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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공동주관 : 차세대BG21사업단 (농생물게놈활용연구사업단, GM작물개발사업단, 식물분자육종사업단),
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후　　원 : 농촌진흥청, 국립식량과학원, 국립산림과학원, 한국농식품생명과학협회,
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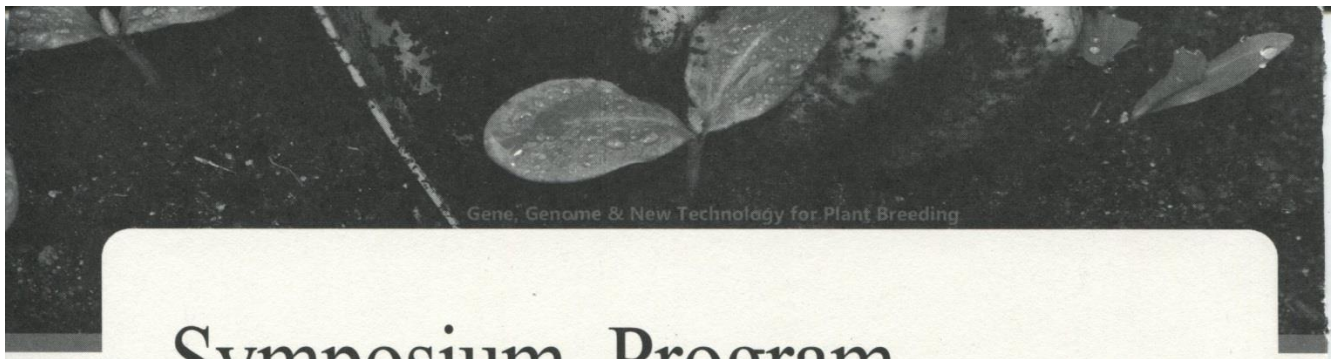
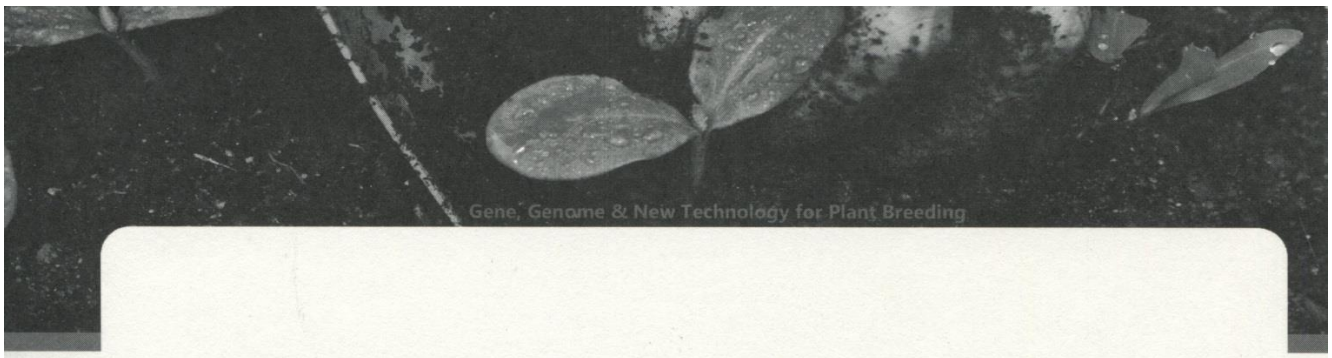


Symposium Program

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

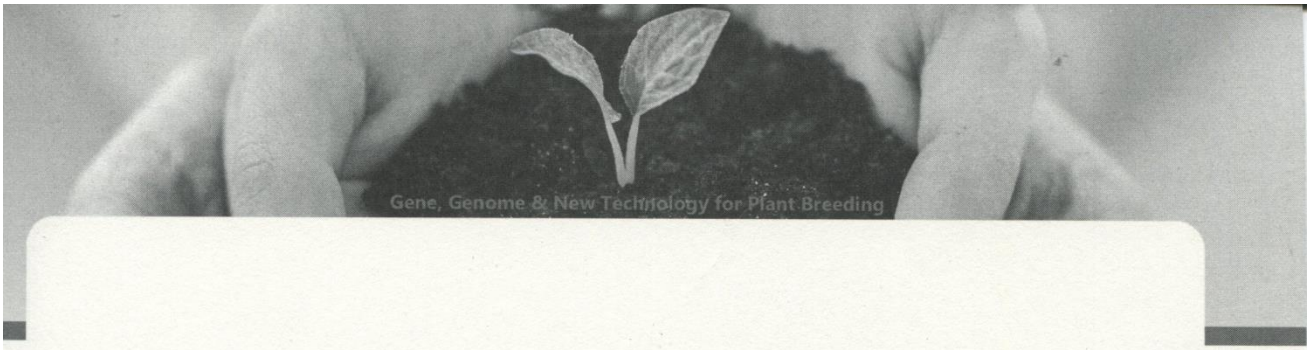
1st day [June 29. Wednesday]	
19:00~	General Meeting of Organizing Committee

