A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Genome-based Fine Mapping of the *Tomato spotted wilt virus* (TSWV) Resistance Gene, *Tsw*, and Identification of a New Source of Resistance Against TSWV in *Capsicum* spp.

고추 *Tomato spotted wilt virus* (TSWV) 저항성 유전자 *Tsw* 에 대한 유전체 기반 유전자 미세 지도 작성 및 새로운 TSWV 저항성 고추 계통의 발굴

AUGUST, 2013

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SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
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ABSTRACT

Tomato spotted wilt virus (TSWV) is an important viral disease affecting pepper production worldwide. A single dominant resistance gene to TSWV, *Tsw*, has been known to originate from *Capsicum chinense*. In this study, comparative mapping, pooled transcritpome analysis and chromosome walking approaches were applied for genome-based fine mapping of the *Tsw* gene. Based on pepper scaffold sequences from the *C. annuum* genome database, four SNP markers including SNP165256, SNP3065253, SNP67464, and SNP151705 showed no recombination in two mapping populations of F_2 'Telmo' (210 individuals) and 'SP' (843 individuals). The *Tsw* gene was defined within 149 kb between two co-segregating markers, SNP165256 and SNP151705. A total of 22 predicted genes were resided in the target region. Of those, five predicted genes including mRNA-6, mRNA-7, mRNA-11, mRNA-12, and mRNA-13 showing annotations of

CC/TIR-NBS-LRR resistance proteins were identified. Gene expression study showed that the mRNA-13 was expressed in ‘PI152225’ but was absent in ‘Special’. This evidence demonstrated that the mRNA-13 could be a strong candidate gene for the *Tsw* gene. This result will be useful for cloning the *Tsw* gene and developing TSWV resistant cultivars.

Discovering a new resistance gene source against TSWV from the *Capsicum* germplasm collection is necessary for pepper breeding programs because the *Tsw* gene has been overcome by field isolates. A new resistance source, *C. chinense* ‘AC09-207’, was identified and characterized when a set of pepper germplasm collections comprising 487 accessions from six *Capsicum* species and 30 commercial F_1 hybrids was evaluated in this study. Molecular marker analyses and genetic allelism tests showed that the resistance gene in *C. chinense* ‘AC09-207’ was a single dominant gene which may be either a novel allele at the *Tsw* locus in *C. chinense* 'PI152225' or controlled by a different gene tightly linked to *Tsw*.

Keyword: *Tomato spotted wilt virus* (TSWV), *Capsicum chinense*, genome-based fine mapping, *Tsw*, germplasm screening, disease resistance

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AMV	*Alfalfa mosaic virus*
AVRDC	Asian Vegetable Research and Development Center
BAC	Bacterial artificial chromosome
BBWV2	*Broad bean wilt virus* 2
BSA	Bulk segregant analysis
CaMV	*Cauliflower mosaic virus*
CAPS	Cleaved amplified polymorphic sequence
CC	Coiled-coil
ChiLCV	*Chilli leaf curl virus*
ChiVMV	*Chilli veinal mottle virus*
CMV	*Cucumber mosaic virus*
cM	CentiMorgan
dpi	Day post inoculation
ER	Extreme resistance
ELISA	Enzyme-linked immunosorbent assay
FAOSTAT	Food and Agriculture Organization statistical database
gDNA	Genomic DNA
GRSV	*Groundnut ringspot virus*
hpi	Hours post inoculation
HR	Hypersensitive response
HRM	High resolution melting

INSV	*Impatiens necrotic spot virus*
LRRs	Leucine-Rich Repeats
MAS	Marker-assisted selection
N	Nucleocapsid protein
NBSs	Nucleotide Binding Sites
NICEM	The National Instrumentation Center for Environmental Management
NSs	Non-structural protein, RNA S
NSm	Viral movement protein, nonstructural protein, RNA M
ORFs	Open reading frames
PCR	Polymerase chain reaction
PepMoV	*Pepper mottle virus*
PMMoV	*Pepper mild mottle virus*
PVMV	*Pepper veinal mottle virus*
PVY	*Potato virus Y*
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
R-genes	Resistance genes
RLK	Receptor-like kinase
RLP	Receptor-like protein
RNA	Ribonucleic acid
SNP	Single nucleotide polymorphism
SGMV	*Serrano golden mosaic virus*
ss	Single-stranded

SSRs	Simple sequence repeats
TCSV	*Tomato chlorotic spot virus*
TEV	*Tobacco etch virus*
TIR	Toll/interleukin-1 receptor
TMV	*Tobacco mosaic virus*
TMGMV	*Tobacco mild green mosaic virus*
ToMV	*Tomato mosaic virus*
TSWV	*Tomato spotted wilt virus*
WGS	Whole genome sequencing

GENERAL INTRODUCTION

Tomato spotted wilt virus (TSWV) is the type member of the plant virus genus *Tospovirus* within the Family *Bunyaviridae* (Francki et al., 1991). It has become a threat in many vegetable and ornamental production areas in tropical, subtropical, and high temperate regions. TSWV was mainly transmitted by two major thrips vectors: *Frankliniella occidentalis* (Pergande) and *F. fusca* (hinds). Some major crops susceptible to TSWV include tomato, pepper, lettuce, potato, papaya, peanut and tobacco (German et al., 1992).

Capsicum species are among the most important vegetable crops worldwide. According to FAOSTAT 2007 (http://faostat.fao.org/), peppers are grown in 77 countries with a total production of more than 29 million tons. TSWV has constituted a severe threat to *Capsicum* cultivation all around the world (Lima et al., 2000). Pepper plants are highly susceptible to TSWV at all developmental stages with severe symptoms, usually necrosis, on leaves and fruits. The main vector, *Frankliniella occidentalis*, which feeds preferentially in flowers, provides an alternative route to leaves for entry of the virus and increases the ability infection of TSWV (Roggero et al., 2002a). TSWV had the wide host range of about 900 plants species (Roselló et al., 1996). It is difficult to control this disease physically, chemically and biologically. Therefore, resistant cultivars against TSWV are greatly required for pepper production. Also, continuous deployment of new resistant cultivars is necessary because the virus is known to evolve and

break down resistance rapidly (Choe et al., 1996; Latham and Jones, 1998; Hobbs et al., 1994; Moury et al., 1997).

Heritable resistance to TSWV based on a hypersensitive reaction in several accessions of *C. chinense Jacquin* and *C. baccatum*. [‘PI152225’, ‘PI159236’ (syn. 'CNPH 679'), the Peruvian cv 'Panca' (syn. 'CNPH-275'), ‘7204’, ‘PI15’ and ‘C00943’] has been identified (Black et al., 1991; Boiteux et al., 1993; Cupertino et al., 1988; Jorda et al., 1994; Hobbs et al., 1994; Diez et al., 1993; Nuez et al., 1994). Boiteux and de Avila (1994), Boiteux (1995) and Moury et al. (1997) confirmed that the resistance in *C. chinense* accessions (‘PI159236’, ‘PI152225’, ‘CNPH-275’ and ‘7204’) is monogenic, dominant and located in the same locus named *Tsw*. The *Tsw* gene in *C. chinense* has been tagged with a random amplified polymorphic DNA (RAPD) marker ($Q\text{-}06_{270}$) that is located 3.45 centiMorgan (cM) from the *Tsw* locus, about 2.1 cM from TG420 and mapped to the distal portion of chromosome 10 (Jahn et al., 2000). In the effort to select this resistance, Moury et al. (2000) used the bulked segregant analysis with 153 F_2 individuals and they found four RAPDs linked to the *Tsw* locus spanning 20 cM. A close RAPD marker was converted into a codominant cleaved amplified polymorphic sequence (CAPS) ($SCAC_{568}$) using specific PCR primers and restriction enzymes. This CAPS marker is tightly linked to the *Tsw* locus (0.9 ± 0.6 cM) and is helpful for marker-assisted selection (MAS) in a wide range of genetic intercrosses (Moury et al., 2000). According to Kim et al. (2008),

$SCAC_{568}$ was applied on seven resistant lines and five susceptible lines, but it did not show polymorphism on all resistant lines.

No distinguishable resistance alleles at the *Tsw* locus in *C. chinense* have been identified (Boiteux, 1995). Black et al. (1996) confirmed that TSWV resistance in pepper was conferred by a single dominant gene, *Tsw*. Apart from the *Tsw* locus, no additional resistance loci in *Capsicum* spp. have been reported (Boiteux et al., 1993; Boiteux, 1995; Moury et al., 1997). Jahn et al. (2000) pointed out that if resistance in 'PI152225' and 'PI159234' is due to alleles at different loci, these loci must be tightly linked.

The management of TSWV using resistant pepper cultivars has been widely discussed because pathogen resistances in plants are often challenged by high temperature and virus isolates can severely and systemically overcome commercial TSWV resistance cultivars. According to Moury et al. (1998), TSWV resistance in pepper conferred by the *Tsw* gene is less stable in continuous high temperature conditions about 32 degrees Celsius (^{o}C) or upon early plant inoculation.

TSWV displays a higher level of biological diversity and a better capacity to generate new strains than any other plant viruses (Moyer and Qiu, 1996). The ability of TSWV to replicate in its thrip vector may increase the opportunity for genetic diversification in the population (Wijkamp, 1995). Moreover, the variability and mutability of TSWV can be further increased

because the virus single-stranded RNA genome, which is subject to error-prone replication, is also tripartite and can allow the formation of pseudo-recombinants between mutants RNAs (Moury et al., 1997). Field isolates virulent towards the *Tsw* gene have been found widely around the world, for example in Louisiana, USA (Hobbs et al.1994), Brazil (Boiteux and Nagata, 1993), Italy (Roggero et al., 2002b), Spain (Margaria et al., 2007), and Australia (Sharman et al., 2006). In Korea, a severe outbreak of *Cucumber mosaic virus* (CMV) and TSWV on bell pepper grown in the greenhouse located in Gwangyang, Jeonnam province occurred in 2006 (Mun et al., 2008). Further, TSWV spread to major vegetables in 2010 such as red pepper, paprika and tomato (Kim et al., 2011).

The serious economical loss due to TSWV all over the world has posed an urgent need to find and use new sources of resistance to TSWV and other *Tospovirus* species infecting *Capsicum* (Cebolla-Cornejo et al., 2003). The purposes of my study are (1) to develop additional markers tightly linked to the *Tsw* locus, (2) to identify a candidate for the *Tsw* gene, (3) to isolate new resistant source(s) against TSWV from the *Capsicum* germplasm, and (4) to study the alleles and inheritance for the new TSWV resistant sources.

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LITERATURE REVIEW

1. Viral diseases in plants

Plant viruses are pathogens that cause diseases in plants. They are responsible for huge losses in crop production and quality in all parts of the world. Viruses invade plant tissues by way of arthropod, nematode or fungal vectors, by vegetative propagation, or they enter through wounds. Over 800 plant viruses have been recognized and characterized. These viruses are classified into six major groups based on the nature of the genome: Double-stranded DNA, single-stranded (ss) DNA, reverse-transcribing viruses, double-stranded RNA, negative-sense ss RNA and positive-sense ss RNA. Most of genomes of plants viruses are positive-sense ss RNAs. *Tobacco mosaic virus* (TMV) and *Cauliflower mosaic virus* (CaMV) are examples of plant viruses which contain infective ss RNAs and double-stranded DNA in its genome, respectively. Plant viruses are composed mainly of two components, a protein coat and a nucleic acid (genome) center. Thus, virus-infected plants are usually detected by an indirect ELISA method (Voller et al., 1976). Viruses can be determined and seen primarily with an electron microscope and X-ray diffraction techniques because most plant viruses range in size from 12 to 50 nanometers in diameter and from 200-2000 nanometers in length. Viruses are intracellular obligate parasites, so their particles cannot replicate on their own without the help of a live plant cell. Viral diseases in plants cannot be cured. Viruses usually cause systemic infection in the host plants.

Some viruses are very host specific, while others colonize many different hosts. Infected plants may show a range of symptoms including stunting, mosaic, ring spots, color breaks, and distortion. The difference among viral, fungal and bacterial diseases may be very clear, but viral diseases are often confused with herbicide injury, nutrient stress, and other environmental stresses. Thus, the pattern of diseased plants is often very important in diagnosing diseases caused by viruses (Nancy, 2008).

Viruses can be transmitted from plant to plant in several ways. Some viruses spread from the parental plants to succeeding generations through the seed. Other modes of viral transmission may be through vegetative propagation, grafting and budding, seed transmission and mechanical spread by arthropods and men. To date no completely effective chemical control over virus diseases has been reported. Therefore, sanitation and the use of resistant varieties of plants have been considered the most effective in controlling plant viruses.

1.1. Dominant plant virus resistance genes

Dominant resistance is often associated with the hypersensitive response (HR) after specific recognition of the virus, leading to the localization of virus spread by rapid programmed cell death surrounding the infection site, which results in visible necrotic local lesions. Thus, HR-mediated resistance is a common resistance mechanism for viruses and for other plant pathogens (Kang et al., 2005). Moreover, most plant disease resistance genes (R-genes) isolated and

characterized to date interact with the invading cognate pathogens in a gene-for-gene manner. Following pathogen recognition, a gene-encoded protein may activate a signaling cascade that coordinates plants defense responses to block pathogen spread, resulting in an incompatible interaction. A number of dominant plant virus R-genes have been isolated from Solanaceous hosts: *Ny*, *Rx1*, *Rx2*, *Nb*, *Ty-2*, *Sw5*, *Tm-1*, *Tm-2*, and $Tm2^2$, *L*, and *cmv 11.1* (Kang et al., 2005). Specially, five R-genes, *Tsw*, *Pvr4*, *Pvr7*, *Cmr1* and *L4*, were mapped and three R genes, *L4*, *Bs2*, and *Bs3*, were mapped, cloned, and characterized in pepper (Liu et al., 2013).

1. 2. Recessive plant virus resistance genes

Many recessive R-genes, in contrast of dominant R-genes, function at the single cell level or affect cell-to-cell movement. More than half of recessive R genes identified to date, for example: pvr^1 (pvr^2), confer resistance to potyviruses (Kang et al., 2005). In fact, plant viruses completely depend on the host to complete their infection cycle. In general, four to ten proteins were encoded by typical plant viruses that organize the complex biochemistry and intermolecular interactions which are required for viral infection cycles. Host factors that are required for susceptibility and mechanisms of plant pathogenesis by the pathogen can be revealed by studying recessive virus resistance in plants (Kang et al., 2005). Several host genes have been identified and characterized in plants, such as: *lsp1*, *cum1*, and *cum2* in *Arabidopsis*; *nsv* in *Cucumis melo*; *rym4/5* in *Hordeum*

vulgare; *sbm1* in *Pisum sativum*; *mo1* in *Lactuca spp.*; *pot1* in *Solanum spp.;* and *pvr2* and *pvr6* in *Capsicum spp.* (Kang et al., 2005).

2. Virus diseases in pepper

The production of pepper is often challenged by biotic and abiotic stresses. Biotic stress by major groups of pathogens (viruses, bacteria, omycetes, fungi and nematodes) can cause significant damage to pepper production worldwide. Among them, viruses are the most important pathogens in pepper. Fourteen common viral species have been reported to predominantly infect pepper crops at the Asian Vegetable Research and Development Center (AVRDC) (http://libnts.avrdc.org.tw/fulltext_pdf/eam0142.pdf): TMV, *Tomato mosaic virus* (ToMV), *Pepper mild mottle virus* (PMMoV), *Cucumber mosaic virus* (CMV), *Chilli veinal mottle virus* (ChiVMV), *Potato virus Y* (PVY), *Tobacco etch virus* (TEV), *Pepper mottle virus* (PeMoV), *Pepper veinal mottle virus* (PVMV), *Pepper mild tigre virus*, *Serrano golden mosaic virus* (SGMV), *Chilli leaf curl virus* (CLCV), *Tomato spotted wilt virus* (TSWV), and *Alfalfa mosaic virus* (AMV). According to a virus incidence survey in Santa Carla and San Benito counties, California, USA in 2004, the incidence ratio of six major viruses infecting peppers worldwide, TMV, TSWV, CMV, PepMoV, PVY, and TEV, were 2.4%, 50%, 38%, 2.4%, 4.8%, 2.4%, respectively. Among them, TSWV was the most serious virus (Aziz, 2005). In Korea, pepper is affected by six main

viruses including CMV, PepMoV, PMMoV, *Broad bean wilt virus* 2 (BBWV2), *Tobacco mild green mosaic virus* (TMGMV) and TSWV. A severe outbreak of TSWV on bell peppers grown in greenhouses occurred in 2006 (Mun et al., 2008).

3. *Tomato spotted wilt virus*

TSWV, first discovered on tomatoes in Australia in 1915 (Brittlebank, 1919), was identified as a virus disease in 1930 by Samuel and co-workers (Samuel et al., 1930). TSWV has the second widest host range among any virus and is now common in temperate, subtropical, and tropical regions around the world. It infects many economically important plants representing 35 plant families, including dicots and monocots, and causes a serious disease in many crops such as tobacco (*Nicotiana tabacum*), peanut (*Arachis hypogaea*), tomato (*Solanum lycopersium*), and pepper (*Capsicum annuum*) (Moyer, 1999). This virus ranks among the ten most detrimental plant viruses worldwide (Prins and Goldbach, 1998).

Recently, TSWV has been causing considerable damage in pepper cultivation. Production losses as high as 69% have been reported in sweet pepper cultivated under open-field conditions (Cupertino et al., 1984). The results of a survey conducted of 14 pepper fields in California, USA showed that TSWV accounted for 72% of the viral infections (Aziz, 2005). The yield loss from infection by TSWV on red pepper in Brazil was 50% to 70% (Boiteux et al., 1993b). TSWV is also one of the most important pathogens causing yield and

quality losses in pepper production in Korea and other Asian countries (Sylvia, 1993; Mun et al., 2008).

3. 1. Morphological phenotypes of TSWV Symptom

The symptoms attributed to TSWV infection are diverse depending on the host, the environmental conditions affecting the host, the individual virus infecting the plant, and the number of virus strains infecting the host plant (German et al., 1992). Local lesions, with chlorosis and necrosis are usually found on non-systemic hosts, while ring-spots, line patterns, wilting, stunting, mottling, chlorosis, and necrosis can often be detected on systemic hosts. Symptoms may appear within two to four days after infection which usually occurs in epidermal cells through wounds made by thrips (Moon, 2006).

In peppers, infected leaves may twist or curl and show chlorotic line patterns or mosaic with necrotic spots which frequently coalesce. The stems of infected pepper plants display severe stunting, lesions and chlorosis and may become distorted. Long necrotic streaks on stems usually extend to the growing tips. Plant death may occur in severe instances. Pepper fruits infected with TSWV may be distorted in shape and may show necrotic spots and streaks, mosaic, and ring patterns. Fruit formed after infection usually possesses large necrotic streaks and spots or may be completely necrotic (Dow AgroSciences, 2008).

3. 2. Biological characteristics of TSWV

TSWV belongs to the genus *Tospovirus,* which encompasses the plant infecting members of the family *Bunyaviridae* (Goldbach and Peters, 1994; Murphy et al., 1995). Moyer (1999) reported that TSWV is an ambisense RNA virus comprising a tripartite genome packaged in a quasi-spherical, enveloped particle of 80–110 nm in size. The virus particle or virion contains 10-20 copies of the ssRNA genome (Adkins et al., 2005). The three ssRNA genome molecules including S (2.9 kb), M (4.9 kb), and L (8.9 kb) RNAs have been characterized (De Haan et al., 1990 and 1991; German et al., 1992). The entire genome codes for six proteins via five different open reading frames (ORFs). The L RNA encodes the viral RNA-dependent RNA polymerase (330 kDa) for viral replication and genome transcription. Two envelope precursor glycoproteins (G1 & G2) and the viral movement protein (NSm) for cell-to-cell viral transfer are encoded by the M RNA. The S RNA encodes the N protein used to construct the virion capsid and a non-structural protein (NSs) (Cortez et al., 2001). The NSs protein of TSWV was responsible for the RNA silencing-suppressing activity (Takeda et al., 2002 and Bucher et al., 2013). The absence of short interfering RNAs in NSs-expressing leaf sectors suggests that the tospoviral NSs protein interferes with the intrinsic RNA silencing present in plants (Bucher et al., 2013).

