene (Table 2). Markers 8F/10R and 10F/10R identified two commercial F_1 hybrids, 'Shinbon-N88' and 'Hapjak906', and line K130763, as heterozygosity, but not 8F/12R. 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). Thomas et al. (1995) have identified two AFLP markers tightly linked to the *Cf-9* gene, it would be appropriate to use in MAS. However, these markers are dominant; they can not differentiate homozygous plants from heterozygous plants. Thus, they are not applicable in MAS. The three codominant markers reported in this study are like-wise perfect and provide new codominant markers for the identification of leaf mold resistance in *Solanum*. The results have demonstrated the feasibility of these markers, and its application for germplasm screening and are being used for screening of leaf mold resistance in breeding programs.

Literature Cited

Dickinson, M., D.A. Jones, and J.D.G. Jones. 1993. Close linkage between the *Cf-2/Cf-5* and *Mi* resistance loci in tomato. Mol. Plant-Microbe Interact. 6:341-347.

Enya, J., K. Ikeda, T. Takeuchi, N. Horikoshi, T. Higashi, T. Sakai, Y. Iida, K. Nishi, and M. Kubota. 2009. The first occurrence of leaf mold of tomato caused by races 4.9 and 4.9.11 of *Passalora fulva* (syn. *Fulvia fulva*) in Japan. J. Gen. Plant Pathol. 75:76-79.

Fulton, T.M., J. Chunwongse, and S.D. Tanksley. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol. Biol. 13:207-209.

Foster, E., D. Schneiderman, M. Cloutier, S. Gleddie, L.S. Robert. 2002. Modifying the pollen coat protein composition in *Brassica*. Plant J. 31:1-12.

Lindhout, P., W. Korta, M. Cislik, I. Vos, and T. Gerlagh. 1989. Further identification of races of *Cladosporium fulvum* (*Fulvia fulva*) on tomato originating from the Netherlands, France and Poland. Neth. J. Plant Pathol. 95:143-148.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucl. Acid Res. 19:4321-4325.

Park, S. and K. Crosby. 2007. Molecular marker-assisted selection of the *Bs2* bacterial spot resistance gene in diverse pepper cultivars and breeding lines. Hortscience 42:1006.

Parniske, M., K.E. Hammond-Kosack, C. Golstein, C.M. Thomas, D.A. Jones, K. Harrison, B.B.H. Wulff, and J.D.G. Jones. 1997. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato. Cell 91:821-832.

Parniske, M. and J.D.G. Jones. 1999. Recombination between diverged clusters of the tomato *Cf-9* plant disease resistance gene family. Proc. Natl. Acad. Sci. USA 96:5850-5855.

Rozen, S. and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers, p. 365-386. In: S. Krawetz and S. Misener (eds). Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, USA.

Sambrook, J., E.F. Fritsch, T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press.

Thomas, C.M., M.S. Dixon, M. Parniske, C. Golstein, and J.D.G. Jones. 1998. Genetic and molecular analysis of tomato *Cf* genes for resistance to *Cladosporium fulvum*. Phil. Trans. R. Soc. Lond. B. 353:1413-1424.

Thomas, C.M., M. Zabeau, D.A. Jones, K.A. Norcott, B.P. Chadwick, and J.D.G. Jones. 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J. 8:785-794.

Tigchelaar, E.C. 1984.Collections of isogenic tomato stocks. Rep. Tomato Genet. Coop. 34:55-57.

Yang, H., D. Renshaw, G. Thomas, B. Buirchell, and M. Sweetingham. 2008. A strategy to develop molecular markers applicable to a wide range of crosses for marker assisted selection in plant breeding: a case study on anthracnose disease resistance in lupin (*Lupinus angustifolius* L.). Mol. Breed. 21:473-483.