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Application of Disease Resistance Markers for Developing Elite Tomato Varieties and Lines

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Abstract. Using the abundant available information about the tomato genome, we developed DNA markers that are linked to disease resistant loci and performed marker-assisted selection (MAS) to construct multi-disease resistant lines and varieties. Resistance markers of Ty-1, T2, and I2, which are linked to disease resistance to *Tomato yellow leaf curl virus* (TYLCV), *Tomato mosaic virus* (ToMV), and Fusarium wilt, respectively, were developed in a co-dominant fashion. DNA sequences near the resistance loci of TYLCV, ToMV, and Fusarium wilt were used for primer design. Reported candidate markers for powdery mildew-resistance were screened and the 32.5Cla marker was selected. All four markers (Ty-1, T2, I2, and 32.5Cla) were converted to cleavage amplification polymorphisms (CAPS) markers. Then, the CAPS markers were applied to 96 tomato lines to determine the phenetic relationships among the lines. This information yielded clusters of breeding lines illustrating the distribution of resistant and susceptible characters among lines. These data were utilized further in a MAS program for several generations, and a total of ten varieties and ten inbred lines were constructed. Among four traits, three were introduced to develop varieties and breeding lines through the MAS program; several cultivars possessed up to seven disease resistant traits. These resistant trait-related markers that were developed for the tomato MAS program could be used to select early stage seedlings, saving time and cost, and to construct multi-disease resistant lines and varieties.

***Additional key words*:** cleavage amplification polymorphisms marker, Fusarium wilt, marker-assisted selection, powdery mildew, *Tomato mosaic virus*, *Tomato yellow leaf curl virus*

Introduction

The first genetic map of the tomato was developed using restriction fragment length polymorphisms (RFLPs) of 57 loci (Bernatzky et al., 1986). Since then, hundreds of genes related to important traits have been localized on tomato genetic maps. For example, the disease resistance loci for leaf mold disease (*Cf-9*; Thomas et al., 1995) and bacterial speck (*Pto*; Martin et al., 1993) have been mapped, and traits such as yield (Eshed and Zamir, 1995) and fruit weight (Alpert and Tanksley, 1996) have also been mapped. In addition to mapping studies, other technologies such as genome sequencing (Shibata, 2005), bacterial artificial chromosomes (BACs; Budiman et al., 2000), expressed sequence tags (ESTs; Yamamoto et al., 2005) and microarrays (Moore et al., 2005) have been used to generate DNA marker information including RFLPs (Bonierbale et al., 1988), simple sequence repeats (SSRs; Areshchenkova and Ganal, 2002) and conserved orthologous sets (COSs; Wu et al., 2006).

DNA markers have been tagged for target loci and used for marker-assisted selection (MAS) in tomato breeding programs. Lindhout et al. (1994) identified three loci associated with earliness-related genes by RFLP analysis and was able to use them to obtain flowers and fruits one month early. Gu et al. (1995) used PCR-based markers for cost-effective, reliable, and rapid screening of a large number of samples for MAS. Therefore, the use of MAS with PCR-based markers, especially co-dominant markers, may efficiently reduce labor and costs, and shorten generations of conventional breeding.

Although MAS has been applied to breeding programs in many crops, a detailed MAS protocol has not been developed for elite tomato varieties. In this study, DNA markers pertinent to disease resistance were identified and used to develop new tomato varieties. We focused on two viruses, TYLCV