3. 3. Transmission and infection of TSWV in plants

TSWV is transmitted by several thrip species in a propagative manner (Ullman et al., 1993; Wijkamp et al., 1993). Presently, at least seven species of thrips, belonging two genera, have been reported as vectors of TSWV: *Frankliniella schultzei* (Trybom), *F. occidentalis*, *F. intonsa* (Trybom), *F. fusca* (Hinds), *F. bispinosa* (Morgan), *Thrips tabaci* Lindeman and *T. setosus* Moulton (Thysanoptera: *Thripidae*) (Mound, 1996; Mumford et al., 1996; Ullman et al., 1997, 2002; Webb et al.,1997). *F. occidentalis*, the western flower thrips, is considered to be the most important vector species because it is globally distributed and can transmit most tospoviruses. Only thrips that acquire the virus in the larval stage when the larvae feed on the virus infected leaves can become transmitters, either as second instars larvae or as adults (Sakimura, 1962; Wijkamp et al., 1993). Winged adult thrips can survive for 30-45 days and lay 150-300 eggs that are inserted into plant tissues (usually flower parts or young leaves). Although the developmental period for thrips to acquire the virus is limited, the wide host range for both virus and thrips facilitates the development of epidemics.

After the insects feed on infected plants, the virus enters the midgut lumen. The midgut epithelial cells of thrips are the original site of TSWV entrance and infection (Ullman et al., 1993; Nagata et al., 1999). Virions must move across the midgut apical membrane of the brush border and this is potentially mediated

via an interaction with the TSWV glycoproteins and a midgut receptor. After the virus replicates in the midgut epithelial cells, it spreads to adjacent midgut cells and eventually moves into the muscle cells surrounding the midgut. TSWV must then move from the gut tissues to the primary salivary glands for transmission to occur. Thus the insect vectors not only transmit the virus, but also serve as a host (Ullman et al., 1993; Wijkamp et al., 1993; Negata et al., 1999). The virus replicates again in the salivary glands and moves across the apical membrane to the plant. After transmission to plant cells, the virus is released of its membrane, and the nucleocapsid enters into the cytoplasm. At this stage, the viral RNA will be either transcribed or replicated by the viral RNA dependent RNA polymerase (Matthews, 1992).

3. 4. Genetic variation and adaption ability of TSWV

The genetic variation in plant virus populations is generated by errors occurring during the replication of genomes because RNA viruses lack proofreading ability (Domingo and Holland, 1997). For viruses, the two main types of errors are mutation, the initial source of variation, and recombination. Re-assortment of genomic segments in viruses with a segmented genome which may cause genetic exchange also plays a major role in virus evolution (White et al. 1995). It is believed that for RNA viruses in the family *Bunyaviridae*, mutation and genome segment reassortment are the major sources of variability (Elliott, 1995). TSWV isolates simply exchanged or re-assort genome segments in a

nonrandom fashion. The intergenic region of the S RNA was associated with competitiveness of the individual segments in reassortant isolates (Qiu et al., 1998).

TSWV is characterized by high genetic variability attributed to error-prone replication, high replication rates, short generation time and large population size (Domingo and Holland, 1997). The high diversity of TSWV explains its ability to rapidly adapt to new or resistant hosts (Feuer et al., 1999). This virus routinely breaks resistance in commercial crops such as pepper within a short period of time (Hobbs et al., 1994; Moury et al., 1997). TSWV is able to overcome transgenic-mediated resistance through natural selection for resistance-mediated isolates in the field (Herrero et al., 2000; Jankolova et al., 1999), and through genomic re-assortment under experimental conditions (Qiu et al., 1998). According to the partial genetic analysis of Hoffman et al. (2001) the M RNA of TSWV rather than the S RNA that encodes the N gene plays a primary role in overcoming host- and transgenic-mediated resistance. Hoffman et al. (2001) also indicated that although elements on the M RNA played a major role, the suppression of transgenic silencing could be a combined effect of different viral genome segments.

3. 5. Control measures of TSWV

Controlling TSWV in crop plants has proven difficult and expensive because of the wide range of plant including many reservoir weeds and effective

spread of TSWV by thrip vectors (Hanssen et al., 2010). The peculiarly persistent and circulative interaction of TSWV thrips, the large number of viruliferous thrips present in TSWV-infected crop residues, the adult thrips' ability to readily disperse and inoculate healthy host plants almost continuously during their life span, and the low efficiency of chemical control and the rapid acquisition of resistance to insecticides by thrips are also reasons which restrain effective controls of TSWV (Boiteux et al., 1993b; Ie, 1970; Cho et al., 1989, Rice et al., 1990). Chemical or biological control of thrips is very difficult in both greenhouse and field conditions (Moury et al., 1997).

Several cultural control methods can be carried out to protect pepper production from TSWV. Conducting effective weed controls in and around pepper fields by maintaining a 10-m plant-free border is necessary to prevent or slow the spread of TSWV to susceptible pepper plants. Planting peppers far away from TSWV susceptible crops (i.e. peanut, tobacco) or keeping infected field areas fallow for 3-4 weeks to allow thrips to emerge from crop debris and disperse from the field are also highly recommended. Despite the fact that TSWV has often spread before symptoms develop, infected plants should be removed and destroyed immediately to reduce secondary infection.

Controlling thrips with chemicals can be difficult because they rapidly develop resistance. Several insecticides specific to vector thrips, not broad-spectrum insecticides that kill natural enemies of thrips like minute pirate bug on

peppers, should be applied to reduce an infestation. Rotating insecticides from different chemical classes may be a way to delay insecticide resistance. Acibenzolar-S-methyl was shown to reduce incidence of TSWV disease when used with other management tools (i.e. UV metalized mulch). Acibenzolar-S-methyl (Actigard) should not be used on pepper due to potential adverse effects.

Moury et al. (1997) emphasized that searching for sources of resistance and development of resistant cultivars appears to be a better way to control the disease than chemical or biological measures. In this way, we can take advantages of their ready-to-use characteristics and their inherent benefit in reducing environmental damages such as those associated with insecticides with regard to spraying abuses (Boiteux, 1995).

3. 6. TSWV resistance genes in crop plants

Plant resistance to TSWV has been intensively studied in tomato (Cho et al., 1989; Boiteux and Giordano, 1992; Stevens et al., 1992) and other crops. Genes conferring a high level of resistance to TSWV have been characterized in tomato. *Sw5,* a single dominant gene originating from *Lycopersicon peruvianum* (now *Solanum peruvianum*), has been introgressed in cultivated tomato plants from the wild species *L. peruvianum* and provides resistance against TSWV isolates from different geographical locations (Boiteux and Giordano, 1993; Stevens et al., 1992). Stevens et al. (1995) mapped *Sw5* between two restriction fragment length polymorphism (RFLP) markers (CT71 and CT220) near a

telomeric area of chromosome 9, and also identified one RAPD marker that is localized within 0.5 cM of the *Sw5* gene.

In tobacco, the *Sw5* gene not only provides resistance to TSWV but also to two related tomato-infecting *topoviruses*, *Groundnut ring spot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) (Boiteux and Giordano, 1993). Two resistance gene candidates, *Sw5-a* and *Sw5-b*, showing highly homologous sequences (95%) were identified within 40 kb. The *Sw5-a* encodes a protein of 1245 amino acid and the *Sw5-b* encodes a 1246 amino acid protein. The two genes which are the members of the coiled-coil, nucleotide-binding-ARC, leucine-rich repeat group of resistance gene candidates have highly homologous promoter and terminator regions. They also significantly resemble the tomato nematode and aphid resistance gene *Mi* and, to a lesser extent, *Pseudomonas syringae* resistance gene, *Prf*. Transformation of *Nicotiana tabacum* cv. Sr1 plants revealed that the *Sw5-b* alone is necessary and sufficient for conferring resistance against TSWV (Spassova et al., 2001).

3. 7. TSWV resistance sources and genes in pepper

There are few, if any, resistant pepper varieties available. Several lines expressing a HR to TSWV have been identified including ‘PI152225’ and ‘PI159236’ (Black et al., 1991), ‘CNPH 275’ (= ‘Panca’) (Boiteux et al., 1993b), ‘PI15’ (Jorda et al., 1994), ‘C00943’ (Hobbs et al., 1994), ‘7204’ (Nuez et al.,

1994), ‘PI159234’ (Jahn et al., 2000), and ‘ECU-973’ (Cebolla-Cornejo et al., 2003). *C. chinense* 'PI159236' was highly resistant to all isolates of TSWV but susceptible to all isolates classified as TCSV and GRSV (Boiteux and de Avila, 1993; Nagata et al., 1993).

Distinct resistant sources to TSWV, 'PI152225', 'PI159236' (syn. 'CNPH 679') and the Peruvian cv 'Panca' (syn. 'CNPH 275'), ‘7204’, ‘PI15’ and ‘C00943’ have been identified in *C. chinense Jacquin*, the most important reservoir of TSWV resistance alleles, and *C. baccatum* (Cupertino et al., 1988; Black et al., 1991; Boiteux et al., 1993, Jorda et al., 1994; Hobbs et al., 1994; Diez et al., 1993; Nuez et al., 1994). Progeny and allelism tests showed that the resistance of these three sources is monogenic, dominant and located at the same locus, *Tsw* (Boiteux, 1995). This locus originated from *C. chinense* and was introgressed into pepper cultivars (Costa et al., 1995). The *Tsw* gene has localized or hypersensitive-like responses to TSWV isolates as its main phenotypic characteristics (Bos, 1978; Fraser, 1990; White and Antoniw, 1991). A RAPD marker ($Q\text{-}06_{270}$) linked to the *Tsw* gene about 3.45 cM away from the gene was developed and mapped to the distal portion of chromosome 10 (Jahn et al., 2000). Moury et al. (2000) developed four RAPD markers linked to the *Tsw* locus spanning 20 cM. A close RAPD marker was converted into a co-dominant CAPS marker ($SCAC_{568}$) linked 0.9 (±0.6) cM away from the gene to facilitate MAS in a wide range of genetic intercrosses. However, $SCAC_{568}$ was applied on seven resistant lines, five

susceptible lines and it did not show polymorphism on all resistant lines (Kim et al., 2008).

Recently, naturally occurring resistance-breaking TSWV strains have been identified. The first TSWV resistance breaking strain in *C. chinense* was reported in 1993 by Boiteux and Nagata. Several other resistance-breaking strains and isolates were also reported in Italy in *Capsicum* spp. (Roggero et al., 1999 and 2002b), in Spain (Margaria et al., 2004) and in Australia (Thomas-Carroll and Jones, 2003). According to Jahn et al. (2000) and Margaria et al. (2007), S RNA of TSWV genome which encodes both the N and the NSs carried the genetic determinant for breakdown of *Tsw* resistance. N protein is the avirulence gene of the *Tsw* resistance gene, and induces DNA fragmentation in *C. chinense* cells, and thus eliciting programmed cell death (Lovato et al., 2008). While Margaria et al. (2007) indicated that NSs protein could prevent newly emerging leaves from recovery from TSWV infection over time, Lovato et al. (2008) identified no evidence of the NSs protein being the avirulence component of the *Tsw*-mediated HR. Margaria et al. (2007) also confirmed that local necrotic HR at the start of infection is not sufficient for resistance in *Capsicum* spp. carrying the *Tsw* gene. By contrast, de Ronde et al. (2013) pointed out that the NSs protein of resistance-inducing isolate triggered a HR in *Tsw*-containing *Capsicum* plants, but not in susceptible *Capsicum*.

4. Molecular markers

Molecular markers have been employed as a useful tool for plant breeding and cultivar identification for many years. Various DNA markers were used for genome mapping and MAS in plant breeding program. Common molecular marker technologies are RFLP, RAPD, amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), CAPSs, and single nucleotide polymorphisms (SNPs). However, each of molecular marker type has its drawbacks. For examples, genotyping with CAPSs requires amplified DNA to be treated with a restriction enzyme. In SSR analysis, the size differences between the products amplified from each allele are usually too small to produce reliable scores by standard agarose gel electrophoresis and other types of RAPD, RFLP, AFLP markers are often low throughput assay. SNPs seem to be the most attractive tool for marker-assisted breeding, genotyping, and mapping. This marker system is highly abundant and distributed throughout the genomes such as one SNP per 1,000 bp in human genome (Wang et al., 1998) and one SNP per 4,000 to 8,500 bp in Arabidopsis (Van Deynze et al., 2007). In addition, high resolution melting (HRM) analysis is an inexpensive, simple, and rapid detection technology for SNP (Park et al., 2009).

5. Genetic fine-mapping in plants

Genetic maps provide a powerful tool for gene identification, study,

utilization and isolation. In fine-mapping, markers tightly linked to a targeted gene are identified. In plants, traditional genetic markers (morphological traits, isozymes, etc.) which were too rare, too widely spaced and often too difficult to use usually did not permit fine mapping. Fine mapping can be done in any plants that can be crossed with the help of DNA markers. A genetic fine map of a specific locus usually aims at the identification and location of markers that flank the targeted gene and are within one or fewer centiMorgans. With a fine map, MAS can be very precise. Also, accurate comparisons of map positions among different species to see if they share similar traits at that chromosomal location are possible. Finally, fine-mapping is usually an essential step in map-based gene isolation for the researcher to acquire the gene for further precise study and crop improvement by transgenic technology (Bennetzen, 2000).

To date, most of dominant R-genes in plants had been mapped and cloned encode proteins characterized by nucleotide-binding site (NBS), leucine-rich repeat (LRR) domains and variable amino- and carboxy-terminal domains (McHale et al., 2006). Classical genetic and molecular data showed that NBS-LRR-encoding genes in plants are frequently clustered in the genome, the result of both segmental and tandem duplication (Meyers et al., 2003).

6. Clusters of resistance genes in plants

Mapping and cloning of plant R-genes are of great importance in controlling common pathogens to diverse agricultural crops for several reasons (McDowell and Woffenden, 2003): identification of genetic markers closely linked to R-genes are useful for breeding resistant cultivars; transgenic expression of R-genes can confer resistance against common pathogens to distantly related crop species; molecular analysis of R-gene products provides us with knowledge on plant defense mechanisms; and structural analysis of alleles of resistance genes is expected to help understand the co-evolution of pathogens and host R-genes.

Previous studies with classical genetic and molecular data have revealed that R-genes in plants are frequently found in a cluster of homologous genes (McDowell and Woffenden, 2003), but the sizes and arrangement of R-gene clusters vary within loci and plant species (Tomita et al., 2008). According to McDowell and Woffenden (2003), R-genes to diverse pathogens encode proteins with common motifs indicating that R-genes are part of signal-transduction systems. NBS-LRR proteins are some of the largest proteins known in plants, ranging from about 860 to about 1900 amino acids. They have at least four distinct domains joined by linker regions: a variable amino-terminal domain, the NBS domain, the LRR region, and variable carboxy-terminal domains. LRRs are multiple and seria1 repeats of a motif about 24 amino acids in length (Kobe and Deisenhofer, 1994). LRRs contain leucines or other hydrophobic residues at

regular intervals and can also contain regularly spaced prolines and asparagines (Bent, 1996). Sequences encoding putative solvent-exposed residues in LRR region are hypervariable and have elevated ratios of nonsynonymous to synonymous substitutions. This suggests that they have evolved to detect variation in pathogen-derived ligands (McDowell and Woffenden, 2003). NBS domains occur in diverse proteins with ATP or GTP binding activity, such as ATP synthase subunits, ras proteins, ribosomal elongation factors, and adenylate kinases (Saraste et al., 1990; Traut, 1994).

Plant NBS-LRR proteins have two major subfamilies: TIR-NBS-LRR proteins (TNLs) and CC-NBS-LRR proteins (CNLs). TIR-NBS-LRR proteins (e.g. encoded by *Bs4* from tomato and *Y-1* from potato) have Toll/interleukin-1 receptor (TIR) motif while CC-NBS-LRR proteins (e.g. encoded by *I2*, *Mi*, *Prf* from tomato and *Rx* from potato) have coiled-coil (CC) motifs in the amino-terminal domain (McHale, 2006). R-genes have high levels of inter- and intra-specific variation but their rates of mutation or recombination are not very high (Kuang et al., 2004). Gene variation is caused by normal genetic mechanisms, including unequal crossing-over, sequence exchange, and gene conversion, but not genetic events particular to NBS-LRR-encoding genes (Mayers et al., 2003; Kuang et al., 2004; Baumgarten et al., 2003; Mondragon-Palomino and Gaut, 2005). Studies have revealed that clusters of R-genes may contain sequences with similar function, but not with similarity in sequence. For example, *Prf*, an NBS-

LRR gene, is within a cluster of five *Pto* homologs encoding protein kinases and *Mi* is also an NBS-LRR gene that is linked loosely with *Cf2,* a LRR-TM gene (McDowell and Woffenden, 2003).

There are two main groups of R gene-mediated resistance: dominant resistance genes and recessive resistance gene. Dominant R-genes are activated on effectors recognition and interact with pathogen molecules, either directly or indirectly (Martin et al., 2003; Soosaar et al., 2005). They limit or block viruses, while the allele for susceptibility does not provide resistance. Most of the dominant resistance genes are involved in resistance manifested by an HR or an extreme resistance (ER) (Maule et al., 2007). Recessive resistance genes show resistance when the host gene is unable to promote virus infection (Maule et al., 2007; Moffett, 2009). Recessive resistance appears to be more frequent for potyviruses than for viruses of other families (Kang et al., 2005).

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CHAPTER I

Genome-based Fine Mapping of the *Tomato spotted wilt virus* Resistance Gene, *Tsw,* in *Capsicum* spp.

ABSTRACT

Tsw, a single dominant resistant gene against *Tomato spotted wilt virus* (TSWV), has been mapped on chromosome 10 in *Capsicum chinense* species. Previously reported molecular markers linked to the *Tsw* gene are not transferable for all pepper breeding materials. To develop additional markers aiming at genome-based fine mapping of the *Tsw* gene, comparative mapping, pooled transcriptome analysis, and genome walking were performed. Eleven additional SNP molecular markers tightly linked to the *Tsw* gene were developed using tomato and pepper whole genome sequencing databases. Among them, four SNP markers SNP165256, SNP3065253, SNP67464, and SNP151705, showed no recombination in two segregating populations of F_2 'Telmo' (210 individuals) and 'SP' (843 individuals). Three scaffold sequences (Pep10kb.0.9_scaffold165256, scaffold67464, and scaffold151705) from the *C. annuum* genome database and two BAC clones (575C14RO and 464C8RF) from the BAC library of *C. annuum* 'CM334' covering the *Tsw* gene were identified. Finally, a scaffold sequence (PGAv.1.1.scaffold3065253) covering the three pepper scaffold sequences was

identified from *C. annuum* genome database. Alignment analysis of two BAC clone sequences and the PGAv.1.1.scaffold3065253 sequence, the *Tsw* gene was delimited within 149 kb between two co-segregating markers, SNP165256 and SNP151705. A total of 22 predicted genes were resided in the target region. Among them, five predicted genes encoding CC/TIR-NBS-LRR proteins mRNA-6, mRNA-7, mRNA-11, mRNA-12, and mRNA-13, were identified. Gene expression study showed that the mRNA-13 is expressed in 'PI152225' but was absent in 'Special'. This evidence demonstrated that the mRNA-13 could be a strong candidate gene for the *Tsw* gene. This result will be useful information for cloning the *Tsw* gene and developing TSWV resistant cultivars.

INTRODUCTION

Tomato spotted wilt virus (TSWV), which is transmitted in nature by thrips, belongs to the plant virus genus *Tospovirus* within the family *Bunyaviridae* (Francki et al., 1991). TSWV has the second widest host ranges of any plant virus infecting as many as 900 plant species (Peters, 1998). TSWV causes significant yield reduction in Solanaceous crops worldwide such as tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum* spp.) (Tomlinson, 1987). Pepper plants are highly susceptible to TSWV at all stages of development with severe symptom on leaves and fruits. The wide host range of TSWV and the occurrence of at least 13 species of thrips that transmit TSWV make eliminating the sources of primary inoculum of the virus impractical (John, 2005). Although controlling weeds, avoiding contaminated host plants near the vegetable crop, and eliminating thrips in greenhouses have been considered to be a good way to manage this problem (Swift, 2010), but cultivar choice has been the most consistent way to suppress TSWV epidemics.

