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Program & Abstracts
2016 Annual Spring Conference of the Korean Society for Horticultural Science

주제 스마트 원예(Smart Horticulture)의 현황과 발전방안
일자 및 장소 2016. 5.25(수)~28(토), 청원컨벤션센터(CECO)
**Program**

- **Title:** 2016 Annual Spring Conference of the Korean Society for Horticultural Science
- **Theme:** Smart Horticulture – Current State and Perspectives
- **Date/Venue:** May 25–28, 2016 / Changwon Exhibition Convention Center, Changwon, Gyeongnam, Korea

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Molecular Mapping of the Root-Knot Nematode (Meloidogyne incognita) Resistance Gene Me7 in Pepper and Histological Characterization of the Parental Lines

Ammoret Changkewon, Jin-Kyung Kwon, Ji-Woong Han, Jeong-Ho Lee, Gyu-Ja Choi, Yong-Ho Kim, and Byoung-Cheol Kang

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The famous pepper accession (Capsicum annum) CM334 is known to be a resistant source against several pathogens including root-knot nematode (Meloidogyne incognita). This line carries the resistance gene Me7 that triggers a hypersensitive response (HR) resulting in brown or petal-egg-mass production on root surface. This study was aimed to map the Me7 gene using the F2 populations derived from a cross between ECW30R, a susceptible line and CM334. The previously developed molecular markers were analyzed to delimit the genomic region of Me7. A YAC clone corresponding to the genomic region was sequenced. To confirm the resistance phenotype, resistant and susceptible plants were compared after root inoculation with M. incognita race 4 at the second juveniles stage (J2). The test plants were inoculated with 1,000 J2 approximately, four weeks of post planting. Disease reaction on plants roots were observed at 5, 10 and 15 days of post inoculation. The roots of infected plants were characterized by examining histological variations, number of gall formation and comparing gall size on root surface. Further study will confirm and distinguish the resistance and susceptibility
Molecular Mapping of the Root-Knot Nematode (Meloidogyne incognita) Resistance Gene Me7 in Pepper and Histological Characterization of the Parental Lines

Amornrat Changkwian1, Jin-Kyung Kwon1, Ji-Woong Han, Jang-Ho Lee, Gyung-Ja Choi1, Yong-Ho Kim2, and Byoung-Cheol Kang2

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The famous pepper accession (Capsicum annuum) CV7003 is known to be a resistant source against several pathogons including root-knot nematode (Meloidogyne incognita). This line carries the resistance gene Me7 which triggers a hypersensitive response (HR) resulting in reduction or halt egg mass production on root surface. This study was aimed to map the Me7 gene using the F1 population derived from a cross between EC100W, a susceptible line and CV7003. The previously developed molecular markers were analyzed to define the genomic region of Me7. A Vac gene corresponding to the disease resistance genes was sequenced. To confirm the resistance phenotype, resistant and susceptible plants were compared after root inoculation with M. incognita race 4 at the second juvenile-stage (L3). The resistant plants were inoculated with 1,000 J3 per plant, approximately four weeks of post planting. Disease reaction on plants was observed at 5, 10 and 15 days of post inoculation. The roots of resistant plants were characterized by examining histological variations, number of gall formation and growing size on root surface. Further study will confirm and distinguish the resistance and susceptibility mechanism at cellular level.

Introduction

Root-knot nematode (RKN) is a major pest of Solanaceae family. M. incognita is one of the most virulent species. A second stage juvenile (J2) can penetrate to roots immediately after hatching. Then, successful nematode is able to induce giant cells close to vascular cylinder and this feeding site is essential for reproduction. The giant cell starts inhibiting transfer of water and nutrient, which causes low yield of crop. M. incognita usually completes life cycle in 45 days after penetration. Uncontrolled feeding site causes incomplete life cycle of the plant. The Me7 gene mediates hypersensitive reaction which causes cell necrosis and inhibits feeding site establishment in resistance pepper plants. This study aims at observation of the cellular level of parental line and its mapping of gene using a second filial generation (F2) of pepper derived from the crossed between Early Calabrese 39R (EC100W) and Criolla de Morelos 374 (CV7003) which are susceptible and resistance lines, respectively.