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and ToMV, and two fungi, Fusarium wilt and powdery mildew, because these pathogens heavily impact tomato yield. Genes conferring resistance to these pathogens have already been characterized. Resistance to TYLCV is controlled by dominant alleles of *Ty-1* (Zamir et al., 1994), *Ty-2* (Hanson et al., 2000, 2006) and *Ty-3* (Ji and Scott, 2006), which were identified using the 'LA1969' (*Solanum chilense*), 'B6013' (*S. habrochatis*) and 'LA2779' (*S. chilense*) strains, respectively, and mapped to chromosomes 6, 11, and 6, respectively. Especially *Ty-1*, among three TYLCV-tolerance loci on the chromosome 3, 6, and 7 detected from LA1969, was found as a major locus (Zamir et al., 1994). The *Tm-2a* gene (also known as *Tm-2*^{2}; Young et al., 1988) from *Lycopersicon peruvianum* (Alexander, 1971) confers resistance to ToMV and is located on the long arm of chromosome 9 near the centromere (Schroedoer el al., 1967). *Tm-2*^{2} gene was isolated via transposon tagging (*Tm-2*^{2}; Lanfermeijer et al., 2003) from *L. esculentum*. The resistance level expressed from *Tm-1* locus on the chromosome 5 from *L. hirsutum* is not durable while the resistance conferred by *Tm-2*^{2} is related to durability, which is useful in tomato cultivation for a long period. Fusarium wilt is caused by *Fusarium oxysporum* f. sp. *lycopersici* races 1, 2, and 3, and is regulated by single dominant genes of races *I1*, *I2*, and *I3*, respectively. Resistance to *Oidium neolycopersici*, which causes powdery mildew, is related to the dominant gene group of *Ol-1*, *Ol-3*, *Ol-4*, *Ol-5*, and *Ol-6* (Bai et al., 2003) located on chromosome 6.

Resistance linked markers for *Ty-1*, *Tm-2*^{2}, *I2*, and *Ol-4* and *Ol-6* loci were developed and selected using available information. We converted *Ty-1*, *Tm-2*^{2}, and *I2* gene- or their linked marker-information into PCR based markers in a co-dominant fashion. These markers were used in rapid MAS of tomato lines and to breed tomato varieties with multi-disease resistance.

Materials and Methods

Primer Design

DNA markers for *Ty-1* and *I2* were developed from RFLP marker sequences near the mapped positions of phenotype markers (Ji et al., 2007; Sarfatti et al., 1989). T2 markers were produced directly from the *Tm-2*^{2} gene (AF536201) sequence, and the 32.5Cla marker co-segregating with *Ol-4* and *Ol-6* loci on the chromosome 6 was selected among the candidate primer sets (Bai et al., 2005). Primer sets were designed for several candidate markers using Primer3 (http://frodo.wi.mit.edu/primer3). All amplified PCR products were sequenced and analyzed using CAPS Designer (http://solgenomics.wur.nl/tools) to develop CAPS markers (Table 1).

PCR Analysis for Screening

Ninety-six tomato breeding lines from Nongwoo Bio Co. were used for MAS (Table 2). Young leaves were detached from tomato lines and ground using a Retsch MM301 Ball Mill (Daigger, USA) for 5 min. Genomic DNA was prepared following the method described by Kang et al. (2001). PCR reactions were conducted in total volumes of 20 μL containing 10 x buffer, 0.2 mM dNTP, 0.5 mM forward and reverse primers, 20 ng genomic DNA, and 0.5 unit Taq DNA polymerase (Genet Bio Inc., Korea). PCR was performed using the GeneAmp PCR System 9700 (Applied Biosystems, USA) with the following conditions: denaturation at 94℃ for 5 min; 35 cycles of 94℃ for 1 min, 55-59℃ (Table 1) for 1 min, 72℃ for 1 min, and final elongation at 72℃ for 5 min.

PCR products were separated in 1.5% agarose gel to assess whether the PCR reaction had amplified the specific bands designed by the primer sets. After restriction enzyme digestion (Table 1) for 2 h at 37℃, PCR bands were separated in ethidium bromide stained 2% agarose gel at 100 V for 40

Table 1. CAPS markers of Ty-1, T2, I2, and 32.5Cla.

Marker	Sequence(5'-3')	Tm (℃)	Length. (R/S[z]; bp)	Restricted enzyme	Chr.[y]	Reference
Ty-1	F[x]: CCTAAAGTGAATGACCCTTTGAGA R : TGATTATATCATGTAGCGAAACTTATA	56	380/400-500	*Hin* fI	6	Ji et al., 2007
T2	F : AGAGAGAAATGAGACACATTCG R : AACCCATTCGGGCTATGAAT	56	1000/1500	*Hae* III	9	Lanfermeijer et al., 2003
I2	F : GCTAGAACAGTTGCAGTTCCAG R : GCATATCGACAGTGCAGGACCT	59	800/550	*Taq* I	11	Sarfatti et al., 1989
32.5Cla	F : ACACGAAACAAAGTGCCAAG R : CCACCACCAAACAGGAGTGTG	56	350/1000	*Hin* fI	4	Bai et al., 2005

[z]Specific band from PCR amplification for resistant (R) and susceptible (S) tomatoes.
[y]Chromosome number of located marker.
[x]F: forward primer; R: reverse primer.