The increasing problem of TSWV in pepper crops has strongly stimulated research for sources of genetic resistance. A single dominant resistance gene (*Tsw*) has been identified by various research groups in several accessions of *C. chinense* ('PI152225', 'PI159234', 'PI159236', 'CNPH 275', '7204', 'PI-15', 'C00943', 'ECU-973' and 'AC09-207') (Black et al., 1991; Boiteux et al., 1993; Jorda et al., 1994; Black et al., 1996; Moury et al., 1997; Jahn et al., 2000;

Cebolla-Cornejo et al., 2003; Hoang et al., 2013). The *Tsw* gene is known to be uneffective at the high temperature (28-32 ^{o}C) as with the *N* gene in *N. tobaccum* (Roggero et al., 1996; Moury et al., 1998). Furthermore, there has been reported that field isolates of TSWV overcome the *Tsw* gene (Roggero et al., 2002; Sharman and Persley, 2006). Recently, outbreaks of TSWV also reported in Jeonnam province in Korea (Mun et al., 2008).

Previously, the *Tsw* gene has been tagged with a random amplified polymorphic DNA (RAPD) ($Q\text{-}06_{270}$) that is located 3.45 cM from the *Tsw* locus, and this marker was mapped to the distal region of chromosome 10 (Jahn et al., 2000). Moury et al. (2000) also developed a co-dominant cleaved amplified polymorphic sequence (CAPS) marker named $SCAC_{568}$. This $SCAC_{568}$ marker is tightly linked to the *Tsw* locus about 0.9 ± 0.6 cM away from the gene. According to Kim et al. (2008), $SCAC_{568}$ applied on seven resistant and five susceptible lines did not show polymorphism between resistant and susceptible lines.

Most of disease resistance genes (R-genes) in plants that had been cloned to date encode proteins characterized by NBS, LRR domains and variable amino- and carboxy-terminal domains (McHale et al., 2006). In addition, the NBS domains are highly conserved in the plant genome (Yue et al., 2012). Classical genetic and molecular data showed that NBS-LRR-encoding genes in plants are frequently clustered in the genome, the result of both segmental and tandem duplication (Meyers et al., 2003). In the Solanaceae family, 33 R genes

have been isolated (Sanseverino et al., 2010). Three R-genes (*Bs2, Bs3,* and *CaMi*) conferring resistance to strains of *Xanthomonas campestris* pv. *vesicatoria* and root-knot nematodes have been identified from pepper (Tai et al., 1999; Römer et al., 2007; Chen et al., 2007). Among them, two genes (*Bs2* and *CaMi*) encode motifs characteristic of the NBS-LRR class of R-genes (Tai et al., 1999; Chen et al., 2007). Recent studies on R-gene homologues in tomato also demonstrated that R-genes frequently occur in clusters of related gene copies including NBS, receptor-like protein (RLP), and receptor-like kinase (RLK) genes. The syntenic position of *Tsw* in tomato contains multiple R-gene homologues (Andolfo et al., 2013). Grube et al. (2000) reported that the *Pvr4*, *Pvr7*, and *Tsw* genes are the first identified cluster of dominant R genes at a position on chromosome 10 in *Capsicum* L. Fine mapping of a gene is an important step for cloning of the gene. The objectives of this study are to develop additional markers tightly linked to the *Tsw* locus and to identify the *Tsw* gene by genome-based fine mapping. This study will provide not only useful information for cloning of the *Tsw* gene but also for developing TSWV resistance cultivars.

MATERIALS AND METHODS

Plant materials and DNA extraction

The two mapping populations used in fine mapping of *Tsw* were an F_2 population derived from F_1 hybrid cultivar 'Telmo' (Enza Zaden, The Netherlands) and an inter-specific F_2 population obtained from a cross between *C. annuum* 'Special' (Enza Zaden, The Netherlands) and *C. chinense* 'PI152225'. 'Telmo' F_2 population were consisted with 210 individuals. The *C. chinense* 'PI152225' contains the *Tsw* gene conferring dominant resistance to the TSWV (Jahn et al., 2000) and the *C. annuum* 'Special' is a susceptible cultivar (Kim et al., 2008). An F_2 population of 843 individuals obtained from a cross *C. annuum* 'Special' and *C. chinense* 'PI152225' were named 'SP' F_2 population. A segregating population of 109 F_2 individuals derived from 'Boojie' (Enza Zaden, The Netherlands) were used for pool transcriptome analysis. The 'Boojie' (Enza Zaden, The Netherlands) is also known to carry the *Tsw* gene. Total DNA was extracted following the cetytrimethylammonium bromide (CTAB) method (Hwang et al., 2009).

Virus material and inoculation

$TSWV_{Pap.}$ isolate was provided by Dr. Bong Nam Chung (National Institute of Horticultural and Herbal Science, Suwon, Korea). The inoculum of $TSWV_{Pap.}$ isolate was prepared from infected leaves of *Nicotiana rustica*. One

gram of diseased leaves was ground in 4 ml of phosphate buffer saline (TARAKA BIO INC., Japan). The pepper seedlings were inoculated with the $TSWV_{Pap.}$ isolate when two cotyledons were fully expanded and two true leaves began to appear. The seedlings were dusted with Carborundum #400 mesh (Hayashi Pure Chemical Ind., Japan) and inoculated by rubbing the virus onto two cotyledons. Control plants were mock-inoculated with phosphate buffer. After 15 to 20 min post-inoculation, the inoculated plants were sprinkled with tap water and kept in a growth chamber at 25^{o}C. The TSWV symptoms were first observed one week after inoculation, and the development of symptoms was monitored continuously until the experiment was completed.

Detecting TSWV by DAS-ELISA

DAS-ELISA was performed to detect the TSWV infection of inoculated plants at 15 days post-inoculation (dpi). The DAS-ELISA was done following the manufacturer's protocol (Agdia, USA). Samples were considered positive for the presence of TSWV if the absorbance value (405 nm) of each sample was three times greater than that of a healthy control plant.

Comparative maps

The TG420 marker linked to *Tsw* was used to identify a corresponding tomato scaffold sequence. Tomato scaffold sequence SL1.50sc06504 of 7,841,215 bp in length covering the corresponding region of the *Tsw* locus was identified in

Sol Genomic Network (SGN) (http//:solgenomics.net). The gene coding regions of the tomato scaffold were predicted by FGENESH (http://linux1.softberry.com). The predicted amino acid sequences were used to search for the annotated genes using the BLASTP program (http://www.ncbi.nlm.nih.gov). The sequences of gene coding regions tomato scaffold sequence were utilized to search for the homologous pepper sequences from *C. annuum* genome database (http://cab.pepper.snu.ac.kr). Two pepper scaffold sequences, Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492, were obtained from *C. annuum* 0.9 10kb 1st Scaffold version of *C. annuum* genome database (http://cab.pepper.snu.ac.kr), by BLASTN search program. Two identified pepper scaffold sequences were employed to develop markers linked to the *Tsw* locus.

Development of molecular markers linked to the *Tsw* gene

First of all, the primers were manually designed based on the intergenic regions of pepper contig/scaffold sequences by PrimerSelect program (DNASTAR, Inc., Madison, WI, USA) with PCR products of about 1,000 bp to 1,200 bp in size. The designed primers were used for parental screening using the high resoluation melting (HRM) analysis. The PCR was conducted on a Rotor-Gene™ 6000 thermocycler (Corbett, Australia) in 20 μl reaction mixtures containing 60 mM KCl, 10 mM Tris–Cl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 5 pmol of each primer, 1 unit *Taq* polymerase, 1.25 μM Syto9 (Invitrogen, USA),

and 50 ng genomic DNA (gDNA). Cycling conditions were at 95℃ for 4 min, followed by 95℃ for 20 s, 58℃ for 20 s, and then 45 cycles of 72℃ for 40 s. HRM analysis was run for every increment of 0.1℃ between 70℃ and 90℃. PCR products showing differences in melting curve pattern were sequenced to identify the SNP(s) position. To sequencing, the PCR products were separated on a 1% agarose gel in 0.5x TAE buffer containing ethidium bromide (EtBr) and visualized under UV light. The amplified bands were excised and purified with a Zymoclean PCR Purification Kit following the manufacturer's protocol (Invitrogen Korea, Seoul, Korea). Purified PCR products were sequenced at the National Instrumentation Center for Environmental Management (NICEM), Seoul National University, Seoul, Korea. Nucleotide sequences were aligned using SeqMan program (DNASTAR, Inc., Madison, WI, USA) to detect SNP(s) position. Secondly, to design the more producible SNP markers, the polymorphic sequences were used and designed primers to amplify PCR products smaller than 250 bp in size. Seven additional SNP markers, SNP246056-1, SNP246056-2, SNP246056-3, SNP246056-4, SNP653492-1, SNP653492-2, and SNP653492-3, were developed and used for mapping on F_2 segregation populations of 'Telmo' and 'SP' (Table 1).

Table 1. Fine mapping results of SNP markers for the *Tsw* gene.

Marker name	Number of recombinants (Recombinants/Total individuals)		Reference
	'Telmo'	'SP'	
SCAC568	-	16/843	Moury et al. (2000)
SNP246056-1	5/210	8/843	This study
SNP246056-2	-	5/843	This study
SNP246056-3	-	4/843	This study
SNP246056-4	4/210	3/843	This study
SNP165256	0/210	0/843	This study
SNP3065253	0/210	0/843	This study
SNP67464	0/210	0/843	This study
SNP151705	0/210	0/843	This study
SNP653492-1	1/210	2/843	This study
SNP653492-2	1/210	5/844	This study
SNP653492-3	1/210	7/844	This study

- No polymorphism

Among them, two SNP markers (SNP246056-4 and SNP653492-1) tightly linked to the *Tsw* locus were located at the lower and upper sites of the *Tsw* gene, respectively (Table 1). However, a gap between two scaffold sequences of Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492 was existed in *C. annuum* genome database. To fill the gap, two approaches were carried out: (1) Pool transcriptome analysis and (2) BAC library screening.

Bulk segregant analysis (BSA) and pooled transciptome analysis

An F_2 population of 'Boojie' was used for BSA instead of using the F_2 'SP' population due to lack of the inter-specific F_2 'SP' population. Thus, 109 individuals of 'Boojie' F_2 population were genotyped by using two tightly linked markers, SNP246056-4 (0.35 cM) and SNP653492-1 (0.23 cM), which located at the upper and lower positions of the *Tsw* locus, respectively. Thirty-one homozygous resistant plants and thirty-three homozygous susceptible plants were identified. Thirty individuals of each homozygous resistant and susceptible genotype were utilized for pooling a resistance bulk (R-bulk) and a susceptible bulk (S-bulk) for transciptome analysis, respectively. For the R-bulk, a young leaf disc of each resistant individual was sampled with the same plant tissue amount and pooled into the R-bulk. The S-bulk was performed the same way as the R-bulk but using susceptible individuals. The homozygous resistant and susceptible pools were used to extract total RNA separately. Total RNAs were extracted using

RNeasy ® Plant Mini Kit (QIAGEN, USA) following the method described by the manufacturer. Total RNA products of R- and S-bulk were sequenced at NICEM. Low-quality transcriptome sequences of R- and S-bulks were filtered and removed using quality trimming program (Q<20). *De novo* programming was carried out to assemble the high-quality transcriptome sequences.

BAC library screening

A bacterial artificial chromosome (BAC) library consisting of 235,000 clones covering 12x pepper genome (99%) constructed from *C. annuum* 'CM334' (Yoo et al., 2003) was used to develop 2D BAC pools. BAC pools were screened using the flanking markers, SNP246056-4 and SNP653492-1, as the probes. Positive BAC clones were sequenced for to obtaine BAC-end sequence using SP6 and T7 primers. BAC-end sequences were aligned with two sequences of Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492 as references by BLASTN program ((http://www.ncbi.nlm.nih.gov). The extended BAC-end sequences were used to search for the homologous pepper sequences in *C. annuum* genome database (http://cab.pepper.snu.ac.kr). The gene-coding regions and amino acid sequences of scaffold sequences were identified by FGENESH program (http://linux1.softberry.com). The amino acid sequences were used to search for the annotated genes using the BLASTP program (http://www.ncbi.nlm.nih.gov).

RESULTS

Development of SNP markers tightly linked to the *Tsw* locus

A tomato scaffold sequence with ID of SL1.50sc06504 of 7,841,215 bp in size corresponding to the region of the *Tsw* locus was obtained from Sol Genomic Network (SGN) (http//:solgenomics.net) using the sequence of *Tsw*-linked marker (TG420). The TG420 sequence of 487 bp in length was located from 4,480,971 bp to 4,481,457 bp on the tomato scaffold sequence. Total of 165 predicted genes were identified by analyzing the flanking region (1,120,822 bp in size) of tomato scaffold sequence between TG420 and TG408 markers (data not shown). Two scaffold sequences of Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492 were obtained from http://cab.pepper.snu.ac.kr using the gene-coding regions of the tomato scaffold sequence. Gene prediction analysis of two pepper scaffold sequences showed 40 and 112 predicted genes residing on two sequences of the Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492, respectively (data not shown). Seven additional SNP markers, SNP246056-1, SNP246056-2, SNP246056-3, SNP246056-4, SNP653492-1, SNP653492-2, and SNP653492-3 showing polymorphisms between resistant (‘Telmo’ and ‘PI152225’) and susceptible (‘Special’) parents, were developed (Table 1). Of those, four SNP markers (SNP246056-1, SNP246056-2, SNP246056-3, and SNP246056-4) were derived from the

Pep10kb.0.9_scaffold246056 sequence and three SNP markers (SNP653492-1, SNP653492-2, and SNP653492-3) were obtained from the Pep10kb.0.9_scaffold653492 sequence. Among seven additional SNP markers, five SNP markers including SNP246056-1, SNP246056-4, SNP653492-1, SNP653492-2, and SNP653492-3, were mapped in F_2 'Telmo' segregating population. SNP246056-1 and SNP246056-4 markers showed five and four recombinations about 2.38 cM and 1.90 cM away from the gene, respectively. SNP246056-1 and SNP246056-4 markers were located at the upper the *Tsw* locus. One recombinant was identified in three SNP653492-1, SNP653492-2, and SNP653492-3 markers, whose position were about 0.47 cM away from the gene and located the other side of SNP246056-1 and SNP246056-4 (Table 1 and Fig. 1).

Molecular markers SNP246056-2, SNP246056-3 and $SCAC_{568}$ (Moury et al. 2000) could not be mapped in 'Telmo' F_2 population due to lack of polymorphism (data not shown). To develop markers more easily, a new inter-specific F_2 population was constructed by crossing *C. annuum* 'Special' and *C. chinense* 'PI152225'. Seven SNP markers (SNP246056-1, SNP246056-2, SNP246056-3, SNP246056-4, SNP653492-1, SNP653492-2, and SNP653492-3) and the $SCAC_{568}$ marker were mapped on 843 individuals of F_2 'SP' population.

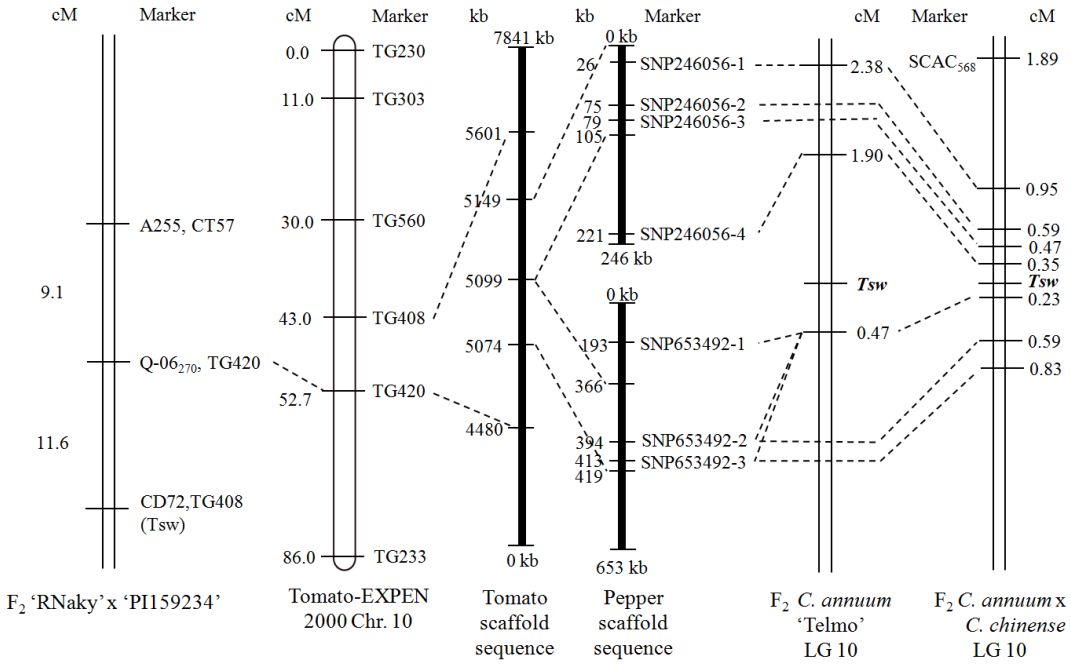


Fig. 1. Comparison analysis of the *Tsw* region in between pepper and tomato maps. The *Tsw* gene was initially mapped near to TG420 marker on LG 10 (Jahn et al. 2000). The TG420 marker is located on Chr. 10 of Tomato-EXPEN 2000 map (http://solgenomics.net). A tomato scaffold sequence with ID of SL1.50sc06504 of 7,841 kb in length was identified based on the sequence of TG420 marker (http//:solgenomics.net). Pepper scaffold sequences were obtained from *Capsicum* genome database (http://cab.pepper.snu.ac.kr). $SCAC_{568}$ is a *Tsw*-linked marker which developed by Moury et al. (2000). *Dotted lines* indicate makers/linkage regions in pepper and tomato maps, cM means centiMorgan, and kb means kilobase.

Eight molecular markers of $SCAC_{568,}$ SNP246056-1, SNP246056-2, SNP246056-3, SNP246056-4, SNP653492-1, SNP653492-2, and SNP653492-3 showed 16, 8, 5, 4, 3, 2, 5, and 7 recombinations, respectively. Among them, markers SNP246056-4, about 0.35 cM above the *Tsw* locus, and SNP653492-1, about 0.23 cM below the *Tsw* locus, were the closest markers to *Tsw* (Table 1 and Fig. 1). These recombinant plants were susceptible to TSWV. Development of additional markers linked to the *Tsw* gene at the end regions of two scaffold sequences (Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492) was failed because the region located from 221 kb to 246 kb of Pep10kb.0.9_scaffold246056 sequence and the sequence region from 0 kb to 193 kb of Pep10kb.0.9_scaffold653492 sequence exhibited highly repetitive nature and multiple copies (data not shown). For example, two markers developed from Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492 sequences showed different recombinations with two closest markers (SNP246056-4 and SNP653492-1) in F_2 'SP' population (data not shown). Sequence analysis of the two markers showed that they contain multiple copy sequences. Analyzing the repetitive regions of Pep10kb.0.9_scaffold246056 (from 95 kb to 246 kb) and Pep10kb.0.9_scaffold653492 (from 0 kb to 234 kb) sequences showed that four and ten predicted genes belonging to NBS-LRR genes, respectively. At that time, the complete *C. annuum* genome database was not available. To fill the gap between those two scaffold sequences of Pep10kb.0.9_scaffold246056 and

Pep10kb.0.9_scaffold653492, two approaches were carried out: (1) fourteen NBS-LRR sequences were used to identify candidate genes in the transcriptome sequence of R-bulk and (2) flanking markers (SNP246056-4 and SNP653492-1) were used as probes for BAC library screening and genome walking.

