XVI. EUCARPIA

Capsicum and Eggplant Meeting

KECSKEMÉT • HUNGARY • 12-14.SEPT.2016

in memoriam
Dr. Alain Palloix

PROCEEDINGS

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PROGRAM

Sunday, 11 September

16:00	**Registration and Poster set up**
19:00-21:30	**Welcome reception**

Monday, 12 September

7:30-	**Registration**
8:30-9:30	**Opening Session** **Chair:** Sergio Lanteri **Lajos Helyes, Katalin Ertsey-Peregi** Welcome on behalf of the Hungarian Local Organizers **Jaime Prohens** Welcome by the EUCARPIA Representative **Véronique Lefebvre** Alain Palloix commemorative lecture **Miklós Fári** **Szent-Györgyi Memorial Lecture** Father of vitamin C, Albert Szent-Györgyi (1893-1986) and his time. Science, creativity and society behind a Nobel Prize winner (1937)
9:30-10:45	**Session 1 – Breeding Strategies** **Chairs:** Paul Bosland, Giuseppe L. Rotino **9:30-9:45** **Jaime Prohens** Utilization of crop wild relatives in eggplant pre-breeding for adaptation to climate change **9:45-10:00** **Gábor Palotás** New hot interspecific hybrid variety between *Capsicum annuum* L. and *Capsicum chinense* Jacq.

10:00-10:15
Sanjeet Kumar
Male sterility research in peppers at AVRDC – The World Vegetable Center

10:15- 10:30
Dario Danojevic
Sweet pepper breeding against bacterial spot *(Xanthomonas euvesicatoria)* in Serbia

10:30-10:45
Miklós Fári
What kind of root should a pepper plant have?

10:45-11:10 **Coffee break and Poster viewing** *sponsored by:* Dél Kertész SZENTES

11:10-11:55 **Session 1 – Breeding Strategies (ctd.)**
Chairs: Paul Bosland, Giuseppe L. Rotino

11:10-11:25
Roeland E. Voorrips
Aphid resistance in a *Capsicum* collection

11:25-11:40
Pál Salamon
Symptoms caused by *Tomato spotted wilt virus* (TSWV) in Pepper (*Capsicum* spp.) and marker assisted selection of TSWV resistant pepper lines for hybrid constructions

11:40-11:55
István Tóbiás
Evergreen question: Whether *Tobamoviruses* are transmitted via pepper seeds or not?

12:00-12:45 **Session 2 – Growing and Seed Production**
Chair: Zsuzsanna Füstös

12:00-12:15
Katalin Ertsey-Peregi
Sweet pepper (*Capsicum annuum* L.) growing on a basis of thermal water with respect of protection the natural environment

12:15- 12:30
John Damicone
Biology and management of bacterial spot of pepper in Oklahoma, United States

12:30- 12:45
András Kovács
Short evaluation of eggplant production and variety usage in Romania

12:45-14:30 **Lunch**

14:30-14:50 **Gathering in the lobby for the technical visit**

15:00-19:30 **Technical visits**
- Pepper and eggplant trials (ZKI)
- Pepper processing plant (UNIVER)

Open field trip

19:30-22:30 **Traditional folklore dinner**

Tuesday, 13 September

8:30-10:00 **Session 3 – Genetic Resources**
Chairs: Jaime Prohens, Marie-Christine Daunay

8:30-8:45
Marie-Christine Daunay
Eggplant resistance to bacterial wilt and to *Fusarium* wilt: Is there a link?

8:45-9:00
Zsuzsanna Füstös
Study of morphological characteristics of eggplant *(Solanum melongena* L.*)* varieties

9:00-9:15
Claudio Dal Zovo
Wild *Capsicum* in the area of the Amboró National Park in Bolivia

9:15-9:30
John Samuels
Solanum insanum L. (Solanaceae): Linnaean species or introgressed hybrid?