Table 2. Description of 96 tomato lines screened for the presence of desirable traits.

Name	Origin	Trait				Name	Origin	Trait			
		Plant shape[z]	Fruit					Plant shape	Fruit		
			Color	Shape	Weight[y]				Color	Shape	Weight
NW1	China	ID	Pink	Round	200	NW49	Europe	ID	Red	Ovate	136
NW2	China	ID	Pink	Round	200	NW50	Europe	ID	Red	Ovate	102
NW3	China	ID	Pink	Round	245	NW51	Europe	ID	Red	Ovate	110
NW4	China	ID	Pink	Flattened	313	NW52	Southwest Asia	ID	Red	Flattened	90
NW5	Japan	ID	Pink	Slightly flattened	110	NW53	China	ID	Red	Ovate	132
NW6	Japan	ID	Pink	Slightly flattened	130	NW54	China	ID	Red	Ovate	93
NW7	Japan	ID	Pink	Slightly flattened	220	NW55	Europe	ID	Red	Ovate	85
NW8	Europe	ID	Pink	Slightly flattened	150	NW56	South America	ID	Red	Round	88
NW9	Europe	ID	Pink	Slightly flattened	150	NW57	South America	ID	Red	Ovate	116
NW10	Europe	ID	Pink	Slightly flattened	120	NW58	South America	ID	Red	Round	117
NW11	Europe	ID	Pink	Slightly flattened	150	NW59	Europe	ID	Red	Round	18
NW12	Europe	ID	Pink	Slightly flattened	140	NW60	Europe	ID	Red	Flattened	16
NW13	Europe	ID	Pink	Slightly flattened	110	NW61	Europe	ID	Red	Flattened	17
NW14	Europe	ID	Pink	Slightly flattened	120	NW62	Europe	ID	Red	Flattened	18
NW15	Europe	ID	Pink	Slightly flattened	130	NW63	Europe	ID	Red	Flattened	19
NW16	Europe	ID	Pink	Slightly flattened	120	NW64	Europe	ID	Red	Flattened	18
NW17	Europe	ID	Pink	Slightly flattened	180	NW65	Europe	ID	Red	Flattened	17
NW18	Europe	ID	Pink	Slightly flattened	170	NW66	Europe	D	Purple	Ovate	18
NW19	Japan	ID	Pink	Slightly flattened	161	NW67	Europe	ID	Red	Round	22
NW20	Korea	ID	Pink	Round	257	NW68	Europe	ID	Red	Round	20
NW21	Japan	ID	Pink	Slightly flattened	310	NW69	Europe	ID	Red	Round	18
NW22	Europe	ID	Pink	Slightly flattened	297	NW70	Europe	ID	Red	Round	22
NW23	Europe	ID	Pink	Flattened	436	NW71	China	ID	Red	Ovate	20
NW24	Europe	ID	Pink	Flattened	425	NW72	China	D	Red	Ovate	14
NW25	Europe	ID	Pink	Slightly flattened	367	NW73	Europe	D	Red	Ovate	15
NW26	Europe	ID	Pink	Round	250	NW74	Europe	D	Yellow	Ovate	15
NW27	Europe	ID	Pink	Flattened	344	NW75	China	D	Yellow	Ovate	16
NW28	Europe	ID	Pink	Flattened	330	NW76	Europe	ID	Red	Round	27
NW29	Japan	ID	Pink	Slightly flattened	184	NW77	Europe	ID	Red	Round	18
NW30	China	ID	Pink	Round	135	NW78	Europe	ID	Red	Round	40
NW31	China	ID	Pink	Round	195	NW79	Europe	ID	Red	Round	29
NW32	Europe	ID	Red	Round	120	NW80	Europe	ID	Red	Flattened	120
NW33	Europe	ID	Red	Flattened	165	NW81	Europe	D	Red	Round	156
NW34	Europe	ID	Red	Round	96	NW82	Europe	D	Red	Flattened	291
NW35	Europe	ID	Purple	Round	101	NW83	South America	D	Red	Ovate	83
NW36	Europe	ID	Purple	Round	104	NW84	Europe	D	Red	Ovate	102
NW37	Europe	ID	Red	Flattened	184	NW85	Southwest Asia	D	Red	Ovate	131
NW38	Europe	ID	Red	Slightly flattened	361	NW86	Southwest Asia	D	Red	Ovate	118
NW39	Europe	ID	Red	Flattened	200	NW87	South America	D	Red	Ovate	109
NW40	Europe	ID	Red	Flattened	187	NW88	Southwest Asia	D	Red	Ovate	96
NW41	Europe	ID	Red	Flattened	203	NW89	Europe	D	Red	Ovate	119
NW42	Europe	ID	Red	Flattened	332	NW90	South America	D	Red	Ovate	82
NW43	Europe	ID	Red	Flattened	129	NW91	Europe	D	Red	Ovate	116
NW44	Europe	ID	Red	Round	219	NW92	Europe	D	Red	Ovate	82
NW45	South America	ID	Red	Flattened	295	NW93	Europe	ID	Pink	Slightly flattened	230
NW46	Europe	ID	Red	Flattened	220	NW94	Europe	ID	Pink	Slightly flattened	225
NW47	Europe	ID	Red	Ovate	80	NW95	Europe	ID	Red	Round	22
NW48	South America	ID	Red	Ovate	65	NW96	Korea	ID	Red	Ovate	23