Pooled transcriptome analysis

A total of 89,616 contig/singlet transcriptome sequences of 163,562,853 bp in length with the largest contig of 19,313 bp in length were obtained by sequencing the R- and S-bulks. Seventy-nine transcriptome sequences were identified using fourteen NBS-LRR sequences blasted to 89,616 transcriptome sequences. Among them, 55 NBS-LRR sequences showed differential expression by digital expression cufflink analysis. Three scaffold sequences (Pep10kb.0.9_scaffold165256, scaffold67464, and scaffold151705) were identified by *in silico* mapping of 55 NBS-LRR sequences to whole genome sequencing of *C. annuum* genome database (Fig.2a).

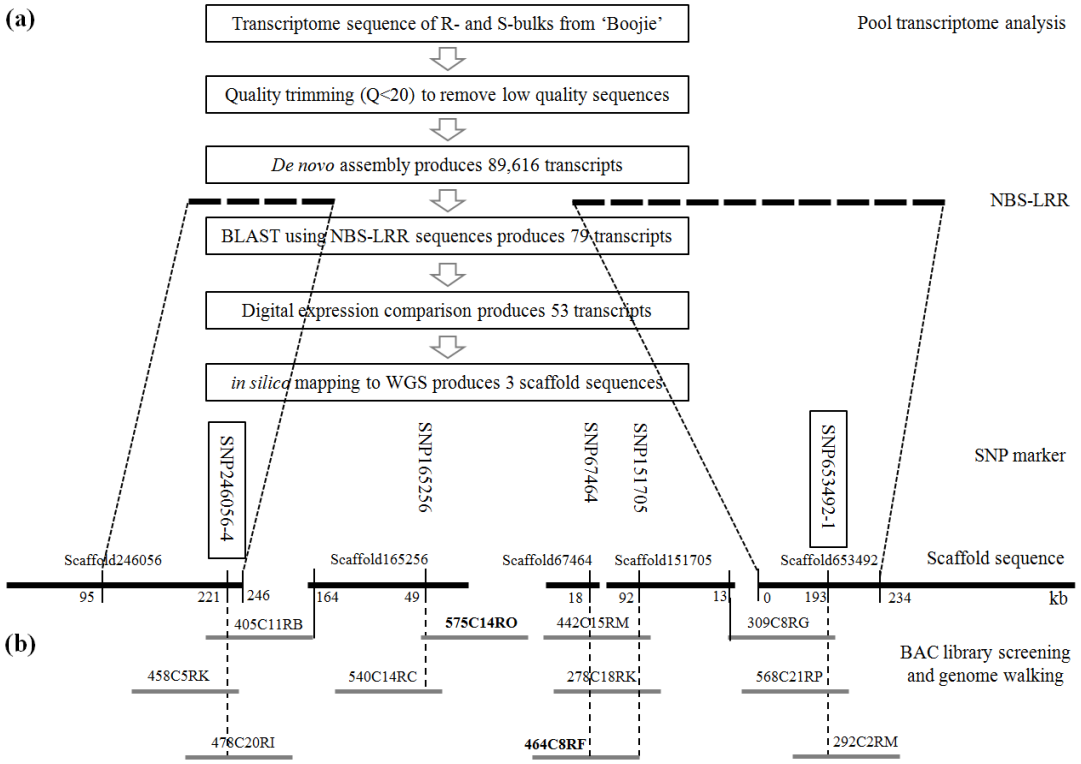


Fig. 2. Analysis of pooled transcriptome and BAC library screening. (a) Pool transcriptome analysis. Two SNP markers with closed frame were flanking markers. Three pepper scaffold sequences located between two flanking markers were identified by transcriptome analysis using NBS-LRR sequences which derived from flanking scaffold regions. *Spotted lines* indicate locations of NBS-LRR genes. (b) BAC library screening and genome walking. The numbers under the scaffold sequences indicate position with unit of kilobase (kb). *Dotted lines* indicate BAC amplification using SNP markers. Lines indicate identical sequences between BAC-end sequence and scaffold sequence (reference sequence). BAC clones with bold indicate selected BAC clones for BAC sequencing.

Three CAPS markers, CAPS20172-2, CAPS17918-1, and CAPS1072-2, which were developed from three scaffold sequences of Pep10kb.0.9_scaffold165256, scaffold67464, and scaffold151705, respectively were provided by Prof. Doil Choi (Department of Plant Science, Seoul National University, Seoul, Korea). Three SNP markers (SNP165256, SNP67464 and SNP151705) that were modified from three CAPS markers (CAPS20172-2, CAPS17918-1, and CAPS1072-2) showed complete co-segregation with the *Tsw* gene (Table 1, Fig. 2a, and Fig.3a).

BAC library screening and genome walking

Six BAC clones were identified with two *Tsw* flanking markers SNP246056-4 and SNP653492-1. Three BAC clones (405C11RB, 458C5RK and 478C20RI) were amplified by SNP246056-4 marker and three BAC clones (309C8RG, 568C21RP, and 292C7RM,) were amplified by SNP653492-1 marker (Table 2 and Fig. 2b). A total of six positive BAC clones were confirmed by sequencing PCR product of two markers SNP246056-4 and SNP653492-1. Sequences of BAC clones that were completely identical between the marker regions and the amplified BAC clones were selected for BAC-end sequencing.

Table 2. Molecular markers used for BAC library screening

Flanking maker/primer	BAC clone
SNP246056-4	405C11RB, 458C5RK, 478C20RI
SNP653492-1	309C8RG, 568C21RP, 292C7RM
SNP165256	540C14RC, 575C14RO
SNP67464	442C15RM, 278C18RK, 464C8RF
SNP151705	442C15RM, 278C18RK, 464C8RF

The analysis of BAC-end sequences of six BAC clones with two scaffold sequences (Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492) as reference sequences revealed that two BAC clones 405C11RB and 309C8RG are the most extended BAC clones of Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492 sequences, respectively. Two additional scaffold sequences (Pep10kb.0.9_scaffold165256 and scaffold151705) were identified from *C. annuum* genome database using BAC-end sequences of BAC 405C11RB and 309C8RG, respectively (Fig.2a). This result demonstrated that approaches used in this study could fill the gap between two scaffold sequences of Pep10kb.0.9_scaffold246056 and Pep10kb.0.9_scaffold653492. However, the gaps still existed between three scaffold sequences (Pep10kb.0.9_scaffold165256, scaffold67464, and scaffold151705) (Fig. 2a). To fill the gaps, genome walking was carried out by second BAC library screening. Three SNP markers, SNP165256, SNP67464 and SNP151705, were used as probes for BAC library screening. Five BAC clones were identified (Table 2 and Fig.2b). BAC-end sequences analysis showed that two BAC clones, 575C14RO and 464C8RF, could fill the gaps between three scaffold sequences, Pep10kb.0.9_scaffold165256, scaffold67464, and scaffold151705 (Fig.2a). Thus, the 575C14RO and 464C8RF clones were selected for BAC sequencing to identify the candidate gene of the *Tsw* gene.

Fine mapping of the *Tsw* gene

Three SNP markers including SNP165256, SNP67464, and SNP151705 were mapped in F_2 'Telmo' and F_2 'SP' populations and no recombinants were identified (Table 1 and Fig. 3a). Eventually, a pepper scaffold sequence of PGAv.1.1.scaffold3065253 covering four scaffold sequences (Pep10kb.0.9_scaffold165256, scaffold67464, scaffold151705, and Pep10kb.0.9_scaffold653492) was identified from Pepper Scaffold V.1.1 version of *C. annuum* genome database. Three co-segregating markers, SNP165256, SNP67464, and SNP151705, were located at 2798 kb, 2678 kb, and 2652 kb on the PGAv.1.1.scaffold3065253 sequence, respectively (Fig. 3b). Polymorphic markers were hard to develop in this target region (between 2652 kb and 2798 kb) of the PGAv.1.1.scaffold3065253 sequence because this region showed highly repetitive sequences. However, a SNP marker (SNP3065253) was developed from the target region at 2718 kb of the PGAv.1.1.scaffold3065253 sequence and no recombination was identified in two F_2 'Telmo' and 'SP' populations (Table 1 and Fig. 3). Furthermore, several incompletely assembled regions located at the target region of the PGAv.1.1.scaffold3065253 sequence showing N nucleotides (nucleotide sequences that could not be determined by NGS sequencing) were identified (Fig. 3b). Thus, the contig sequences of 575C14RO and 464C8RF BAC clones were used to obtain more precise sequence of the PGAv.1.1.scaffold3065253.

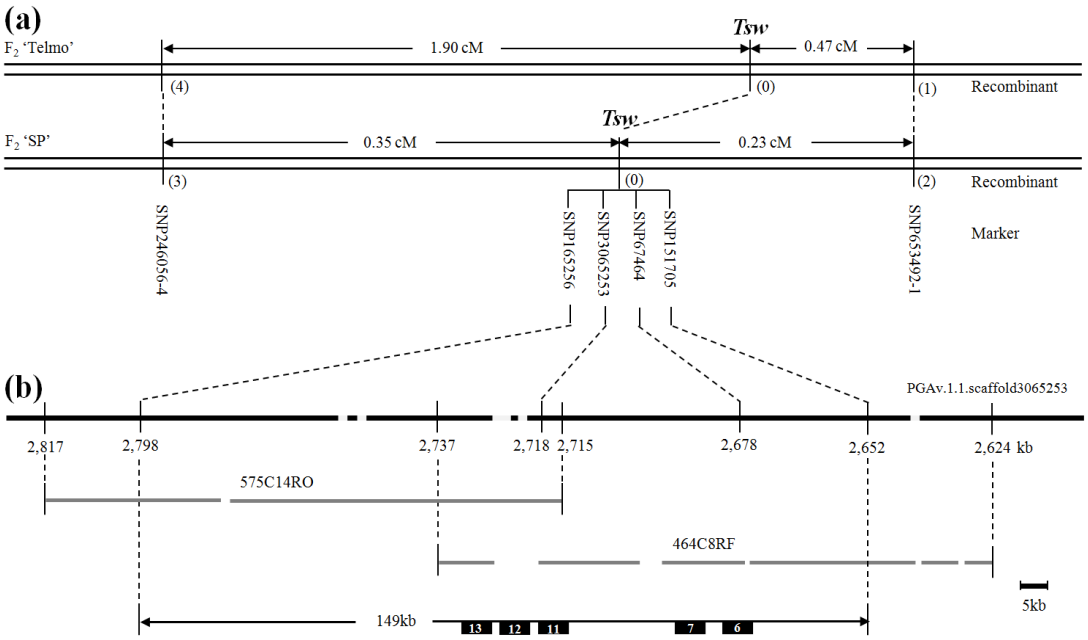


Fig. 3. Genetic and physical maps of the *Tsw* gene. (a) Genetic map of the *Tsw* gene on two F_2 'Telmo' and 'SP' populations. The numbers in the parentheses indicate the recombinantion(s) between co-segregating markers and the *Tsw* gene. The numbers in the two-way arrow indicate the genetic distance between flanking markers and the *Tsw* gene (cM). (b) Physical map of the *Tsw* locus on pepper scaffold sequences. The numbers below the PGAv.1.1.scaffold3065253 scaffold sequence indicate the position with unit of kilobase (kb). 575C14RO and 464C8RF were two BAC clones. *The number in the two-way arrow* indicates the physical distance of the *Tsw* region (kb). The short and white bars on sequence indicate regions with the N nucleotides. The short- and long- grey bars indicate the BAC contig sequences. The thick black bars with white numbers indicate the candidate genes of the *Tsw* gene.

BAC clone sequencing and analysis

Sequences of BAC clones of 575C14RO and 464C8RF were obtained. BAC contig sequence of the 575C14RO clone was about 104,771 bp in length with the largest contig of 58,096 bp in length and 25 BAC contig sequences of the 464C8RF clone was about 118,954 bp in length with the largest contig of 41,379 bp in length. The BAC contig sequences were used to align with the PGAv.1.1.scaffold3065253 as a reference. The regions with N nucleotides in the target region of the PGAv.1.1.scaffold3065253 sequence were replaced by sequences from BAC contig sequences. The *Tsw* gene was defined within 149 kb between SNP165256 and SNP151705 co-segregating markers (Fig. 3b). A total of 22 predicted genes were identified with FGENESH program. Four predicted genes, mRNA-6, mRNA-7, mRNA-12 and mRNA-13, showing annotations of CC/TIR-NBS-LRR resistance proteins were identified by BLASTP program in NCBI. Two predicted genes (mNRA-11 and mRNA-14) showed homology with disease resistance protein RGH5 in *Solanum chacoense* and disease resistance protein *Bs2* in *C. chacoense*, respectively (Table 3). However, the predicted gene of mRNA-14 was 345 bp in length, so it may be a part of the disease resistance gene *Bs2* in *C. chacoense*. Thus, five predicted genes including the mRNA-6 (3,573 bp), mRNA-7 (3,486 bp), mRNA-11 (2,874 bp), mRNA-12 (4,623 bp) and mRNA-13 (2,718 bp) were selected as candidate genes of the *Tsw* gene (Fig. 3b).

Table 3. Annnotations of predicted genes in the target region of the *Tsw* gene

Predicted gene	Length		Description
	bp	aa	
mRNA-1	2355	785	PREDICTED: probable disease resistance protein At4g27220-like [*Solanum lycopersicum*]
mRNA-2	231	76	No significant similarity found
mRNA-3	837	278	Hypothetical protein VITISV_001383 [*Vitis vinifera*]
mRNA-4	2190	729	Hypothetical protein VITISV_017889 [*Vitis vinifera*]
mRNA-5	3780	1259	Putative gag-pol polyprotein, identical [*Solanum demissum*]
mRNA-6	3573	1190	CC-NBS-LRR resistance protein [*Medicago truncatula*]
mRNA-7	3486	1161	TIR-NBS-LRR type disease [*Arachis hypogaea*]
mRNA-8	654	217	PREDICTED: putative germin-like protein 2-3-like [*Solanum lycopersicum*]
mRNA-9	2511	836	Hypothetical protein KNAG_0A03960 [*Kazachstania naganishii* CBS 8797]
mRNA-10	504	167	PREDICTED: putative germin-like protein 2-3-like [*Solanum lycopersicum*]
mRNA-11	2874	957	Disease resistance protein RGH5 [*Solanum chacoense*]
mRNA-12	4623	1540	CC-NBS-LRR resistance protein [*Medicago truncatula*]
mRNA-13	2718	905	CC-NBS-LRR resistance protein [*Medicago truncatula*]
mRNA-14	345	114	Disease resistance protein *Bs2* [*Capsicum chacoense*]
mRNA-15	198	62	No significant similarity found
mRNA-16	909	302	PREDICTED: twitchin-like [*Metaseiulus occidentalis*]
mRNA-17	246	81	PREDICTED: DNA-directed RNA polymerase III subunit RPC2-like [*Solanum lycopersicum*]
mRNA-18	2754	917	PREDICTED: U5 small nuclear ribonucleoprotein 40 kDa protein-like [*Solanum lycopersicum*]
mRNA-19	3705	1234	PREDICTED: DNA-directed RNA polymerase III subunit RPC2-like [*Solanum lycopersicum*]
mRNA-20	3129	1042	PREDICTED: DNA-directed RNA polymerase III subunit RPC2-like [*Solanum lycopersicum*]
mRNA-21	996	331	PREDICTED: peroxidase 15-like [*Solanum lycopersicum*]
mRNA-22	1257	418	PREDICTED: uncharacterized protein LOC101220497 [*Cucumis sativus*]

Gene expression analysis of candidate genes

Five selected candidate genes from *C. annuum* 'CM334' genome were used to BLASTN with 55 NBS-LRR sequences from *C. chinense* R-bulk. The results showed that the mRNA-6, mRNA-7, mRNA-11, mRNA-12, and mRNA-13 were 31, 1, 2, 42 and 33 of NBS-LRR sequences with 0.0 Evalue, respectively. Among them, the mRNA-12 and mRNA-13 showed higher identities from 85% to 93% than others (mRNA-6, mRNA-7, mRNA-11) (Table 4). In addition, the mRNA-12 and mRNA-13 contain 8 and 1 exons, respectively (data not shown). One complete open reading frame (ORF) in the mRNA-12 and mRNA-13 candidate genes was identified by the translate tool (http://web.expasy.org/translate/), respectively (Table 4). The mRNA-13 showed expression in 'PI152225' but was not in 'Special' (Fig. 4), while the mRNA-12 did not showed expression both 'PI152225' and 'Special' (data not shown).

Table 4. Transcriptome analysis and domain types of the candidate genes

Candidate gene	Length		Number of transcriptome sequence	Identities (%)	Domain type	ORF
	(bp)	(aa)				
mRNA-6	3573	1190	33	76-82	2 NB-ARCs	1
mRNA-7	3486	1161	2	78-80	ERT	1
mRNA-11	2874	957	1	81	NB-ARC	1
mRNA-12	4623	1540	42	87-93	NB-ARC	1
mRNA-13	2718	905	31	85-89	NB-ARC	1

ORF, open reading frame
NB, nucleotide binding and ARC, Apaf-1, R proteins, and CED-4
ERT, Endonuclease-reverse transcriptase

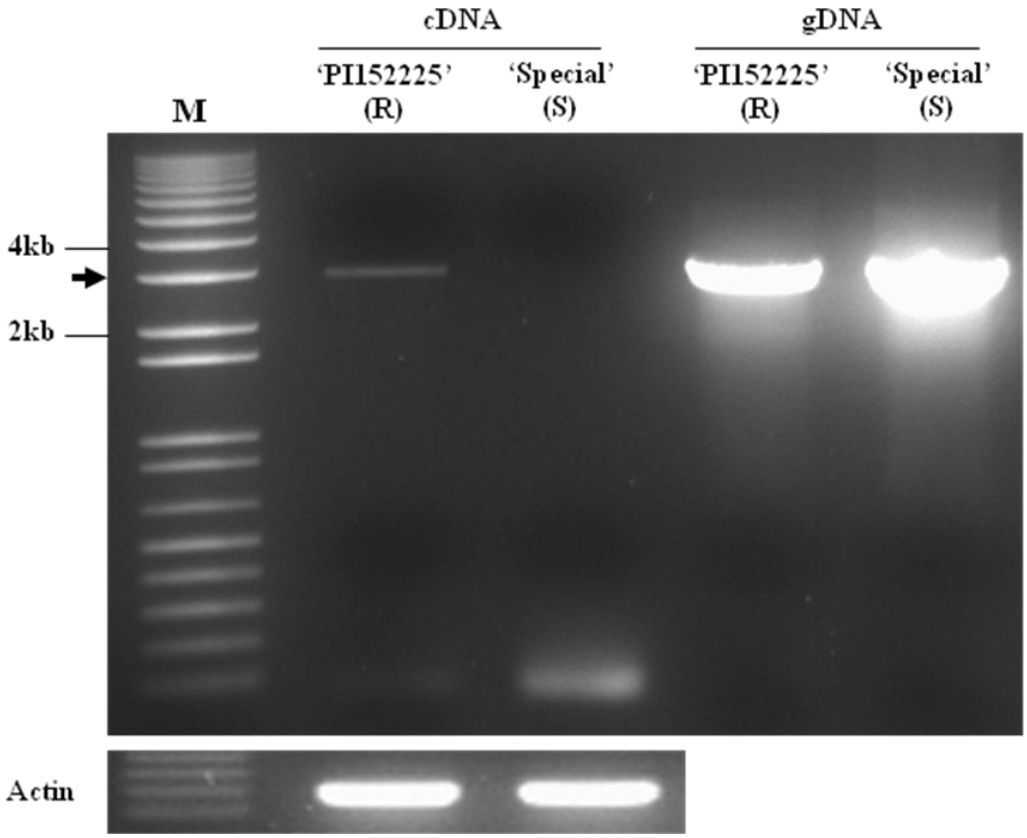
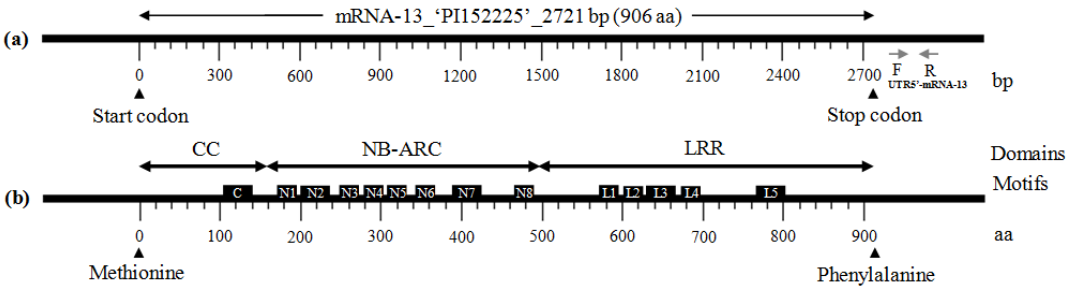


Fig. 4. Expression of the mRNA-13 candidate gene. ORF was expressed in cDNA of 'PI152225' but was absent in cDNA of 'Special'. R and S indicate resistance and susceptibility, respectively. M is molecular ladder. Black arrow indicates the target size of the ORF. Actin was a control expression.

