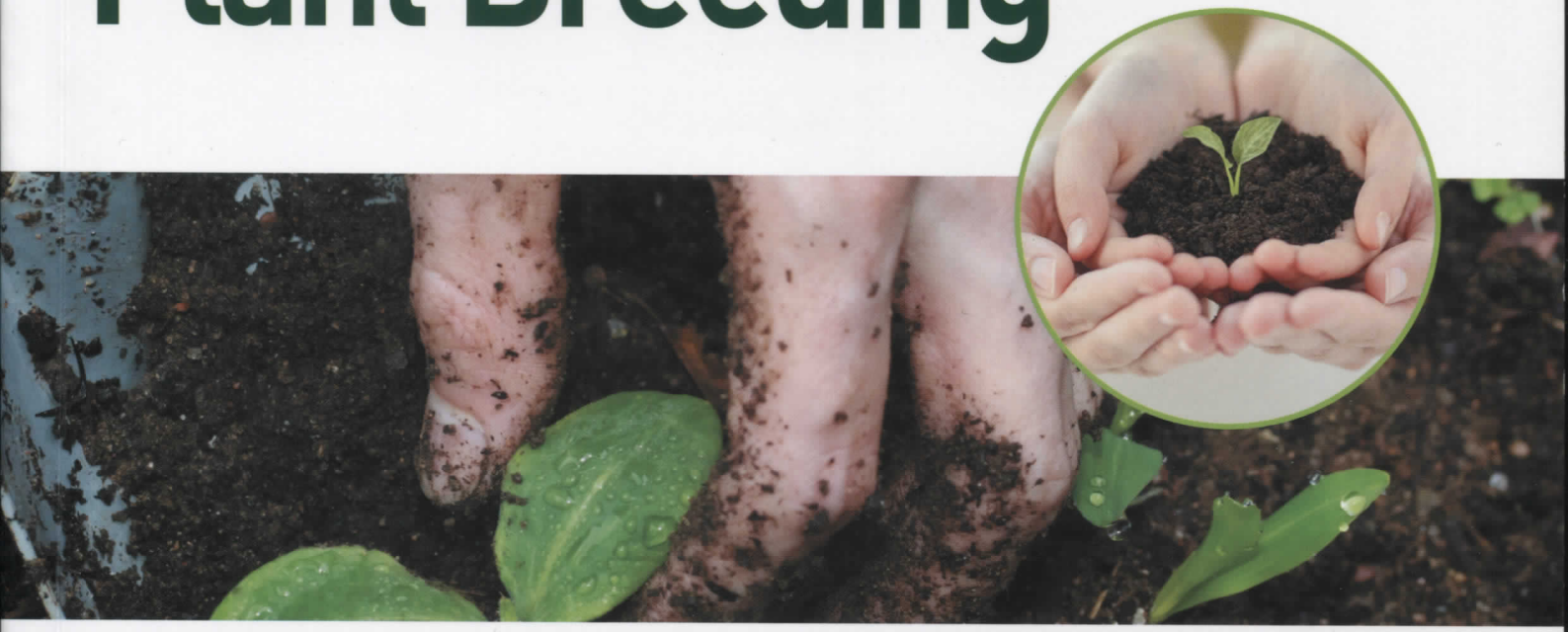
2016

한국육종학회-차세대BG21사업단-GSP사업단 공동심포지엄

Gene, Genome & New Technology for Plant Breeding



일시: 2016년 6월 29(수)~7월 1(금)