9:30-9:45
Olga Babak
Development of DNA-markers to fruit quality genes of sweet pepper *(Capsicum annuum* L.*)*

9:45-10:00
Rosana Rodrigues
A breeding program for resistance to anthracnose in sweet and chili pepper

10:00-10:15
François Villeneuve
Screening of solanaceous wild relatives for graft affinity with eggplant *(Solanum melongena* L.*)*

P4-08
5-5' dicapsiate: Product of the oxidation of capsiate by cationic peroxidases from pepper (*Capsicum annuum* L.) **500**
Alberto Lema, Teresa Martínez-Cortés, Ana Garcés, Cristina Mallor, Oreto Fayos, Gerardo F. Barbero, Cristina Silvar, Federico Pomar

P4-09
Phenylpropanoid biosynthesis and regulation in *Solanum melongena* cv. "Lunga Napoletana" **506**
T. Docimo, G. Francese, A. Ruggiero, G. Batelli, M. De Palma, L. Bassolino, L. Toppino, G.L. Rotino, G. Mennella, M. Tucci

P4-10
Exogenous 6-benzylaminopurine application protects eggplant seedling against low temperature-induced oxidative stress **514**
Xuexia Wu, Lian Yong

SESSION 5 ..... **515**
Molecular genetics and biotechnology

P5-01
Identification of *Capsicum* pentatricopeptide repeats via in-silico analysis provides insights into fertility restoration **517**
Derek W. Barchenger, Joseph I. Said, Franchesca A. Ortega, Paul W. Bosland

P5-02
Fine mapping of the *Me7* gene controlling resistance to Root-Knot Nematode (*Meloidogyne incognita*) in chili pepper **522**
Amornrat Changkwian, Ji-Woong Han, Joung-Ho Lee, Gyung-Ja Choi and Byoung-Cheorl Kang

P5-03
Plant genetic background increasing the efficiency and durability of major resistance genes to root knot nematodes can be resolved into a few resistance QTLs **523**
A. Barbary, C. Djian-Caporalino, P. Castagnone-Sereno, N. Marteu, A. Fazari, B. Caromel, A. Palloix

P5-04
Highly efficient genome doubling method for haploid paprika (*Capsicum annuum* L.) plants **528**
Anikó Gémesné Juhász, Zoltán Kristóf

P5-05
Isolation and characterization of pepper genes involved in CMV-P1 infection **532**
Yeaseong Ha, Joung-Ho Lee, Yoomi Choi, Min-Young Kang, JeeNa Hwang, Won-Hee Kang, Jin-Kyung Kwon and Byoung-Cheorl Kang

P5-06
Genetic mapping of the *Powdery Mildew Resistance* (*PMR1*) gene in pepper (*Capsicum annuum* L.) **533**
Jinkwan Jo, Wonhee Kang, Gyung JaChoi, Jin-Kyung Kwon and Byoung-Cheorl Kang

10:15-10:40 **Coffee break and Poster viewing** *sponsored by:* ÁRPÁD - AGRÁR ZRT. SZENTES

10:40-11:40 **Session 3 – Genetic Resources (ctd.)**
Chairs: Jaime Prohens, Marie-Christine Daunay

10:40-10:55
Awang Maharijaya
Antixenosis and antibiosis based resistance of chilli pepper to melon aphid

10:55-11:10
Orarat Mongkolporn
Genetic diversity of Thai native chili using diversity arrays technology

11:10-11:25
Lucie Tamisier
Quantitative trait loci in pepper genome control the effective population size of two RNA viruses at inoculation

11:25-11:40
Helena Stavělíková
Germplasm of pepper *(Capsicum annuum* L.*)* in Czech Republic

11:40-12:55 **Session 4 – Physiology and Nutritional Value**
Chair: Lajos Helyes

11:40-11:55
Zsuzsanna Füstös
The nutrition value and storage of eggplant *(Solanum melongena* L.*)* varieties

11:55-12:10
I. Kutalmis Kutsal
Effects of mychorriza on pepper plant growth parameters and nutrient uptake under salinity stress

12:10-12:25
Kietsuda Luengwilai
Does anthracnose resistance associate with cuticle characteristics and spore attachment?