[z]ID: indeterminate; D: determinate.
[y]Mean of five fruits in grams.

min. Polymorphic bands such as R (homozygous resistant), S (homozygous susceptible), and H (heterozygous resistant) were scored using a UV transilluminator.

Examination for Disease

The levels of resistance or susceptibility to TYLCV and powdery mildew were evaluated in an infested field in Indonesia and in the infested plastic-house in the breeding institute of Nongwoo Bio Co., respectively. ToMV and Fusarium wilt were inoculated in a separated plastic-house to analyze the levels of resistance or susceptibility. The concentration of inoculums was prepared according to the published protocols (Lanfermeijer et al., 2003; Sarfatti et al., 1989) and the examination methods for measuring symptom levels of four diseases were conducted based on the manuals belong to Nongwoo Bio Co.

Clustering Analysis

All band patterns of the Ty-1, T2, I2, and 32.5Cla markers that were obtained from the 96 tomato lines were scored and used for clustering analysis. Their genetic distances were calculated by NTSYS-pc version 2.2 (Rohlf, 2005) according to the manufacturer's instructions, using the unweighted pair-group method with arithmetic averaging (UPGMA) clustering analysis of the Nei-Li genetic similarity coefficient matrices.

Results and Discussion

Development of Co-dominant Markers for Disease Resistance

To develop resistance-linked markers, PCR primers were designed using basic genome information for the *Ty-1*, *Tm-2*^{2}, and *I2* loci and PCR was performed to produce amplified bands (data not shown). The PCR products were sequenced and aligned to identify DNA sequence polymorphisms for conversion into CAPS markers (Table 1 and Fig. 1). Three enzymes (*Hin* fI, *Hae* III, and *Taq* I) were used to digest the PCR products to obtain CAPS markers for *Ty-1*, *Tm-2*^{2} and *I2*, respectively. *Hin* fI was used to digest the fragment amplified by the 32.5Cla marker, which denotes powdery mildew resistance loci of *Ol-4* and *Ol-6*. Each CAPS marker showed co-dominant band patterns (Fig. 1), and the markers were confirmed by studies of breeding lines containing resistant or susceptible sources (Table 3). The digested band scores from Ty-1, T2, I2, and 32.5Cla were matched with resistant and susceptible tomato phenotypes, indicating close relationships between the developed markers and resistant loci.