장소: 청주 라마다플라자 호텔

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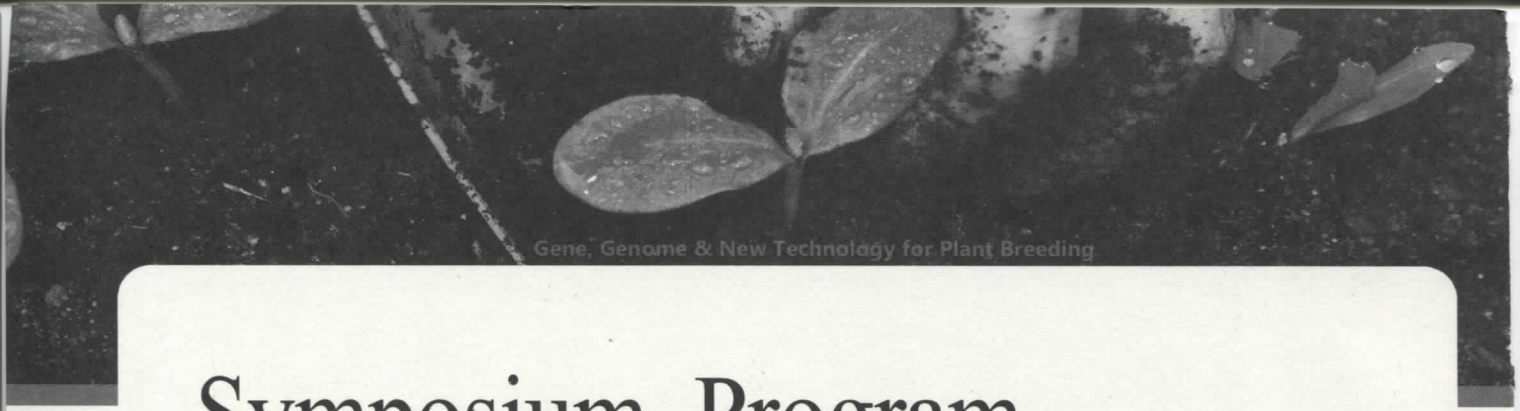

Symposium Program

2016 Annual Symposium of Korean Society of Breeding Science
Date and Place : June 29 - July 1, 2016 & Ramada Hotel at Cheongju

1st day [June 29. Wednesday]	
19:00~	General Meeting of Organizing Committee

2nd day [June 30. Thursday]	
09:00~09:50	Registration
09:50~10:00	Opening Ceremony ▸ Dr. Kang-Sup Lee (Secretary General, RDA, Korea)
	Opening Address – Dr. Young-soo Chung (Organizer, Dong-A University, Korea)
	Welcome Address – Dr. Yong-Gu Cho (President of KSBS, Chungbuk National University, Korea)
〈〈 Plenary Session 〉〉	
	▸Chair : Prof. Hee-Jong Koh (Seoul National University)
10:00~10:40	▸Doubled Haploid (DH) Technology in Maize Breeding: Application and Technology for Production of DH Lines – Dr. Chaikam, CIMMYT, Mexico
10:40~11:20	▸Finding Mineral Element Transporters for Better and Safe Production of Rice – Dr. Jian Feng Ma, Okayama University, Kurashiki, Japan
11:20~12:00	▸Function of Fibrillin Protein in Photosynthetic Metabolism – Dr. Hyun Wook Kim, SeJong University, Korea
12:00~13:20	Lunch & Poster
	▸Chair: Prof. Deun-Gun Oh (Korea National College of Agricultural and Fisheries)
13:20~14:00	▸Revisiting Domestication to Revitalize Crop Improvement: The Florigen Revolution – Dr. Zach Lippman, Cold Spring Harbor Laboratory, USA
14:00~14:40	▸Manipulating Fruit and Vegetable Quality Traits – Dr. David Brummell, Plant & Food Research, New Zealand
14:40~15:20	▸Designing Crops for Global Food Security: Digitizing Plant Phenotypes – Dr. Maurice Moloney, University of Saskatchewan, Canada

PC-81

Exploring Resistance Against *PHYTOPHTHORA CAPSICI* in Pepper

Muhammad Irfan Siddique, Koeun Han, Byoung-Cheorl Kang*

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Pepper (*Capsicum annuum*) is an economically important vegetable grown in tropical and temperate regions for fresh and processed use. *Phytopthtora* blight and root rot caused by the oomycete pathogen *Phytophthora capsici* is the most devastating pepper disease in the world. Although many controlling methods are available including fungicide and resistance cultivars, the pathogen affects yield and quality adversely. Furthermore, breeding resistance against Pc is complicated because of complex mode of inheritance and progressive evolution in pathogen races. In present study, we detected two QTLs; *QTL5-1* and *QTL5-2* on chromosome P5 using an F_2 population cross between resistant accession CM334 and susceptible parent ECW30R, conferring resistance to low and high virulence Pc isolates, respectively, and evaluated their interaction with molecular markers anchoring in close vicinity. In addition, 548 F_4 lines were genotyped with 3 different SNPs markers linked to the two QTLs. Among these, 79 selected lines were subjected to genotyping-by-sequencing (GBS) analysis and screened with highly aggressive Pci solate. As a result, a new genetic linkage map was constructed using 1170 SNPs identified by GBS across 12 pepper chromosomes. QTLs effective against highly aggressive Pc isolate were mapped. The resistant QTLs and linked SNPs will be validated in recombinant inbred line and commercial cultivars. These markers will expedite the breeding programs for resistance introgression in high-yielding Pc susceptible cultivars.