12:25-12:40
Ozlem Altuntas
Effects of michorriza on alleviating salt stress of *Capsicum annuum* L. by ion regulation

12:40-12:55
Lajos Helyes
Correlation between carotenoid components of chili pepper fruits and VIS/NIR reflectance

13:00-14:45 **Lunch and Poster viewing**

14:45-16:15 **Session 5 – Molecular Genetics and Biotechnologies**
Chairs: Sergio Lanteri, Anikó Gémes Juhász

14:45-15:00
Ezio Portis
A high quality eggplant *(Solanum melongena* L.*)* genome sequence

15:00-15:15
Zoltán Kristóf
Ultrastructural study of *in vitro* and *in situ* pepper embryo development

15:15-15:30
Giuseppe L. Rotino
MicroRNA156/7-Mediated control of anthocyanin pigment accumulation in eggplant fruit skin

15:30-15:45
Rodrigo A. VALVERDE
Interactions between *Bell pepper endornavirus*, bell pepper, and acute plant viruses

15:45-16:00
Santiago Vilanova
The transcriptomes of *Solanum incanum* and *S. aethiopicum* provide information of relevance for common eggplant breeding

16:00-16:15
Sylvia E. Salgon
Genetic mapping of broad-spectrum QTLs and strain-specific major QTL for resistance to *Ralstonia solanacearum* in eggplant using GBS

16:15-16:30
Yoshiyuki Tanaka
Multiple mutated putative aminotransferase alleles contribute to low pungency and capsinoid biosynthesis in *Capsicum chinense*

16:30-16.50 **Coffee break** *sponsored by:* ÁRPÁD - AGRÁR ZRT. SZENTES

16:50-19:30 **Optional cultural program**

20:00-23:00 **Gala dinner**

Wednesday, 14 September

8:30-10:30	**Poster session** **Chair:** Katalin Ertsey-Peregi
10:30-10:45	**Coffee break** *sponsored by:*
10:45-11:45	**Session 5 – Molecular Genetics and Biotechnologies (ctd.)** **Chairs:** Sergio Lanteri, Anikó Gémes Juhász

10:45-11:00
Laura Toppino
QTLs mapping for *Fusarium oxysporum* and *Verticillium dahliae* resistance in eggplant *(Solanum melongena* L.*)*

11:00-11:15
Pasquale Tripodi
Genotyping by sequencing for population structure and genome-wide association analysis for fruit shape and size in pepper (*C. annuum* L.) germplasm

11:15-11:30
Jana Leide
Cutin deficiency of bell pepper *(Capsicum annuum* L.*)* results in gluey berries

11:30-11:45
Hatira Taskin
Comparison of pepper genotypes originated from Turkey and the other countries for anther culture response

11:45-12:15	**Conclusion and closing**
12:15-14:00	**Lunch**
14:30-	**Optional post-conference tours**

LIST OF POSTERS

Session 1 – Breeding Strategies

Poster number	Presenting author	Title of poster
P1-01	**András Andrásfalvy**	István Túri – The innovative pepper breeder
P1-02	**Andrea Moór**	Lambert Angeli, pioneering breeder of the first white, sweet variety of bell pepper was born a hundred years ago
P1-03	**Zoltán Timár**	The life and work of a paprika breeder Ferenc Márkus
~~P1-04~~ WITHDRAWN	~~**Saadet Büyükalaca**~~	~~Investigation of obtaining fertile *S. melongena x S. torvum* hybrid populations~~
P1-05	**Ros Caridad**	Could quantitative resistance increase the durability of major genes conferring nematode resistance in pepper?
P1-06	**Dilek Kandemir**	Determination of reaction of *Solanum aethiopium* and *Solanum incanum* genotypes against *Fusarium oxysporum f. sp. melongenae*
P1-07	**Gábor Palotás**	20 years of non-hypersensitive, non-specific, recessive resistance in pepper – review
P1-08	**Mariola Plazas**	Screening for drought tolerance in eggplant relatives and interspecific hybrids
P1-09	**Claudia Ribeiro**	Breeding Calabrian pepper lines (*Capsicum annuum* L.) for Brazilian agriculture from *sui generis* introduction of germplasm
P1-10	**Claudia Ribeiro**	Synthesis of a base population of Habanero chile pepper and initial assessment of derived F_3 lines *(Capsicum chinense)*
P1-11	**Attila Rózsás**	Conservation, landscape and home garden varieties in South part of Hungary
P1-12	**Zsolt Sági**	Higher quality traits – breeding strategies in pepper
P1-13	**Csaba Sebesi**	High quality apple peppers for the canning industry
P1-14	**Olga Timina**	Breeding use of *Capsicum annuum* L. mutant genepool
P1-15	**Péter Varró**	Breeding of a high yielding white waxy hibrid ZKI 113485 for the Mediterranean region
P1-16	**Lajos Zatykó**	The role of general and specific combining abilities in pepper hybrid breeding