The structure of candidate gene and marker analysis

A set of primers (UTR3'-mRNA-13F and UTR5'-mRNA-13R) was developed at untranslates regions of 3 and 5 primes (UTR3' and UTR5') of the mRNA-13 gene. The PCR was carried out by using cDNA of 'PI152225' as tamplate. The mRNA-13 gene was coloned using T-Blunt vector following the manufacturer's protocol (SolGent, Daejeon, Korea). The mRNA-13 gene was sequenced at NICEM. Nucleotide sequence alignment between mRNA-13 candidate genes, which isolated from *C. annuum* 'CM334' genome database (2,718 bp) and *C. chinense* 'PI152225' (2,721 bp and Fig. 5a), showed 89% of identities (data not shown). This result indicated that the mRNA-13 gene in *C. chinense* 'PI152225' is significantly different from the mRNA-13 gene in *C. annuum* 'CM334'. To confirm the candidate gene, a set of primers (UTR5'-mRNA-13F and UTR5'-mRNA-13R) was developed at UTR5' of the mRNA-13 gene using the sequence of 'PI152225' (Fig. 5a). Marker analysis showed that UTR5'-mRNA-13 was co-segregation on F_2 'SP' population (Fig. 5c). Furthermore, the structure analysis of mRNA-13 gene showed that the mRNA-13 of 'PI152225' genotype belonged to the CC-NBS-LRR resistant protein (Fig. 5b). Transcriptome analysis, gene expression, sequence alignment, and marker and structure analysis suggested that the mRNA-13 gene could be a strong candidate gene for the *Tsw* gene.



(c)

F_2 'SP' individuals	1-18	3-14	3-39	4-3	4-22	4-24	4-39	4-45	4-66	5-1	5-26	5-40	6-1	6-8	6-18	6-23	6-103	7-5	7-30	8-2	8-19	9-37	9-41
Phenotype	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	S	R	R	R	S	S
SCAC568	R	S	R	S	S	R	S	R	R	R	R	S	R	S	R	S	S	R	R	S	S	R	R
SNP246056-1	S	H	H	S	S	H	S	H	S	H	H	S	H	S	H	H	H	H	H	H	S	S	S
SNP246056-2	S	H	S	S	S	H	S	H	S	H	H	S	S	S	H	H	H	H	H	H	H	S	S
SNP246056-3	S	H	S	S	S	H	S	H	S	H	H	S	S	S	S	H	H	H	H	H	H	S	S
SNP246056-4	S	H	S	S	S	H	S	H	S	S	H	S	S	S	S	H	H	H	H	H	H	S	S
SNP165256	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	S	R	R	R	S	S
UTR5'-mRNA-13	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	S	R	R	R	S	S
SNP3065253	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	S	R	R	R	S	S
SNP67464	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	S	R	R	R	S	S
SNP151705	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	S	R	R	R	S	S
SNP653492-1	S	H	S	S	S	S	H	H	S	S	S	H	S	S	S	H	H	S	H	H	H	S	S
SNP653492-2	S	H	S	H	S	S	H	H	S	S	S	H	S	H	S	H	H	S	S	H	H	S	S
SNP653492-3	S	H	S	H	H	S	H	S	S	S	S	H	S	H	S	H	H	S	S	H	H	S	S

Fig. 5. The structure of mRNA-13 and markers analysis. (a) Sequence of mRNA-13 gene isolated from *C. chinense* 'PI152225' accession. UTR5'-mRNA-13 was a SNP marker which developed from untranslated region at 5' of mRNA-13 gene. F and R indecated forward and reverse primers, respectively. (b) Gene structure of mRNA-13. CC indicated coiled coil domain and C was coiled coil motif. NB-ARC was domain type with NB (nucleotide binding) and ARC (Apaf-1, R proteins, and CED-4). N1, N2, N3, N4, N5, N6, N7, and N8 indiacted P-loop, RNBS-A, Kinase2, RNBS-B, RNBS-C, GLPL, RNBS-D, and MHD motifs, respectively. LRR was domain type with L (leucine), R (rich), and R (repeat). L1, L2, L3, L4, and L5 indicated LRR-Sol1, LRR-Sol1/2, LRR-Sol3, LRR-Sol1, and LRR-Sol4 motifs, respectively. (c) Markers genotype. S, R, and H indicated susceptible ('Special'), resistant ('PI152225'), and heterozygous genotypes, respectively.

DISCUSSION

This is the first study of genome-based fine mapping of the *Tsw* gene using the whole genome sequencing of *C. annuum* genome database. In this study, the *Tsw* were fine mapped using two segregating populations and SNP markers. SNP markers were developed based on comparative mapping approach of tomato and pepper. A limitation of comparative mapping approach was that tomato scaffold sequences lack the corresponding region to the *Tsw* gene in pepper. Park et al. (2011) reported that the genome size of tomato is three times smaller than that of pepper and some sequences are pepper specific. Thus, bulked segregant transcriptome analysis using a *Tsw* resistance segregating population was also carried out to identify the *Tsw* gene.

Tsw, a single dominant resistant gene (R-gene) against to TSWV, was characterized with HR phenotype in several *C. chinense* species (‘PI152225’, ‘PI159234’, ‘PI159236’, and ‘AC09-207’ (Hoang, et al. 2013). The *Tsw* gene has been mapped on chromosome 10 in *C. chinense* species (Jahn et al., 2000; Moury et al., 2000). Recently, an alternative method to map-based gene cloning is the candidate gene approach utilizing a hypothesis-based method of gene identification. This approach is a useful and rapid method because a known gene controlling its trait in one organism may often have the same function in a related organism. Particularly, the genome sequence of tomato is much similar to the

genome of pepper. Thus, the candidate gene approach was applied in pepper and successfully isolated several resistance genes in pepper such as the *L* locus for tobamovirus resistance, the *pvr1* and *pvr2* looci for potyvirus resistance and the *A* and *cl* loci for fruit color (Mazourek and Wyatt, 2013). The *Sw5* gene against TSWV in tomato was mapped on chromosome 9 (Stevens et al., 1995) whereas the *Tsw* gene resistance to TSWV in pepper was mapped on chromosome 10 (Janh et al., 2000; Moury et al., 2000). Thus, map-based cloning approach was used in this study. This approach has been successfully identified several R-genes from Solanaceae such as *Sw5* (Brommonschenkel and Tanksley, 1997), *Bs3* (Jordan et al., 2006), and *Bs2* (Thai et al., 1999).

According to Mazourek and Wyatt (2013) reported that plant genomes contain regions with highly repetitive sequences and suppressed recombination where map-based cloning of the target genes is much more difficult. Furthermore, the duplication of segment and tandem of plant genome makes NBS-LRR genes frequently clustered (Meyers et al., 2003) and NBS domain highly conserved in plant genome (Yue et al., 2012). Most of R-genes in the plants that have been cloned to date encode proteins characterized by NBS and LRR domains (McHale et al., 2006). Recently, analysis of genome-wide arrangement of R-gene homologues in tomato also demonstrated that R-genes frequently occur in clusters of related gene copies that include NBS, RLP, and RLK genes. The syntenic position of *Tsw* in tomato contains multiple R-gene homologues (Andolfo et al.,

2013). Grube et al. (2000) reported that *Tsw* together with *Pvr4* and *Pvr7* (dominant genes resistance to potyvirus) are clustered within a 30 cM interval. Kang et al., (2005) pointed out that one type of R-gene cluster contains a set of genes, showing similar inheritance and resistance phenotypes that control very closely related viral genotypes. Those evidences support idea that known R-genes of a plant species could be used to identify known or novel R-genes in other plant species. Wan et al. (2012) successfully isolated R-gene analogues in pepper using highly conserved motifs within the NBS domain from other crops. The *Tsw* gene was defined within 149 kb in region with highly repetitive sequences of *C. annuum* 'CM334' genome database.

A predicted gene of the mRNA-13 belonged to CC-NBS-LRR resistance protein was selected as a strong candidate for *Tsw* by gene expression study. However, genotype of *C. annuum* 'CM334' was susceptible to TSWV. Thus, the mRNA-13 of 'CM334' cannot be the *Tsw* gene. The transcriptome sequencing provides a rapid, inexpensive approach to access gene sequences, gene expression abundances, and gene expression patterns in any species. Applications of RNA sequencing in conjunction with *de novo* transcriptome assembly have been successfully enabled the identification of new genes in an array of biochemical pathways in plants. While sequencing technologies are well developed, challenges remain in the handling and analysis of transcriptome sequences (Góngora-Castillo and Buell, 2013). RNA-sequences often rely on aligning short reads to a reference

genome and are thus unsuitable for analyzing resistance to most plant pathogens, as their genomes have not been fully sequenced (Yazawa et al., 2013). The transcriptome sequences of *C. chinense* 'Boojie' were hard to assemble *de novo* due to lack of a reference genome of *C. chinense*. However, the transcriptome sequences of *C. chinense* 'Boojie' were aligned to the reference genome of *C. annuum* 'CM334' due to the NBS domain showing highly conservation in plant genome (Yue et al., 2012).

The original resistance source of the *Tsw, Pvr4* and *Pvr7* genes is from *C. chinense* 'PI159236' (Grube et al., 2000). Thus, genome of *C. chinense* 'PI159236' is being sequenced. The full genome sequences of *C. chinense* 'PI159236' will be useful not only to reveal the structure of the *Tsw* gene but also identify locations and reveal the structure of the *Pvr4* and *Pvr7* loci. Further study, co-expression assay using mRNA-13 candidate gene and the avirulence factor of *Tsw* should be done to confirm the mRNA-13 candidate gene.

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CHAPTER II

Identification and Inheritance of a New Source of Resistance Against *Tomato spotted wilt virus* (TSWV) in *Capsicum* spp.

ABSTRACT

Tomato spotted wilt virus (TSWV) is an important viral disease affecting pepper production worldwide. A single dominant resistance gene, *Tsw*, originating from *Capsicum chinense* has been identified and utilized during the last several decades. However, there have been reports that *Tsw* resistance can be overcome by new field isolates of TSWV. This has necessitated the identification of a new source of resistance. Here, a set of pepper germplasm collections comprising 487 accessions from six *Capsicum* species and 30 commercial F_1 hybrids was evaluated for resistance to $TSWV_{Pap}$. A new resistance source, *C. chinense* 'AC09-207', was identified and characterized. Genetic analysis showed that the resistance in *C. chinense* 'AC09-207' was conferred by a single dominant gene. The resistance responses of 'AC09-207' were compared with other known resistance sources. The timing and number of necrotic response were similar to *C. chinense* 'PI152225', whereas the premature abscission of inoculated cotyledons and leaves were significantly different from other resistance sources, 'PI152225' and 'PI159236'. To compare genome locations between the new resistance gene

and *Tsw*, an allelism test was conducted. No recombinants were found in all F_1, F_2 and reciprocal backcross populations derived from the new resistance source and three known resistance sources ('PI152225', 'PI159236', and 'PI159234') demonstrating that the new resistance gene may be a unique allele at the *Tsw* locus or be controlled by a different gene tightly linked to *Tsw*.

INTRODUCTION

Tomato spotted wilt virus (TSWV) causes significant yield reduction in Solanaceous crops worldwide. Effective control of TSWV is difficult due to the wide host range of both the virus and thrips vectors, its transmissibility by several thrips species, the particularly persistent and circulative interaction of TSWV thrips, the low efficiency of chemical control and the rapid acquisition of resistance to insecticides by thrips (Ie, 1970; Cho et al., 1989; Rice et al., 1990; Boiteux et al., 1993a). Resistant cultivars, which are ready to use and can help reduce the environmental damages caused by insecticide abuses, have been considered as one of the most efficient tools to control the disease in pepper (Boiteux, 1995).

Heritable resistance to TSWV based on a hypersensitive response has been identified in several accessions of *C. chinense* [‘PI152225’, ‘PI159234’, ‘PI159236’ (syn. 'CNPH 679'), the Peruvian cv 'Panca' (syn. 'CNPH 275'), ‘7204’, ‘PI-15’, ‘C00943’ and ‘ECU-973’] (Cupertino et al., 1988; Black et al., 1991; Boiteux et al., 1993a; Dìez et al., 1993; Hobbs et al., 1994; Nuez et al., 1994; Jorda et al., 1994; Jahn et al., 2000; Cebolla-Cornejo et al., 2003). Boiteux and De Avila (1994), Boiteux (1995) and Moury et al. (1997) found that the resistance in several *C. chinense* accessions (‘PI159236’, ‘PI152225’, ‘CNPH-275’ and ‘7204’) is due to either a single dominant gene (*Tsw*) or a tightly linked

group of genes. Black et al. (1996) reported that the resistance in *C. chinense* 'PI152225' and 'PI159236' accessions was controlled by the same gene, *Tsw*. Until now, no distinguishable resistance alleles at the *Tsw* locus in *C. chinense* have been identified (Boiteux, 1995) and no additional resistance loci in *Capsicum* spp. have been reported (Boiteux, 1995; Moury et al., 1997).

The *Tsw* gene has been tagged by molecular markers, and Jahn et al. (2000) and Moury et al. (2000) mapped the *Tsw* gene to chromosome 10. The resistance conferred by the *Tsw* gene is overcome by high temperature (28-33 ^{o}C) and early plant inoculation (two- to four-true-leaf stages) (Pennazio, 1995; Roggero et al., 1996; Moury et al., 1998). Field isolates virulent towards accessions with the *Tsw* gene have also been found widely around the world, for example, in Brazil (Boiteux and Nagata, 1993b), the United States (Hobbs et al., 1994), Italy (Roggero et al., 2002), Spain (Margaria et al., 2004), and Australia (Sharman and Persley, 2006). In Korea, a severe outbreak of TSWV on bell peppers grown in greenhouses has been reported since 2006 (Mun et al., 2008). The resultant serious economic loss has created an urgent need to find and use new sources of resistance to TSWV. This study was aimed at isolating new source(s) resistant to TSWV in pepper, and examining the allelism relationships and inheritance of the new sources of resistance.

MATERIALS AND METHODS

Plant materials

A total of 517 accessions and lines of *Capsicum* species were used comprising 435 accessions of *C. annuum*, 14 of *C. chinense*, 18 of *C. frutescens*, 15 of *C. baccatum*, two of *C. pubescens*, three of *C. chacoense* and 30 lines of the *C. annuum* F_1 hybrid (see supplementary data). *C. annuum* 'Jeju' variety and *C. chinense* ('PI152225', 'PI159234' and 'PI159236') accessions were used as susceptible and resistant control plants, respectively. A new resistant accession of *C. chinenese* 'AC09-207' was found from the screening of *Capsicum* germplasm by observation of TSWV symptoms and DAS-ELISA analysis (data not shown).

For allelism study, three *C. chinense* accessions resistant to TSWV ('PI152225', 'PI159234', 'PI159236') and the newly isolated resistant accession 'AC09-207' were crossed to develop the F_1, F_2, and reciprocal backcross populations. For an inheritance study, *C. annuum* 'Jeju' was used as a susceptible female parent for an interspecific cross. F_2 populations were derived from F_1 plants by self-pollination. The F_1 plants produced a small amount of F_2 seed, only around one to five seeds per fruit. Reciprocal backcross populations were constructed by two crosses between F_1 plants and their parents.

Those plant materials were sown and grown in a greenhouse at the experimental farm of Seoul National University (Suwon, Korea). Twenty seeds of

each accession were soaked first in a solution of 2% sodium chlorate and 10% trisodium phosphate for 10 min and second in 50% Clorox for 30 min. The germinated seeds were sown in plastic trays with 50 holes and then grown in a greenhouse to assess seedling responses to TSWV through artificial inoculation.

Virus materials and inoculation

The $TSWV_{Pap.}$ isolate was kindly provided by Dr. Bong Nam Chung, National Institute of Horticultural and Herbal Science (Suwon, Korea). Ten to fifteen plants per accession were used for overall screening against the $TSWV_{Pap}$. isolate (Table 1). Plant materials were inoculated twice to avoid any escape that potentially occurred during the screening for resistance sources against TSWV. In the first inoculation, the seedlings were inoculated with $TSWV_{Pap.}$ isolate when two cotyledons were fully expanded and two true leaves began to appear. The inoculum of $TSWV_{Pap.}$ isolate was prepared from infected leaf material of *Nicotiana rustica* plants. One gram of infected leaves was ground in 4 ml phosphate buffer. The seedlings were dusted with Carborundum #400 mesh (Hayashi Pure Chemical Ind., Japan) and inoculated by rubbing the virus onto two cotyledons. Control plants were mock-inoculated with phosphate buffer. After 15 to 20 min, the inoculated plants were rinsed in tap water and then maintained in the greenhouse. For the second inoculation, the plants were re-inoculated at 7 days post-inoculation (dpi) on the surfaces of two upper leaves following the

inoculation processes described for the first inoculation. The TSWV-induced symptoms were recorded at 15, 30 and 60 dpi.

Symptom observation of TSWV-inoculated plants

The first observation of TSWV symptoms was recorded at 15 dpi and the virus-infected leaves were sampled from each accession/line for detecting the presence of TSWV by DAS-ELISA (following the protocol of the antiserum manufacturer, Agdia, USA). Samples were considered positive for the presence of TSWV if the absorbance value (405 nm) of each sample was three times greater than that of a healthy control plant.

The second and the third observations of TSWV symptoms were recorded at 30 and 60 dpi, respectively. At 60 dpi, the virus-infected leaves were sampled from the accessions/lines that had not been sampled in the first and the second observations for the DAS-ELISA test.

At the end of the overall screening, five selected healthy plants (free from TSWV infection) with a completely resistant phenotype were grown for the studies of allelism relationships and inheritance.

Characterization of resistance responses of new resistance source 'AC09-207'

The seedlings of new resistance source 'AC09-207' and other known resistance sources ('PI152225', 'PI159236', 'PI159234') were inoculated with $TSWV_{Pap.}$ isolate on two fully expanded cotyledons and two true leaves 15 days

after transplantation. The necrotic local lesions and premature abscissions (inoculated cotyledons and leaves) were scored by counting necrotic spots and the number of fallen cotyledons and leaves (see supplementary Table 1, Table 2, and Table 3). ANOVA/Duncan's multiple range test ($P<0.05$) analysis was performed with SPSS software, Version 17.0 (IMB, USA).

Identification of *Capsicum* species using a molecular marker

An HRM marker for the *Waxy* gene (Jeong et al., 2010) was used to determine the species of 'AC09-207'. As control plants, 12 accessions were used, representing four *Capsicum* species including five *C. chinense* accessions ('PI152225', 'PI159234', 'PI159236', 'Habanero', and 'AC09-207'), five *C. annuum* accessions ('Dempsey', 'NuMex RNaky', 'Special', 'Jeju', and 'Early California Wonder'), *C. frutescens* 'Tabassco', and *C. chacoense* 'PI260429'. A Rotor-Gene 6000 PCR thermocycler (Corbett Research, Sydney, Australia) was used to detect a single nucleotide polymorphism (SNP) via a high resolution melting analysis (HRM). The PCR amplification conditions were as described by Jeong et al. (2010).

