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Development of a high-resolution melting marker for selecting *Fusarium* crown and root rot resistance in tomato

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Abstract

Fusarium crown and root rot is a severe fungal disease of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). In this study, the genomic location of the FORL-resistance locus was determined using a set of molecular markers on chromosome 9 and an F₂ population derived from FORL-resistant inbred ‘AV107-4’ (*Solanum lycopersicum*) × susceptible ‘L3708’ (*Solanum pimpinellifolium*). Bioassay performed using Korean FORL strain KACC 40031 showed single dominant inheritance of FORL resistance in the F₂ population. In all, 13 polymerase chain reaction-based markers encompassing approximately 3.6–72.0 Mb of chromosome 9 were developed based on the Tomato-EXPEN 2000 map and SolCAP Tomato single nucleotide polymorphism array analysis. These markers were genotyped on 345 F₂ plants, and the FORL-resistance locus was found to be present on a pericentromeric region of suppressed chromosomal recombination in chromosome 9. The location of the FORL-resistance locus was further confirmed by testing these markers against diverse commercial tomato and stock cultivars resistant to FORL. A restriction fragment length polymorphism marker, PNU-D4, located at approximately 6.1 Mb of chromosome 9 showed the highest match with the resistance locus and was used for conducting high-resolution melting analysis for marker-assisted selection of FORL resistance.

Keywords: disease resistance, *Fusarium* crown and root rot, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, molecular marker, *Solanum lycopersicum*

Introduction

Fusarium oxysporum (Sacc.) Snyder & Hans., a soil-borne haploid fungal plant pathogen causes vascular wilt or root rot in various agricultural crops and ornamental plants (Olivain and Alabouvette 1997, Recorbet et al. 2003). Pathogenic isolates of *F. oxysporum* showing a high level of host specificity are classified into more than 120 *formae speciales* and races depending on the host plant species and cultivars (Armstrong 1981). Among them, the *Fusarium oxysporum* f. sp. *radicis-lycopersici* W. R. Jarvis & R. A. Shoemaker (FORL) is a saprophytic fungus occurring in the rhizosphere of many plant species and is known to cause Fusarium crown and root rot (FCRR) by forming wounds and natural openings in newly forming roots in tomato. FCRR is one of the most destructive soil-borne diseases of tomatoes in greenhouses and fields (Rowe 1980, Kim et al. 2001, Can et al. 2004,).

The optimum environmental conditions for the outbreak of FCRR are a temperature of 18°C and low humidity (Kouki et al. 2012). The following symptoms of FCRR in tomato were circumstantially described by Szczechura et al. (2013): just above the soil level, necrotic injury appears on the outer surface of the shoot involving the neck of the root and the stem base along with the formation of a pink mass of the fungus on the dead tissue. Practical approaches to control FCRR in the field include eliminating FORL spores and natural suppression of the fungus by soil-steaming with chemicals such as methyl bromide/chloropicrin and captafolin (Sivan and Chet 1993). Biological control using antagonistic fungi or bacteria is an alternative to using fungicides (Ristaino and Thomas 1997, De Ceuster and Hoitink 1999). However, developing tomato cultivars that are genetically resistant to FORL is the most promising potential method for the long-term and environmentally safe control of FCRR. Identification of selective molecular markers for resistance to FORL might promote the development of tomato varieties that are resistant to this disease.

FORL resistance was observed in a wild species *Solanum peruvianum* and has been introduced into *Solanum lycopersicum* via breeding programs that were primarily aimed at the introgression of resistance genes (*Tm-2* and *Tm-2²*) for tobacco mosaic virus (TMV) (Alexander 1963; Yamakawa and Nagata 1975). In Japan, *Tm-2* and FORL resistance were introgressed from plant introduction (PI) 126944 into an *S. lycopersicum* line, 'IRB #301' (Yamakawa and Nagata 1975). Similarly, these two genes were introgressed into a cultivar 'Moperou' from another resistance source, PI126926, in France (Elkind et al. 1988; Laterrot

and Pecaute 1969). In Ohio, USA, *Tm-2*², another allele of the *Tm-2* locus, was introgressed from PI128650 (Alexander 1963). However, PI128650 was susceptible to FORL, and the Japanese line ‘IRB #301’ was used to develop the FORL-resistant inbred ‘Ohio 89-1’ (Scott and Farley 1983). Subsequent genetic inheritance studies (Berry and Oakes 1987; Vakalounakis 1988) showed that FORL resistance in these resistance sources was conferred by a single dominant gene, designated *Frl*, and was closely linked to *Tm-2* (or *Tm-2*²) in the TMV-resistant sources (Elkind et al. 1988; Laterrot and Couteaudier 1989).

Molecular mapping studies suggested that *Tm-2*² and *Frl* were located near the centromere on the long arm of chromosome (Chr.) 9 and was tightly linked to a restricted fragment length polymorphism (RFLP) marker, TG101 (Young and Tanksley 1989, Laterrot and Moretti 1991, Frary et al. 1996). Recently, molecular mapping based on a backcross (BC₁) population [(Ohio 89-1 × Fla7482B) × Fla7482B] of 960 plants determined the genetic distance between *Frl* and *Tm-2* to be approximately 5.1 ± 1.07 cM (Vakalounakis et al. 1997). In another mapping study based on a backcross population of 950 plants (Fazio et al. 1999), TG101 and a set of random-amplified polymorphic DNA (RAPD) markers and UBC primers were linked to *Frl* in the order TG101-UBC655-UBC116-UBC194-*Frl*, with a genetic distance of 5.1 cM between UBC194 and *Frl*. By using an F₂ population, Staniaszek et al. (2014) identified a tight linkage of 3 cM between *Frl* and a conserved ortholog set II (COSII) marker C2_At2g38025 that was positioned at 45 cM of Chr. 9 on Tomato-EXPEN 2000 map. Most recently, Mutlu et al. (2015) reported two COSII markers that were flanking *Frl* by using F₂ and BC₁ populations. One of the COSII markers C2_At3g63200 was linked to *Frl* by 8.5 cM, and the other marker C2_At4g28660 was linked by 0.016 cM. These two markers were positioned at 52 cM and approximately 51 cM of Chr. 9 on the Tomato-EXPEN 2000 map, respectively. These previous studies suggested that *Frl* might be located at a pericentromeric region of 45–52 cM on Chr. 9.

