RESEARCH ARTICLE https://doi.org/10.12972/kjhst.20170050

Development of Fluidigm SNP Type Genotyping Assays for Marker-assisted Breeding of Chili Pepper (*Capsicum annuum* L.)

Haein Kim[1], Jae Bok Yoon[2], and Jundae Lee[1*]

[1]Department of Horticulture, Chonbuk National University, Jeonju 54896, Korea
[2]Research and Development Unit, Pepper and Breeding Institute, Hwaseong 18422, Korea

*Corresponding author: ajfall@jbnu.ac.kr

Abstract

Chili pepper (*Capsicum annuum* L.) is an economically important horticultural crop in Korea; however, various diseases, including *Phytophthora* root rot, anthracnose, powdery mildew, *Cucumber mosaic virus* (CMV), *Pepper mild mottle virus* (PMMoV), and *Pepper mottle virus* (PepMoV), severely affect their productivity and quality. Therefore, pepper varieties with resistance to multiple diseases are highly desired. In this study, we developed 20 SNP type assays for three pepper populations using Fluidigm nanofluidic dynamic arrays. A total of 4,608 data points can be produced with a 192.24 dynamic array consisting of 192 samples and 24 SNP markers. The assays were converted from previously developed sequence-tagged-site (STS) markers and included markers for resistance to *Phytophthora* root rot (M3-2 and M3-3), anthracnose (CcR9, CA09g12180, CA09g19170, CA12g17210, and CA12g19240), powdery mildew (Ltr4.1-40344, Ltr4.2-56301, and Ltr4.2-585119), bacterial spot (Bs2), CMV (Cmr1-2), PMMoV (L4), and PepMoV (pvr1 and pvr2-123457), as well as for capsaicinoids content (qcap3.1-40134, qcap6.1-299931, qcap6.1-589160, qdhc2.1-1335057, and qdhc2.2-43829). In addition, 11 assays were validated through a comparison with the corresponding data of the STS markers. Furthermore, we successfully applied the assays to commercial F_1 cultivars and to our breeding lines. These 20 SNP type assays will be very useful for developing new superior pepper varieties with resistance to multiple diseases and a higher content of capsaicinoids for increased pungency.

Additional key words: disease resistance, foreground selection, high-throughput genotyping, molecular marker, SNP

Received: April 4, 2017
Revised: May 23, 2017
Accepted: May 24, 2017

HORTICULTURAL SCIENCE and TECHNOLOGY
35(4):465-479, 2017
URL: http://www.kjhst.org

pISSN : 1226-8763
eISSN : 2465-8588

This research was supported by the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011 14501 and PJ01114502)" of the Rural Development Administration, Republic of Korea.

Introduction

Chili pepper (*Capsicum annuum* L.) is one of the most important vegetable crops in Korea (Lee et al., 2004). However, annual production and cultivation has gradually declined due, in part, to pepper diseases that usually occur in the summer season.

Major pepper diseases include *Phytophthora* root rot (*Phytophthora capsici*; Liu et al., 2014), anthracnose (*Colletotrichum scovillei* and *C. truncatum*, formerly *C. acutatum* and *C. capsici*, respectively;

Mahasuk et al., 2016), powdery mildew (*Leveillula taurica*; Lefebvre et al., 2003), bacterial wilt (*Ralstonia solanacearum*; Mimura et al., 2009), bacterial spot (*Xanthomonas campestris* pv. *vesicatora*; Truong et al., 2011), *Cucumber mosaic virus* (CMV; Eun et al., 2016), *Pepper mild mottle virus* (PMMoV; Yang et al., 2012), *Tomato spotted wilt virus* (TSWV; Kim et al., 2017), and *Pepper mottle virus* (PepMoV; Kim et al., 2011, 2017). These diseases are difficult to control, even with the use of agrichemicals. Therefore, pepper varieties with multiple resistance are highly desired. In addition, higher levels of pungency are also desired by the pepper processing industry (Lee et al., 2016a). The pungency of chili pepper is related to the content of capsaicinoids such as capsaicin and dihydrocapsaicin (Aza-González et al., 2011).

Molecular markers are widely used to improve the efficiency of plant breeding programs, to construct genetic linkage maps, and to detect genes or quantitative trait loci (QTL) controlling specific traits (Collard et al., 2005; Collard and Mackill, 2008; Xu and Crouch, 2008). Marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) are commonly used to improve the efficiency of selection in plant breeding. MAS involves foreground selection of a target trait or traits of interest, while MABC involves background selection of overall genomic regions in BC generations (Collard and Mackill, 2008). MAS and MABC can reduce the breeding period and the number of generations needed in a breeding program compared to conventional phenotypic selection because codominant markers allow breeders to distinguish between homozygotes and heterozygotes and to detect early the desired traits in seeds or seedlings without having to grow the plants to maturity or inoculate them.

Several molecular markers have been developed in pepper for disease resistance and capsaicinoids content. Two dominant markers, OpD04-717-SCAR and P5-SNAP, for the detection of a major QTL, *Phyto.5.2*, for resistance to *P. capsici* have been reported (Quirin et al., 2005), and codominant markers M3-CAPS and Phyto5NBS1-HRM for the trait have also been developed (Lee et al., 2012; Liu et al., 2014). The markers CaR12.2M1-CAPS and CcR9M1-SCAR have been linked to the major QTLs for resistance to *C. scovillei* and *C. truncatum*, respectively (Lee et al., 2010, 2011). Bacterial spot resistance genes (*Bs2* and *Bs3*) have been cloned in pepper (Tai et al., 1999b; Römer et al., 2007), and gene-based codominant markers 14F/14R and 25-1 for *Bs2* and PR-Bs3 for *Bs3* were subsequently developed (Römer et al., 2010; Truong et al., 2011). Three SNP markers, CaTm-int3HRM, CaT1616BAC, and 240H02sp6, were identified and linked to a single dominant gene, *Cmr1*, that controls CMV resistance (Kang et al., 2010). Two CAPS markers, pvr1-R1 and pvr1-R2, were developed to detect *pvr1* and *pvr1*^{2} alleles for potyvirus resistance in *C. chinense* accessions (Yeam et al., 2005). *Pvr4* and *Tsw* genes have also been cloned: *Pvr4* is a potyvirus resistance gene originating from *C. annuum* 'CM334' and *Tsw* is a TSWV resistance gene from *C. chinense* accessions 'PI159236' and 'PI152225' (Kim et al., 2017). Markers 61786 and NB575m were found to cosegregate with the *Pvr4* and *Tsw* genes, respectively. In addition, high-resolution DNA melting (HRM) markers linked to the QTLs responsible for high capsaicin and dihydrocapsaicin content in *C. chinense* 'Bhut Jolokia' have been developed (Lee et al., 2016a). Many other trait-linked markers have been reported in pepper. Therefore, high-throughput screening methods are needed for the simplified and cost-effective analysis of multiple molecular markers in a single reaction.

Remarkable technological achievements have occurred over the past few decades in the field of DNA sequencing and SNP genotyping, including next-generation sequencing (NGS) and high-throughput SNP genotyping (Varshney et al., 2009; Kumar et al., 2012; Poland and Rife, 2012; Thomson, 2014). High-throughput SNP genotyping is particularly useful in crop breeding (Thomson, 2014). Molecular markers can be rapidly developed for SNPs, which are the most abundant polymorphisms with unlimited nucleotide variations between individual organisms, even within the same species (Rafalski, 2002). Moreover, analysis of SNP markers is accurate, rapid, and inexpensive.

Several high-throughput SNP genotyping platforms have been reported, including: Illumina Infinium iSelect HD array,

Affymetrix Axiom array, Douglas Array Tape, Fluidigm dynamic arrays, restriction-enzyme-based genotyping-by-sequencing (GBS), and amplicon sequencing (Thomson, 2014). Among them, Fluidigm dynamic arrays adopt a flexible, PCR-based SNP platform using a nanofluidic integrated fluid circuit (IFC; Wang et al., 2009). There are three different types of Fluidigm dynamic arrays: a 48.48 dynamic array, which yields 2,304 data points with 48 samples and 48 markers, as well as 96.96 and 192.24 dynamic arrays, which yield 9,216 and 4,608 data points, respectively (Wang et al., 2009; Thomson, 2014). This system can save both resources and time by reducing the reaction volumes to 7-10 nL and producing 2,304-9,216 data points within 2-4 hours (Thomson, 2014), while 384- and 96-well PCR systems require 5-20 μL of the reaction volumes per well and it takes 4-8 days to produce the same number of data points with the PCR systems.