Genotype analysis of the new resistance source

A $SCAC_{568}$ marker linked 0.9 ± 0.6cM away from the *Tsw* locus developed by Moury et al. (2000) was used to analyze the genotypes of four resistance sources ('AC09-207', 'PI152225', 'PI159234' and 'PI159236') and the

susceptible ‘Jeju’ using HRM analysis. The segregation populations were also analyzed with this marker. The PCR was conducted on a Rotor-Gene™ 6000 thermocycler (Corbett, Australia) in 20 µL reaction mixtures containing 60 mM KCl, 10 mM Tris–Cl, 2.5 mM $MgCl_2$, 0.25 mM each dNTP, 5 pmol each primer, 1 unit *Taq* polymerase, 1.25 µM Syto9 (Invitrogen, USA), and 50 ng genomic DNA. Cycling conditions were 95 ℃ for 4 min, followed by 95 ℃ for 20 s, 58 ℃ for 20 s, and then 45 cycles of 72 ℃ for 40 s. HRM was run for every increment of 0.1 ℃ between 70 ℃ and 95 ℃.

RESULTS

TSWV resistance screening using *Capsicum* germplasm

Susceptible control plants *C. annuum* 'Jeju' started to show TSWV symptoms from 5 to 7 dpi and suffered completely systemic infection by 10 to 15 dpi (Fig. 1). These TSWV symptoms developed continuously until the end of the experiment at 60 dpi. By contrast, necrotic local lesions, a typical hallmark of the hypersensitive response (HR), were detected on the inoculated cotyledons and leaves of resistant control plants 'PI152225', 'PI159234' and 'PI159236' from 3 to 5 dpi (Fig.1). These inoculated cotyledons and leaves started to fall off from 4 to 7 dpi. Resistant control plants, 'PI152225', and 'PI159236' showed no systemic TSWV infection through 60 dpi; however, 'PI159234' showed HR on the upper leaves and died within 15 dpi especially when plants were inoculated at younger stages.

At 15 dpi, symptoms of systemic TSWV infection were detected in 385 out of 517 accessions and F_1 lines. Among them, 324 accessions of *C. annuum*, 10 accessions of *C. chinense*, six accessions of *C. frutescens*, 11 accessions of *C. baccatum*, one accession of *C. pubescens*, three accessions of *C. chacoense*, and all 30 F_1 lines showed 100 % systemically infected plants. In particular, all 30 of the F_1 *C. annuum* lines and all three *C. chacoense* accessions showed complete susceptibility at 15 dpi (Table 1). These were called susceptible accessions.

Fig. 1. Hypersensitive responses (HRs) to the $TSWV_{Pap.}$ isolate in different resistant accessions. (A-E) show the inoculated leaf at 69 hpi and (F-J) show an upper leaf at 240 hpi. (A and F) new resistant ‘AC09-207’, (B and G) resistant control ‘PI152225’, (C and H) resistant control ‘PI159236’, (D and I) resistant control ‘PI159234’ and (E and J) ‘Jeju’ susceptible control. Red arrow indicates HR symptom.

Table 1. Summary of *Capsicum* germplasm screening against the $TSWV_{Pap.}$ isolate

Capsicum species	Total number of accessions[1]	Number of accessions								
		At 15 dpi			At 30 dpi			At 60 dpi		
		Sus.[a]	Seg.[b]	Res.[c]	Sus.[a]	Seg.[b]	Res.[c]	Sus.[a]	Seg.[b]	Res.[c]
C. annuum	435	324	108	3	344	89	2	357	78	0
C. chinense	14	10	3	1	12	1	1	12	1	1
C. frutescens	18	6	12	0	12	6	0	13	5	0
C. baccatum	15	11	4	0	12	3	0	12	3	0
C. pubescens	2	1	1	0	1	1	0	1	1	0
C. chacoense	3	3	0	0	3	0	0	3	0	0
C. annuum F_1	30	30	0	0	30	0	0	30	0	0
Total	517	385	128	4	414	100	3	428	88	1

[1] 10 to 15 plants per accession were inoculated with the $TSWV_{Pap.}$ isolate

[a] Susceptible accessions (100% of the tested plants with TSWV symptoms)

[b] Resistance segregating accessions (less than 80% of the tested plants with TSWV symptoms)

[c] Resistant accessions (more than 81% of the tested plants with no TSWV symptoms)

There were 128 resistance segregating accessions, which showed between 1% and 80% of the inoculated plants with TSWV symptoms, and 4 resistant accessions (with more than 80% resistant plants) (Table 1). Three to five individuals of the virus-infected leaves of each accession/line were sampled for DAS-ELISA. The ELISA results confirmed the phenotype observations (data not shown).

At 30 dpi, the total numbers of susceptible, resistance segregating and resistant accessions were 414, 100, and 3 accessions, respectively (Table 1). At the end of the experiment (60 dpi), 428 accessions and F_1 lines showed complete systemic infection, 88 accessions still showed resistance segregating and one *C. chinense* accession was completely resistant to TSWV (Table 1). The virus-infected leaves from susceptible and resistance segregating accessions that had not been sampled in the first (15 dpi) and the second (30 dpi) observations were sampled for DAS-ELISA tests. The results of the ELISA tests matched the phenotypic observations (data not shown). Among the accessions, only one accession, 'AC09-207', was resistant to TSWV. Upper un-inoculated leaves of 15 inoculated plants of 'AC09-207' were sampled and performed DAS-ELISA and confirmed *C. chinenes* accession 'AC09-207' was free of TSWV infection (data not shown).

Comparison of responses among resistant accessions

The HR started to appear at 49 hours post inoculation (hpi) on the inoculated leaves of new resistance source 'AC09-207' and other known resistance sources including 'PI152225', 'PI159236' and 'PI159234'. The number and size of necrotic local lesions on the inoculated leaves rapidly increased during 5 hours after the emergence of necrosis and did not changed largely afterwards (Table 2). The average number of necrotic local lesions per plant in 'AC09-207' was similar to 'PI152225' but significantly different from 'PI159236' and 'PI159234' at 69 hpi (Table 5). Premature abscission of inoculated cotyledons of resistance sources ('AC09-207', 'PI152225', and 'PI159236') started to occur at 75 hpi (Table 3). There was significant differences in the number of abscised cotyledons between new resistance source 'AC09-207' and two resistance sources ('PI152225' and 'PI159236') at 86 hpi (Table 5). Inoculated leaves also started to drop off at 129 hpi (Table 4) and significant differences in the number of abscised leaves were detected at 165 hpi between resistance accessions (Table 5). Unlike other resistant plants, systemic HR started to appear on the upper leaves of 'PI159234' at 165 hpi and the plants eventually died at 189 hpi (data not shown).

Table 2. Timing of necrotic local lesions in new resistance ‘AC09-207’ and other known resistance sources

Accession	Number of individuals	Average number of necrotic local lesions per plant				
		49 hpi	54 hpi	60 hpi	69 hpi	81 hpi
'Jeju'	10	0.0	0.0	0.0	0.0	0.0
'AC09-207'	24	4.04	24.50	31.17	38.21	40.08
'PI152225'	25	4.54	23.54	28.60	35.12	36.00
'PI159234'	25	0.72	4.36	6.00	8.56	9.36
'PI159236'	23	1.96	7.87	10.22	12.13	13.17

hpi, hours post inoculation

Table 3. Timing of premature abscission of inoculated cotyledons in new resistance ‘AC09-207’ and other known resistance sources

Accession	Number of individuals	Average number of premature abscission of inoculated cotyledons per plant				
		75 hpi	81 hpi	86 hpi	105 hpi	129 hpi
'Jeju'	10	0.0	0.0	0.0	0.0	0.0
'AC09-207'	24	0.13	0.42	0.79	1.42	2.00
'PI152225'	25	0.72	1.32	2.00	2.00	2.00
'PI159236'	23	0.09	0.09	0.09	0.26	0.96

Table 4. Timing of premature abscission of inoculated leaves per plant in new resistance ‘AC09-207’ and other known resistance sources

Accession	Number of individuals	Average number of premature abscission of inoculated leaves per plant				
		129 hpi	141 hpi	153 hpi	165 hpi	189 hpi
'Jeju'	10	0.0	0.0	0.0	0.0	0.0
'AC09-207'	24	0.04	1.38	1.54	1.63	2.00
'PI152225'	25	1.28	1.60	1.76	2.00	2.00
'PI159236'	23	0.00	0.04	0.13	0.22	0.22

Table 5. Comparison of resistance responses of new resistance ‘AC09-207’ and other known resistance sources

Accession	Number of individuals	Average number of necrotic local lesions per leaf at 69 hpi	Average number of premature abscission of inoculated cotyledons per plant at 86 hpi	Average number of premature abscission of inoculated leaves per plant at 165 hpi
'AC09-207'	24	38.2[a]	0.79[b]	1.63[b]
'PI152225'	25	35.1[a]	2.00[c]	2.00[c]
'PI159236'	23	12.1[b]	0.08[a]	0.22[a]
'PI159234'	25	8.6[b]	[i]	[ii]

[a, b, c] The same letter indicates no significant difference at $p < 0.05$

[i] All inoculated cotyledons died by 48 hpi

[ii] Inoculated leaves started to die at 141 hpi and all plants eventually died due to systemic necrosis

The TSWV accumulation levels in both inoculated leaves and un-inoculated upper leaves of ‘Jeju’ and resistant plants were evaluated with DAS-ELISA. The levels in the inoculated and upper leaves of the four resistant accessions, ‘PI159234’, ‘PI152225’ ‘PI159236’ and ‘AC09-207’ at 5 dpi were lower than those of ‘Jeju’ (Fig. 2). TSWV accumulated to the lowest levels in ‘AC09-207’.

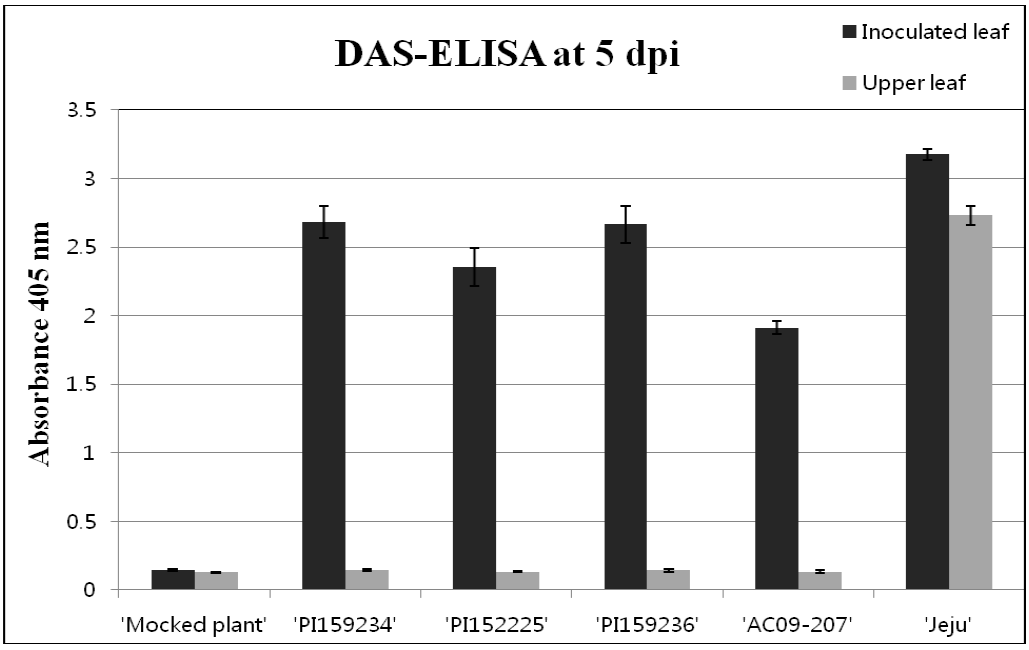

Fig. 2. Detection of TSWV accumulation by a double antibody sandwich (DAS)-enzyme-linked immunosorbent assay (ELISA). Two leaf discs of the inoculated leaves and the un-inoculated leaves of inoculated plants were sampled at 5 dpi. Mocked plant with buffer was a negative control. Error bars indicate standard.

Species identification and morphological characteristics of 'AC09-207'

To identify the species of 'AC09-207', HRM analysis was performed using the *Waxy* marker, which can discriminate four *Capsicum* species including *C. chinense, C. anuum, C. frutescens* and *C. chacoense* (Jeong et al, 2010). The melting curves of the new resistance source showed the same pattern as *C. chinense* (Fig. 3A) demonstrating that 'AC09-207' is a *C. chinense* accession.

To investigate the differences between 'AC09-207' and *C. chinense* 'PI152225', morphological characteristics were compared. There were fewer branches in 'AC09-207' than in 'PI152225'. Leaves of 'AC09-207' were thicker, longer and wider than those of 'PI152225'. In addition, 'AC09-207' fruits were rounder and larger than those of 'PI152225' (Fig. 4). These characteristics together with marker analysis demonstrated that source of *C. chinense* 'AC09-207' is different from *C. chinense* 'PI159234', 'PI152225', and 'PI159236'.

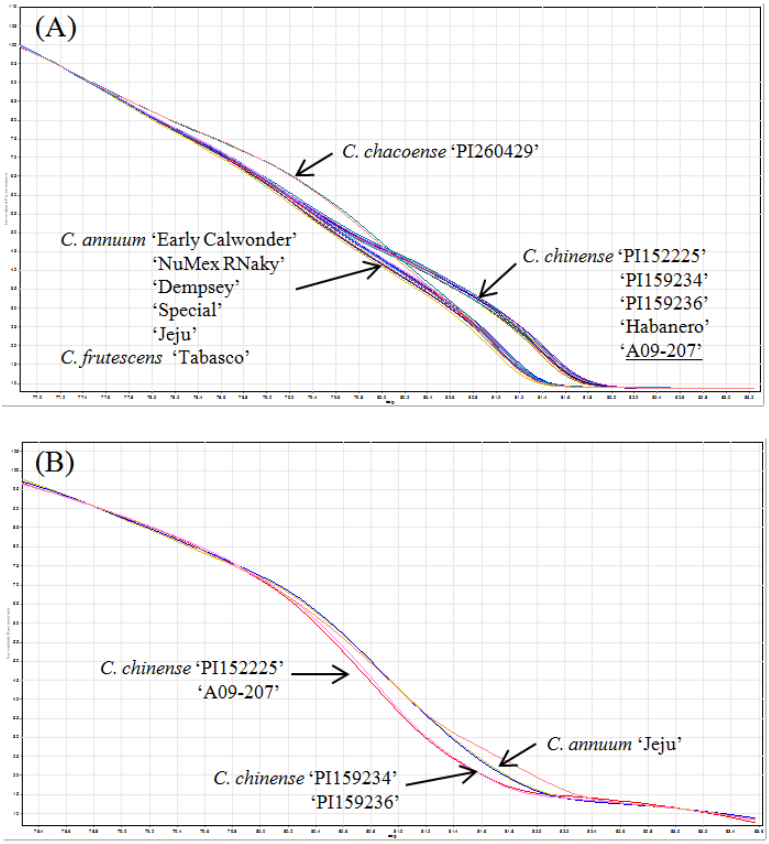

Fig. 3. High resolution melting analysis (HRM) of specific and *Tsw*-linked markers. (A) Application of the HRM *Waxy* molecular marker for the identification of *Capsicum* species (Jeong et al., 2010). A total of twelve accessions was included. *C. chinense* 'AC09-207' (underlined) is a new resistance source identified in this study. (B) Analysis of the TSWV linked marker, 'SCAC$_{568}$' (Moury et al., 2000). *C. chinense* accessions are resistant and *C. annuum* is susceptible to TSWV.

Fig. 4. Comparison of morphological phenotypes of *C. chinense* ‘PI152225’ and *C. chinense* ‘AC09-207’. (A) ‘PI152225’ plant, (B) ‘AC09-207’ plant, (C) leaves of ‘PI152225’, (D) leaves of ‘AC09-207’, (E) fruits of ‘PI152225’, (F) fruits of ‘AC09-207’.

Inheritance study of the new resistance source

All of the susceptible control *C. annum* 'Jeju' plants showed TSWV symptoms, but all plants of the *C. chinense* 'AC09-207' accession showed resistance to the $TSWV_{Pap.}$ isolate at 15 dpi. All plants of the F_1 hybrid progeny derived from a cross between *C. annuum* 'Jeju' and *C. chinense* 'AC09-207' showed complete resistance to $TSWV_{Pap.}$ (Table 6). The F_2 population segregation was consistent with a 3R:1S ratio (R=resistance; S=susceptible). The backcross population obtained from a cross between an F_1 'Jeju' x 'AC09-207' plant and 'Jeju' (BC population) was consistent with a 1R:1S segregation ratio (Table 6). This indicates that the resistance in 'AC09-207' was controlled by a single dominant gene. The genotype analysis of F_2 and BC populations using the $SCAC_{568}$ marker showed one and two recombinants out of 104 individuals of the F_2 and 140 individuals of the BC population, respectively (Table 7). These results demonstrate that the resistance gene in the *C. chinense* "AC09-207' accession is located at chromosome 10 where *Tsw* is located.

Table 6. Inheritance study of the new source of resistance against the $TSWV_{Pap.}$ isolate

Parent lines and populations	Number of plants			Expected ratio (R:S)	X^a	P^b
	Total	R	S			
'Jeju'	30	0	30	0:1	-	-
'AC09-207'	30	30	0	1:0	-	-
F_1 'Jeju' x 'AC09-207'	82	82	0	1:0	-	-
F_2 'Jeju' x 'AC09-207'	104	74	30	3:1	0.8205	0.37
BC_1 F_1 ('Jeju' x 'AC09-207') x 'Jeju'	140	72	68	1:1	0.1143	0.74
BC_1 F_1 ('Jeju' x 'AC09-207') x 'AC09-207'	55	55	0	1:0	-	-

R, Resistance; S, Susceptible

[a] Chi-square test, [b] Probability value

Table 7. Analysis of the new resistance source, *C. chinense* 'AC09-207', with a molecular marker linked to the *Tsw* gene

Marker name	Product size (bp)	Genetic distance (cM)	Polymorphism with three known resistance sources and one susceptible accession				No. of recombinants/ total F_2 and BC_1F_1 individuals*		Reference
			'PI152225'	'PI159234'	'PI159236'	'Jeju'	F_2	BC_1F_1	
$SCAC_{568}$	568	0.9±0.6	-	+	+	+	1/104	2/140	Moury et al. (2000)

-, no polymorphism with new resistance source; +, polymorphism with new resistance source

Allelism test between the new resistance source and three known resistance sources

All F_1 hybrids of 'PI152225' x 'AC09-207', 'PI159234' x 'AC09-207', and 'PI159236' x 'AC09-207' showed complete resistance to the $TSWV_{Pap.}$ isolate. None of the F_2 populations derived from the F_1 hybrids segregated for susceptibility. The reciprocal backcross populations obtained from crosses between F_1 ('PI15225' x 'AC09-207) with their parents did not segregate either (Table 8). In addition, no susceptible plants were found in the F_1 and F_2 populations of a cross between 'PI152225' and 'PI159236' accessions (Table 8). These results demonstrate that resistance in 'AC09-207' is controlled by a gene at the same locus as *Tsw* or is very tightly linked to *Tsw*.

To examine differences between the resistance gene in 'AC09-207' and *Tsw*, the molecular marker $SCAC_{568,}$ which is linked to the *Tsw* gene (Moury et al. 2000), was used. There was no polymorphism between the genotypes of the *C. chinense* 'AC09-207' and *C. chinense* 'PI152225' accessions, whereas polymorphisms were identified among the genotype of *C. chinense* 'AC09-207' and the genotypes of *C. chinense* 'PI159234' and 'PI159236' (Table 7 and Fig. 3B). This demonstrates that the resistance gene in the new resistant accession 'AC09-207' of *C. chinense* species may be the same as the *Tsw* gene in *C. chinense* 'PI152225' or tightly linked to *Tsw*.