The present study aimed to evaluate the genetic inheritance of FORL resistance observed in an elite inbred ‘AV107-4’ (*S. lycopersicum*) line and determine its genomic location. For this, an F₂ population derived from an interspecific cross ‘AV107-4’ × ‘L3708’ (*Solanum pimpinellifolium*) was evaluated for disease resistance to an FORL strain isolated in Korea, and *Frl* was mapped using a set of markers genetically and physically anchored on Chr. 9. The association between these markers and *Frl* was also evaluated based on diverse commercial hybrid cultivars and breeding lines in order to develop a marker for marker-assisted

selection (MAS) of FORL resistance.

Materials and methods

Plant materials and DNA extraction

For the marker-trait association study, an FORL-resistant line, 'AV107-4' (*S. lycopersicum*), and an FORL-susceptible line, 'L3708' (*S. pimpinellifolium*), were used to produce F₂. 'AV107-4' is known to be originally derived from a tomato germplasm (CLN2071C) that was introduced to Korea from the Asian Vegetable Research Development Center (AVRDC). However, the detailed pedigree record for 'AV107-4' is not available. Recently, 'AV107-4' was identified to be highly resistant to FORL isolates in Korea, but whether the resistance was introgressed from *S. peruvianum* is not yet known. The F₁ generation was produced by hand pollination by using line 'AV107-4' as the maternal parent and line 'L3708' as the paternal parent. The F₂ generation was produced by controlled self-pollination of a random F₁ plant. The 345 F₂ plants so produced were used for an FCRR bioassay and for marker genotyping to localize the FORL resistance locus.

The commercial cultivars and breeding lines were evaluated by obtaining the seeds of 43 commercial F₁ hybrid cultivars from private seed companies. This array of cultivars included 29 resistant and 14 susceptible cultivars, as described by seed companies in terms of their response to FORL infection. Additional 17 cultivars were grown to evaluate FORL resistance by using the same methodology as that used for the F₂ population. These 60 cultivars were used to study the association of selected molecular markers with FORL resistance. The leaf samples of 55 breeding lines and cultivars known to be resistant to FORL were provided by a seed company (Nongwoo Bio, Icheon, Korea); they were used to validate the high-resolution melting (HRM) marker developed for MAS of FORL resistance. Genomic DNA was extracted and purified using true leaves, according to the method described by Park et al. (2013).

Bioassay of FORL resistance

A bioassay for the disease severity of FCRR was performed for 40 'AV107-4', 20 'L3708', 20 F₁ plants, 345 F₂ plants, and 17 commercial cultivars (10 plants per cultivar). Pathogen inoculation and disease evaluation were conducted in a greenhouse at Gangneung-Wonju National University (Gangneung, Korea).

For inoculation, an FORL isolate, KACC 40031, obtained from the Korean Agriculture Culture Collection (KACC) was cultured on potato dextrose agar (PDA) plates at 25°C for five days, after which the hypothallus was shifted to a potato dextrose broth for seven days at 25°C on a rotary shaker at 150 rpm. Mycelial fragments were removed by filtering through a triple layer of sterile gauze, and the conidial suspension was diluted in sterile distilled water to yield a final concentration of 1×10^7 conidia/mL. Tomato seeds were sown in horticultural soil (Green, Nongwoo Bio, Korea) in seedling plates (96-hole plug trays) and grown in a glasshouse at $25 \pm 5^\circ\text{C}$ for 3 weeks. Each seedling root was washed to remove soil, and the cut tips were inoculated with FORL- 1×10^7 conidia/mL suspension for 15 min. The inoculated plants were transferred to a 15-cm diameter pot and maintained at approximately 90% humidity and $25 \pm 5^\circ\text{C}$ for 4 weeks in a greenhouse. After 4 weeks, each root was cut with a scalpel and assessed using a FORL disease-severity scale (DSS) of 0–3, based on the degree of vascular tissue browning (Rowe 1980); 0 = no internal browning, 1 = slight internal browning, 2 = moderate to severe internal browning of the entire tap root, and 3 = severe internal browning extending from the tap root to the lower stem above the soil-line. The sensitivity of each F₂ plant to FORL was determined based on the value of DSS; a value of 0 was considered to indicate resistance and that of 1–3 was considered to indicate susceptibility.

Evaluation of RAPD markers linked to *Frl*

A set of RAPD markers previously reported (Fazio et al. 1999) to be linked to FORL resistance were evaluated on our plant materials. PCR analyses were performed using RAPD 10-mer primers UBC655, UBC116, and UBC194, according to the procedure of Operon Technology Inc., and reaction conditions were as follows: 1 cycle of 30 s at 94°C, followed by 45 cycles of 20 s at 92°C, 50 s at 36°C, 2 min at 72°C, 1 cycle of 7 min at 72°C, and 10 min at 4°C. Gel electrophoresis was performed on 1.5% agarose gel containing Tris-acetate EDTA (TAE) at 160 V for 1 h, and the bands were visualized under ultraviolet light after ethidium bromide staining.

The polymorphic DNA fragment of UBC116 was obtained from ‘Youngmuja’ (a resistant commercial cultivar) and sequenced as follows. The PCR band was excised from the agarose gel and purified using ExpinTM Gel SV (GeneAll Biotechnology, Seoul, Korea); subsequently, the DNA fragment was directly sequenced using the dye-termination method of Genotech (Daejeon, Korea). A Basic Local Alignment Search Tool (BLAST) analysis of the DNA sequence was performed using the tomato reference genome (<http://solgenomics.net/>, ITAG2.3 release) to predict the physical location of UBC116.

Development of DNA markers anchored on Chr. 9

Molecular markers spanning the genetic distance of 40–55 cM that harbor UBC116 were selected based on the Tomato EXPEN-2000 map (<http://solgenomics.net/>). This marker set included two simple sequence repeat (SSR) markers—SSR70 and SSR237—located at 42 and 50.37 cM, respectively, and three RFLP markers—T1212, cLET-2-D4, and CD3—located at 48 cM, 50 cM, and 52.1 cM, respectively. These markers were tested on ‘AV107-4’ and ‘L3708’ for the detection of polymorphism.

PCR analysis was performed for the markers in a total volume of 20 μ L consisting of 20 ng genomic DNA, 0.3 μ M of each forward and reverse primer, 1 $\times$ PCR buffer, 0.2 mM dNTPs, and 0.6 U of Taq polymerase (Solgent, Daejeon, Korea). The PCR conditions were as follows: 1 cycle of 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at the annealing temperature (AT; Table 1), 1 min at 72°C, and 7 min at 72°C. Gel electrophoresis of the PCR amplicons from the SSR markers was performed using 2% agarose gel containing TAE at 160 V for 1 h 20 min; the bands were then visualized under ultraviolet light after ethidium bromide staining. For the RFLP markers, PCR amplicons were digested with 12 restriction enzymes according to the manufacturer’s instructions (NEB; Ipswich, Suffolk, England) to identify polymorphisms before agarose gel electrophoresis; these markers were designated as PNU-T1212, PNU-D4, and PNU-CD3.