These markers were considered to be appropriate for use in MAS. Due to the inoculation difficulty of the powdery mildew pathogen, the identification of resistance-linked marker for powdery mildew is particularly desirable. In our tomato breeding, co-dominant CAPS markers have proven more informative than other markers for selecting tomato samples

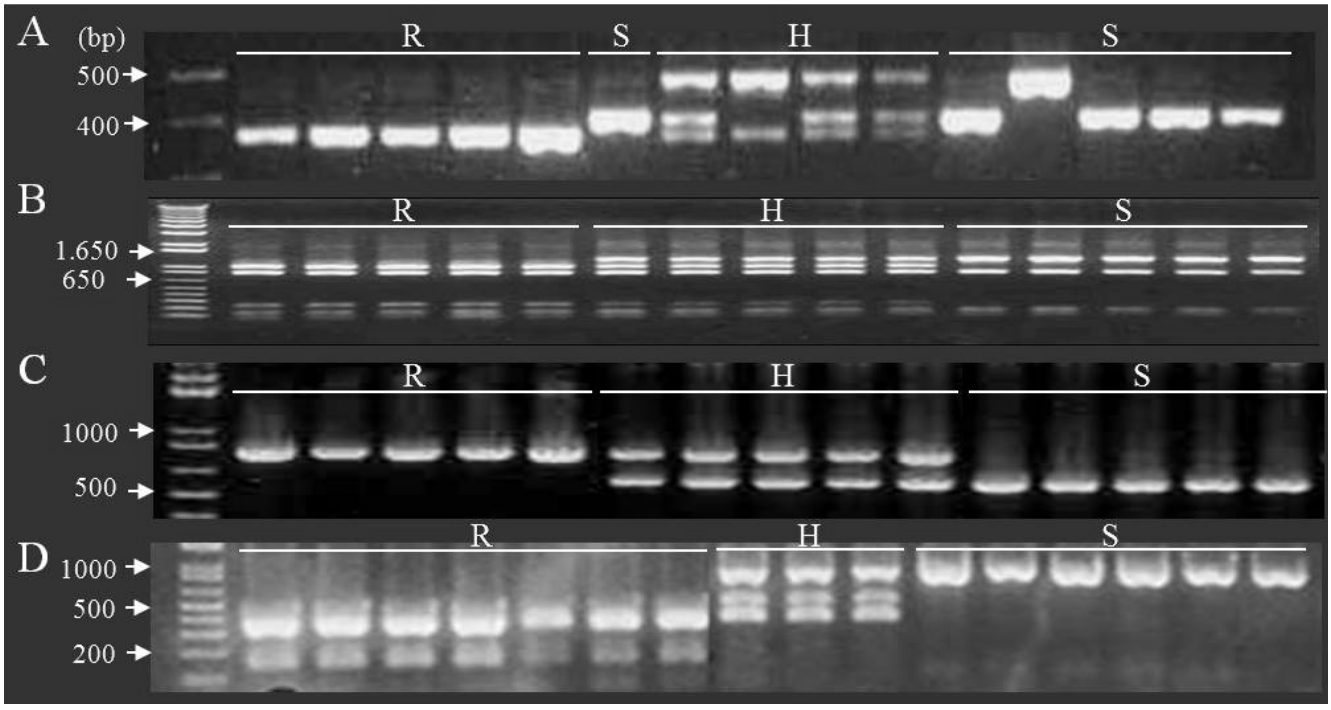

Fig. 1. Digested band patterns of the PCR fragments amplified with Ty-1 (A), T2 (B), I2 (C), and 32.5Cla (D) markers. R: homozygous resistance line; H: heterozygous resistance line; S: homozygous susceptible line.

Table 3. Scores obtained after screening 96 tomatoes with four CAPS markers.

Used marker	Line no.																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Ty-1	S[z]	R	S	S	R	S	R	S	S	S	S	S	S	S	S	S	R	H	S	R	S	S	S	S	H	H	S	H	S	S	S	H
T2	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	H	S	S	H	R	R	R	R
I2	S	H	R	R	R	S	S	S	H	S	S	H	H	H	S	S	S	S	R	R	R	R	R	R	R	R	R	R	S	H	R	R
32.5Cla	S	S	S	S	S	S	S	S	H	H	H	H	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Used marker	Line no.																															
	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Ty-1	S	S	S	S	R	R	S	H	S	R	H	R	S	R	R	R	R	R	S	R	S	H	H	R	R	S	R	S	S	S	S	S
T2	R	S	S	R	R	R	R	H	H	H	R	R	R	R	H	R	R	R	S	R	H	R	S	H	H	H	R	R	R	R	R	R
I2	R	R	R	R	R	R	R	R	R	S	H	H	H	R	S	R	R	H	R	R	R	S	R	R	R	H	R	R	R	R	R	R
32.5Cla	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R
Used marker	Line no.																															
	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
Ty-1	S	S	S	H	H	S	S	S	R	H	S	S	R	H	R	S	S	S	S	S	R	S	S	S	S	R	H	R	H	S	H	S
T2	R	S	R	R	R	R	R	S	R	H	R	R	R	R	R	H	S	S	R	R	R	S	S	R	S	R	S	H	H	H	R	R
I2	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	H	H	R	R
32.5Cla	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[z]R: homozygous resistance type; H: heterozygous resistance type; S: homozygous susceptible type.