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PC-60

Genetic Mapping of Resistance Sources Against ChiVMV (*Chili veinal mottle virus*) in Hot Pepper

Joung-Ho Lee, Jeong-Tak An, Koeun Han, Seula Choi, Muhammad Irfan Siddique, Byoung-Cheorl Kang*

Department of Plant Science, Plant Genomics & Breeding Institute, and Research Institute of Agriculture and Life Sciences, Seoul National University, Korea

Chili veinal mottle virus (ChiVMV) is the notorious virus affecting the severe loss of pepper production in Asia and Africa. To map the positions of ChiVMV resistance genes, F_2 mapping populations were constructed by crossing each resistance accession, *Capsicum annuum*, 'CV3', 'CV4', and 'CV8' with susceptible *C. annuum*, 'Jeju'. In the 'CV3' and 'CV8' mapping populations, resistance genes were inherited by a single dominant manner and located on the short arm of pepper chromosome 6. The ChiVMV resistance marker reported in the previous study was co-segregated with these genes. Through allelism test of resistance genes from 'CV3' and 'CV8', it was revealed that the resistance gene in each line was originated from the same locus, and we named this gene, *Cvr1* (ChiVMV resistance 1) locus. We developed several SNP markers linked to *Cvr1* using pepper genome information and pepper bacterial artificial chromosome (BAC) sequences. By contrast, the inheritance mode of ChiVMV resistance in CV4 was different from those of CV3 or CV8. The inheritance study showed that two independent complementary genes involved in ChiVMV resistance in CV4. To map the resistance genes in CV4, SNP-based linkage map was constructed by genotyping-by-sequencing (GBS) method. The result of this study will accelerate the ChiVMV resistance research and breeding resistance cultivar to ChiVMV in pepper.

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Exploring Resistance against *Phytophthora capsici* in Pepper

Muhammad Irfan Siddique[1], Koeun Han[1] and Byoung-Cheorl Kang[1]*

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Abstract

Pepper (*Capsicum annuum*) is an economically important vegetable grown in tropical and temperate regions for fresh and processed use. *Phytopthtora* blight and root rot caused by the oomycetes pathogen *Phytophthora capsici* (Pc) is the most devastating pepper disease in the world. Although many controlling methods are available including fungicide and resistance cultivars, the pathogen affects yield and quality adversely. Furthermore, breeding resistance against Pc is complicated because of complex mode of inheritance and progressive evolution in pathogen races. In present study, we detected two QTLs; *QTL5-1* and *QTL5-2* on chromosome P5 using an F_2 population cross between resistant accession CM334 and susceptible parent ECW30R, conferring resistance to low and high virulence Pc isolates, respectively, and evaluated their interaction with molecular markers anchoring in close vicinity. In addition, 548 F_4 individuals were genotyped with different SNPs markers linked to the two QTLs. Among these, 79 selected lines were subjected to genotyping-by-sequencing (GBS) analysis and their subsequent generations were screened with highly aggressive Pc isolate. As a result, a new genetic linkage map was constructed using 1170 SNPs identified by GBS across 12 pepper chromosomes. QTLs effective against highly aggressive Pc isolate were mapped. The resistant QTLs and linked SNPs will be validated in recombinant inbred line and commercial cultivars. These markers will expedite the breeding programs for resistance introgression in high-yielding Pc susceptible cultivars.

Introduction

Peppers, *Capsicum spp.*, are important vegetable crop grown all over the world for fresh consumption, dried condiments, medicinal and ornamental uses. It is a rich source of vitamins, particularly vitamins A and C containing considerable amount of calcium, phosphorus, and iron as well. It has high economic and cash value to farmers.

Phytophthora capsici Leonian, a soil born pathogen (oomycetes), which causes multiple disease symptoms including; damping off, root rot, leaf blight and fruit and crown rot, has been identified as a critical limiting factor in pepper production areas (Kim et al., 2012; Ristaino and Johnston, 1999).It causes significant economic losses and also reduces the market quality of edible fruit.