Additional molecular markers on Chr. 9 were developed by genotyping the genomic DNA of ‘AV107-4’ and ‘L3708’ by using the SolCAP Tomato SNP array on the Infinium platform (Illumina Inc., San Diego, CA, USA). The SolCAP array was genotyped by SNP Genetics Inc. (Seoul, Korea). The annotation information matrix for SolCAP SNPs was downloaded from the SGN database. In all, 8 SNPs [two SNPs

(solcap_snp_sl_100431 and solcap_snp_sl_53169) physically located between PNU-D4 and SSR70, two SNPs (solcap_snp_sl_100181 and solcap_snp_sl_100461) between SSR237 and PNU-CD3, and four SNPs (solcap_snp_sl_43011, 4 solcap_snp_sl_6840, solcap_snp_sl_58307, and solcap_snp_sl_63662) between PNU-CD3 and the telomere of Chr. 9] were converted to cleaved amplified polymorphic sequence (CAPS) markers by using the CAPS designer software (<http://solgenomics.net/>; Table 1). PCR amplification, restriction enzyme digestion, and electrophoresis were performed as described above for RFLP markers.

Development of an HRM marker linked to *Frl*

For the detection of SNP of D4 CAPS marker (PNU-D4), PCR amplicons were sequenced as described above for RAPD. An HRM marker was developed by designing a 3'-blocked and unlabeled oligonucleotide probe (Luna Probe; BioFire Defense, Salt Lake, Utah, USA) based on the targeted SNP. PCR was performed using saturating dye Eva Green® Plus to generate melting curve characteristics of the genotype under the probe. Melting curves were generated and analyzed using the LightScanner® Instrument System (Roche, Basel, Switzerland). PCR involved pre-denaturation at 95°C for 5 min, followed by cycles of denaturation at 95°C for 20 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s, and then a final extension for 40 s at 72°C. Primer and probe sets for SNP detection are described in Table 2.

Results

Development of markers for marker-phenotype association studies

As previously reported by Fazio et al. (1999), *Frl* was mapped on the long arm of Chr. 9 and was closely linked to the RAPD marker sets that included TG101 (map order: TG101-UBC655-UBC116-UBC194-*Frl*). In this map, UBC194 was the most tightly linked to *Frl* at 5.1 cM. These markers were evaluated for polymorphism between 'AV107-4' and 'L3708', but polymorphic PCR bands were reproducible only for UBC655 and UBC116. The PCR band for UBC116 was cloned, and the sequence of the DNA fragment was BLASTed to the reference genome (<http://solgenomics.net/>, ITAG2.3 release). This 482-bp

sequence (data not shown) was physically located on Chr. 9 at 57.8 Mb, which corresponded to 50–52 cM of the Tomato EXPEN-2000 map (<http://solgenomics.net/>).

Two SSR and three RFLP markers were selected as genetically and physically anchored markers, respectively, that were mapped within 40–55 cM of Chr. 9 of the Tomato EXPEN-2000 map. The selected SSR markers were then evaluated by PCR, and polymorphisms were detected between ‘AV107-4’ and ‘L3708’. For RFLP markers, PCR amplicons were digested with 12 restriction enzymes that were randomly chosen, and polymorphic fragments were detected using specific enzymes shown in Table 1. In addition to these publicly available markers, CAPS markers were designed for SNPs on Chr. 9, which were detected by genotyping ‘AV107-4’ and ‘L3708’ by using the SolCAP SNP array. A total of 219 SNPs were discovered on Chr. 9, and eight SNPs that were widely distributed in this chromosome and were harbored in restriction enzyme sites were selected for converting to CAPS. Thus, a total of 13 PCR-based markers that covered 3.6–72.0 Mb of the pseudochromosome 9 genome (SL2.50ch09) were developed and used for genotyping of the F₂ populations (Table 1).

Marker-phenotype association of the F₂ progeny

Genomic location of *Frl* was assessed by evaluating an F₂ progeny derived from ‘AV107-4’ × ‘L3708’. A total of 345 F₂ plants were assayed for resistance to the FORL isolate ‘KACC 40031’ and genotyped using 13 markers physically anchored on Chr. 9. A high level of resistance was observed for ‘AV107-4’ (mean DSS = 0.23) and F₁ plants (mean DSS = 0.18), whereas ‘L3708’ was highly susceptible (mean DSS = 2.43; Fig. 1). For the F₂ progeny, DSS ranged from 0 to 3, and 254 F₂ plants were resistant (DSS = 0) and 91 were susceptible (DSS ≥ 1). The ratio of the number of resistant to susceptible F₂ plants followed a 3:1 Mendelian ratio ($\chi^2 = 0.35$, $P < 0.05$), suggesting that resistance to FORL was controlled by a single dominant gene.

The F₂ progeny was genotyped using 13 markers, including five on the Tomato EXPEN-2000 map and eight CAPS markers derived from the SNPs between ‘AV107-4’ and ‘L3708’ (Fig. 2). The first marker, SSR70, located at 3.6 Mb (42 cM at Tomato EXPEN-2000 map) showed a relatively low marker-phenotype match of 89.5%. The next markers, PNU-T1212 and PNU-D4, which were located at 5.0 Mb and 6.0 Mb

(48.0 cM and 50.0 cM), respectively, showed the highest marker-phenotype matches of 93.0% and 92.8%, respectively. For the next marker, PNU-100431 (25.1 Mb), the match rate decreased to 92.8% and, interestingly, remained at the same level for the following four markers, PNU-53169 (28.0 Mb), SSR237 (46.4 Mb, 50.37 cM), PNU-100181 (48.7 Mb), and PNU-100461 (56.9 Mb), which covered a large physical region of Chr. 9 (SL2.50ch09: 3,626,502–56,948,073 bp; ITAG2.4 <http://solgenomics.net/>). This result and that of a previous mapping study (Young and Tanksley 1989, Sim et al. 2012a) suggest that this large chromosomal block belongs to a pericentromeric region of Chr. 9, which normally shows a discrepancy between genetic and physical distance due to severely suppressed genetic recombination. However, for the next downstream markers, PNU-CD3 (63.1 Mb), PNU-43011 (64.5 Mb), PNU-46840 (67.1 Mb), PNU-58307 (68.8 Mb), and PNU-63662 (72.0 Mb), the match rate gradually reduced from 90.7% to 60.9%, indicating that crossing-over between the marker and the resistance locus gradually increased in this genomic range.