during breeding line cultivation to identify resistance or susceptibility, and whether resistant samples were homozygous or heterozygous. In contrast, if dominant rather than co-dominant markers are used for MAS, more generations are required to produce homozygous tomato genotypes. Recently, co-dominant single nucleotide amplified polymorphism (SNAP) markers (Kim et al., 2005), single strand conformational polymorphisms (SSCP; Bertin et al., 2005), and high resolution melting (HRM; Jung et al., 2010) technology-based markers have been studied, but CAPS markers remain a good, and cost-effective choice because they are visible in agarose gel.

Phenetic Relationships

Clustering analysis (Fig. 2) was performed by calculating genetic distances using four CAPS markers in 96 tomato lines (Table 2) to examine levels of disease resistance and susceptibility. Genetic similarity coefficients varied from 0.37 to 1.00, indicating the multiple evolutionary origins of modern tomato cultivars. Four major clusters (I, II, III, and IV) and eight subgroups (a to h) were defined based on Nei-Li genetic similarity coefficients. The members of clusters I, II, and IV were divided into two (a and b), four (c to f), and two (g and h) subgroups, respectively. The powdery mildew locus, which is detected using the 32.5Cla marker, splits the 96 lines into resistant (IV) and susceptible tomato clusters (I, II, and III). Cluster III contains tomato lines that are susceptible to powdery mildew, those with heterozygous type scores from T2 and I2, and heterozygous or susceptible type scores from Ty-1 (Table 3). Cluster I contains lines that are susceptible to TYLCV and powdery mildew. In subgroup a of cluster I, homozygous lines that are resistant to ToMV originating from China, Europe, Japan, South America, Southwest Asia, and Korea were grouped together, and most of the tomato lines in subgroup a belong to the beef-steak type, except for the cherry tomato NW76. Subgroup b was further divided into two subgroups, one containing homozygous resistant lines to Fusarium wilt and lines susceptible to TYLCV and powdery mildew from China, Europe, and Southwest Asia. The other subgroup consists of NW66 and NW82, which are susceptible to all four diseases. The cherry-type tomato NW66 is one of three lines with purple fruit among the 96 tomatoes and both NW66 and NW82, introduced from Europe, exhibit determinate growth of the apical meristem (Table 2). Cluster II consists of four subgroups: subgroups c and d are TYLCV and ToMV resistant, subgroup d contains heterozygous resistant lines to ToMV that probably require further breeding steps to stabilize their homozygous genotype. NW18, NW54, and NW91 in subgroup e possess either TYLCV or Fusarium wilt resistance but are susceptible to the other three diseases, and subgroup f consists of TYLCV and Fusarium wilt resistant lines originating from Europe.

The introgression of homozygous resistant traits to susceptible lines is important for breeding programs. Three tomato lines in cluster III with beef-steak type fruit weight and indeterminate apical meristem growth require further fixation toward homozygosity of TYLCV, ToMV, and Fusarium wilt