Table 8. Allelism test between the new resistance source and three known resistance sources

Parent lines and populations	Number of plants			Ratio (R:S)
	Total	R	S	
'Jeju'	50	0	50	0:1
'PI152225'	54	54	0	1:0
'PI159234'	45	45	0	1:0
'PI159236'	63	63	0	1:0
'AC09-207'	67	67	0	1:0
F_1 'PI152225' x 'AC09-207'	178	178	0	1:0
F_2 'PI152225' x 'AC09-207'	386	386	0	1:0
BC_1 F_1 ('PI152225' x 'AC09-207') x 'PI152225'	179	179	0	1:0
BC_1 F_1 ('PI152225' x 'AC09-207') x 'AC09-207'	89	89	0	1:0
F_1 'PI159234' x 'AC09-207'	152	152	0	1:0
F_2 'PI159234' x 'AC09-207'	197	197	0	1:0
F_1 'PI159236' x 'AC09-207'	140	140	0	1:0
F_2 'PI159236' x 'AC09-207'	168	168	0	1:0
F_1 'PI152225' x 'PI159236'	67	67	0	1:0
F_2 'PI152225' x 'PI159236'	186	186	0	1:0

R, Resistance; S, Susceptible

DISCUSSION

Discovering a new resistance gene (R gene) source against TSWV from the *Capsicum* germplasm collection would be useful for pepper breeding programs because the resistance previously identified in several accessions of *Capsicum* species has been overcome by abiotic and biotic factors (Moury et al., 1998; Roggero et al., 2002). No resistance to TSWV has been found in *C. annuum* species so far (Sharman and Persley, 2006). No *C. annuum* resistance source was found in this study either, which demonstrates that R gene(s) rarely exist in *C. annuum* species. In this study, we found a new resistant accession 'AC09-207' in *C. chinense* that was similar to two other known resistant accessions, 'PI152225' and 'PI159236', but different from the susceptible control plants *C. annuum* 'Jeju' until the end of the experiment (60 dpi).

'AC09-207' is different from other resistance sources, especially from 'PI152225', in many aspects. Phenotypic characteristics such as the number of branches and morphology of leaves and fruits were distinct from other TSWV-resistant accessions in *C. chinense.* Leaves of 'AC09-207' were thicker, longer and wider and fruits were rounder and bigger than those of *C. chinense* 'PI152225' (Fig. 4). The HR response has been reported to be related to resistance response in plants. According to Goodman and Novacky (1994), the HR is characterized as the rapid death of a limited number of cells in the vicinity of the invading pathogen that is often associated with a block on the progression of the

infection. Plants carrying the *Tsw* gene usually show necrotic local lesions on the inoculated leaf, followed by premature abscission and plants do not become systemically infected by TSWV (Boiteux, 1995). In contrast, the infection in 'PI152225' by TSWV resistance-breaking strain ($p166^{RB}$) produced necrotic local lesions on the inoculated leaf, followed by systemic necrotic local lesions. This result demonstrated that HR response alone may not be sufficient to restrict infection on the inoculated leaf (Margaria et al., 2007). In our study, we observed the necrotic local lesions on the inoculated leaves of 'AC09-207' and other known resistance sources ('PI152225', 'PI159236', and 'PI159234') caused by a TSWV isolate ($TSWV_{Pap.}$), and no TSWV symptoms were detected in the un-inoculated leaves of 'AC09-207' through the end of the experiments. Further, we observed the HR on the upper leaves of 'PI159234', but no TSWV infection. This showed that HR response in 'AC09-207' is different from that of Margaria et al. (2007). The various resistance responses of different resistance sources demonstrated that there might be a number of TSWV-resistance mechanisms. Boiteux et al. (1993a) also proposed that there might be a number of resistance mechanisms against TSWV in *C. chinense* accessions ('CNPH275' and 'PI159236') based on responses to different TSWV isolates. Further experiments using other TSWV isolates should be performed to elucidate the differences of 'AC09-207' from other *C. chinense* resistant accessions. However, no *Tsw*-overcoming isolate has been available in Korea to date.

It is not clear whether the resistance to TSWV in *C. chinense* species is controlled by different alleles at the same locus or by a tightly linked group of genes (Boiteux and De Á vila, 1994; Boiteux, 1995; Moury et al., 1997). It appears that resistance genes in *C. chinense* species may be different alleles at the *Tsw* locus. Our failure to discriminate between resistance sources using populations obtained from crosses between *C. chinense* 'AC09-207' and other accessions supports this notion. Black et al. (1996) demonstrated that the resistance in *C. chinense* 'PI152225' and 'PI159236' accessions was governed by the same gene, *Tsw*. However, clear differences in the number of necrotic local lesions on the inoculated leaves and number of premature abscission on the inoculated cotyledons and leaves in our study demonstrate that R genes in 'PI152225' and 'PI159236' may be controlled by different alleles. Jahn et al. (2000) showed that the R gene in *C. chinense* 'PI159234' is also located at a similar position to *Tsw*. Again, the resistance response of *C. chinense* 'PI152225' was clearly distinct from that of *C. chinense* 'PI159234', where the HR response often resulted in systemic necrosis. The average number of necrotic local lesions in 'AC09-207' was similar to 'PI152225' but different from 'PI159236' and 'PI159234'. When we used the CAPS marker ($SCAC_{568}$) linked to *Tsw* (Moury et al., 2000) to discriminate *Tsw* alleles, the $SCAC_{568}$ genotype of 'AC09-207' was the same as that of 'PI152225' but different from 'PI159236' and 'PI159234'. These results indicated that the resistance in 'AC09-207' may be a unique allele at the *Tsw*

locus. However, we cannot rule out the possibility these R genes are actually a group of genes that are tightly linked. It is well known that a definitive proof of allelism is not possible for dominant genes via genetic complementation analysis if two loci are tightly linked. The *Tsw* locus is located at a position on pepper chromosome 10 where other tightly linked virus resistance genes, *Pvr4* and *Pvr7*, are also located. *Pvr4* and *Pvr7* confer dominant resistances to potyvirus that are similar in both inheritance and resistance phenotype. These genes are clustered together with *Tsw* within a 30 cM interval (Grube et al., 2000). The R gene cluster containing *Tsw* was the first defined cluster in pepper. Recent analysis of genome-wide arrangement of R gene homologues in tomato also demonstrated that R genes frequently occur in clusters of related gene copies that include nucleotide binding site (NBS), receptor-like protein (RLP), and receptor-like kinase (RLK) genes. The syntenic position of *Tsw* in tomato contains multiple R gene homologues (Andolfo et al., 2013). Therefore, it is possible that different copies of these R genes within a cluster in the pepper genome will render specificity to resistance against pathogens. Ultimately, cloning of the *Tsw* gene and genome analysis of this R gene cluster will reveal the identity of R gene(s) in 'AC09-207' and other TSWV-resistance genes.

In conclusion, a new resistance source, *C. chinense* 'AC09-207', was identified and characterized. This R gene was controlled by a single dominant gene. The results of resistance response and marker test demonstrated that the new

resistance source ‘AC09-207’ may contain the same allele at *Tsw* as in ‘PI152225’, but not in ‘PI159236’ and ‘PI159234’, or the resistance in ‘AC09-207’ may be a unique allele at the *Tsw* locus. The results of allelism tests indicated that the new R gene may be the same gene at *Tsw* or that there is a group of R genes that are tightly linked. This new resistance source will be useful for the development of TSWV-resistant cultivars in pepper.

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초록

토마토반점시듦바이러스 (*Tomato spotted wilt virus*; TSWV)는 전 세계적으로 고추 생산량에 영향을 주는 중요한 바이러스에 해당된다. TSWV 에 대한 단일 우성 저항성 유전자인 *Tsw* 는 *Capsicum chinense* 에서 유래한 것으로 알려져 왔다. 그 가운데 *Tsw* 유전자에 연관된 분자표지들이 개발되었으나 다양한 고추 육종 소재에 대한 분자 육종에는 적용될 수 없었다. 본 연구에서는 토마토 및 고추 CM334 계통의 유전체 정보를 활용한 유전자 지도 작성, 저항성 및 이병성 계통 각각의 RNA 풀(pool)을 활용한 전사체 분석 및 염색체 워킹(chromosome walking) 방법을 통해 다양한 계통에 적용이 가능한 분자표지를 개발하였고 *Tsw* 유전자에 대한 유전자미세지도 작성을 수행하였다. *C.annuum* BAC 클론 서열로부터 선별된 고추 CM334 계통의 스캐폴드(scaffold)로부터 SNP165256, SNP3065253, SNP67464, SNP151705 등 4 개의 단일염기다형성(SNP) 분자표지를 개발하였으며 이들 분자표지는 210 개체로 이루어진 'Telmo' F_2 집단 및 843 개체로 이루어진 'SP' F_2 집단에서 TSWV 저항성 특성과 공동분리하였다. 이를 통해 *Tsw* 유전자는 SNP165256 와 SNP151705 분자표지 간 149kb 길이의 DNA 구역에 위치하는 것으로 확인되었다. 이 중 CC/TIR-NBS-LRR 류의 저항성 유전자로 예측되는 5 개의 유전자인 mRNA-6,

mRNA-7, mRNA-11, mRNA-12, mRNA-13 가 동정되었다. 유전자 발현 실험을 통해 mRNA-13 은 'PI152225' 계통에서는 발현되나 'Special' 계통에서는 발현되지 않음을 확인할 수 있었다. 또한 후보유전자인 mRNA-13 은 *C.annuum* 인 'CM334'와 *C.chinense* 인 'PI152225'에서 89%의 상동성을 나타내었다. 이를 통해 mRNA-13 은 *Tsw* 유전자의 강력한 후보 유전자로 판단할 수 있었다. 위와 같은 결과는 *Tsw* 유전자 동정 및 TSWV 저항성 품종 육성에 유용하게 활용될 수 있을 것으로 판단된다.

Capsicum 속의 6 개 고추 종에 속하는 487 개의 유전자원 계통 및 30 개의 상용 F_1 품종에서 TSWV 에 대한 저항성을 평가해 본 결과 *C. chinense* 에 속하는 'AC09-207' 계통이 새로운 저항성 소재로서 발굴되었다. 유전 분석, 분자표지 활용 분석 및 대립유전자 간 상관관계 분석을 수행한 결과 'AC09-207'의 저항성 유전자는 *Tsw* 유전자좌에 위치하는 새로운 대립유전자이거나 *Tsw* 유전자좌에 매우 가깝게 위치하는 새로운 유전자일 것으로 판단할 수 있었다.

핵심 단어: 토마토반점시듦바이러스, *Capsicum chinense*, 유전체 기반 유전자미세지도 작성, *Tsw*, 유전자원 탐색, 병저항성

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APPENDIX

List of accessions tested in the overall resistance screening for TSWV

Accession	Species	Cultivar name	Character
PI152225	*Capsicum chinense*	PI152225	Resistance
PI159234	*Capsicum chinense*	PI159234	Resistance
PI159236	*Capsicum chinense*	PI159236	Resistance
AC09207	*Capsicum chinense*	Cajamarca	Resistance
AC002012	*Capsicum chinense*	Jalapeno	Susceptibility
AC03043	*Capsicum chinense*	Bangkok//Thailand	Susceptibility
AC061582	*Capsicum chinense*	Habanero(red)	Susceptibility
AC070041	*Capsicum chinense*	Early Red sweet//USA	Susceptibility
AC072732	*Capsicum chinense*	Malagueta	Susceptibility
AC99192	*Capsicum chinense*	KC 217 triploid offspring//Korea	Susceptibility
AC99193	*Capsicum chinense*	KC 217 triploid offspring//Korea	Susceptibility
AC99199	*Capsicum chinense*	KC 217 triploid offspring//Korea	Susceptibility
AC09183	*Capsicum chinense*	Bisbas	Susceptibility
AC09186	*Capsicum chinense*	Bisbas rotfruechtig	Susceptibility
AC09208	*Capsicum chinense*	Numex Suave Orange	Susceptibility
AC09209	*Capsicum chinense*	Bhut Jolokia	Susceptibility
AC09230	*Capsicum chinense*	Numex Suave Red	Susceptibility
AC002463	*Capsicum annuum*	PI 271322	Susceptibility
AC02130	*Capsicum annuum*	PP1993	Susceptibility
AC021332	*Capsicum annuum*	Sweet Banana//USA	Susceptibility
AC02135	*Capsicum annuum*	Unknown	Susceptibility
AC021491	*Capsicum annuum*	PBC 458//Taiwan	Susceptibility
AC02150	*Capsicum annuum*	9852-193 AVRDC 211//Taiwan	Susceptibility
AC02152	*Capsicum annuum*	Chocolate Beauty//Taiwan	Susceptibility
AC02168	*Capsicum annuum*	Unknown	Susceptibility
AC03002	*Capsicum annuum*	Kathmandu-2//Nepal	Susceptibility
AC03045	*Capsicum annuum*	Malaysia1//Malaysia	Susceptibility
AC03053	*Capsicum annuum*	India1//India	Susceptibility
AC030641	*Capsicum annuum*	Thailand1//Thailand	Susceptibility
AC040012	*Capsicum annuum*	KG	Susceptibility
AC050022	*Capsicum annuum*	Yuhan pepper(4-3)	Susceptibility
AC060113	*Capsicum annuum*	Unknown 43	Susceptibility

Accession	Species	Cultivar name	Character
AC06019	*Capsicum annuum*	Unknown 45	Susceptibility
AC06101	*Capsicum annuum*	PBC 122//Taiwan	Susceptibility
AC061213	*Capsicum annuum*	Criollos de Morelos 334	Susceptibility
AC06122	*Capsicum annuum*	PI 342948//USA	Susceptibility
AC061253	*Capsicum annuum*	Jupiter	Susceptibility
AC06127	*Capsicum annuum*	Unknown	Susceptibility
AC061291	*Capsicum annuum*	Piquillo	Susceptibility
AC061331	*Capsicum annuum*	Verdel//USA	Susceptibility
AC061354	*Capsicum annuum*	Carolina Wonder	Susceptibility
AC061362	*Capsicum annuum*	CharLston Belle(OP)//USA	Susceptibility
AC061382	*Capsicum annuum*	Hot Portugal(OP)//Portugal	Susceptibility
AC061391	*Capsicum annuum*	Jamaican Yellow//USA	Susceptibility
AC061402	*Capsicum annuum*	Thai Hot (OP)//USA	Susceptibility
AC06141	*Capsicum annuum*	Super Hot//USA	Susceptibility
AC061444	*Capsicum annuum*	Del Ray Bell	Susceptibility
AC061461	*Capsicum annuum*	Agronomico 10	Susceptibility
AC061484	*Capsicum annuum*	Flavr2	Susceptibility
AC061522	*Capsicum annuum*	VR-4	Susceptibility
AC061572	*Capsicum annuum*	Choco Pepper	Susceptibility
AC061632	*Capsicum annuum*	Thai Hot(OP)//USA	Susceptibility
AC06166	*Capsicum annuum*	Scotch Bonnet//USA	Susceptibility
AC06173	*Capsicum annuum*	Japoneb//USA	Susceptibility
AC06174	*Capsicum annuum*	Merah//USA	Susceptibility
AC06175	*Capsicum annuum*	Pequin//USA	Susceptibility
AC061762	*Capsicum annuum*	Pujab small Hot//USA	Susceptibility
AC061772	*Capsicum annuum*	Red chilli	Susceptibility
AC061782	*Capsicum annuum*	Yatsufusa	Susceptibility
AC061792	*Capsicum annuum*	Red chilli	Susceptibility
AC061843	*Capsicum annuum*	Avelar 2001	Susceptibility
AC061853	*Capsicum annuum*	Jmavelara 12/99	Susceptibility
AC061931	*Capsicum annuum*	Yolo Y	Susceptibility
AC0619610	*Capsicum annuum*	JMAV-B	Susceptibility
AC061992	*Capsicum annuum*	JMAV-C	Susceptibility
AC062001	*Capsicum annuum*	Golden Cal Wonder//USA	Susceptibility
AC064261	*Capsicum annuum*	Twilight//USA	Susceptibility

Accession	Species	Cultivar name	Character
AC064272	*Capsicum annuum*	Perennial(Cornell)	Susceptibility
AC070012	*Capsicum annuum*	Pretty Purple//USA	Susceptibility
AC07002	*Capsicum annuum*	Poinsettia//USA	Susceptibility
AC07003	*Capsicum annuum*	Filus blue//USA	Susceptibility
AC07014	*Capsicum annuum*	Starburst//USA	Susceptibility
AC070182	*Capsicum annuum*	5502//USA	Susceptibility
AC072021	*Capsicum annuum*	Szeged1cseresznye//Hungary	Susceptibility
AC07203	*Capsicum annuum*	Karkovskij//Rusia	Susceptibility
AC07204	*Capsicum annuum*	CO 0725//Italy	Susceptibility
AC07205	*Capsicum annuum*	CO 0726//Italy	Susceptibility
AC072072	*Capsicum annuum*	Chilli serrano//Mexico	Susceptibility
AC07208	*Capsicum annuum*	CO 1436//Mexico	Susceptibility
AC072091	*Capsicum annuum*	Huay sithon	Susceptibility
AC072102	*Capsicum annuum*	CO 2028//Turkey	Susceptibility
AC07211	*Capsicum annuum*	Black prince//England	Susceptibility
AC072121	*Capsicum annuum*	409 7586//China	Susceptibility
AC072131	*Capsicum annuum*	411 7593//China	Susceptibility
AC072141	*Capsicum annuum*	412 5110//China	Susceptibility
AC072162	*Capsicum annuum*	Chinda2//Thailand	Susceptibility
AC072172	*Capsicum annuum*	NARC-4//Pakistan	Susceptibility
AC072182	*Capsicum annuum*	PBC646/PBC378*5	Susceptibility
AC07219	*Capsicum annuum*	Cipanas	Susceptibility
AC072222	*Capsicum annuum*	Paul smith & quot's serrano 1534	Susceptibility
AC07223	*Capsicum annuum*	Guajilo ancho//Mexico	Susceptibility
AC072252	*Capsicum annuum*	Redlands sweet sue//Australia	Susceptibility
AC07226	*Capsicum annuum*	Nose gay//USA	Susceptibility
AC072271	*Capsicum annuum*	CO 1857//Iran	Susceptibility
AC07229	*Capsicum annuum*	CO 1727//El Salvador	Susceptibility
AC072302	*Capsicum annuum*	FIPS//Netherlands	Susceptibility
AC07231	*Capsicum annuum*	Szegeda csip-mentes 47	Susceptibility
AC072322	*Capsicum annuum*	CO 0799	Susceptibility
AC07235	*Capsicum annuum*	C05010//Germany	Susceptibility
AC072391	*Capsicum annuum*	387-ONG// Malasia	Susceptibility
AC072402	*Capsicum annuum*	388-ONG// Malasia	Susceptibility
AC07242	*Capsicum annuum*	Beldi//Tunisia	Susceptibility

Accession	Species	Cultivar name	Character
AC07244	*Capsicum annuum*	MONTEGO	Susceptibility
AC072451	*Capsicum annuum*	AZETH//Brazil	Susceptibility
AC07246	*Capsicum annuum*	PBC725// Papua New Guinea	Susceptibility
AC072472	*Capsicum annuum*	BIRD&QUOT'S EYE//Italy	Susceptibility
AC07248	*Capsicum annuum*	FECSKE//Hungary	Susceptibility
AC07250	*Capsicum annuum*	Baramashi//Japan	Susceptibility
AC07252	*Capsicum annuum*	PBC977//Israel	Susceptibility
AC07253	*Capsicum annuum*	Peter pepper//USA	Susceptibility
AC07254	*Capsicum annuum*	Guasillo//USA	Susceptibility
AC07255	*Capsicum annuum*	Canada cheese//Canada	Susceptibility
AC07256	*Capsicum annuum*	Cascabella//USA	Susceptibility
AC07257	*Capsicum annuum*	Chocolate cherry//USA	Susceptibility
AC07261	*Capsicum annuum*	Puerto rico wonder//Puerto Rico	Susceptibility
AC07263	*Capsicum annuum*	Mak Phed Lepmevnang//Laos	Susceptibility
AC072641	*Capsicum annuum*	Pepperoncini//USA	Susceptibility
AC072662	*Capsicum annuum*	CITRINA//Slovakia	Susceptibility
AC07267	*Capsicum annuum*	DE ARBOL//USA	Susceptibility
AC07268	*Capsicum annuum*	JOVA//Czech Republic	Susceptibility
AC072692	*Capsicum annuum*	Bolivian rainbow// Bolivia	Susceptibility
AC07270	*Capsicum annuum*	Chile picnte 1045//Costa Rica	Susceptibility
AC072743	*Capsicum annuum*	Dove italiana	Susceptibility
AC07276	*Capsicum annuum*	Califonia wonder	Susceptibility
AC072802	*Capsicum annuum*	Redsweet//Rusia	Susceptibility
AC072812	*Capsicum annuum*	Sweish//Rusia	Susceptibility
AC072822	*Capsicum annuum*	Tura	Susceptibility
AC07283	*Capsicum annuum*	HRF	Susceptibility
AC07284	*Capsicum annuum*	Yolo Wonder	Susceptibility
AC07285	*Capsicum annuum*	Tuzgolyo	Susceptibility
AC07287	*Capsicum annuum*	Edesalma	Susceptibility
AC07289	*Capsicum annuum*	Kalocsa V-2	Susceptibility
AC07290	*Capsicum annuum*	Macskasarga	Susceptibility
AC072932	*Capsicum annuum*	EU 016	Susceptibility
AC072942	*Capsicum annuum*	EU 017	Susceptibility
AC072952	*Capsicum annuum*	EU 018	Susceptibility
AC072963	*Capsicum annuum*	EU 019	Susceptibility