Marker-phenotype association in commercial cultivars

Further delimitation of the *Frl* locus by using more markers was not promising in our biparental populations, since *Frl* was found to be present in a linkage block having highly limited crossing over. Instead, the association of markers with *Frl* was evaluated in a population consisting of diverse commercial hybrid tomato cultivars (Table 3). According to the descriptions by seed companies, this population included 43 FORL-resistant and 17 FORL-susceptible cultivars. A subset (17 cultivars) of these cultivars was assayed for disease resistance by using the method described above. Of the 17 cultivars inoculated, 14 were resistant (mean DSS = 0) and three were susceptible (mean DSS > 1), which was in accordance with their description by the seed companies (Table 3).

Sixty cultivars were genotyped using PNU-T1212, PNU-D4, PNU-100431, PNU-53169, and SSR237 encompassing approximately 5.0–46.4 Mb (41 Mb) of Chr. 9. Unexpectedly, PNU-T1212, which showed the highest association with the phenotype in the F₂ progeny, showed only 46.5% match to the resistant cultivars tested, whereas all susceptible cultivars showed marker-phenotype matches except for ‘Marune TY’ (Table 3). Conversely, the highest match rate was detected for marker PNU-D4, which showed a 95.0% match for resistant cultivars and an 88.0% match for susceptible cultivars. Therefore, frequencies of

crossing over (and thus, better estimation of genetic linkage) between PNU-T1212 and PNU-D4 were higher when diverse commercial cultivars were used than when the biparental populations of F₂ were used. The other three downstream markers showed decreased match rates for both resistant and susceptible cultivars (Table 3). Our results indicated that the linkage between PNU-T1212 and PNU-D4 was subjected to frequent genetic recombination, particularly during the breeding process for FORL resistance, by private seed companies.

HRM marker development for MAS

The evaluation of marker-phenotype association, especially in commercial cultivars, indicated PNU-D4 as the marker of choice for MAS of FORL resistance. In order to convert PNU-D4 to an HRM marker, PCR fragments amplified using the primer set for PNU-D4 were directly sequenced from 'AV107-4' and 'L3708'. The alignment of both sequences revealed 21 polymorphic nucleotide sequences, among which one site contained a single-nucleotide mismatch (A/G) between the two cloned sequences (Fig. S1). Sequence-based prediction indicated that the A/G mismatch was a causal variation resulting in fragment-size variation after digestion and was thus ideal for designing an HRM marker.

The SNP region was confirmed using 3'-blocked and unlabeled oligonucleotide probes for melting curve analysis. This HRM marker was evaluated using 'AV107-4', 'L3708', and F₁, and the three different melting curves were distinguishable with two homozygous types (A/A and G/G) and a heterozygous type (A/G; Fig. 3). This marker was further used for genotyping 55 breeding lines and cultivars (data not shown) provided by a private seed company (Nongwoo Bio Inc., Yeosu, Korea). The genotyping results showed that marker genotype and phenotype were perfectly matched (Fig. 3).

Discussion

In this study, we confirmed that resistance to FORL was conferred by a single dominant gene that was located on Chr. 9, as reported earlier (Vakalounakis et al. 1997, Fazio et al. 1999, Mutlu et al. 2015), indicating that the FORL resistance in 'AV107-4' might have originated from the *Frl* of an *S. peruvianum* accession. However, the results on the genomic location of *Frl* were contradictory between recently published

studies and our study: by using an F_2 population, Staniaszek et al. (2014) mapped the COSII marker C2_At2g38025 at 3 cM distal to *FrI* in line '3070'. The C2_At2g38025 was positioned at 45 cM of Chr. 9 on Tomato-EXPEN 2000, and its genomic location was 5.89 Mb (possibly short arm of Chr. 9; Sim et al. 2012a). Recently, Mutlu et al. (2015) reported that SCAR_{FrI} marker derived from a COSII marker (C2_At4g28660) cosegregated with FORL resistance in 'Fla. 7781'. The SCAR_{FrI} marker was located approximately at 51 cM of Chr. 9 on Tomato-EXPEN 2000 map with a genomic location of 61.7 Mb (long arm of Chr. 9). These two studies show significant discrepancy in physical (genomic) distance versus map distance in Chr. 9 and in locations linked to FORL resistance (Mutlu et al. 2015). However, similar to the findings of Staniaszek et al. (2014), our present results strongly suggested that FORL resistance in 'AV107-4' was closely linked to the PNU-D4 marker, which is in the vicinity of 6.0 Mb (50.0 cM of Chr. 9 on Tomato-EXPEN 2000 map) for which genetic recombination (crossing over) is severely suppressed. Furthermore, the SCAR_{FrI} marker was evaluated using our 43 FORL-resistant commercial cultivars; 55.3% mismatches were found between phenotype and marker genotype (data not shown). This indicates that FORL resistance in 'AV107-4' and 'Fla.7781' is possibly controlled by two different genes, or a same gene (*FrI*) for which genomic location was changed by chromosomal rearrangement such as inversion (Mutlu et al. 2015). Further studies are required to conduct allelism test and physical mapping of those resistance loci in order to clarify this assumption. Genome reorganization by inversion in tomato species has been reported between *S. lycopersicum* and *Lycopersicon pennellii* for a region on Chr. 7 that carries the *SUN* gene (Knaap et al. 2004). Two chromosomal inversions between *S. lycopersicum* and *Solanum chilense* were also reported in *Ty-1* gene introgression, and recombination was severely precluded in the inverted region (Verlaan et al. 2011).