P5-02

Fine mapping of the *Me7* gene controlling resistance to Root-Knot Nematode (*Meloidogyne incognita*) in chili pepper

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

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Meloidogyne incognita is a root-knot nematode causing severe yield reduction to pepper (*Capsicum annuum)* cultivation worldwide. The *Me7* gene has been reported as a resistance gene localized on long arm of the chromosome P9. The objective of this study was to fine map the *Me7* gene using 503 F2 individuals derived from a cross between ECW30R and CM334. Phenotype screening was performed by inoculating 1,000 second-stage juveniles per individual. The disease was scored 45 days of post inoculation using gall index system. The screening results depicted 391 resistant and 112 susceptible plants, which fit into 3:1 ratio confirming the resistance is controlled by a single dominant gene. The fine mapping was initiated using previously published PCR-based markers that are located at 4.3 and 2.7 cM from the *Me7* gene. To further narrow down the candidate gene interval, additional SNP markers were developed using CM334 reference genome information, yet no closer marker has identified. Therefore, Ren-Seq analysis was performed to locate the NB-LRR candidates that clustered between and nearby the flanking markers. At present, 22 NB-LRR candidates were identified in the flanking region. In addition, an F5 recombinant inbred lines carrying resistant gene are being screened to obtain a solid phenotype inheritance pattern. The candidate gene discovery will facilitate the breeding programs for the crop improvement against root-knot nematode in pepper.

Keywords: root-knot nematode, resistance gene, fine map, SNP marker, NB-LRR candidate

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Fine Mapping of the *Me7* Gene Controlling Resistance to Root-Knot Nematode (*Meloidogyne incognita*) in Chili Pepper

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ABSTRACT

Meloidogyne incognita is a root-knot nematode causing severe yield reduction in pepper (*Capsicum annuum)* worldwide. The *Me7* gene has been reported as a resistance gene localized on long arm of the chromosome P9. The objective of this study was to fine map the *Me7* gene using 503 F_2 population derived from a cross between ECW30R and CM334. Phenotype screening was performed in pepper plants by inoculating 1,000 second-stage juveniles per individual. Resistance to nematode was scored 45 days of post inoculation using gall index system. The screening results showed 391 resistant and 112 susceptible plants, which fit to 3:1 ratio confirming the resistance is controlled by a single dominant gene. The fine mapping was initiated using previously published PCR-based markers that are located at 4.3 and 2.7 cM from the *Me7* gene. To further narrow down the target region, additional SNP markers were developed using CM334 reference genome information, yet no closer markers have been identified. Therefore, Ren-Seq analysis was performed to locate the NB-LRR candidates that clustered between and nearby the flanking markers. At present, 22 NB-LRR candidates were identified in the target region. In addition, an F_3 recombinant inbred lines carrying resistant gene are being screened to obtain a solid phenotype inheritance pattern. The candidate gene discovery will facilitate the breeding programs for the crop improvement against root-knot nematode in pepper.

Keywords: root-knot nematode, resistance gene, fine map, SNP marker, NB-LRR candidate

INTRODUCTION

Root-knot nematode (RKN) is a major pest of Solanaceae family. Among them *M. incognita* is one of the most virulent species. The successfully penetrated pathogen is able to induce giant cells in host tissues closer to vascular cylinder and establishes feeding site. The giant cell inhibits transfer of water and nutrient, resulting in poor growth, and yield reduction. The *Me* gene mediates hypersensitive response causing cell necrosis and inhibition of feeding site establishment in resistance pepper plants. The resistance gene was previously reported to located on chromosome P9 (Djian-Caporalino et al., 2007: Fazari et al., 2012). This study aims at mapping of the *Me7* gene using a F_2 pepper population derived from the cross between Early Calwonder 30R (ECW30R) and Criollo de Morelos 334 (CM334) the susceptible and resistance lines, respectively (**Figure 1**).

Figure 1. Comparison between A) resistant (CM334) and B) susceptible (ECW30R) phenotypes at 45 days after inoculation of *M. incognita,* Bar = 1 CM.