In this study, we developed SNP type assays linked to disease resistance or capsaicinoids content for use in Fluidigm 192.24 dynamic arrays that could simultaneously analyze 24 SNP markers. The CAPS, SCAR, and SNP markers previously developed in chili pepper were successfully converted into the Fluidigm SNP type assays.

Materials and Methods

Plant Materials

Three populations (PG, CHB-F_3, and JN-F_5) were used to develop the SNP type assays. The PG population consisted of 51 *Capsicum* accessions including six species (*C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, *C. pubescens*, and *C. chacoense*), which were obtained from the National Agrobiodiversity Center, Rural Development Administration, Republic of Korea (Table 2; http://genebank.rda.go.kr/). The CHB-F_3 population was made up of 48 individuals originating from a cross of *C. annuum* 'A1' × *C. annuum* '2602', which was used for QTL analysis of CMV_{P1} resistance (Table 3; Eun et al., 2016). The JN-F_5 population consisted of 50 individuals of an F_5 single seed descent (SSD) population derived from a cross of *C. annuum* 'NB1' × *C. chinense* 'Bhut Jolokia', which was used for QTL analysis of capsaicinoids content (Table 4; Lee et al., 2016a). In addition, 33 commercial cultivars, 30 breeding lines, and 7 genetic sources for pepper were used for validation of the newly developed SNP type assays (Tables 5 and 6).

Primer Design for the Fluidigm SNP Type Assays

The following target sequence criteria were employed to design primers for the SNP type assays. Length of the target sequences: a minimum of 60 bp (including both upstream and downstream of the target SNP site) and a maximum of 250 bp. For SNPs, only one SNP was present in the target sequence. For insertions/deletions (In/Dels), the length of the In/Del was shorter than 10 bp. The G/C content of the target sequence was < 65%. A total of 43 primers were designed using D3 Assay Design (https://d3.fluidigm.com/; Fluidigm, South San Francisco, CA, USA). Primer information is listed in Table 1. Each assay consisted of three types of primers: a specific target amplification (STA) primer, a locus-specific (LS) primer, and an allele-specific (AS) primer (Wang et al., 2009).

DNA Extraction

Genomic DNA was prepared from fresh leaves using the miniprep method described by Eun et al. (2016). The DNA concentration was measured using a BioDrop μLITE (BioDrop UK Ltd., Cambridge, UK) and adjusted to 50 ng·μL^{-1}. The DNA

was used for the SNP type assays and HRM analysis.

Specific Target Amplification

Before performing the SNP type assay, specific target amplification (STA), which is used to enrich the amplicon including the targeted SNP sequences, was performed to increase the probability of success of the SNP type assay (Wang et al., 2009). First, a 10× STA primer pool was prepared comprising a mixture of 2 μL of STA primer for each of the 24 markers, 2 μL of LS primer for each of the 24 markers, and 304 μL of DNA suspension buffer (Teknova, Holister, CA, USA). For each of the 191 samples, STA was executed using a LightCycler 96 Real-Time PCR (Roche, Basel, Switzerland) in a total volume of 5 μL per reaction, which contained 2.5 μL of master mix (Qiagen, Hilden, Germany), 0.5 μL of the 10× STA primer pool, 0.75 μL of PCR-certified water, and 1.25 μL of genomic DNA with the following PCR profile: pre-denaturation for 900 s at 95°C followed by 14 cycles of a 2-step amplification of 15 s at 95°C and 240 s at 60°C. Then, 3 μL of amplified product was diluted in 97 μL of PCR-certified water and then used for the SNP type assay.

SNP Type Assay

To perform the SNP type assays using the 192.24 IFC, the assay mix and sample mix were prepared. The assay mix contained 1.2 μL of PCR-certified water, 2 μL of 2× assay loading reagent, and 0.8 μL of the assay pre-mix, which was comprised of 3 μL of each AS primer, 8 μL of each LS primer, and 29 μL of DNA suspension buffer (Teknova, Holister, CA, USA). The sample pre-mix contained 540 μL of 2× Fast Probe Master Mix (Biotium, Fremont, CA, USA), 54 μL of SNP type 20× sample loading reagent, 18 μL of SNP type 60× reagent, 6.48 μL of 50× ROX dye (Invitrogen, Waltham, MA, USA), and 11.52 μL of PCR-certified water. Subsequently, the sample mix was prepared by mixing 1.9 μL of each STA product and 2.6 μL of the sample pre-mix in each well of two 96-well plates. Finally, 3 μL of each sample mix and 3 μL of each assay mix were loaded into 192 sample inlets and 24 assay inlets of the 192.24 IFC, respectively.

The SNP type assays were performed in series using three machines, the IFC controller RX (Fluidigm, South San Francisco, CA, USA), the IFC cycler (Fluidigm, South San Francisco, CA, USA), and the EP1 system (Fluidigm, South San Francisco, CA, USA) according to the manufacturer's instructions (Wang et al., 2009).

Scoring of SNPs

In each SNP type assay, two types of fluorescence, FAM (red, Y axis) and HEX (green, X axis), were analyzed and each fluorescence was linked to each SNP (Table 1). Using Fluidigm SNP genotyping analysis version 4.1.3 (Fluidigm, South San Francisco, CA, USA), three different genotypes (A, H, and B) were identified: A and B refer to a specific homozygous SNP; H refers to a heterozygous SNP (Fig. 1).

Analysis of HRM Markers

HRM analysis was performed using a LightCycler 96 Real-Time PCR machine (Roche, Basel, Switzerland). The reaction solution for HRM analysis was prepared and PCR reactions were performed according to Lee et al. (2016b). High-Resolution Melt software version 1.1 (Roche, Basel, Switzerland) was used to analyze the marker types of HRM. HRM marker information was derived from the references in Table 1.

Table 1. List of Fluidigm SNP type assays used in this study and relevant information.