The centromeric region of Chr. 9 has been mapped using numerous F_2 plants derived from two interspecific crosses, *S. lycopersicum* (E) $\times$ *S. pennellii* (P) (1,620 F_2 plants) and *S. lycopersicum* (E) $\times$ *S. pimpinellifolium* (PM) (1,640 F_2 plants) (Frary et al. 1996). This map localized the centromere at the junction of the short and long arms, which was situated in a cluster of cosegregating markers. Further, they suggested that recombination suppression was equally strong in crosses between closely related (E $\times$ PM) and remotely related (E $\times$ P) parents; therefore, centromeric suppression is not due to DNA sequence mismatches within homologous chromosomes, but attributed to some other mechanism (Frary et al. 1996). In a comparison of

genetic and physical distances in the tomato genome, physical mapping around the *Tm-2a* near the centromere of Chr. 9 revealed that a genetic distance of 1 cM was equivalent to at least 4 Mb of DNA, whereas 1 cM on the tomato molecular map corresponded to approximately 750 Kb on an average (Ganal et al. 1989). In addition, the *S. peruvianum* BC₁ map indicated that 1 cM of the centromeric region of Chr. 9 corresponded to at least 6 Mb (Pillen et al. 1996). The comparisons of genetic and physical distances by using several thousand markers also revealed that similar patterns of recombination suppression are noted in the large pericentromeric regions of each chromosome (Sim et al. 2012a). These regions represent repeat-rich (satellite DNA) and gene-poor heterochromatin, encompassing 77% of the tomato genome. Such recombination suppression has also been found in many other plant species, although it is not usually as pronounced as in tomato (Sidhu and Gill 2005, Yan et al. 2005, Ma and Bennetzen 2006, Wu and Tanksley 2010).

With the availability of complete genome sequences, genetic mapping might assume secondary importance. However, physical distance is not an accurate predictor of genetic distance in areas of suppressed recombination, and the precise localization of genes and the cost-efficient selection of traits for crop improvement are still largely dependent on genetic distance. Therefore, genetic mapping with a maximized degree of recombination remains an essential tool in plant biology and plant breeding (Sim et al. 2012b). In our study, the physical distance between markers PNU-D4 and PNU-1000461 was more than 52.8 Mbp (accounting for 72.9% of the entire length of Chr. 9, which is 72.5-Mb long), but phenotype-genotype match was constantly maintained at 92.8%. This discrepancy can lead to misjudgment of the applicability of markers for MAS, especially when markers were developed only by testing a narrow genetic background, such as a biparental population. Conversely, association mapping can potentially increase the efficiency of MAS by identifying markers tightly linked to traits of interest in germplasm panels that are directly relevant to plant breeders. In our study, recombination between the markers was markedly detected using 60 commercial cultivars derived from diverse breeding programs and parentages, and thus, further precise prediction was possible for the location of *Frl*. In this context, evaluating the marker-gene association by using diverse germplasm, including natural populations, can be more powerful than using biparental populations derived from the crossing of specific inbreds. This is especially true under circumstances where the genes are located on recombination-suppression regions, as was found for the genes used in this study.

We screened for disease-resistance genes (R genes) located within 5–25 Mb of Chr. 9; this genomic region shows high level of marker-*Frl* association based on Jbrowse at Sol Genomics Network. We found clusters of receptor-like kinase homologous gene family near 5, 6, 6.8, 8.2, and 11 Mb and a coiled-coil nucleotide-binding site–leucine-rich repeat (CC-NBS-LRR) homologous gene near 13 Mb of the reference genome (ITAG2.3, <http://solgenomics.net/>; data for gene list not shown). A vast majority of functionally defined R-genes are receptor-like proteins containing the (NBS-LRR) ligand-binding domain (Pan et al. 2000), as particularly noted in tomato for the *I-2* and *Cf* genes (*Cf-2*, *Cf-4*, *Cf-5*, and *Cf-9*) conferring resistance to race 2 of *Fusarium oxysporum* f. sp. *lycopersici* and *Cladosporium fulvum*, respectively (Ori et al. 1997, Simons et al. 1998). R-genes of this class were also found in the genomic region of FORL resistance by Mutlu et al. (2015); they were suggested as putative candidate genes for *Frl*.

In conclusion, a high significant association between the marker PNU-D4 and FORL resistance was found in ‘AV107-4’. Physical and genetic map locations of PNU-D4 and other previously reported FORL-linked markers (Staniaszek et al. 2014) indicated that FORL resistance genes other than *Frl* might exist. Further, genomic rearrangement events in Chr. 9, such as inversion, might have resulted in the discrepancy in the chromosomal location of *Frl* in different FORL resistance tomato germplasm. Allelism among genes needs to be evaluated to address this issue.

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Table 1. Molecular markers anchored on chromosome 9 of *Solanum lycopersicum* L. that were used to map the Fusarium crown root rot resistance locus (*Frl*)

Marker	Marker type ^a	Location		Primer sequence (5'-3')	Tm ^d (°C)	Enzyme	Product size ^e (AV107-4/L3708)
		Reference genome	genetic map				
		(bp) ^b	(cM) ^c				
SSR70	SSR	3626502-3629252	42	F: TTTAGGGTGTCTGTGGGTCCC R: GGAGTGCGCAGAGGATAGAG	TD	-	120/104
	RFLP	5090038-5092652	48	F: GGAGGAGTTAAGTGCATGGC R: GTGACCATCGGATCCTTTGC	TD	<i>MspI</i>	2014 (1100/1200)
PNU-D4	RFLP	6097378-6097754	50	F: CAGCTGAAAGATGTCACCCA R: TGATCATTTACAAGGCGGCA	54	<i>MboI</i>	938 (465/387)
PNU-100431	CAPS	25170985-25173931	-	F: GATCACTAGTCGTCCTGGTCAA R: TTCACGATGGTCTTTATTCGT	54	<i>HpyI88III</i>	513 (340/292)
PNU-53169	CAPS	28023613-28029028	-	F: AGAGACGGTACATGCTTTGACA R: GTTCATTTGCATGGCTTCCT	54	<i>AluI</i>	408 (167/103)
SSR237	SSR	46426906-46426925	50.4	F: GTGGTAACGGCAAAGGGACT R: CTTATGGCCTTAGCAGCCAG	TD	-	161/191
PNU-100181	CAPS	48783965-48806510	-	F: TGACCAAGTTGTGAGCAAGG	55	<i>ApoI</i>	662 (405/291)

				R: GCTTCACTTGGTGCTGGTTT			
PNU-100461	CAPS	56943553-56948073	-	F: AAGCGTCGACCTGATGTTGT	60	<i>TaqαI</i>	603 (470/603)
				R: ATGAAATGGGACCAAATGGA			
PNU-CD3	RFLP	63108341-63111046	52.1	F: CGAGTGGTGCAAGTTGAGAAG	56	<i>MspI</i>	2042 (1170/2042)
				R: CGCTCCTTCAAATCTTCCGG			
PNU-43011	CAPS	64520600-64528534	-	F: GATGGATATCCCGCTCAAGA	53	<i>AluI</i>	702 (240/145)
				R: GGCTTAGGCCCTGGTTAGTC			
PNU-46840	CAPS	67134247-67142334	-	F: GGAGTGGATAATGTTGCCTGT	55	<i>AluI</i>	893 (680/396)
				R: TTGCAAGAAGATTGCTGCTC			
PNU-58307	CAPS	68877546-68881816	-	F: TTTGCAAATGTCCAAACACAA	53	<i>RsaI</i>	764 (501/764)
				R: TACCTCAACCCGAATTGGTC			
PNU-63662	CAPS	72029006-72032743	-	F: TTCCATCTCCCAAAAAGACG	53	<i>ApoI</i>	866 (866/647)
				R: AAGGACTGCCCCTAGTGTT			

^aSSR, simple sequence repeat; RFLP, restriction fragment length polymorphism; CAPS, cleaved amplified polymorphic sequence.