METHODOLOGY

Plant Material and Inoculation: A total of 503 F_2 plants were used for phenotype screening and fine mapping. And 76 recombinant F_5 inbred lines (RIL) were used in introgression analysis. *M. incognita* (race 4) egg masses were extracted using Baermann funnel technique. The four week-old plants were inoculated with approx. 1,000 J_2. The nematode was provided by the Clinical Plant Pathology and Nematology Lab, Seoul National University.

Phenotype Screening: The gall formation enumeration was done 60 days of post inoculation. Resistance were evaluated using gall index system; ≤10% was considered as a resistant, 11-25% was moderate resistant, and >25% was scored as a susceptible.

Histological Study: The inoculated plants were kept in the growth chamber at 24 ℃. After 5, 10 and 15 days after inoculation (dai) The infected roots tissue were section and observed under magnified microscope (Seo et al, 2013).

Genotype Screening: The genotype screening was done initially using 192 F_2 population with references 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 (V1.55) and CM334 reference scaffold (V1.5), (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).

BAC Library Screening: Using *C. annuum* 'CM334' BAC library of 12X genome coverage with 235,000 clones (99%) and an average insert size of 130 kb (Yoo et al. 2003).

RESULTS

1. Histological Study

External root characteristic study of the parental lines ECW30R (**Figure 2A, C,** and **E**) and CM334 (**Figure 2B, D,** and **F**) were conducted at 5, 10, and 15 dai, respectively. At 5 dai, gall formation was hardly identified in both lines (**A**; ECW30R, **B**; CM334) due rather small. At 10 dai, the galls from susceptible root (**C**) were clearly distinguished with bigger size than the resistant (**D**). At 15 dai larger galls were observed in ECW30R (**E**), whereas in CM334 small sized galls were observed (**F**).

The tissue sectioning of infected roots illustrated normal vascular cylinder in non-infected roots (**Figure 3**; **A, D,** and **H**). At 5 dai, nematode were detected in both parents (ECW30R; **B**, CM334; **C**) without either giant cell formation or cell necrosis. At 10 dai, giant cell development were showed in both parents. Nonetheless, only in ECW30R (**E**) the cells close to the feeding sites were observed to be narrow and dense compared with the CM334 (**F**). At 15 dai, the infected cells in ECW30R (**H**) grow much larger than CM334 (**I**) and causes compact cell layers in vascular tissue.

Figure 2 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 galls development on the infected roots, Bar= 1 cm.

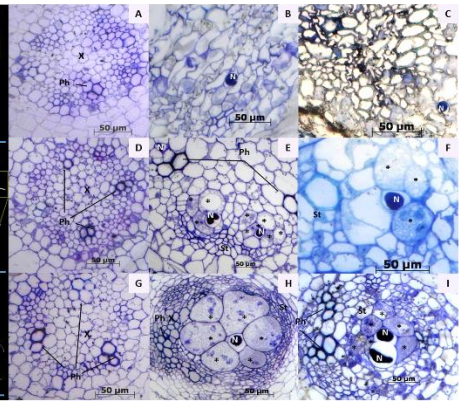

Figure 3. The cross sections of roots of a susceptible female parent ECW30R (B, E, H) and a resistant male parent CM334 (C, F, I) at 5, 10, and 15 dpi; nematode (N), enlarged multinucleate giant cells (*), phloem (Ph) , xylem (X), stele (St).

2. Phenotype Screening

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

Parent lines and population	Number of plants			Expected ratio (R:S)	χ^2	*P value*
	Total	Resistant	Susceptible			
CM334	38	38	0			
ECW30R	52	0	52			
CM334 X ECW30R (F_1)	20	3	0			
CM334 X ECW30R (F_2)	503	391	112	3:1	2.005	0.1568

- No significant difference between the observed and expected ratio
- The 3:1 phenotypic ratio confirmed that resistance phenotype is controlled by a single dominant gene

3. Mapping Analysis

The screening was initially performed using PCR based markers, CAPS_F4R4 and SCAR_PM6a (Djian-Caporalino et al., 2007: Fazari et al., 2012). Based on linkage and genetic analysis these two markers were located at 4.3 and 2.7 cM from the *Me7* gene. To narrow down the target region, newly developed SNP based markers were analyzed with 192 F_2 population. The fine mapping analysis found the closest flanking markers, HRM_SF1-3 and HRM_NBLRR7, located at 0 and 1 cM away from the target locus (**Figure 4-A and B**). BAC library screening has been performed to fill the gaps between the non-overlapping pepper scaffold sequences, scaffold A and B (**Figure4-C**). Newly developed BAC-end markers from the clone BAC1-4 showed no recombinant (**Table 2**).