Assay No.	SNP type assay	Trait	Target gene or QTL	Position	SNP	SNP(color of dye[z])	SNP(phenotype[y])	Fluidigm assay ID	Reference
A1	M3-2	*Phytophthora* root rot resistance	*Phyto.5.2*	Chr.5	...GTA[C/T]GTA...	C(R):T(G)	T(R):C(S)	GTA0120128	Lee et al., 2012; Liu et al., 2014
A2	M3-3	*Phytophthora* root rot resistance	*Phyto.5.2*	Chr.5	...TGT[CAGA/GAGT]GAT...	CAGA(R):GAGT(G)	CAGA(R):GAGT(S)	GTA0130825	Lee et al., 2012; Liu et al., 2014
A3	CcR9	Anthracnose resistance	*CcR9*	Chr.9	...ACA[A/C]TTA...	A(R):C(G)	C(R):A(S)	GTA0120131	Lee et al., 2010, 2011
A4	CA09g12180	Anthracnose resistance	*CcR9*	Chr.9	...TAT[A/C]GTG...	A(R):C(G)	A(R):C(S)	GTA0130466	Lee et al., 2010, 2011
A5	CA09g19170	Anthracnose resistance	*CcR9*	Chr.9	...GGT[C/T]GTA...	C(R):T(G)	C(R):T(S)	GTA0130465	Lee et al., 2010, 2011
A6	CA12g17210	Anthracnose resistance	*CaR12.2*	Chr.12	...CAT[T/G]GAA...	T(R):G(G)	T(R):G(S)	GTA0130462	Lee et al., 2010, 2011
A7	CA12g19240	Anthracnose resistance	*CaR12.2*	Chr.12	...GAT[CGCGAA/AGCGAG]AAA...	CGCGAA(R):AGCGAG(G)	CGCGAA(R):AGCGAG(S)	GTA0130463	Lee et al., 2010, 2011
A8	Ltr4.1-40344	Powdery mildew resistance	*Ltr4.1*	Chr.4	...ATC[AAAAC/GAAAT]TTG...	AAAAC(R):GAAAT(G)	AAAAC(R):GAAAT(S)	GTA0130479	Yoon, 2003
A9	Ltr4.2-56301	Powdery mildew resistance	*Ltr4.2*	Chr.4	...TTA[A/C]GAG...	A(R):C(G)	A(R):C(S)	GTA0130480	Yoon, 2003
A10	Ltr4.2-585119	Powdery mildew resistance	*Ltr4.2*	Chr.4	...CGA[C/T]ATT...	C(R):T(G)	C(R):T(S)	GTA0130475	Yoon, 2003
A11	Bs2	Bacterial spot resistance	*Bs2*	Chr.2	...CTC[A/T]GTG...	A(R):T(G)	T(R):A(S)	GTA0121581	Truong et al., 2011
A12	Cmr1-2	CMV resistance	*Cmr1*	Chr.2	...GAA[G/T]GAG...	G(R):T(G)	T(R):G(S)	GTA0121482	Kang et al., 2010
A13	L4	TMV resistance	L_4	Chr.11	...AAC[A/T]CTC...	A(R):T(G)	A(L_4):T(not L_4)	GTA0121486	Tomita et al., 2011; Yang et al., 2012
A14	pvr1	Potyvirus resistance	*pvr1*	Chr.4	...AAT[A/C]CAG...	A(R):C(G)	A(*pvr1*):C($pvr1^+$)	GTA0130482	Yeam et al., 2005; Charron et al., 2008
A15	pvr2-123457	Potyvirus resistance	*pvr2*	Chr.4	...CAG[T/A]GGC...	T(R):A(G)	A($pvr2^{123457}$) T($pvr2^{not\ 123457}$)	GTA0130485	Yeam et al., 2005; Charron et al., 2008
A16	qcap3.1-40134	Capsaicinoid content	*qcap3.1*	Chr.3	...CTT[A/C]AGA...	A(R):C(G)	A(H):C(L)	GTA0130484	Lee et al., 2016a
A17	qcap6.1-299931	Capsaicinoid content	*qcap6.1*	Chr.6	...CAG[G/A]AGG...	G(R):A(G)	G(H):A(L)	GTA0130483	Lee et al., 2016a
A18	qcap6.1-589160	Capsaicinoid content	*qcap6.1*	Chr.6	...AGG[G/A]AAA...	G(R):A(G)	G(H):A(L)	GTA0130481	Lee et al., 2016a
A19	qdhc2.1-1335057	Capsaicinoid content	*qdhc2.1*	Chr.2	...ATT[A/G]GCA...	A(R):G(G)	A(H):G(L)	GTA0130478	Lee et al., 2016a
A20	qdhc2.2-43829	Capsaicinoid content	*qdhc2.2*	Chr.2	...CCG[G/A]ACC...	G(R):A(G)	G(H):A(L)	GTA0130477	Lee et al., 2016a

[z]R, red (FAM dye); G, green (HEX dye).
[y]R, resistant; S, susceptible; H, allele for high capsaicinoid content; L, allele for low capsaicinoid content; $pvr2^{123457}$ indicates $pvr2^1$, $pvr2^2$, $pvr2^3$, $pvr2^4$, $pvr2^5$, or $pvr2^7$.

Results and Discussion

Development of the SNP Type Assays

A total of 43 primer sets were designed for the SNP type assays and 20 assays were clearly analyzed via classification into three or two groups (Fig. 1). In Fig. 1, red, green, and blue points indicate XX (fluorescence of only FAM dye), YY (only HEX dye), and XY (both FAM and HEX dyes) marker types, respectively. The marker type of each SNP type assay was divided into resistant (R), heterozygous (H), or susceptible (S) types for disease resistance, and high (H), heterozygous (M), or low (L) types for capsaicinoids content (Fig. 1and Table 1). The 20 successful SNP type assays are listed in Table 1, which includes the name of the original genes or QTLs for capsaicinoids content (*qcap3.1, qcap6.1, qdhc2.1*, and *qdhc2.2*) and for resistance to diseases such as *Phytophthora* root rot (*Phyto.5.2*), anthracnose (*CcR9* and *CaR12.2*), powdery mildew (*Ltr4.1* and *Ltr4.2*), bacterial spot (*Bs2*), CMV (*Cmr1*), TMV (L_4), and potyvirus (*pvr1* and *pvr2*). A total of 5 192.24 ICFs were analyzed using the 43 primer sets and the three segregating populations (Tables 2, 3, and 4). The 20 successful SNP type assays showed clear results that were polymorphic in at least one population (Fig. 1and Table 1). In addition, 11 assays were compared with the corresponding original HRM markers to determine whether the marker types cosegregated (Tables 2, 3, and 4).

Two SNP type assays, M3-2 (Fig. 1A) and M3-3 (Fig. 1B), were converted from the M3-CAPS marker tightly linked to

Phyto.5.2, a major QTL for resistance to root rot caused by *P. capsici* (Table 1, A1, and A2; Quirin et al., 2005; Lee et al., 2012). These assays revealed that the resistance allele was widely distributed among domesticated *Capsicum* species except for *C. annuum* (Table 2, A1 and A2). Thus, the resistance of the five domesticated species should be analyzed via inoculation with *P. capsici* because the resistance allele originated from *C. annuum* accessions, including 'CM334', 'PI201232', 'PI201234', and 'AC2258' (Liu et al., 2014). The marker types for the M3-2 assay were perfectly matched with those of the previously developed M3-HRM marker in two segregating populations, CHB-F_3 and JN-F_5 (Tables 3 and 4, A1), while the M3-3 assay revealed only one recombinant in the CHB-F_3 population (Tables 3 and 4, A2). Therefore, the M3-2 assay can be used to select *Phytophthora* root rot resistance because it cosegregated perfectly with the M3-HRM marker.

Three assays, CcR9 (Fig. 1C), CA09g12180 (Fig. 1D), and CA09g19170 (Fig. 1E), were derived from the CcR9M1-SCAR marker to detect *CcR9*, a major QTL for resistance to anthracnose caused by *C. truncatum* (Table 1, A3, A4, and A5), while two assays, CA12g17210 (Fig. 1F) and CA12g19240 (Fig. 1G), originated from the CaR12.2M1-CAPS marker to select *CaR12.2*, a