^bPhysical locations of the primer sequences on the reference genome sequence ITAG2.4 (<http://solgenomics.net/>).

^cLocations on the Tomato EXPEN-2000 map (<http://solgenomics.net/>); -, unknown

^dT_m, PCR annealing temperatures; TD, touchdown PCR

^ePCR fragment sizes specific to A107-4 and L3708 are presented in parentheses preceded by a virgule.

Table 2. List of primers and probe used to sequence the restricted fragment length polymorphism (RFLP) marker PNU-D4 and develop a high-resolution melting (HRM) marker

Name	Sequence (5'-3')	Tm (°C)	Remarks
PNU-D4-F	CAGCTGAAAGATGTCACCCA	60	forward primer for sequencing
PNU-D4-R	TGATCATTTACAAGGCGGCA		reverse primer for sequencing
J3-D4-aF1	TCTTCGATATGATTTTCGGTATACTCCCATAAATCT	60	forward primer for HRM
J3-D4-aR1	AATTAAGTGGACCAATACCGGAGTCATTTGGCA		reverse primer for HRM
J3-D4-pF1	GTCCTTGACAGATCAATTGTGTCA		probe for HRM

Table 3. Genotyping of 60 commercial hybrid cultivars by using molecular markers anchored on chromosome 9 of *Solanum lycopersicum* L. that were used to study their association with the Fusarium crown root rot (FCRR) resistance locus *Frl*

Cultivar ^a	Seed Provider	Phenotype ^b (mean DSS)	Marker Genotype ^c				
			PNU-T1212	PNU-D4	PNU-100431	PNU-53169	SSR237
			5090038	6097378	25170985	28023613	46426906
Alexander Deluxe	PPS	R	S	H	H	H	H
All Round	Nongwoobio	R	R	R	R	R	R
AS6*	Daeyoun	R(0.0)	S	R	R	R	R
AS7*	Daeyoun	R(0.1)	S	R	R	R	R
ASD-152*	Daeyoun	R(0.0)	S	H	R	R	R
ASD-312*	Daeyoun	R(0.2)	S	H	H	H	H
B Blocking*	Koregon	R (0.0)	H	H	R	R	R
Bacchus	Monsanto Korea	R	S	H	H	H	H
Beta Alexander	PPS	R	S	H	H	H	H
Block*	Daeyoun	R(0.0)	S	H	R	R	R
Doctor Q	Nongwoobio	R	R	R	R	R	R
Dotaerang Dia	Koregon	R	S	H	H	H	H
Dotaerang Gourmet	Takii	R	S	H	H	H	H
Dotaerang Master	Koregon	R	S	H	H	H	H
Dotaerang Season	Koregon	R	S	H	H	H	H
Fighting	Takii	R	S	H	R	R	R
Ganbarune 11*	Daeyoun	R(0.1)	S	H	H	H	H
Green Guard*	Daeyoun	R(0.0)	R	R	R	R	R
Green Saver*	Daeyoun	R(0.2)	R	R	R	R	R
Harmony	Sakata Korea	R	R	R	R	R	R
High Power*	Daeyoun	R(0.0)	R	H	R	R	R
Kyupirang	Nongwoobio	R	S	H	H	H	H
Magnet	Sakata Korea	R	H	R	R	R	R
MB*	Daeyoun	R(0.2)	H	H	R	R	H
Prime Alexander	PPS	R	S	H	H	H	H
Rafito	Monsanto Korea	R	H	H	R	R	R
Rex AD*	Daeyoun	R(0.0)	S	R	R	R	R

Shincheonggang	Monsanto Korea	R	S	H	H	H	H
Special	Koregon	R	H	H	R	R	R
Spider	Takii	R	R	R	R	R	R
Spike 23*	Daeyoun	R(0.0)	H	R	R	R	R
Suhosin	PPS	R	R	R	R	R	H
Super 334	Sakata Korea	R	H	H	H	H	H
Super sun road	Sakata Korea	R	H	H	H	H	S
Superprime	PPS	R	S	H	H	H	H
Support	Sakata Korea	R	H	R	R	R	R
Tosama*	Sakata Korea	R(0.1)	H	H	H	H	S
TY Altorang	Nongwoobio	R	S	H	H	H	H
Ultra	PPS	R	R	R	R	R	R
Zuiken	Sakata Korea	R	S	H	H	H	H
Ace Kkul	Bunong seed	S	S	S	S	S	S
Biolight	Tomato Life Science Institute	S	S	S	S	S	R
Charming	Konong seed	S	S	H	S	S	S
Dream 900*	Daeyoun	S(2.4)	S	S	H	H	H
Galaxy	Hyundai seed	S	S	S	S	S	S
House Doterang	Takii	S	S	S	H	H	H
Karysma	Bunong	S	S	S	H	H	R
Koko	Koregon	S	S	S	H	H	S
Marune TY*	Daeyoun	S(2.0)	H	S	H	H	H
Maskara	Bunong seed	S	S	S	H	H	R
All keeper*	Bunong seed	S(2.3)	S	H	R	R	R
Sinchunggang	Monsanto Korea	S	S	S	H	H	H
Super Ace	Tomato Life Science Institute	S	S	S	S	S	S
Super Dotaerang	Takii	S	S	R	H	H	R
Tenten	Takii	S	S	S	H	H	S
Tiara	PPS	S	S	S	H	H	H
Umgi	Bunong seed	S	S	S	H	H	H

“Cultivars for which bioassays were conducted and disease resistance validated are marked by an asterisk. Phenotype information of all other cultivars is based on the description by seed providers.

^bDSS, disease severity scale; R, resistance to FCRR; S, susceptibility to FCRR.

^cR, homozygous marker type for resistance line ‘AV107-4’; S, homozygous marker type for susceptible line ‘L3708’; and H, heterozygous marker type.