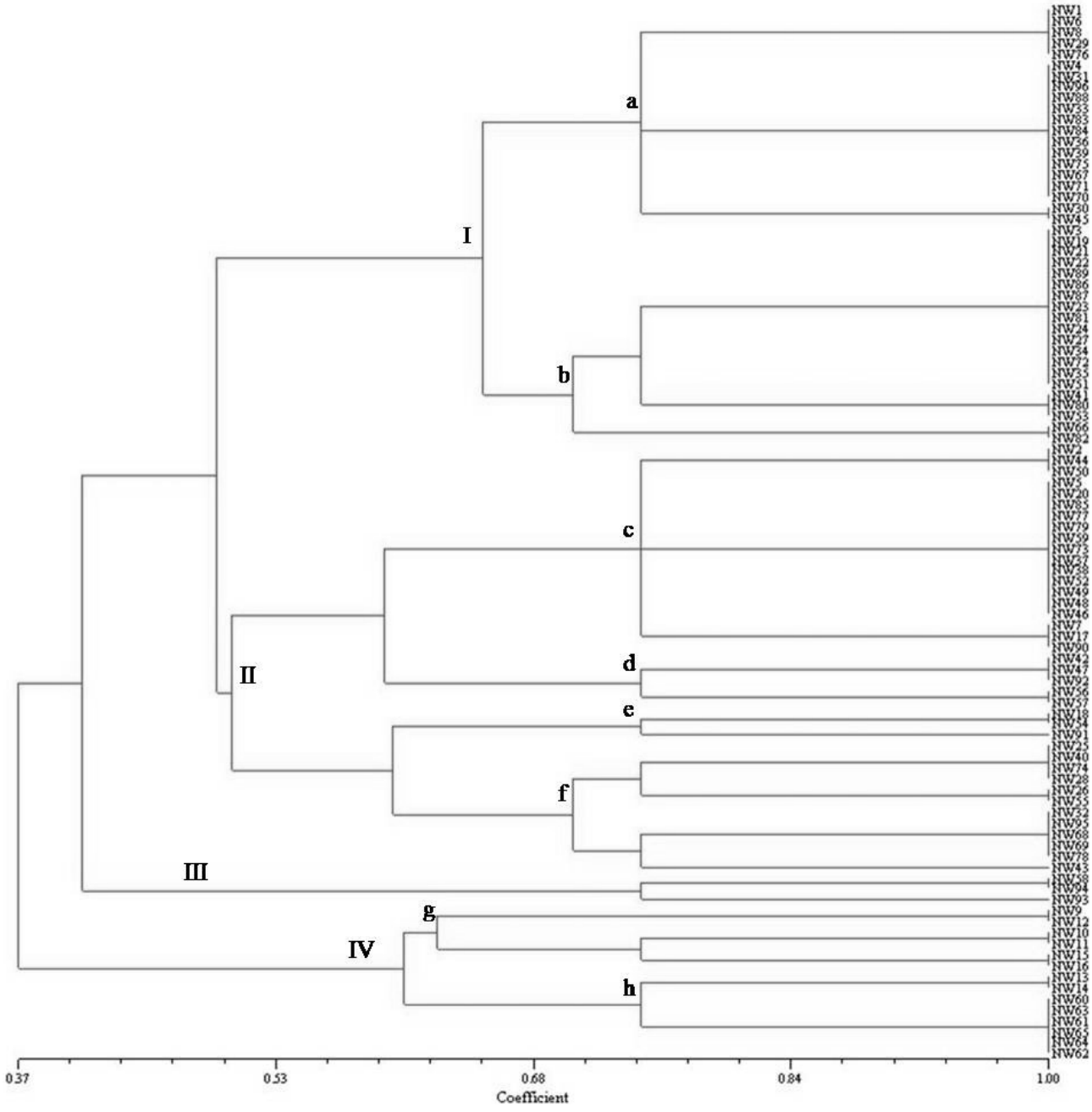

Fig. 2. Phenetic relationships among 96 tomato lines produced by UPGMA cluster analysis using data obtained from four CAPS markers. Four main clusters were designated as I, II, III, and IV, and eight subgroups as a to h.

resistance loci. Both subgroup g and h of cluster IV, which contain powdery mildew resistant lines originating from Europe, would be additively improved if the TYLCV resistance locus was transferred.

The phenetic relationship among 96 tomato lines based on the genetic distances using four CAPS markers and the levels of disease resistance and susceptibility was useful for tomato breeders to design breeding programs. Actually tomato lines were selected based on the resistance levels, the horticultural characteristics and the regional origin, and utilized to generate breeding lines and varieties (Table 4).