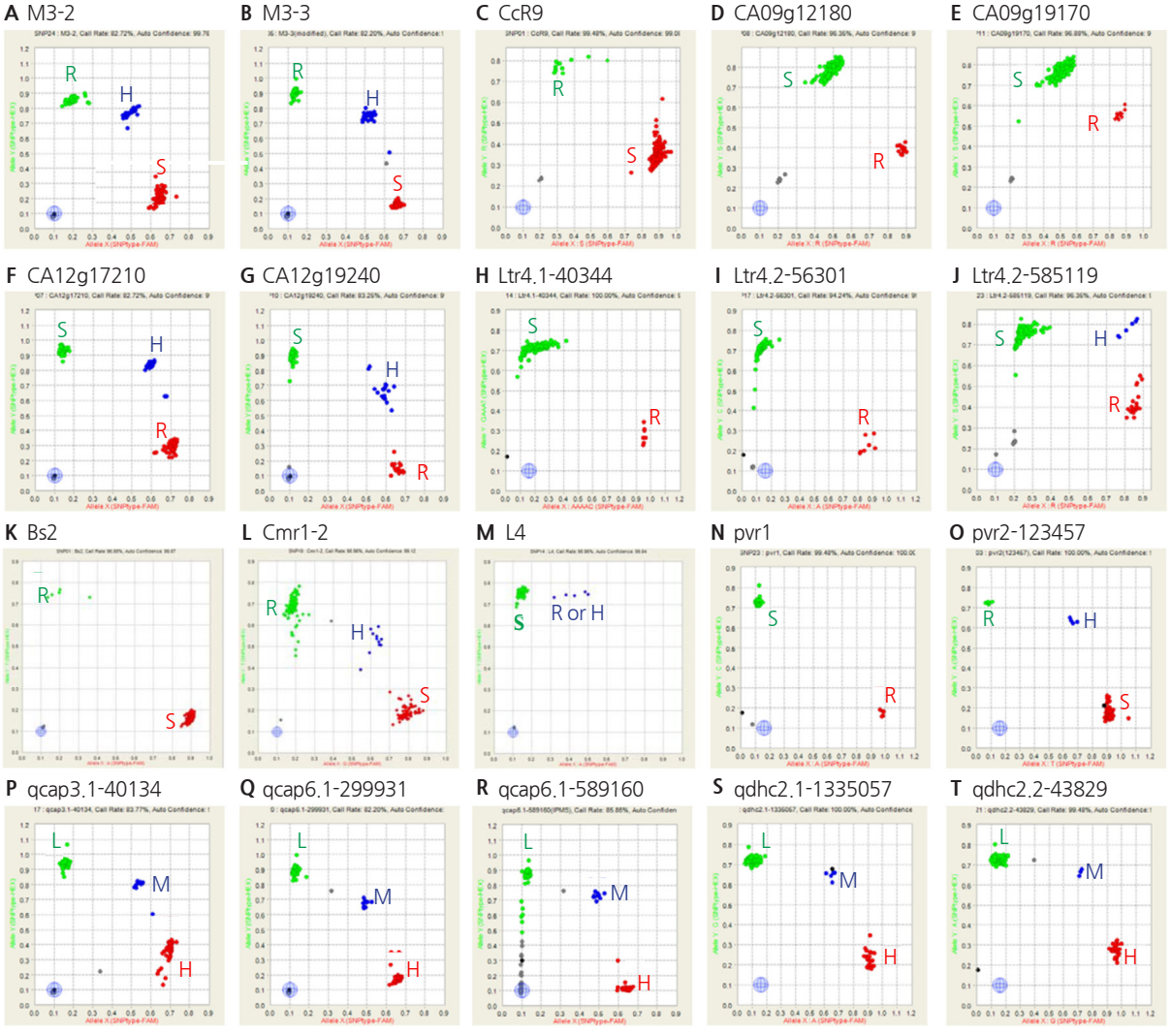

Fig. 1. Scatter plots of 20 SNP type assays. R, resistant; S, susceptible; H, heterozygous for A-O, high capsaicinoid content allele for P-T; L, low capsaicinoid content allele.

Table 2. Marker types of 18 SNP type assays and two HRM markers in 51 pepper accessions including six *Capsicum* species.

Plant material	IT No.	Species	Original name	Origin	Marker type[z]																		
					A1[y]	A2	A3	A4	A5	CA09g12180-HRM	CA09g19170-HRM	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20
PG1	IT 032414	*C. annuum*	(426)(59-1S)	South Korea	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PG3	IT 032418	*C. annuum*	Nongdae(451) (76-1)	South Korea	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PG4	IT 264053	*C. annuum*	11PC7	South Korea	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	L	L	L	L	L
PG5	IT 264054	*C. annuum*	11PC6	South Korea	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	L	L	L	L	L
PG6	IT 264055	*C. annuum*	11PC5	South Korea	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	L	L	L	L	L
PG7	IT 264056	*C. annuum*	11PC2	South Korea	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	L	L	L	L	L
PG8	IT 267516	*C. annuum*	11PC 3	South Korea	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	L	L	L	L	L
PG9	IT 286135	*C. annuum*	Andong 1	South Korea	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	L	L	L	L	L
PG10	IT 286136	*C. annuum*	Andong 2	South Korea	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG16	IT 264042	*C. annuum*	04H092	South Korea	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PG18	IT 264045	*C. annuum*	04H043	South Korea	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PG19	IT 264046	*C. annuum*	04H039	South Korea	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	L	L	L	L	L
PG22	IT 224968	*C. annuum var. annuum*	JATILABA	Indonesia	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG24	IT 224949	*C. annuum var. annuum*	Tetenyi Aprocseresznye	Hungary	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG27	IT 224965	*C. annuum var. annuum*	PBC174	Senegal	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG31	IT 163513	*C. annuum var. annuum*	VR-2	India	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PG34	IT 270368	*C. annuum*	0305348-2-1	China	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG35	IT 270483	*C. annuum*	12041015	China	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PG36	IT 286107	*C. annuum*	TONG AN XIAD MI	China	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG37	IT 286137	*C. annuum*	China 1	China	S	S	S	S	S	S	S	S	S	S	S	H	S	S	S	H	L	L	L	L
PG38	IT 286138	*C. annuum*	China 2	China	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG39	IT 270409	*C. annuum*	30R	Australia	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG68	IT 270768	*C. baccatum*	Spain 6	Spain	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	H	H	L	H	H
PG69	IT 284411	*C. baccatum*	Spain 10	Spain	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	H	H	L	H	H
PG70	IT 290121	*C. baccatum var. pendulum*	PI 224440	Costa Rica	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	H	H	L	H	H
PG71	IT 270672	*C. baccatum var. pendulum*	CGN 23206	Brazil	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	H	H	L	H	H
PG72	IT 229146	*C. chinense*	09G060	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG73	IT 229193	*C. chinense*	09G169	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	H	H	H	H	H
PG74	IT 229195	*C. chinense*	09G174	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG75	IT 229198	*C. chinense*	09G179	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG76	IT 229201	*C. chinense*	09G182	Hungary	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG77	IT 229203	*C. chinense*	09G185	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG78	IT 229205	*C. chinense*	09G187	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG79	IT 229207	*C. chinense*	09G192	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG80	IT 229209	*C. chinense*	09G197	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG81	IT 229210	*C. chinense*	09G198	Hungary	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	H	H	H	H	H
PG82	IT 286109	*C. chinense*	C O4410-1	Bolivia	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	H	H	H	H	H
PG83	IT 286110	*C. chinense*	C O4410-2	Bolivia	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	H	H	H	H	H
PG84	IT 286111	*C. frutescens*	C O4742	Puerto Rico	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	H	H	L	H	H
PG86	IT 221660	*C. frutescens*	Catumandu-1	Nepal	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PG87	IT 264081	*C. frutescens*	IT221660-1	Nepal	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
PG88	IT 270560	*C. frutescens*	Indonesia 16	Indonesia	R	R	S	S	S	S	S	S	S	S	S	S	S	S	H	H	H	L	H	H
PG89	IT 270597	C. frutescens	CGN 22817	Indonesia	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	H	H	L	H	H
PG90	IT 290042	C. frutescens	Aji chile dulce	Cuba	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	H	H	L	H	H

Table 2. Continued.

Plant material	IT No.	Species	Original name	Origin	Marker type[z]																			
					A1[y]	A2	A3	A4	A5	CA09g12180-HRM	CA09g19170-HRM	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20
PG91	IT 261294	*C. frutescens*	SVK 278	Laos	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	H	L	L	L	L
PG92	IT 267517	*C. frutescens*	ATP 24	Laos	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	L	L	L	L	L
PG93	IT 158741	*C. pubescens*	NUM-113-ROJO	Guatemala	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	L	L	L	L	L
PG95	IT 207297	*C. pubescens*	Locoto	Bolivia	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	H	H	H	H	H
PG96	IT 283281	*C. chacoense*	BOL-AWS-1999-358	Unknown	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	H	H	L	H	H
PG97	IT 283500	*C. chacoense*	G2280	Germany	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	H	H	L	H	H
PG98	IT 283501	*C. chacoense*	BERKMORTEL	Unknown	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	H	H	L	H	H

[z]R, resistant; S, susceptible; H, heterozygous for A1-A14, high capsaicinoid content allele for A16-A20; L, low capsaicinoid content allele.
[y]A1, M3-2; A2, M3-3; A3, CcR9; A4, CA09g12180; A5, CA09g19170; A6, CA12g17210; A7, CA12g19240; A8, Ltr4.1-40344; A9, Ltr4.2-56301; A10, Ltr4.2-585119; A11, Bs2; A12, Cmr1-2; A13, L4; A14, pvr1; A15, pvr2-123457; A16, qcap3.1-40134; A17, qcap6.1-299931; A18, qcap6.1-589160; A19, qdhc2.1-1335057; A20, qdhc2.2-43829.

major QTL for resistance to anthracnose caused by *C. scovillei* (Table 1, A6 and A7; Lee et al., 2011). These assays showed that resistance alleles only appeared in *C. baccatum* and *C. chacoense* (Table 2, A3-A5). Resistance was already known to be present in *C. baccatum*, including 'PBC80', 'PBC81', 'Cbp', and 'PI594137' (Park et al., 2009). However, the resistance of *C. chacoense* has not been analyzed with inoculation assays, and thus needs to be examined. The marker types of the CA12g17210 and CA12g19240 assays perfectly coincided with those of the CA12g17210-HRM and CA12g19240-HRM markers, respectively, in the CHB-F_3 and JN-F_5 populations (Table 2, A6; Table 3, A6 and A7).