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



3rd day [July 1. Friday]

〈〈 Concurrent Session 〉〉	
	– Golden Seed Project for Vegetable & Horticulture –
	▸Chair : Prof. Yong-Pyo Lim (GSP Vegetable Seed Center)
09:00~10:40	▸Seed Industry Development by Strengthening Plant Breeder's Right and Seed Market Control – Dr. Yi Seung In (Korea Seed & Variety Service)
	▸Current condition and strategy for expansion of seed testing service in KSVS – Dr. Eun Hee Soh (Korea Seed & Variety Service)
	▸ Chair : Prof. Ill-Sup Nou (GSP Horticulture Seed Center)
	▸Breeding for Pyramiding Target-genes and Selection of F1 Hybrids by Marker Assisted Selection in Tomato – Dr. Myung Kwon Kim (Tomato Life Science & Research)
	▸Gene Identification, Expression Analysis and Breeding for Enhanced Glucosinolate Biosynthesis in Brassica – Dr. Arif Hasan Khan Robin (Sunchon National University)
10:40~11:00	Coffee Break
〈〈 4부 Plenary Session 〉〉	
	– Golden Seed Project for Cereal –
	▸Chair : Dr. Young-Chan Cho (National Institute of Crop Science)
09:00~10:40	▸Specialty Corn Breeding at Sweet Seeds in Thailand to The Tropical World – Dr. Taweesak Pulam (Sweet Seeds Co., Ltd., Thailand)
	▸The Status of Rice Production and Breeding in China – Dr. Han Longzhi (Institute of Crop Sciences of Chinese Academy of Agricultural Sciences)
	▸Application of Biotechnology in Developing New Rice Varieties For High Temperature Tolerance in the Philippines – Dr. Norvie Manigbas (PhilRice, Philippines)
	▸Introduction of rice in rice production and the change of major diseases in rice during the period of climate change in Vietnam – Dr. Lai Tien Dung (Plant Protection Institute Research, Vietnam)
10:40~11:00	Coffee Break
〈〈 Plenary Session 〉〉	
	▸ Chair: Prof. Yong-Won Seo (Korea University)
11:00~11:40	▸RNA-guided genome editing in stem cells, animals, and plant – Dr. Jin Soo Kim, Seoul National University, Korea
11:40~12:20	▸CRISPR Genome Editing in Outcrossing Woody Perennials – Dr. CJ Tsai, UGA, USA
12:20~13:00	Awards Ceremony & Closing Remark



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Fine Mapping of the Root-Knot Nematode (*Meloidogyne incognita*) Resistance Gene *Me7* in Pepper (*Capsicum annuum*)

Amornrat Changkwian[1], Ji-Woong Han, Joung-Ho Lee, Gyung-Ja Choi[2], Byoung-Cheorl Kang[1*]

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Root-knot nematode (*Meloidogyne incognita*) is an endoparasitic roundworm that causes major damage to chili pepper worldwide. The pepper accession *Capsicum annuum* CM334 is known to carry a single dominant gene (*Me7*) against this parasite. The resistant gene is presumed to be located on the distal end of chromosome 9. In this study, several PCR based *Me*-specific markers as well as newly developed markers have been used to narrow down the target region of the *Me7*. An F_2 population derived from a cross between ECW30R and CM334 was used for construction of a linkage map. One of the *Me7* flanking markers was found to be located on a scaffold belong to chromosome 9 (1.7 Mb) and another one was located on chromosome 0 (0.3 Mb), which is yet to be assigned to a chromosome. The root-knot nematode resistant gene (*Mi*) in tomato belongs to a nucleotide binding and leucine-rich repeat (NB-LRR) gene family. Therefore, additional SNP markers will be developed from mRNA sequences coding for NB-LRR and nematode resistance-like proteins to facilitate mapping of the *Me7*.