Development of Lines and Varieties Using Diagnostic Markers in MAS

The introgression of targeted traits into elite lines usually requires four to six generations of backcrossing for phenotype stabilization, and at least three generations for genotypic homogeneity (Fig. 3A). MAS may be used to efficiently decrease the number of generations required for genetic fixation of desired trait loci (Peleman and van der Voort, 2003). Using several markers is more efficient than using single trait markers, especially for pyramiding elite traits. Moreover, marker-assisted backcrossing with genomic markers

Table 4. Introduction of traits to varieties and inbred lines using the MAS program.

Resistance[z]	Variety									
	Qyupirang	Pinktop	Minichal	DoctorQ	219	251	518	Eureka	Omnia	Carmen
T2	o[y]	o	o	o	o	o	o	o	o	o
Ty-1						o		o	o	
I2	o	o		o	o	o		o	o	o
32.5Cla					o		o			
Ve	□[x]	□		□	□	□		□	□	□
Frl	□	□		□	□	□			□	
Cf-9	□		□		□	□	□			
Mi	□	□		□	□	□	□	□		□
BW				□						
Sw-5								□		
Pto								□		□
I3										
	Domestic beef-steak	Domestic beef-steak	Domestic saladette	Rootstock	Domestic beef-steak	Domestic beef-steak	Domestic cherry	Middle East	India	Middle East

Resistance	Inbred line									
	1059	1069	1155	1302	1439	1908	1985	2566	2569	2590
T2	o	o	o	o	o	o	o	o	o	o
Ty-1		o		o	o		o	o	o	o
I2			o		o			o	o	
32.5Cla	o					o				
Ve		□	□	□	□			□	□	
Frl					□				□	
Cf-9	□		□		□	□				
Mi			□		□		□			□
BW										
Sw-5		□	□	□				□		□
Pto										□
I3			□				□			
	Domestic beef-steak	Domestic beef-steak	Domestic beef-steak	Europe	Europe	Cherry	Cherry	Middle East	India	Middle East

[z]Ve: Verticillium wilt; Frl: Fusarium crown and root rot; Cf-9: Leaf mold; Mi: Root-knot nematode; BW: Bacterial wilt; Sw-5: Tomato spotted wilt virus; Pto: Bacterial speck; I3: Fusarium wilt.
[y]o is a newly introduced trait while.
[x]□ is the trait that was previously presented in the lines.

that are used to eliminate undesirable gene segments would reduce the duration of breeding generations (Prigge et al., 2009).

The T2 marker was used in a MAS program to select ToMV-resistant seedlings to develop varieties of Qyupirang and Minichal that are ToMV resistant (Figs. 3B and 3C). Then, the Fusarium wilt resistance trait was added to Qyupirang using the I2 marker. Since the parent lines of Qyupirang contained four other disease resistant traits, Ve (Verticillium wilt), Frl (Fusarium crown and root rot), Cf-9 (Leaf mold) and Mi (Root-knot nematode), the domestic beef-steak type Qyupirang exhibited six disease resistant characters in total (Table 4).

A total of 10 varieties and 10 inbred lines were constructed using four CAPS markers. The T2 marker was introduced to all tomatoes, and therefore all of the resulting tomato varieties were expected to be ToMV-resistant. The 32.5Cla marker was used in the MAS program, and resistant traits were successfully transferred to varieties 219 and 518 and inbred lines 1059 and 1908. Using four CAPS markers, up to three traits were introduced to develop four varieties (219, 251, Eureka, and Omnia) and three inbred lines (1439, 2566,

Fig. 3. Diagram (A) for MAS program for introducing elite traits to construct tomato breeding lines. Qyupirang (B) and Minichal (C) are tomato F_1 varieties developed by crossing the breeding lines.

and 2569). Among these cultivars, 219 and 251 are characterized by seven disease resistant traits, including four other resistant traits (Ve, Frl, Cf-9, and Mi). Eureka also contains seven disease resistant traits, including four other resistant traits (Ve, Sw-5 [*Tomato spotted wilt virus*], Pto [Bacterial speck], and Mi).

In this study, four CAPS markers were selected and evaluated in 96 tomato lines to assess the phenetic relationships of resistant and susceptible tomato lines. MAS was then successfully performed to construct inbred lines and varieties with multi-disease resistance traits. Based on the performance of MAS over a breeding program lasting several years, the implementation of a progeny selection process using CAPS markers that are tightly linked to disease resistance loci was able to save time, labor, and costs.

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