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Figure legends

Figure 1. Bioassay of two parental lines for resistance to Fusarium crown and root rot (FCRR). Resistant line 'AV107-4' shows no internal browning, whereas susceptible line 'L3708' shows severe internal browning extending from the tap root to the lower stem.

Figure 2. Genetic and physical location of 14 markers used for studying their association with Fusarium crown and root rot (FCRR) resistance. Despite the large physical distance between PNU-D4 and SSR237, the genetic distance is only 0.4 cM, indicating severely suppressed crossing over in this region. The previously reported possible location of *Frl* and the linked random amplified polymorphic DNA are marked by a blue dotted box and a yellow dot, respectively. In this study, PNU-T1212 and PNU-D4 showed the highest match with resistance (Match %; pink dotted box), which implies that *Frl* is possibly located on a pericentromeric region of the short arm of Chr. 9.

Figure 3. (A) F₁ hybrid cultivars were evaluated for Fusarium crown and root rot (FCRR). In this Figure, 12 cultivars were tested, of which 10 cultivars were resistant, whereas 2 (#11 and 12) were susceptible. For susceptible cultivars, disease symptoms were indicated in red boxes. (B) Markers PNU-T1212 and PNU-D4 were tested on these cultivars, and PNU-D4 (CAPS) genotype was perfectly matched with the disease response, whereas PNU-T1212 showed 50% match. This result indicated that a high level of genetic recombination occurred between these markers in this population and PNU-D4 is possibly more closely linked to *Frl*. (C) PNU-D4 was converted to high-resolution meting (HRM) marker and tested on 55 breeding lines that were resistant or susceptible to FCRR. A perfect match between PNU-D4 HRM marker genotype and FCRR resistance was observed.

1 Table 1. Molecular markers anchored on chromosome 9 of *Solanum lycopersicum* L. that were used to map the Fusarium crown root rot resistance locus
2 (*FrI*).
3

Marker	Marker type ^a	Location		Primer sequence (5'-3')	Tm ^d (°C)	Enzyme	Product size ^e (AV107-4/L3708)
		Reference genome (bp) ^b	genetic map (cM) ^c				
SSR70	SSR	3626502-3629252	42	F: TTTAGGGTGTCTGTGGGTCCC R: GGAGTGCGCAGAGGATAGAG	TD	-	120 / 104
PNU-T1212	RFLP	5090038-5092652	48	F: GGAGGAGTTAAGTGCATGGC R: GTGACCATCGGATCCTTTGC	TD	<i>MspI</i>	2014(1100/1200)
PNU-D4	RFLP	6097378-6097754	50	F: CAGCTGAAAGATGTCACCCA R: TGATCATTTACAAGGCGGCA	54	<i>MboI</i>	938 (465/387)
PNU-100431	CAPS	25170985-25173931	-	F: GATCACTAGTCGTCCTGGTCAA R: TTCACGATGGTCTTTATTCGT	54	<i>Hpy188III</i>	513 (340/292)
PNU-53169	CAPS	28023613-28029028	-	F: AGAGACGGTACATGCTTTGACA R: GTTCATTTGCATGGCTTCCT	54	<i>AluI</i>	408 (167/103)
SSR237	SSR	46426906-46426925	50.4	F: GTGGTAACGGCAAAGGGACT	TD	-	161 / 191

				R: CTTATGGCCTTAGCAGCCAG			
PNU-100181	CAPS	48783965-48806510	-	F: TGACCAAGTTGTGAGCAAGG	55	<i>ApoI</i>	662 (405/291)
				R: GCTTCACTTGGTGCTGGTTT			
PNU-100461	CAPS	56943553-56948073	-	F: AAGCGTCGACCTGATGTTGT	60	<i>TaqαI</i>	603 (470/603)
				R: ATGAAATGGGACCAAATGGA			
PNU-CD3	RFLP	63108341-63111046	52.1	F: CGAGTGGTGCAGTTGAGAAG	56	<i>MspI</i>	2042 (1170/2042)
				R: CGCTCCTTCAAATCTTCCGG			
PNU-43011	CAPS	64520600-64528534	-	F: GATGGATATCCCGCTCAAGA	53	<i>AluI</i>	702 (240/145)
				R: GGCTTAGGCCCTGGTTAGTC			
PNU-46840	CAPS	67134247-67142334	-	F: GGAGTGGATAATGTTGCCTGT	55	<i>AluI</i>	893 (680/396)
				R: TTGCAAGAAGATTGCTGCTC			
PNU-58307	CAPS	68877546-68881816	-	F: TTTGCAAATGTCCAAACACAA	53	<i>RsaI</i>	764 (501/764)
				R: TACCTCAACCCGAATTGGTC			
PNU-63662	CAPS	72029006-72032743	-	F: TTCCATCTCCCAAAAAGACG	53	<i>ApoI</i>	866 (866,647)
				R: AAGGACTGCCCCTAGTGGTT			

4

5 ^aSSR, simple sequence repeat; RFLP, restriction fragment length polymorphism; CAPS, cleaved amplified polymorphic sequence.

6 ^bPhysical locations of the primer sequences on the reference genome sequence ITAG2.4 (<http://solgenomics.net/>).

- 7 ^cLocations on the Tomato EXPEN-2000 map (<http://solgenomics.net/>); -, unknown
- 8 ^dT_m, PCR annealing temperatures; TD, touchdown PCR
- 9 ^ePCR fragment sizes specific to A107-4 and L3708 are presented in parentheses preceded by a virgule.

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1 Table 2. List of primers and a probe used to sequence the restricted fragment length polymorphism (RFLP) marker PNU-D4 and develop a high-resolution
2 melting (HRM) marker.
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Name	Sequence (5'-3')	Tm (°C)	Remarks
PNU-D4-F	CAGCTGAAAGATGTCACCCA	60	forward primer for sequencing
PNU-D4-R	TGATCATTTACAAGGCGGCA		reverse primer for sequencing
J3-D4-aF1	TCTTCGATATGATTTTCGGTATACTCCCATAAATCT	60	forward primer for HRM
J3-D4-aR1	AATTA ACTGGACCAATACCGGAGTCATTTGGCA		reverse primer for HRM
J3-D4-pF1	G TTCCTTGACAGATCAATTGTGTCA		probe for HRM

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Table 3. Genotyping of 60 commercial hybrid cultivars by using molecular markers anchored on chromosome 9 of *Solanum lycopersicum* L. that were used to study their association with the Fusarium crown root rot (FCRR) resistance locus *Frl*