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[1]Department of Plant Science, Plant Genomics and Breeding Institute, and Vegetable Breeding Research Center, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea, [2] Research Center for Biobased Chemistry, Korea Research Institute of Chemical Technology, Daejoon 305-600, Republic of Korea

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Abstract

Root-knot nematode (*Meloidogyne incognita*) is an endoparasitic roundworm that causes major damage to chili pepper worldwide. The pepper accession *Capsicum annuum* CM334 is known to carry a single dominant gene (*Me7*) conferring resistance against *M. incognita*. The resistant gene is presumed to be located on the distal end of chromosome 9. In this study, several PCR based *Me*-specific markers as well as newly developed markers have been used to narrow down the target region of the *Me7*. An F_2 population derived from a cross between ECW30R and CM334 was used for construction of a linkage map. One of the *Me7* flanking markers was found to be located on a scaffold belong to chromosome 9 (1.7 Mb) and another one was located on chromosome 0 (0.3 Mb), which is yet to be assigned to a chromosome. The root-knot nematode resistant gene (*Mi*) in tomato belongs to a nucleotide binding and leucine-rich repeat (NB-LRR) gene family. Therefore, additional SNP markers will be developed from mRNA sequences coding for NB-LRR and nematode resistance-like proteins to facilitate mapping of the *Me7*.

Introduction

Root-knot nematode (RKN) is a major pest of Solanaceae family. Among them *M. incognita* is one of the most virulent species. A second stage juvenile (J_2) can penetrate to roots immediately after hatching. The successful pathogen is able to induce giant cells into host closer to vascular cylinder and established feeding site (**Figure 1, 2**). The giant cell inhibits transfer of water and nutrient, resulting in yield reduction. Life cycle of *M. incognita* usually completes in 20-45 days after penetration. Failure in feeding site establishment restrain the reproduction of the nematode. The *Me* gene mediates hypersensitive response which induces cell necrosis and inhibits feeding site establishment in resistance pepper plants. This study aims at mapping of the *Me7* gene using a second filial generation (F_2) of pepper derived from the cross between Early Calwonder 30R (ECW30R) and Criollo de Morelos 334 (CM334) the susceptible and resistance lines, respectively.

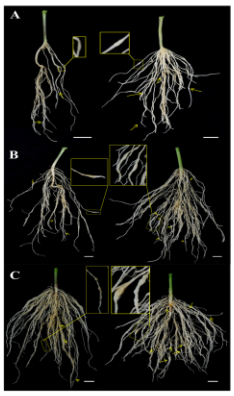

Figure 1. The physical appearance of ECW30R (A, C, E) and CM334 (B, D, F) roots and galls formation at 5, 10, and 15 dpi. Arrow pointing the clear well-developed gall on the roots, Bar= 1 cm.

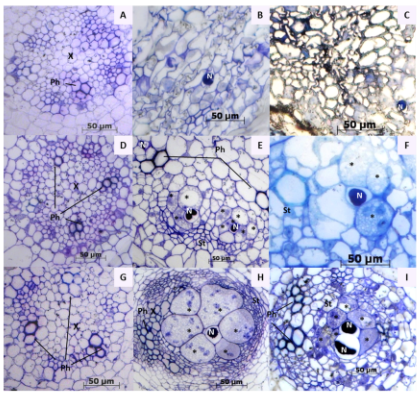

Figure 2. The cross sections of roots of a susceptible female parent ECW30R (B, E, H) and a resistant male parent CM334 (C, F, I) at 5, 10, and 15 dpi; nematode (N), enlarged multinucleate giant cells (*), phloem (Ph) , xylem (X), stele (St).

Methodology

Plant Material and Inoculation: A total of 199 F_2 plants derived from a cross between C. annuum 'Early Calwonder 30R (ECW30R)' and 'Criollo de Morelos 334 (CM334)' were used for phenotype screening and fine mapping. *M. incognita* (race 4) egg masses were extracted using 1% NaOCl solution, and J_2 nematode was collected by using Baerman funnel technique. The four week-old plants were inoculated with approx 1,000 J_2.

Phenotype Screening: The gall formation enumeration was done 60 days of post inoculation (**Figure 1**). An uprooted plant was washed and observed for gall formation. Resistance were evaluated using gall index system, which is categorized on percentages; equal or less than 10% was considered as a resistant, 11-25% was moderate resistant, and over 25% was scored as a susceptible.

Genotype Screening: The genotype screening was done initially using SCAR and CAPS markers (Djian-Caporalino et al., 2007; Fazari et al., 2012). To develop closer marker, Basic Local Alignment Search Tool (BLAST) option of the pepper CM334 reference genome, Pepper.v.1.55 (http://peppergenome.snu.ac.kr/) were used. Further SNP markers were developed based on PCR and high resolution melting (HRM) techniques.

Mapping Analysis: The Me7 linked markers were analyzed by Carthagene ActiveTcl 8.4 (software) and mapping distance was calculated by Kosambi's mapping functions at LOD threshold 3.0 and distance threshold 0.5. The genetic linkage map was drawn by MapChart 2.2 (software).