Methodology

Plant Material and Inoculation

A total of 199 F2 plants derived from a cross between C. annuum ‘Early Calabrese 39R’ (EC100W) and ‘Criolla de Morelos 374’ (CV7003) were used in phenotype screening and fine mapping analysis. M. incognita egg masses were extracted using 1% NaOCl solution, and nematodes were collected using Baerman funnel technique. The four-week-old plants were inoculated with 1,000 J3.

Histological Study

The inoculated plants were kept in the growth chamber at 24°C. After 5, 10 and 15 days after inoculation (dai), plants were sprayed and fixed with modified Karnovsky’s fixative and post fixation steps followed according to Moon, et al. (2010) procedure. The tissue sections, were done using ultra-microtome and stained with 1% toluidine blue O in 1% potassium and observed under a light microscope.

Phenotype Screening

The galls formation was assessed after 60 dai. An inoculated plant was washed and measured (Figure 3). Criteria of resistance was done out using gall index system, which was categorized on percentage equal to less than 10%, was presented highly resistant, 11-30% moderate resistant, and over 35% was susceptible.

Genotype Screening

The genotype screening was done initially using SCAR and CAPS markers (Quiroga-Capadona et al., 2007; Patra et al., 2012). To develop closer map, we used BLAST with the pepper CMS49 genome reference (PepX, http://www.peppergenomics.esci.ac.co). Further development of SNP markers were based on PCR and high resolution melting (HRM) techniques.

Mapping Analysis

The Me7 linked markers were analyzed by Cardiovate Affilx 8.4 and mapping distance was calculated by Kosambi’s mapping functions, LOD threshold 3.0 and distance threshold 0.3. The genetic linkage chart was organized with MapChart 2.2 software.

Results and Discussion

Histological Study

External root characteristic study (Figure 1) of the parental lines EC100W (A, C, E) and CMS49 (B, D, F) were conducted at 5, 10, and 15 days, respectively. At 5 dai, gall formation was clearly identified in both lines (A, C, E, B, D, F) due to reduced root. At 10 and 15 dai, susceptible root (C, F) were clearly distinguished with more xylem than the resistant root (A, B, D, E). At 15 dai, there were large well-developed gall in EC100W (C, F) and small galls in CMS49 (B, D). Those galls roots were selected for further study at tissue level. The tissue dissected (Illustrated normal vascular cylinder in non-infected roots) (Figure 2A, B, D, H). At 5 dai, both parents (EC100W, CMS49) were infected but significantly different in size and cell development. At 10 dai, giant cell development was showed in both parents. Nonetheless, only in EC100W (C) the cells close to the feeding site were composed of dense tissue and compared with the CMS49 (F). At 15 dai, the infected cells in EC100W (H) grow much larger than CMS49 (F) and causes compact cell layers in vascular tissue.

Phenotype Observation

From total 199 F2 population the phenotyping showed 103 resistant and 96 susceptible plants (X2 = 5.687 and P > 0.01). The 11 phenotype sets confirmed that resistance phenotype is controlled by a single dominant gene. The gall counts were categorized in 3 groups, highly resistant, moderate resistant and susceptible (Figure 3). The resistant plant roots usually showed no or very small number of gall (CMS49). Plants with moderate resistance showed around 3-5 galls (C). The number of gall formed varied from 10 to 140 in susceptible parent (B) and (F).

Genotype Screening and Map Analysis

The screening was performed using PCR based markers which included SCAR, CAPS and HRM. All of the markers were co dominant markers. The closest flanking markers, SCAR, Primers (Patra et al., 2012) and newly developed HRM (Figure 11) were 3.9 and 4.5 cM away from the Me7 gene (Figure 8, respectively). In the previous research, a RFLP marker was mapped 4.4 cM from the Me7 gene in the population derived from CMS49 (Patra et al., 2012), and the RFLP was mapped to a locus on a Vac gene (PepX, 90 C) YC2022 (Tao et al., 1999). Therefore, in order to see how this could influence the Me7 gene sequence information, the Vac gene was sequenced. Amongst YAC-oriigins used for marker development for fine mapping, the fine mapping showed that the molecular markers, CAPS22B, HRM_YC2023, YAC30, and YAC235 were found to be located about 35 cM away from the Me7 gene. Besides the Vac gene marker sequencing, SNP-LDR candidate gene markers from the phenotyping screening (HRM, NBR201, NBR8035 and NBR134) were also analyzed, nevertheless, none were found to be in the vicinity of the target gene. Further studies with more markers will be developed from the RFLP scaffold.

References