Three assays, Ltr4.1-40344 (Fig. 1H), Ltr4.2-56301 (Fig. 1I), and Ltr4.2-585119 (Fig. 1J), were developed to identify two QTLs, *Ltr4.1* and *Ltr4.2*, for powdery mildew resistance, which were derived from *C. baccatum* 'PBC81' (Table 1, A8, A9, and A10; Yoon, 2003). Like the anthracnose resistance assays, the resistance marker types of these assays were also specific to accessions of *C. baccatum* and *C. chacoense* (Table 2, A8-A10).

The Bs2 (Fig. 1K) assay was based on the *Bs2* gene conferring resistance to most *Xanthomonas campestris* pv *vesicatora* races including 0, 1, 2, 3, 7, and 8 (Table 1, A11; Stall et al., 2009; Truong et al., 2011). The resistance marker type of this assay was found only in *C. chacoense* accessions (Table 2, A11). This result was supported by Tai et al. (1999a) who described *Bs2* resistance from a wild species of pepper, *C. chacoense* 'PI260435'.

The Cmr1-2 (Fig. 1L) assay was derived from the CaTm-int3-HRM marker closely linked to *Cmr1*, a dominant resistance gene against CMV_{Korean} and CMV_{FNY} strains (Table 1, A12; Kang et al., 2010). The resistance marker type of this assay was widely distributed throughout *Capsicum* spp. except for *C. baccatum* and *C. chacoense* (Table 2, A12), and its segregation pattern was the same as that of the CaTm1-HRM marker in the CHB-F_3 and JN-F_5 populations (Tables 3 and 4, A12). Therefore, this assay can be used to select the *Cmr1* gene.

The L4 (Fig. 1M) assay was developed to select L_4 alleles resistant to most pathotypes including ToMV (P_0), PaMMV (P_0 and P_1), and PMMoV ($P_{1,2}$ and $P_{1,2,3}$) (Table 1, A13; Tomita et al., 2011). Similar to the *Bs2* gene, the resistance marker type of the L_4 assay was present only in *C. chacoense* accessions (Table 2, A13). This result was consistent with the findings of Boukema (1984) who reported that L_4 resistance was found in *C. chacoense* accessions 'PI260429' and 'SA185'.

Two assays, pvr1 (Fig. 1N) and pvr2-123457 (Fig. 1O), were created to detect *pvr1* and *pvr2* genes, respectively, which were allelic and encoded an eIF4E protein, but originated from different genetic resources: *pvr1* from *C. chinense* and *pvr2* from *C. annuum* and *C. frutescens* (Table 1, A14 and A15; Kang et al., 2005; Charron et al., 2008). A resistant marker type of the *pvr1*

Table 3. Comparison of marker types between the SNP type assays and HRM markers in the CHB-F_3 population.

Plant material	*Phytophthora* root rot resistance (*Phyto.5.2*)			Anthracnose resistance (*CaR12.2*)				CMV resistance (*Cmr1*)	
	A1[z]	A2	M3-HRM	A6	CA12g17210-HRM	A7	CA12g19240-HRM	A12	CaTm1-HRM
CHB5	S[y]	S	S	H	H	H	H	R	R
CHB9	R	R	R	R	R	R	R	R	R
CHB10	H	H	H	R	R	R	R	R	R
CHB11	H	H	H	S	S	S	S	R	R
CHB12	S	S	S	H	H	H	H	H	H
CHB13	S	S	S	R	R	R	R	S	S
CHB14	S	S	S	H	H	H	H	H	H
CHB15	R	R	R	S	S	S	S	S	S
CHB16	R	R	R	H	H	H	H	S	S
CHB17	R	R	R	H	H	H	H	S	S
CHB19	R	R	R	H	H	H	H	S	S
CHB20	H	H	H	R	R	R	R	S	S
CHB21	R	R	R	R	R	R	R	H	H
CHB23	S	S	S	H	H	H	H	H	H
CHB24	R	R	R	R	R	R	R	R	R
CHB25	R	R	R	S	S	S	S	H	H
CHB26	S	S	S	R	R	R	R	H	H
CHB29	R	R	R	R	R	R	R	S	S
CHB32	S	S	S	R	R	R	R	S	S
CHB34	R	R	R	R	R	R	R	S	S
CHB36	H	H	H	S	S	S	S	S	S
CHB37	S	S	S	S	S	S	S	H	H
CHB40	H	H	H	S	S	S	S	S	S
CHB41	S	S	S	S	S	S	S	R	R
CHB42	H	H	H	S	S	S	S	S	S
CHB43	H	H	H	S	S	S	S	S	S
CHB44	R	R	R	S	S	S	S	S	S
CHB45	S	S	S	R	R	H	H	S	S
CHB46	H	H	H	R	R	R	R	R	R
CHB51	S	S	S	H	H	H	H	H	H
CHB52	H	H	H	S	S	S	S	R	R
CHB54	H	H	H	R	R	R	R	S	S
CHB56	S	S	S	R	R	R	R	S	S
CHB59	S	S	S	H	H	H	H	S	S
CHB60	R	R	R	S	S	S	S	H	H
CHB61	H	H	H	R	R	R	R	R	R
CHB62	H	H	H	H	H	H	H	S	S
CHB65	H	H	H	S	S	S	S	H	H
CHB66	R	H	R	S	S	S	S	R	R
CHB67	R	R	R	S	S	S	S	R	R
CHB68	H	H	H	H	H	H	H	R	R
CHB69	H	H	H	R	R	R	R	H	H
CHB71	S	S	S	H	H	H	H	S	S
CHB72	S	S	S	H	H	H	H	H	H
CHB75	R	R	R	R	R	R	R	R	R
CHB80	H	H	H	H	H	H	H	H	H
CHB64	S	S	S	H	H	H	H	R	R
CHB84	S	S	S	R	R	H	H	H	H

[z]A1, M3-2; A2, M3-3; A6, CA12g17210; A7, CA12g19240; A12, Cmr1-2.

[y]R, resistant; S, susceptible; H, heterozygous.

assay was mainly distributed in *C. chinense* accessions (Table 2, A14), while that of the pvr2-123457 assay was found in *C. annuum* and *C. frutescens* accessions (Table 2, A15). The pvr2-123457 assay was based on a specific SNP of potyvirus-resistance genes, *pvr2*[1], *pvr2*[2], *pvr2*[3], *pvr2*[4], *pvr2*[5], and *pvr2*[7] (Charron et al., 2008).

Five assays, qcap3.1-40134 (Fig. 1P), qcap6.1-299931 (Fig. 1Q), qcap6.1-589160 (Fig. 1R), qdhc2.1-1335057 (Fig. 1S), and qdhc2.2-43829 (Fig. 1T), were linked to four QTLs, *qcap3.1* and *qcap6.1* for capsaicin content and *qdhc2.1* and *qdhc2.2* for dihydrocapsaicin content, which were identified in *C. chinense* 'Bhut Jolokia' (Table 1, A16-A20; Lee et al., 2016a). The qcap6.1-589160 assay was specific to only *C. chinense* accessions, while the other four assays were widely polymorphic in five *Capsicum* species, with the exception of *C. annuum* (Table 2, A16-A20). This result implied that the QTL *qcap6.1* might have a greater effect on capsaicinoid content than the other three QTLs. In addition, linkage analysis in the JN-F_5 population showed that the four assays cosegregated with corresponding HRM markers (Table 4, A16, A17, A18, and A20). This result suggests that the assays can be used to detect corresponding QTLs.