Generally, it has been accepted that two kind of resistance against *Phytophthora* is existed. Oligogenic or specific resistance following gene-for-gene relationship, it is active against specific isolates of pathogen (Monroy-Barbosa & Bosland, 2008; Sy et al., 2008). Other type is quantitative resistance which is polygenic (Lefebvre & Palloix, 1996; Thabuis et al., 2003 Truong et al., 2012). Numerous Quantitative trait loci (QTLs) linked to Phytophthora root rot resistance have been described (Thabuis et al., 2003, 2004; Ogundiwin et al., 2005; Bonnet et al., 2007), in which few are resistant to specific isolates (Truong et al., 2012) . Hence, identifying the number and position of the resistance genes in the pepper genome and exploiting the genetic relation between the host and parasite, is imperative to develop resistance cultivars.

The utilization of a recombinant inbred line (RIL) population has several plus points than other populations that are used for QTL analysis and genetic mapping. A RIL can provide a permanent mapping source that will allow replicated tests either in various environments or with diverse pathogen isolates (Truong et al., 2012). In the present study we detected two QTLs; *QTL5-1* and *QTL5-2* on chromosome P5 conferring resistance to low and high virulence *P. capsici* isolates, respectively, and evaluated their interaction with the help of molecular markers anchoring in close vicinity of QTLs, identified by GBS approach and systematic screening of mapping population.

Materials and Methods

Plant material

The mapping population was constructed by crossing resistant source 'Criollo de Morelos-334' (CM334) with susceptible pepper accession ECW30R. 274 F_2 plants were selected and the generation advancement was carried out by single seed descend method.

Disease assay and resistance scoring

P. capsici isolate KPC-7 was kindly provided by the (Dr. Choi, KRICT). The disease severity was scored at 7 and 14 days of post inoculation based on the disease scale 1-4 described by Kim et al., (2012) where 1 = no visible symptoms observed; 2 = dark lesion visible on the base of stem, but surviving without wilting; 3 = wilting with dark lesion on the base of stem; 4 = dead and dried.

Marker development and genotyping

Phyto5 NBS1 a SNP marker (Liu et al., 2014) pinpointing major resistant QTL and 2 other newly developed fluidigm markers were used to genotype the 548 individuals. The individuals were categorized into 6 groups on the basis of genotype analysis. The subsequent generations of the individuals carrying these groups were then evaluated for the contribution of the phenotypic variance.

Preparation of library for GBS and sequencing

ECW30R a susceptible control along with, a total of 80 individuals including eight individuals from group1, fifty individuals from group 2, three individuals from each of the group 3,4, 5 and nine individuals from group 6, selected on the basis of explained phenotypic variance for GBS analysis.

SNP discovery, linkage mapping and QTL analysis

After GBS analysis total 1170 SNPs were identified. Genetic map was constructed using Carthagene software with the total map length of 2751 cM and an average of 1.8 minimum and 3.6 maximum intervals between adjacent markers across all chromosomes. QTL mapping was performed using WinQTLCart.

Results and Discussion

Genotyping and marker recombination groups

Based on our preliminary study of QTL detection using F_2 segregating population, two QTLs; QTL 5-1 and 5-2 were mapped in close vicinity on P5. Focusing on those QTLs, SNP markers from previously published map, one marker linked with QTL 5-1 (Liu et al., 2014) and two newly developed markers linked with QTL 5-2 (Fig. 1) were converted into KASP markers for genotyping using Lightcycler ® 480 system. The genotyping results are presented in (Table 1). Genotyping of 548 individuals with SNP markers assorted all the individuals into six diverse groups with the above two mentioned QTLs segments corresponding to anchoring markers (Fig. 1).

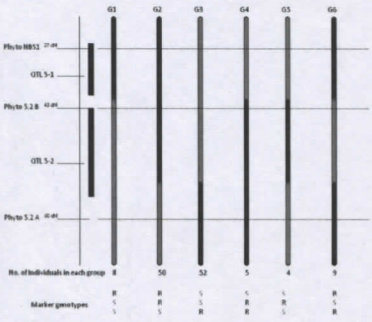

Figure 1. Recombination groups of the test plants and QTLs positions. Genotyping was carried out with SNP markers. Phyto 5NBS1 is located in the peak of QTL 5-1 while Phyto 5.1B and 5.2A are located at the upper and lower ends of QTL 5-2. Black portion indicates genomic segment from resistant genotype CM334, while grey represents susceptible genomic segment from the ECW30R background. Total 128 individuals were sorted out with three different SNPs. R, resistant; S, susceptible.