Cultivar ^a	Seed Provider	Phenotype ^b (mean DSS)	Marker Genotype ^c				
			PNU-T1212	PNU-D4	PNU-100431	PNU-53169	SSR237
			5090038	6097378	25170985	28023613	46426906
Alexander Deluxe	PPS	R	S	H	H	H	H
All Round	Nongwoobio	R	R	R	R	R	R
AS6*	Daeyoun	R(0.0)	S	R	R	R	R
AS7*	Daeyoun	R(0.1)	S	R	R	R	R
ASD-152*	Daeyoun	R(0.0)	S	H	R	R	R
ASD-312*	Daeyoun	R(0.2)	S	H	H	H	H
B Blocking*	Koregon	R (0.0)	H	H	R	R	R
Bacchus	Monsanto Korea	R	S	H	H	H	H
Beta Alexander	PPS	R	S	H	H	H	H
Block*	Daeyoun	R(0.0)	S	H	R	R	R
Doctor Q	Nongwoobio	R	R	R	R	R	R
Dotaerang Dia	Koregon	R	S	H	H	H	H
Dotaerang Gourmet	Takii	R	S	H	H	H	H
Dotaerang Master	Koregon	R	S	H	H	H	H
Dotaerang Season	Koregon	R	S	H	H	H	H
Fighting	Takii	R	S	H	R	R	R
Ganbarune 11*	Daeyoun	R(0.1)	S	H	H	H	H
Green Guard*	Daeyoun	R(0.0)	R	R	R	R	R
Green Saver*	Daeyoun	R(0.2)	R	R	R	R	R
Harmony	Sakata Korea	R	R	R	R	R	R
High Power*	Daeyoun	R(0.0)	R	H	R	R	R
Kyupirang	Nongwoobio	R	S	H	H	H	H
Magnet	Sakata Korea	R	H	R	R	R	R
MB*	Daeyoun	R(0.2)	H	H	R	R	H
Prime Alexander	PPS	R	S	H	H	H	H
Rafito	Monsanto Korea	R	H	H	R	R	R

Rex AD*	Daeyoun	R(0.0)	S	R	R	R	R
Shincheonggang	Monsanto Korea	R	S	H	H	H	H
Special	Koregon	R	H	H	R	R	R
Spider	Takii	R	R	R	R	R	R
Spike 23*	Daeyoun	R(0.0)	H	R	R	R	R
Suhosin	PPS	R	R	R	R	R	H
Super 334	Sakata Korea	R	H	H	H	H	H
Super sun road	Sakata Korea	R	H	H	H	H	S
Superprime	PPS	R	S	H	H	H	H
Support	Sakata Korea	R	H	R	R	R	R
Tosama*	Sakata Korea	R(0.1)	H	H	H	H	S
TY Altorang	Nongwoobio	R	S	H	H	H	H
Ultra	PPS	R	R	R	R	R	R
Zuiken	Sakata Korea	R	S	H	H	H	H
Ace Kkul	Bunong seed	S	S	S	S	S	S
Biolight	Tomato Life Science Institute	S	S	S	S	S	R
Charming	Konong seed	S	S	H	S	S	S
Dream 900*	Daeyoun	S(2.4)	S	S	H	H	H
Galaxy	Hyundai seed	S	S	S	S	S	S
House Doterang	Takii	S	S	S	H	H	H
Karysma	Bunong	S	S	S	H	H	R
Koko	Koregon	S	S	S	H	H	S
Marune TY*	Daeyoun	S(2.0)	H	S	H	H	H
Maskara	Bunong seed	S	S	S	H	H	R
All keeper*	Bunong seed	S(2.3)	S	H	R	R	R
Sinchunggang	Monsanto Korea	S	S	S	H	H	H
Super Ace	Tomato Life Science Institute	S	S	S	S	S	S
Super Dotaerang	Takii	S	S	R	H	H	R
Tenten	Takii	S	S	S	H	H	S
Tiara	PPS	S	S	S	H	H	H
Umgi	Bunong seed	S	S	S	H	H	H

*Cultivars for which bioassays were conducted and disease resistance validated are marked by an asterisk. Phenotype information of all other cultivars is based on the description by seed providers.

^bDSS, disease severity scale; R, resistance to FCRR; S, susceptibility to FCRR.

^cR, homozygous marker type for resistance line 'AV107-4'; S, homozygous marker type for susceptible line 'L3708'; and H, heterozygous marker type.

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Figure 1. Bioassay of two parental lines for resistance to Fusarium crown and root rot (FCRR). Resistant line 'AV107-4' shows no internal browning, whereas susceptible line 'L3708' shows severe internal browning extending from the tap root to the lower stem.
36x29mm (300 x 300 DPI)

Figure 2. Genetic and physical location of 14 markers used for studying their association with Fusarium crown and root rot (FCRR) resistance. Despite the large physical distance between PNU-D4 and SSR237, the genetic distance is only 0.4 cM, indicating severely suppressed crossing over in this region. The previously reported possible location of Frl and the linked random amplified polymorphic DNA are marked by a blue dotted box and a yellow dot, respectively. In this study, PNU-T1212 and PNU-D4 showed the highest match with resistance (Match %; pink dotted box), which implies that Frl is possibly located on a pericentromeric region of the short arm of Chr. 9

71x62mm (300 x 300 DPI)

Figure. 3. (A) F1 hybrid cultivars were evaluated for Fusarium crown and root rot (FCRR). In this Figure, 12 cultivars were tested, of which 10 cultivars were resistant, whereas 2 (#11 and 12) were susceptible. For susceptible cultivars, disease symptoms were indicated in red boxes. (B) Markers PNU-T1212 and PNU-D4 were tested on these cultivars, and PNU-D4 (CAPS) genotype was perfectly matched with the disease response, whereas PNU-T1212 showed 50% match. This result indicated that a high level of genetic recombination occurred between these markers in this population and PNU-D4 is possibly more closely linked to Frl. (C) PNU-D4 was converted to high-resolution melting (HRM) marker and tested on 55 breeding lines that were resistant or susceptible to FCRR. A perfect match between PNU-D4 HRM marker genotype and FCRR resistance was observed.

71x58mm (300 x 300 DPI)

Gen-2015-0115.R1Supplement 1. Sequence alignment of the polymerase chain reaction (PCR) fragment amplified using PNU-D4 primers from 'AV107-4' and 'L3708'. Single nucleotide polymorphisms (SNPs) are shown in blue, and a causal SNP for restriction fragment length polymorphism (RFLP) and high resolution melting (HRM) marker is marked by a green rectangle

223x148mm (150 x 150 DPI)