Application of SNP Type Assays to Pepper Cultivars and Breeding Lines

Successful SNP type assays were performed on 33 commercial cultivars, 30 breeding lines, and 7 genetic sources of chili pepper (Tables 5 and 6). Only five assays, M3-2 (A1), M3-3 (A2), Cmr1-2 (A12), pvr2-123457 (A15), and qcap3.1-40134 (A16), exhibited polymorphic results for the 33 commercial cultivars (Table 5). All cultivars except for 'Geumsugangsan', 'Nokkwang', and 'Matkwang' were *Phytophthora*-resistant varieties. The results of M3-2 and M3-3 assays were consistent with the resistance phenotypes except for those of 'Gangcheolhong', 'Ganghantopstar', and 'PR Chengyang', which might have developed with another resistance gene(s) unrelated to the *Phyto.5.2* gene because the M3-CAPS marker was perfectly matched with the resistance in the *C. annuum* resources including 'AC2258', 'CM331', 'CM334-INRA', 'CM334-KBU', 'PBC602', 'YCM334', 'PI201234', 'PBC280', and 'PBC495' (Table 5, A1 and A2; Lee et al. 2012). A comparison of the assays with M3-HRM markers showed that the M3-3 assay was more accurate than the M3-2 assay, which had one recombinant ('Meetinggochudaemok'). The other three polymorphic assays, Cmr1-2, pvr1-123457, and qcap3.1-40134, indicated that cultivars with the resistance marker type might have CMV and potyvirus resistance and that cultivars having the high capsaicinoid content marker type might be more pungent (Table 5, A12, A15, and A16). In an analysis of pepper breeding lines, 9 assays demonstrated polymorphic results among the cultivars (Table 6). Resistance marker types of three assays, Ltr4.1-40344 (A8), Ltr4.2-56301 (A9), and Ltr4.2-585119 (A10), were present in PM breeding lines that were resistant to powdery mildew (Table 6, A8, A9, and A10). In the analysis of the 7 genetic sources, a *C. chinense* accession, 'Bhut Jolokia', one of the world's hottest peppers, had all high capsaicinoid content marker types on the five capsaicinoid content assays (Table 6, A16-A20; Lee et al., 2016a). Six anthracnose-resistant *C. baccatum* accessions, including 'PBC81' and 'PI594137', had all of the resistance marker types for the three anthracnose resistance assays (Table 6, A3, A4, and A5; Park et al., 2009).

Fluidigm dynamic arrays, a high-throughput SNP genotyping method, include three formats for IFCs: 96 samples × 96 SNPs, 48 samples × 48 SNPs, or 192 samples × 24 SNPs. They can be used with three types of assays: TaqMan, KASP, or SNP type assays (Wang et al., 2009; Thomson, 2014). In this study, we used Fluidigm dynamic arrays for the first time for foreground selection of targeted genes or QTLs in pepper by combining 192.24 IFCs with SNP type assays to analyze 24 SNP markers at a time. This system can save both resources and time by reducing the reaction volume and producing 4,608 data points at a time (Wang et al., 2009). Of the 43 primer sets designed, 20 SNP type assays were successfully developed (Fig. 1 and Table 1). The accuracy of 11 assays was confirmed by comparing with the corresponding original HRM markers (Tables 2, 3, 4, and 5). These

Table 4. Comparison of marker types between the SNP type assays and HRM markers in the JN-F_5 population.

Plant material	*Phytophthora* root rot resistance (*Phyto.5.2*)			Anthracnose resistance (*CaR12.2*)				Capsaicinoid content								
	A1[z]	A2	M3-HRM	A6	CA12g17210-HRM	A12	CaTm1-HRM	A16	qcap3.1-40134-HRM	A17	qcap6.1-299931-HRM	A18	qcap6.1-589160-HRM	A19	A20	qdhc2.2-43829-HRM
JN1-1	R[y]	R	R	R	R	S	S	H	H	H	H	H	H	L	L	L
JN5-1	S	S	S	R	R	S	S	M	M	H	H	H	H	L	L	L
JN5-2	S	S	S	R	R	S	S	M	M	H	H	H	H	L	L	L
JN16-1	S	S	S	S	S	S	S	H	H	L	L	L	L	L	L	L
JN21-3	S	S	S	R	R	S	S	H	H	L	L	L	L	L	L	L
JN23-1	R	R	R	H	H	R	R	H	H	H	H	H	H	L	L	L
JN23-2	R	R	R	R	R	R	R	H	H	M	M	H	H	L	L	L
JN24-2	S	S	S	R	R	S	S	H	H	L	L	L	L	L	L	L
JN29-3	S	S	S	S	S	R	R	L	L	L	L	L	L	L	L	L
JN31-1	S	S	S	S	S	H	H	H	H	M	M	M	M	L	H	H
JN33-2	S	S	S	S	S	R	R	H	H	H	H	H	H	L	L	L
JN34-3	S	S	S	R	R	S	S	H	H	L	L	L	L	L	L	L
JN37-3	S	S	S	R	R	R	R	L	L	L	L	L	L	L	L	L
JN43-1	S	S	S	S	S	R	R	L	L	H	H	H	H	L	L	L
JN44-3	R	R	R	S	S	R	R	H	H	H	H	H	H	L	L	L
JN48-1	S	S	S	R	R	S	S	H	H	M	M	M	M	L	L	L
JN49-2	S	S	S	H	H	R	R	L	L	L	L	L	L	L	L	L
JN52-3	S	S	S	R	R	R	R	L	L	H	H	L	L	L	L	L
JN56-1	R	R	R	S	S	S	S	L	L	L	L	H	H	H	H	H
JN62-2	H	H	H	R	R	R	R	H	H	H	H	H	H	H	H	H
JN70-3	S	S	S	S	S	R	R	H	H	H	H	H	H	H	H	H
JN73-2	R	R	R	S	S	S	S	H	H	H	H	H	H	H	H	H
JN73-4	S	S	S	S	S	R	R	H	H	H	H	H	H	L	L	L
JN79-2	S	S	S	S	S	S	S	H	H	L	L	L	L	L	L	L
JN87-3	S	S	S	R	R	R	R	H	H	H	H	H	H	L	L	L
JN91-1	R	R	R	R	R	R	R	L	L	L	L	L	L	M	L	L
JN93-1	S	S	S	S	S	R	R	L	L	L	L	L	L	H	H	H
JN94-2	S	S	S	S	S	S	S	L	L	H	H	H	H	L	L	L
JN96-2	S	S	S	S	S	R	R	L	L	H	H	H	H	L	L	L
JN100-2	S	S	S	S	S	R	R	H	H	L	L	L	L	L	L	L
JN103-2	S	S	S	R	R	S	S	M	M	M	M	M	M	L	L	L
JN109-1	R	R	R	S	S	R	R	H	H	H	H	H	H	L	L	L
JN110-2	S	S	S	S	S	S	S	H	H	L	L	L	L	L	L	L
JN110-3	S	S	S	S	S	S	S	M	M	H	H	L	L	L	M	M
JN112-3	H	H	H	S	S	R	R	M	M	L	L	L	L	L	L	L
JN115-3	S	S	S	S	S	R	R	H	H	H	H	H	H	L	L	L
JN117-1	S	S	S	R	R	R	R	H	H	H	H	H	H	H	H	H
JN120-2	S	S	S	S	S	R	R	H	H	L	L	L	L	L	L	L
JN120-3	S	S	S	S	S	R	R	H	H	H	H	H	H	L	L	L
JN131-1	H	H	H	H	H	R	R	L	L	L	L	L	L	L	L	L
JN146-1	S	S	S	H	H	R	R	H	H	H	H	H	H	L	L	L
JN147-1	R	R	R	S	S	R	R	L	L	L	L	L	L	L	L	L
JN164-1	S	S	S	S	S	H	H	L	L	L	L	L	L	H	H	H
JN177-1	S	S	S	R	R	R	R	M	M	L	L	L	L	L	L	L
JN178-1	S	S	S	S	S	S	S	H	H	H	H	H	H	L	L	L
JN183-1	S	S	S	R	R	S	S	M	M	L	L	M	M	L	L	L
JN204-1	R	R	R	S	S	H	H	L	L	L	L	L	L	H	M	M
JN220-1	S	S	S	R	R	R	R	H	H	M	M	M	M	H	H	H
JN220-2	S	S	S	H	H	R	R	M	M	L	L	L	L	L	L	L
JN254-2	R	R	R	S	S	R	R	L	L	H	H	H	H	L	L	L

[z]A1, M3-2; A2, M3-3; A6, CA12g17210; A12, Cmr1-2; A16, qcap3.1-40134; A17, qcap6.1-299931; A18, qcap6.1-589160; A19, qdhc2.1-1335057; A20, qdhc2.2-43829.
[y]R, resistant; S, susceptible; H, heterozygous for A1, A2, A6, A12, high capsaicinoid content allele for A16-A20; L, low capsaicinoid content allele.

results suggest that the newly developed SNP type assays can be used in place of the original markers. The other 9 assays also need to be compared to original markers using polymorphic populations to confirm their accuracy. These SNP type assays will be useful in molecular breeding programs for developing new pepper varieties resistant to multiple diseases and with increased pungency.

Table 5 . Marker types of 17 SNP type assays and an M3-HRM marker in 33 commercial pepper cultivars.