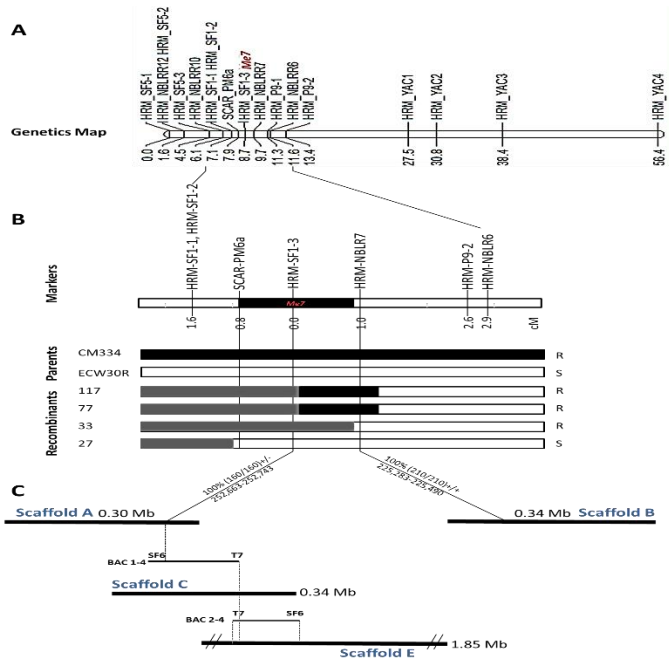

Figure 4. Fine mapping of the *Me7* locus. A) the location of the *Me7* locus on pepper chromosome 9 between HRM_SF1-3 and HRM_NBLRR7. B) the introgression fragment between three flanking markers locus from each side of the gene detected four recombinants from 192 plants. C) BAC library screening aimed at fill up the gap between the two scaffolds that localize the nearest flanking markers. The first BAC library screening were started used HRM_SF1-3 (0 cM). Four clones, BAC 1-1 to BAC 1-4, were detected in the first round screening and other four clones, BAC2-1 to BAC 2-4, were detected from the second round. The BAC end were sequencing by T7 promoter and SF6 primers and BLAST to CM334 reference scaffold (V1.5) to predict the physical position of the clones.

4. Marker Development and Recombinant Screening

To find more recombinant plants, phenotype screening were performed with larger F_2 population consisting of 503 plants. Six recombinants were detected in an interval between HRM_SF1-3 and HRM_NBLRR7 markers. HRM_SF1-3 and HRM_NBLRR7 showed three and six recombinants, respectively whereas no recombinants were found with BAC1-4 marker (**Table 2**).

Table 2. Genotyping and segregation analysis of the 503 F_2 population

Marker Name	ECW30R X CM334 F2 Population						Recombinant
	33	364	499	500	502	503	
Phenotype (*Me7*)	R	S	R	R	R	R	
HRM_SF1-3	H	S	S	S	S	R	3
HRM_BAC1-4	H	S	H	H	H	H	0
HRM_NBLRR7	S	H	S	S	S	S	6

5. Screening of F_5 RIL Population

The phenotype analysis of the root-knot nematode resistance in 76 F_5 lines found 45 resistant, 22 susceptible and 9 segregating (**Table 3**). Although this resistance trait is known to be controlled by a single gene (*Me7*), but the segregation ratio of resistant and susceptible did not fit 1:1 ration (P<0.05). The data suggest that either the F_5 lines have significant heterozygosity or could be due to the phenotyping error. The confirm the F_5 population phenotypes, F_6 population will be further phenotype screening.

Table 3. Inheritance of the *Me7* resistance in 'ECW30R X CM334' F_5 recombinant inbred line

Number of F5 RIL			Total	Expected Ratio (R:S)
Resistant	Segregating	Susceptible		
45	9	22	76	1:1

ACKNOWLEDMENT

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