Results & Discussion

Phenotype Screening

Table 1. Inheritance of Me7 resistance in 'CM334 X ECW30R' F_2 population

Parent lines and population	Number of plants			Expected ratio (R:S)	χ^2	*P.value*
	Total	Resistant	Susceptible			
CM334	3	3	-			
ECW30R	19	-	19			
CM334 X ECW30R (F_1)	3	3	-			
CM334 X ECW30R (F_2)	199	163	36	3:1	5.067	0.0244

Results & Discussion

Mapping Analysis

- The genotyping was performed using 19 co-dominant PCR based markers (SCAR, CAPS, and HRM).
- The introgressed region containing the *Me7* gene was delimited by 6 closest flanking markers (**Table 2**)
- The most closest flanking markers show 3 and 2 recombinants (**Table 3**)

Table 2. Genotyping analysis of Introgressed fragments in F_2 population

	CM334 X ECW30R F2 population																												
	50	70	152	176	8	20	146	158	27	102	13	127	151	65	66	90	133	140	149	171	159	190	192	164	115	138	123	161	25
Phenotype	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
HRM_SF551458	R	H	H	H	R	R	R	R	R	H	H	H	H	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	H
HRM_SF16406	H	R	H	H	R	R	R	R	R	H	H	H	H	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	H
SCAR_PM6a	R	R	H	R	H	R	R	R	R	H	H	H	H	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S
HRM_SF55-511	H	H	R	R	H	R	R	R	R	R	R	R	R	R	R	R	R	R	R	H	R	R	S	S	S	H	H	S	
HRM_NBLRR12	H	H	H	R	H	H	R	R	R	nd	S	S	S	R	R	R	R	R	R	H	S	S	S	S	S	H	H	S	
HRM_SF551177	H	H	H	R	nd	H	H	H	S	S	S	S	S	R	R	nd	R	R	R	R	H	S	S	H	H	H	H	H	S

Marker name

nd: not identify

Table 3. The number of recombinants in accordance with 6 SNP markers

Population	Marker Name					
	HRM_SF551458	HRM_SF16406	SCAR_PM6a	HRM_SF551511	HRM_NBLRR12	HRM_SF551177
CM334 X ECW30R (F2)	12	4	3	2	7	10

- The flanking markers, SCAR_PM6a (Fazari et al., 2012) and a newly developed HRM_SF551511 were mapped at 2.8 and 3.1 cM away from the *Me7* (**Figure 3**), respectively.
- Three scaffolds (scaffold A,B and C) were observed with high sequence matching rate with markers sequences.
- At the target region between the flanking markers, 26 NB-LRR candidates were detected and 15 candidates with 100% homology to the target scaffolds.
- Three markers in NB-LRR regions were developed (HRM_NBLRR6b, NBLRR7b1 and NBLRR12).
- Furthermore, new markers in target regions will be developed using scaffold A and NB-LRR candidates. The markers will be validated in RIL population and commercial cultivars.

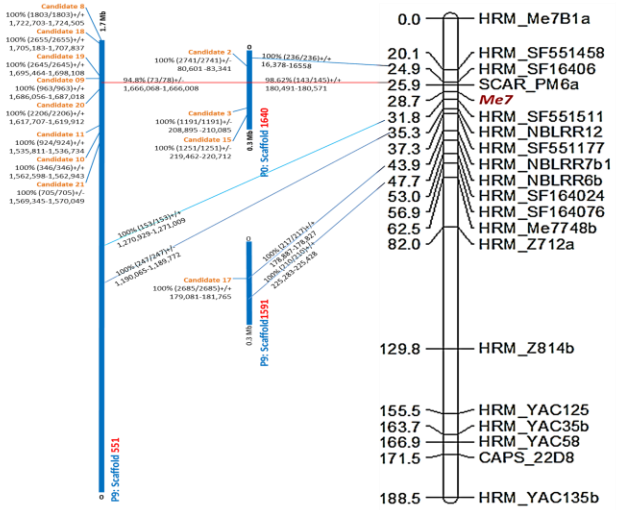

Figure 3. The mapping of the *Me7* gene linkage markers to CM334 scaffold assembly v1.5

Acknowledgement

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References

Djian-Caporalino C, Fazari A, Arguel MJ, Vernie T, Vandecasteele C, Faure I, Brunoud G, Pijarowski L, Palloix A, Abad P (2007) Root-Knot Nematode (*Meloidogyne* Spp.) *Me* Resistance Genes in Pepper (*Capsicum annuum* L.) are Clustered on the P9 Chromosome. *Theoretical and Applied Genetics* 114:473–86.

Fazari A, Palloix A, Wang LH, Hua M, Sage-Palloix AM, Zhang BX, Djian-Caporalino C. (2012). The Root-Knot Nematode Resistance *N* -Gene Co-Localizes in the *Me* -Genes Cluster on the Pepper (*Capsicum annuum* L.) P9 Chromosome. *Plant Breeding* 131:665–73.