Plant material	*Phytophthora* root rot resistance			Anthracnose resistance			Powdery mildew resistance			CMV resistance	TMV resistance	Potyvirus resistance		Capsaicinoid content				
	A1[z]	A2	M3-HRM	A3	A4	A5	A8	A9	A10	A12	A13	A14	A15	A16	A17	A18	A19	A20
Anseongmachum	H[y]	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
Tantandaemok	H	H	H	S	S	S	S	S	S	H	S	S	R	L	L	L	L	L
PR Power	H	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
Konesianhot	H	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
Meetinggochudaemok	H	R	R	S	S	S	S	S	S	R	S	S	H	M	L	L	L	L
Kataguruma	H	H	H	S	S	S	S	S	S	H	S	S	S	L	L	L	L	L
PR Smart	H	H	H	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
Muhanjilju	H	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
Illpyundansim	H	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
AR Legend	H	H	H	S	S	S	S	S	S	H	S	S	S	L	L	L	L	L
Josaengace	H	H	H	S	S	S	S	S	S	R	S	S	H	L	L	L	L	L
Gisedeungdeung	H	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
Bitgoeul	H	H	H	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
Geumgangseok	H	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
PR Hwanhoseoung	H	H	H	S	S	S	S	S	S	R	S	S	H	L	L	L	L	L
Gangcheolhong	S	S	S	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
Yebbeundokyacheongcheong	H	H	H	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
Supergeumdang	H	H	H	S	S	S	S	S	S	H	S	S	H	L	L	L	L	L
PR Cheonmyung	H	H	H	S	S	S	S	S	S	R	S	S	H	L	L	L	L	L
Ganghantopstar	S	S	S	S	S	S	S	S	S	R	S	S	H	L	L	L	L	L
PR Chengyang	S	S	S	S	S	S	S	S	S	H	S	S	H	M	L	L	L	L
PR Pungnyunga	H	H	H	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PR Jeolsemiin	H	H	H	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
Buldojang	H	H	H	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
Mubyungjidae	R	R	R	S	S	S	S	S	S	H	S	S	S	L	L	L	L	L
Mutanjidae	R	R	R	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
Manmul	H	H	H	S	S	S	S	S	S	H	S	S	S	L	L	L	L	L
Chungseong	H	H	H	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
Sinbi	H	H	H	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
Haemalgeungochu	H	H	H	S	S	S	S	S	S	S	S	S	S	M	L	L	L	L
Geumsugangsan	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
Nokkwang	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
Matkwang	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L

[z]A1, M3-2; A2, M3-3; A3, CcR9; A4, CA09g12180; A5, CA09g19170; A8, Ltr4.1-40344; A9, Ltr4.2-56301; A10, Ltr4.2-585119; A12, Cmr1-2; A13, L4; A14, pvr1; A15, pvr2-123457; A16, qcap3.1-40134; A17, qcap6.1-299931; A18, qcap6.1-589160; A19, qdhc2.1-1335057; A20, qdhc2.2-43829.

[y]R, resistant; S, susceptible; H, heterozygous for A1-A15, high capsaicinoid content allele for A16-A20; L, low capsaicinoid content allele.

Table 6 . Marker types of 17 SNP type assays in 30 breeding lines and 7 genetic sources for pepper.

Plant material	*Phytophthora* root rot resistance		Anthracnose resistance			Powdery mildew resistance			CMV resistance	TMV resistance	Potyvirus resistance		Capsaicinoid content				
	A1[z]	A2	A3	A4	A5	A8	A9	A10	A12	A13	A14	A15	A16	A17	A18	A19	A20
PM4	H[y]	H	S	S	S	R	R	R	S	S	S	S	L	L	L	L	L
PM8	R	R	S	S	S	H	R	H	S	S	S	S	L	L	L	L	L
PM16	H	H	S	S	S	H	R	H	S	S	S	S	L	L	L	L	L
PM33	H	H	S	S	S	H	R	H	S	S	S	H	L	L	L	L	L
PM36	S	S	S	S	S	H	R	H	S	S	S	H	L	L	L	L	L
PM43	H	H	S	S	S	R	R	R	H	S	S	S	L	L	L	L	L
PM52	H	H	S	S	S	H	R	H	S	S	S	S	L	L	L	L	L
PM57	R	R	S	S	S	R	R	R	S	S	S	S	L	L	L	L	L
PM67	R	R	S	S	S	H	R	H	H	S	S	S	L	L	L	L	L
PM68	H	H	S	S	S	R	R	R	S	S	S	S	L	L	L	L	L
PM95	H	H	S	S	S	R	R	R	S	S	S	S	L	L	L	L	L
PM108	S	S	S	S	S	R	R	R	S	S	S	S	L	L	L	L	L
PM122	S	S	S	S	S	R	R	R	S	S	S	S	L	L	L	L	L
6538-8	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6542-3	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6542-6	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6542-7	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6542-10	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6544-2	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6545-3	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6545-5	S	S	S	S	S	S	H	S	S	S	S	S	L	L	L	L	L
6545-13	R	R	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PBI285-5-1	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PBI285-5-2	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
PBI286-3-5	H	H	S	S	S	S	S	S	H	S	S	S	L	H	L	L	L
PBI286-7-3	S	S	S	S	S	S	S	S	R	S	S	S	L	L	L	L	L
10-271-1-5	R	R	S	S	S	S	S	S	S	S	S	H	L	L	L	L	L
10-271-1-6	R	R	S	S	S	S	S	S	S	S	S	H	L	L	L	L	L
10-271-2-2	H	H	S	S	S	S	S	S	S	S	S	S	M	L	L	L	L
10-271-2-3	H	H	S	S	S	S	S	S	S	S	S	S	L	L	L	L	L
Bhut Jolokia	R	R	S	S	S	S	S	S	R	S	S	S	H	H	H	H	H
PBC81	R	NA	R	R	R	R	R	R	S	S	S	S	H	H	L	H	H
6548	R	NA	R	R	R	R	R	R	S	S	S	S	H	H	L	H	H
6550	R	NA	R	R	R	R	R	R	S	S	S	S	H	H	L	H	H
6551	R	NA	R	R	R	R	R	R	S	S	S	S	H	H	L	H	H
6557	R	NA	R	R	R	R	R	R	S	S	S	S	H	H	L	H	H
PI594137	R	NA	R	R	R	R	R	R	S	S	S	S	H	H	L	H	H

[z]A1, M3-2; A2, M3-3; A3, CcR9; A4, CA09g12180; A5, CA09g19170; A8, Ltr4.1-40344; A9, Ltr4.2-56301; A10, Ltr4.2-585119; A12, Cmr1-2; A13, L4; A14, pvr1; A15, pvr2-123457; A16, qcap3.1-40134; A17, qcap6.1-299931; A18, qcap6.1-589160; A19, qdhc2.1-1335057; A20, qdhc2.2-43829.

[y]R, resistant; S, susceptible; H, heterozygous for A1-A15, high capsaicinoid content allele for A16-A20; L, low capsaicinoid content allele

Literature Cited

Aza-González C, Núñez-Palenius HG, Ochoa-Alejo N (2011) Molecular biology of capsaicinoid biosynthesis in chili pepper (*Capsicum* spp.). Plant Cell Rep 30:695-706. doi:10.1007/s00299-010-0968-8

Boukema IW (1984) Resistance to TMV in *Capsicum chacoense* Hunz. is governed by allele of the L-locus. Capsicum Newsl 3:47-48

Charron C, Nicolai M, Gallois JL, Robaglia C, Moury B, Palloix A, Caranta C (2008) Natural variation and functional analyses provide evidence for co-evolution between plant eIF4E and potyviral VPg. Plant J 54:56-68. doi:10.1111/j.1365-313X.2008.03407.x

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142:169-196. doi:10.1007/s10681-005-1681-5

Collard BCY, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Phil Trans R Soc B 363:557-572. doi:10.1098/rstb.2007.2170

Eun MH, Han JH, Yoon JB, Lee J (2016) QTL mapping of resistance to the *Cucumber mosaic virus* P1 strain in pepper using a genotyping-by-sequencing analysis. Hortic Environ Biotechnol 57:589-597. doi:10.1007/s13580-016-0128-3

Kang BC, Yeam I, Frantz JD, Murphy JF, Jahn MM (2005) The *pvr1* locus in *Capsicum* encodes a translation initiation factor eIF4E that interacts with Tobacco etch virus VPg. Plant J 42:392-405. doi:10.1111/j.1365-313X.2005.02381.x