Table 1. Genotyping analysis of EC population with 3 SNP markers carried out using KASP genotyping technology

Total samples	Marker[a]	Physical position[b]	Genetic position[c]	QTL[d]	Resistance	Susceptible	Heterozygous	Not-Detected
548	Phyto5 NBS1	28	27	Phyto 5-1	214	200	63	71
548	Phyto 5.2 B	33	43	Phyto 5-2	181	211	75	81
548	Phyto 5.2 A	223	68	Phyto 5-2	219	183	67	79

[a] Markers located in the 2 QTLs region
[b] Physical position of the marker in Mbp on reference genome
[c] Genetic position of the marker in cM on chromosome 5
[d] Two QTLs Phyto 5-1 and 5-2 conferring resistance to low and high virulence isolates respectively

Evaluation of resistance to Phytophthora root rot

Test plants were screened against highly aggressive isolate of *P. capsici* (KPC-7) with two different level of inoculum densities. Concentrations of spore suspensions were adjusted to 5 x 10^4/ml for higher and 3 x 10^3/ml for moderate using Hemocytometer. Higher disease indices were observed when inoculated with the higher inoculums density as compared with moderate inoculum (Table 2). Individuals from the group having 2 QTLs showed higher resistance levels, while groups with only one QTL showed less resistance (Fig. 2).

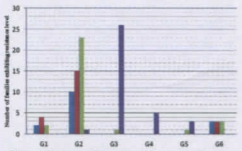

Figure 2. Frequency distribution of *P. capsici* resistance. G = groups. Along Y-axis are the families tested in each groups (each family contain 8 replications). Blue = highly resistance; Red = moderately resistance while Green and Purple are susceptible.

Table 2. Recombinant groups on the basis of marker genotypes and their corresponding phenotypic variance

Recombinant groups	Phenotype with high inoculum density[a]	Phenotype with moderate inoculum density[b]	Genotype / marker		
			Phyto5 NBS1	Phyto 5.2 B	Phyto 5.2 A
Group 1	R (29.2%)	R (80%)	R	S	S
Group 2	R (54.1%)	R (81%)	R	R	S
Group 3	S	S	S	S	R
Group 4	S	S	S	R	R
Group 5	S	S	S	R	S
Group 6	R (46.0%)	R (65%)	R	S	R

[a] Sporangial suspension density adjusted at 5 x 10^4
[b] Sporangial suspension density adjusted at 3 x 10^3

QTL analysis

QTL analysis was carried out with 183 F_2 individuals using 142 SNP markers which revealed the presence of two QTLs on P5 (Fig. 3). Further genotyping of RILs with GBS-SNPs confirmed the presence of those two QTLs on P5 at similar location (Fig. 4). The both QTLs explained different phenotypic variation (data not shown). Moreover, the interaction study of these QTLs will reveal their role in resistance against different isolates with varying aggressiveness.

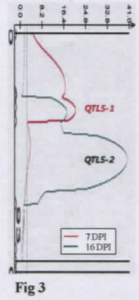

Fig 3

Figure 3. QTL mapping on P5 using F_2 segregating population. QTL analysis was carried out using 142 SNPs. Two QTLs; QTL 5-1 and 5-2 were detected on 7 days and 14 days of post inoculation, respectively.

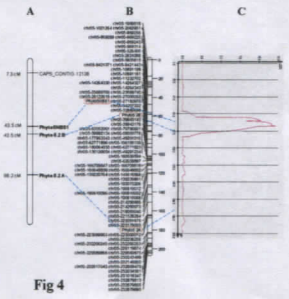

Fig 4

Figure 4. QTLs mapping with GBS-SNPs using RIL lines. **A.** is the chromosome 5 with location of SNPs markers used for genotyping and selection of different groups. **B.** Genetic linkage map of GBS-SNPs markers on P5 used for the QTL analysis. **C.** QTL results of RIL line using GBS-SNPs and screened against highly aggressive Pc isolate KPC-7.

Literature Cited

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Acknowledgement

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