Kang WH, Hoang NH, Yang HB, Kwon JK, Jo SH, Seo JK, Kim KH, Choi D, Kang BC (2010) Molecular mapping and characterization of a single dominant gene controlling CMV resistance in peppers (*Capsicum annuum* L.). Theor Appl Genet 120:1587-1596. doi:10.1007/s00122-010-1278-9

Kim HJ, Han JH, Kim S, Lee HR, Shin JS, Kim JH, Cho J, Kim YH, Lee HJ, Kim BD, Choi D (2011) Trichome density of main stem is tightly linked to PepMoV resistance in chili pepper (*Capsicum annuum* L.). Theor Appl Genet 122:1051-1058. doi:10.1007/s00122-010-1510-7

Kim SB, Kang WH, Huy HN, Yeom SI, An JT, Kim S, Kang MY, Kim HJ, Jo YD, Ha Y, Choi D, Kang BC (2017) Divergent evolution of multiple virus-resistance genes from a progenitor in *Capsicum* spp. New Phytologist 213:886-899. doi:10.1111/nph.14177

Kumar S, Banks TW, Cloutier S (2012) SNP discovery through next-generation sequencing and its application. Int J Plant Genomics 831460. doi:10.1155/2012/831460

Lee J, Do JW, Yoon JB (2011) Development of STS markers linked to the major QTLs for resistance to the pepper anthracnose caused by *Colletotrichum acutatum* and *C. capsici*. Hortic Environ Biotechnol 52:596-601. doi:10.1007/s13580-011-0178-5

Lee J, Hong JH, Do JW, Yoon JB (2010) Identification of QTLs for resistance to anthracnose to two *Colletotrichum* species in pepper. J Crop Sci Biotechnol 13:227-233. doi:10.1007/s12892-010-0081-0

Lee J, Park SJ, Hong SC, Han JH, Choi D, Yoon JB (2016a) QTL mapping for capsaicin and dihydrocapsaicin content in a population of *Capsicum annuum* 'NB1' x *Capsicum chinense* 'Bhut Jolokia'. Plant Breeding 135:376-383. doi:10.1111/pbr.12355

Lee SH, Lee JB, Kim SM, Choi HS, Park JW, Lee JS, Lee KW, Moon JS (2004) The incidence and distribution of viral diseases in pepper by cultivation types. Res Plant Dis 10:231-240. doi:10.5423/RPD.2004.10.4.231

Lee WP, Lee J, Han JH, Kang BC, Yoon JB (2012) Validity test for molecular markers associated with resistance to *Phytophthora* root rot in chili pepper (*Capsicum annuum* L.). Korean J Hortic Sci Technol 30:64-72. doi:10.7235/hort.2012.11112

Lee YR, Yoon JB, Lee J (2016b) A SNP-based genetic linkage map of *Capsicum baccatum* and its comparison to the *Capsicum annuum* reference physical map. Mol Breeding 36:61. doi:10.1007/s11032-016-0485-8

Lefebvre V, Daubèze AM, Rouppe van der Voort J, Peleman J, Bardin M, Palloix A (2003) QTLs for resistance to powdery mildew in pepper under natural and artificial infections. Theor Appl Genet 107:661-666. doi:10.1007/s00122-003-1307-z

Liu WY, Kang JH, Jeong HS, Choi HJ, Yang HB, Kim KT, Choi D, Choi GJ, Jahn M, Kang BC (2014) Combined use of bulked segregant analysis and microarrays reveals SNP markers pinpointing a major QTL for resistance to *Phytophthora capsici* in pepper. Theor Appl Genet 127:2503-2513. doi:10.1007/s00122-014-2394-8

Mahasuk P, Struss D, Mongkolporn O (2016) QTLs for resistance to anthracnose identified in two *Capsicum* sources. Mol Breed 36:10. doi:10.1007/s11032-016-0435-5

Mimura Y, Kageyama T, Minamiyama Y, Hirai M (2009) QTL analysis for resistance to *Ralstonia solanacearum* in *Capsicum* accession 'LS2341'. J Jpn Soc Hort Sci 78:307-313. doi:10.2503/jjshs1.78.307

Park SK, Kim SH, Park HG, Yoon JB (2009) *Capsicum* germplasm resistant to pepper anthracnose differentially interact with *Colletotrichum* isolates. Hortic Environ Biotechnol 50:17-23

Poland JA, Rife TW (2012) Genotyping-by-sequencing for plant breeding and genetics. Plant Genome 5:92-102. doi:10.3835/plantgenome2012.05.0005

Quirin EA, Ogundiwin EA, Prince JP, Mazourek M, Briggs MO, Chlanda TS, Kim KT, Falise M, Kang BC, Jahn MM (2005) Development of sequence characterized amplified region (SCAR) primers for the detection of *Phyto.5.2*, a major QTL for resistance to *Phytophthora capsici* Leon. in pepper. Theor Appl Genet 110:605-612. doi:10.1007/s00122-004-1874-7

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5:94-100. doi:10.1016/S1369-5266(02)00240-6

Römer P, Hahn S, Jordan T, Strauβ T, Bonas U, Lahaye T (2007) Plant pathogen recognition mediated by promoter activation of the pepper *Bs3* resistance gene. Science 318:645-648. doi:10.1126/science.1144958

Römer P, Jordan T, Lahaye T Identification and application of a DNA-based marker that is diagnostic for the pepper (*Capsicum annuum*) bacterial spot resistance gene *Bs3*. Plant Breeding 129:737-740. doi:10.1111/j.1439-0523.2009.01750.x

Stall RE, Jones JB, Minsavage GV (2009) Durability of resistance in tomato and pepper to Xanthomonads causing bacterial spot. Annu Rev Phytopathol 47:265-284. doi:10.1146/annurev-phyto-080508-081752

Tai T, Dahlbeck D, Stall RE, Peleman J, Staskawicz BJ (1999a) High-resolution genetic and physical mapping of the region containing the *Bs2* resistance gene of pepper. Theor Appl Genet 99:1201-1206. doi:10.1007/s001220051325

Tai TH, Dahlbeck D, Clark ET, Gajiwala P, Pasion R, Whalen MC, Stall RE, Staskawicz BJ (1999b) Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato. Proc Natl Acad Sci 96:14153-14158. doi:10.1073/pnas.96.24.14153

Thomson MJ (2014) High-throughput SNP genotyping to accelerate crop improvement. Plant Breeding Biotech 2:195-212. doi:10.9787/ PBB.2014.2.3.195

Tomita R, Sekine KT, Mizumoto H, Sakamoto M, Murai J, Kiba A, Kikichi Y, Suzuki K, Kobayashi K (2011) Genetic basis for the hierarchical interaction between Tobamovirus spp. and *L* resistance gene alleles from different pepper species. Mol Plant Microbe Interact 24:108-117. doi:10.1094/MPMI-06-10-0127

Truong HTH, Kim KT, Kim S, Cho MC, Kim HR, Woo JG (2011) Development of gene-based markers for the *Bs2* bacterial spot resistance gene for marker-assisted selection in pepper (*Capsicum* spp.). Hortic Environ Biotechnol 52:65-73. doi:10.1007/ s13580-011-0142-4

Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends Biotechnol 27:522-530. doi:10.1016/j.tibtech.2009.05.006

Wang J, Lin M, Crenshaw A, Hutchinson A, Hicks B, Yeager M, Berndt S, Huang WY, Hayes RB, Chanock SJ, Jones RC, Ramakrishnan R (2009) High-throughput single nucleotide polymorphism genotyping using nanofluidic Dynamic Arrays. BMC Genomics 10:561. doi:10.1186/1471-2164-10-561

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Sci 48:391-407. doi:10.2135/ cropsci2007.04.0191

Yang HB, Liu WY, Kang WH, Kim JH, Cho HJ, Yoo JH, Kang BC (2012) Development and validation of *L* allele-specific markers in *Capsicum*. Mol Breeding 30:819-829. doi:10.1007/s11032-011-9666-7

Yeam I, Kang BC, Lindeman W, Frantz JD, Faber N, Jahn MM (2005) Allele-specific CAPS markers based on point mutations in resistance alleles at the *pvr1* locus encoding eIF4E in *Capsicum*. Theor Appl Genet 112:178-186. doi:10.1007/s00122-005-0120-2

Yoon JB (2003) Identification of genetic resources, interspecific hybridization and inheritance analysis for breeding pepper (*Capsicum annuum*) resistant to anthracnose. PhD Diss., Seoul National Univ., Seoul, Korea