Accession	Species	Cultivar name	Character
AC07297	*Capsicum annuum*	EU 021	Susceptibility
AC072982	*Capsicum annuum*	Guundilla larga amarilla	Susceptibility
AC073001	*Capsicum annuum*	Macskapires	Susceptibility
AC08095	*Capsicum annuum*	Yeongyang pepper test site//97-2005	Susceptibility
AC08096	*Capsicum annuum*	Yeongyang pepper test site//97-2040	Susceptibility
AC080971	*Capsicum annuum*	Yeongyang pepper test site//97-2003	Susceptibility
AC08098	*Capsicum annuum*	Yeongyang pepper test site//97-2011	Susceptibility
AC08099	*Capsicum annuum*	Yeongyang pepper test site//97-2012	Susceptibility
AC08100	*Capsicum annuum*	Yeongyang pepper test site//97-2004	Susceptibility
AC08101	*Capsicum annuum*	Yeongyang pepper test site//97-2026	Susceptibility
AC08102	*Capsicum annuum*	Yeongyang pepper test site//97-2033	Susceptibility
AC08103	*Capsicum annuum*	Yeongyang pepper test site//97-2023	Susceptibility
AC08104	*Capsicum annuum*	Yeongyang pepper test site//97-2028	Susceptibility
AC081051	*Capsicum annuum*	Ibam//Yeongyang pepper test site	Susceptibility
AC08106	*Capsicum annuum*	Yeongyang pepper test site//97-2010	Susceptibility
AC08107	*Capsicum annuum*	Yeongyang pepper test site//97-2020	Susceptibility
AC08108	*Capsicum annuum*	Yeongyang pepper test site//97-2025	Susceptibility
AC081091	*Capsicum annuum*	Cheonggi//Yeongyang pepper test site	Susceptibility
AC081101	*Capsicum annuum*	Cheonggi//Yeongyang pepper test site	Susceptibility
AC08111	*Capsicum annuum*	Yeongyang pepper test site//06-1077	Susceptibility
AC08112	*Capsicum annuum*	Yeongyang pepper test site//06-1078	Susceptibility
AC08113	*Capsicum annuum*	Yeongyang pepper test site//06-1079	Susceptibility
AC081141	*Capsicum annuum*	Anjeunbaengi//Yeongyang pepper test	Susceptibility
AC081151	*Capsicum annuum*	Punggakcho//Yeongyang pepper test	Susceptibility
AC08117	*Capsicum annuum*	Yeongyang pepper test site//06-1083	Susceptibility
AC08118	*Capsicum annuum*	Tomato growers co.//9005	Susceptibility
AC08119	*Capsicum annuum*	Tomato growers co.//9007	Susceptibility
AC081211	*Capsicum annuum*	Tomato growers co.//9022	Susceptibility
AC08122	*Capsicum annuum*	Tomato growers co.//9025	Susceptibility
AC081241	*Capsicum annuum*	Tomato growers co.//9044	Susceptibility
AC08126	*Capsicum annuum*	Tomato growers co.//9054	Susceptibility
AC08127	*Capsicum annuum*	Tomato growers co.//9071	Susceptibility
AC08128	*Capsicum annuum*	Tomato growers co.//9079	Susceptibility
AC08129	*Capsicum annuum*	Tomato growers co.//9123	Susceptibility
AC08131	*Capsicum annuum*	Tomato growers co.//9133	Susceptibility

Accession	Species	Cultivar name	Character
AC08132	*Capsicum annuum*	Tomato growers co.//9146	Susceptibility
AC08133	*Capsicum annuum*	Tomato growers co.//9148	Susceptibility
AC08134	*Capsicum annuum*	Tomato growers co.//9177	Susceptibility
AC081351	*Capsicum annuum*	Tomato growers co.//9202	Susceptibility
AC08136	*Capsicum annuum*	Tomato growers co.//9218	Susceptibility
AC08137	*Capsicum annuum*	Tomato growers co.//9224	Susceptibility
AC081381	*Capsicum annuum*	Tomato growers co.//9240	Susceptibility
AC081391	*Capsicum annuum*	Tomato growers co.//9250	Susceptibility
AC08141	*Capsicum annuum*	Tomato growers co.//9272	Susceptibility
AC08143	*Capsicum annuum*	Tomato growers co.//9290	Susceptibility
AC08145	*Capsicum annuum*	Tomato growers co.//9303	Susceptibility
AC08146	*Capsicum annuum*	Tomato growers co.//9309	Susceptibility
AC08147	*Capsicum annuum*	Tomato growers co.//9313	Susceptibility
AC081481	*Capsicum annuum*	Tomato growers co.//9318	Susceptibility
AC08149	*Capsicum annuum*	Tomato growers co.//9320	Susceptibility
AC08150	*Capsicum annuum*	Tomato growers co.//9329	Susceptibility
AC08153	*Capsicum annuum*	Tomato growers co.//9342	Susceptibility
AC08154	*Capsicum annuum*	Tomato growers co.//9353	Susceptibility
AC08155	*Capsicum annuum*	Tomato growers co.//9359	Susceptibility
AC08156	*Capsicum annuum*	Tomato growers co.//9362	Susceptibility
AC08157	*Capsicum annuum*	Tomato growers co.//9377	Susceptibility
AC08159	*Capsicum annuum*	Tomato growers co.//9393	Susceptibility
AC08160	*Capsicum annuum*	Tomato growers co.//9400	Susceptibility
AC08161	*Capsicum annuum*	Tomato growers co.//9403	Susceptibility
AC08162	*Capsicum annuum*	Tomato growers co.//9417	Susceptibility
AC08163	*Capsicum annuum*	Tomato growers co.//9425	Susceptibility
AC08164	*Capsicum annuum*	Tomato growers co.//9437	Susceptibility
AC081652	*Capsicum annuum*	Tomato growers co.//9452	Susceptibility
AC08166	*Capsicum annuum*	Tomato growers co.//9456	Susceptibility
AC08167	*Capsicum annuum*	Tomato growers co.//9457	Susceptibility
AC08169	*Capsicum annuum*	Tomato growers co.//9499	Susceptibility
AC08171	*Capsicum annuum*	Tomato growers co.//9503	Susceptibility
AC08172	*Capsicum annuum*	Tomato growers co.//9506	Susceptibility
AC08173	*Capsicum annuum*	Tomato growers co.//9510	Susceptibility
AC08174	*Capsicum annuum*	Tomato growers co.//9516	Susceptibility

Accession	Species	Cultivar name	Character
AC08175	*Capsicum annuum*	Tomato growers co.//9520	Susceptibility
AC081771	*Capsicum annuum*	Tomato growers co.//9526	Susceptibility
AC08178	*Capsicum annuum*	Tomato growers co.//9532	Susceptibility
AC08179	*Capsicum annuum*	Tomato growers co.//9557	Susceptibility
AC08180	*Capsicum annuum*	Tomato growers co.//9560	Susceptibility
AC081812	*Capsicum annuum*	Tomato growers co.//9591	Susceptibility
AC08182	*Capsicum annuum*	Tomato growers co.//9596	Susceptibility
AC08184	*Capsicum annuum*	Tomato growers co.//9603	Susceptibility
AC081851	*Capsicum annuum*	Tomato growers co.//9609	Susceptibility
AC08186	*Capsicum annuum*	Tomato growers co.//9615	Susceptibility
AC08187	*Capsicum annuum*	Tomato growers co.//9623	Susceptibility
AC081881	*Capsicum annuum*	Tomato growers co.//9626	Susceptibility
AC08189	*Capsicum annuum*	Tomato growers co.//9629	Susceptibility
AC081901	*Capsicum annuum*	Tomato growers co.//9638	Susceptibility
AC08193	*Capsicum annuum*	Tomato growers co.//9723	Susceptibility
AC08196	*Capsicum annuum*	Tomato growers co.//9763	Susceptibility
AC08197	*Capsicum annuum*	Tomato growers co.//9800	Susceptibility
AC08198	*Capsicum annuum*	Tomato growers co.//9831	Susceptibility
AC08199	*Capsicum annuum*	Tomato growers co.//9835	Susceptibility
AC08200	*Capsicum annuum*	Tomato growers co.//9836	Susceptibility
AC082011	*Capsicum annuum*	Tomato growers co.//9857	Susceptibility
AC08202	*Capsicum annuum*	Tomato growers co.//9567	Susceptibility
AC08203	*Capsicum annuum*	Tomato growers co.//9580	Susceptibility
AC08210	*Capsicum annuum*	Tomato growers co.//9051	Susceptibility
AC08211	*Capsicum annuum*	Tomato growers co.//9217	Susceptibility
AC08212	*Capsicum annuum*	Yeongyang pepper test site	Susceptibility
AC08213	*Capsicum annuum*	Yeongyang pepper test site	Susceptibility
AC08215	*Capsicum annuum*	Vietnam06	Susceptibility
AC08216	*Capsicum annuum*	Vietnam07	Susceptibility
AC082171	*Capsicum annuum*	Vietnam08	Susceptibility
AC08218	*Capsicum annuum*	Vietnam09	Susceptibility
AC08219	*Capsicum annuum*	Vietnam10	Susceptibility
AC08220	*Capsicum annuum*	Vietnam11	Susceptibility
AC08221	*Capsicum annuum*	Vietnam12	Susceptibility
AC99008	*Capsicum annuum*	KC296	Susceptibility

Accession	Species	Cultivar name	Character
AC99009	*Capsicum annuum*	26-7-4-2	Susceptibility
AC990502	*Capsicum annuum*	ORB(Y)	Susceptibility
AC990531	*Capsicum annuum*	ancho	Susceptibility
AC99190	*Capsicum annuum*	KC 217 triploid offspring//Korea	Susceptibility
AC991942	*Capsicum annuum*	KC 217 triploid offspring//Korea	Susceptibility
AC99198	*Capsicum annuum*	KC 217 triploid offspring//Korea	Susceptibility
AC99204	*Capsicum annuum*	KC 217 triploid offspring//Korea	Susceptibility
AC99207	*Capsicum annuum*	KC 217 triploid offspring//Korea	Susceptibility
AC992092	*Capsicum annuum*	KC 217 triploid offspring//Korea	Susceptibility
AC09001	*Capsicum annuum*	Su-Tsu	Susceptibility
AC09002	*Capsicum annuum*	Fushhimikara	Susceptibility
AC09003	*Capsicum annuum*	Goshiki	Susceptibility
AC09006	*Capsicum annuum*	Hontaka	Susceptibility
AC09007	*Capsicum annuum*	Kozij Rogij	Susceptibility
AC09008	*Capsicum annuum*	Kubanskij Rannij 70/60	Susceptibility
AC09009	*Capsicum annuum*	Nagayatsubusa	Susceptibility
AC09010	*Capsicum annuum*	Nikko	Susceptibility
AC09011	*Capsicum annuum*	Nitrianska Krajova	Susceptibility
AC09013	*Capsicum annuum*	Ohyatsubua	Susceptibility
AC09014	*Capsicum annuum*	Sapporofuto	Susceptibility
AC09015	*Capsicum annuum*	ShiShi	Susceptibility
AC09031	*Capsicum annuum*	Da-Chun-Pao	Susceptibility
AC09047	*Capsicum annuum*	Keystone Wonder Giant	Susceptibility
AC09048	*Capsicum annuum*	Keystone Resistant	Susceptibility
AC09050	*Capsicum annuum*	Five Colour Pepper	Susceptibility
AC09056	*Capsicum annuum*	Siau-Fung-Tsong	Susceptibility
AC09057	*Capsicum annuum*	Siau-Fung-Tsong	Susceptibility
AC09087	*Capsicum annuum*	Sok Hu	Susceptibility
AC09088	*Capsicum annuum*	Sopul	Susceptibility
AC09089	*Capsicum annuum*	Nam Cju	Susceptibility
AC09187	*Capsicum annuum*	BISBAS	Susceptibility
AC09194	*Capsicum annuum*	Early Jalapeno	Susceptibility
AC09195	*Capsicum annuum*	NuMex Sweet	Susceptibility
AC09196	*Capsicum annuum*	Ornamental Chile Pepper	Susceptibility
AC09197	*Capsicum annuum*	Chile Pepper Institute	Susceptibility

Accession	Species	Cultivar name	Character
AC09198	*Capsicum annuum*	Numex St.Patrick's Day	Susceptibility
AC09199	*Capsicum annuum*	Takanotsume	Susceptibility
AC09200	*Capsicum annuum*	Santaka	Susceptibility
AC09201	*Capsicum annuum*	Numex Heritage 6-4	Susceptibility
AC09202	*Capsicum annuum*	Numex Big Jim	Susceptibility
AC09203	*Capsicum annuum*	Pimenta De Espelette	Susceptibility
AC09204	*Capsicum annuum*	Numex Joe E.Parker	Susceptibility
AC09205	*Capsicum annuum*	Numex 6-4	Susceptibility
AC09206	*Capsicum annuum*	NuMex Espanila Improved	Susceptibility
AC09210	*Capsicum annuum*	Numex Garnet	Susceptibility
AC09211	*Capsicum annuum*	Numex Sunrise	Susceptibility
AC09212	*Capsicum annuum*	Barker Hot	Susceptibility
AC09214	*Capsicum annuum*	Numex Primavera	Susceptibility
AC09215	*Capsicum annuum*	Numex Centennial	Susceptibility
AC09216	*Capsicum annuum*	Numex Pinata	Susceptibility
AC09217	*Capsicum annuum*	Omnicolor	Susceptibility
AC09218	*Capsicum annuum*	Numex Conquistador	Susceptibility
AC09219	*Capsicum annuum*	Numex Bailey Piquin	Susceptibility
AC09220	*Capsicum annuum*	Numex Christmas	Susceptibility
AC09221	*Capsicum annuum*	Numex Thanksgiving	Susceptibility
AC09222	*Capsicum annuum*	Numex Valentine	Susceptibility
AC09223	*Capsicum annuum*	Numex Halloween	Susceptibility
AC09224	*Capsicum annuum*	Messilla Cayenne	Susceptibility
AC09225	*Capsicum annuum*	Numex Sunglo, Sunburst	Susceptibility
AC09227	*Capsicum annuum*	Numex Mirasol	Susceptibility
AC09228	*Capsicum annuum*	Black cuban	Susceptibility
AC09229	*Capsicum annuum*	deArbol	Susceptibility
AC09231	*Capsicum annuum*	Poblano	Susceptibility
AC09232	*Capsicum annuum*	Floral Gem	Susceptibility
AC09233	*Capsicum annuum*	Spanish Piquillo	Susceptibility
AC09234	*Capsicum annuum*	Santa fe grande	Susceptibility
AC09235	*Capsicum annuum*	Mulato	Susceptibility
AC09236	*Capsicum annuum*	Piqunin	Susceptibility
R09029	*Capsicum annuum*	Unknown14	Susceptibility
R09031	*Capsicum annuum*	Unknown16	Susceptibility

Accession	Species	Cultivar name	Character
R09040	*Capsicum annuum*	Unknown25	Susceptibility
R09041	*Capsicum annuum*	H.Wax	Susceptibility
R09042	*Capsicum annuum*	H.Wax No.2	Susceptibility
R09043	*Capsicum annuum*	New Mexico	Susceptibility
R09044	*Capsicum annuum*	Cascavel	Susceptibility
R09045	*Capsicum annuum*	Agronomico NO.8	Susceptibility
R09046	*Capsicum annuum*	Agronomico 10G	Susceptibility
R09047	*Capsicum annuum*	Agronomico 10G	Susceptibility
R09048	*Capsicum annuum*	Hot Portugal	Susceptibility
R09049	*Capsicum annuum*	San ta Fe Grand	Susceptibility
R09050	*Capsicum annuum*	PBC413 TAM Mildjalapeno	Susceptibility
R09051	*Capsicum annuum*	PBC416 YJ81032	Susceptibility
R09052	*Capsicum annuum*	PBC120 HDA336	Susceptibility
R09053	*Capsicum annuum*	PBC427 NuMex Eclipse	Susceptibility
R09054	*Capsicum annuum*	PBC429 NuMex Sunrise	Susceptibility
R09055	*Capsicum annuum*	PBC828 Papri Queen	Susceptibility
R09056	*Capsicum annuum*	Guajillo ancho	Susceptibility
R09057	*Capsicum annuum*	China pepper	Susceptibility
R09058	*Capsicum annuum*	Jeongseonnyanggakcho	Susceptibility
R09059	*Capsicum annuum*	Bogariaralcho	Susceptibility
R09061	*Capsicum annuum*	Pepperoncini	Susceptibility
R09062	*Capsicum annuum*	pepper from Hongkong	Susceptibility
R09063	*Capsicum annuum*	97 from China1	Susceptibility
R09064	*Capsicum annuum*	Unknown26	Susceptibility
R09065	*Capsicum annuum*	Lac-301	Susceptibility
R09066	*Capsicum annuum*	llica 256	Susceptibility
R09067	*Capsicum annuum*	MilesFlavor se	Susceptibility
R09068	*Capsicum annuum*	Unknown27	Susceptibility
R09069	*Capsicum annuum*	Malang Pujon Local17	Susceptibility
R09070	*Capsicum annuum*	Szechwan4	Susceptibility
R09071	*Capsicum annuum*	Tit Super	Susceptibility
R09072	*Capsicum annuum*	Jatilaba	Susceptibility
R09073	*Capsicum annuum*	Unknown28	Susceptibility
R09074	*Capsicum annuum*	IN,JA,VM4	Susceptibility
R09075	*Capsicum annuum*	97H.B offype EmCu-22	Susceptibility

Accession	Species	Cultivar name	Character
R09076	*Capsicum annuum*	Hu-33 AVRCD94187	Susceptibility
R09077	*Capsicum annuum*	Sacheon94187	Susceptibility
R09078	*Capsicum annuum*	Szechwan 2	Susceptibility
R09080	*Capsicum annuum*	Unknown29	Susceptibility
R09081	*Capsicum annuum*	Huaruar	Susceptibility
R09082	*Capsicum annuum*	LongChilli	Susceptibility
R09083	*Capsicum annuum*	IN.JA.VM5	Susceptibility
R09084	*Capsicum annuum*	96IN F1se	Susceptibility
R09085	*Capsicum annuum*	IN. Keriting	Susceptibility
R09086	*Capsicum annuum*	Unknown30	Susceptibility
R09087	*Capsicum annuum*	S.N PrigkeeNuu	Susceptibility
R09088	*Capsicum annuum*	Cabe Keriting	Susceptibility
R09089	*Capsicum annuum*	Medan(Sumatra)	Susceptibility
R09090	*Capsicum annuum*	Unknown31	Susceptibility
R09091	*Capsicum annuum*	Unknown32	Susceptibility
R09092	*Capsicum annuum*	Unknown33	Susceptibility
R09093	*Capsicum annuum*	97JA VM 4	Susceptibility
R09094	*Capsicum annuum*	Malang Pujonlocal17	Susceptibility
R09095	*Capsicum annuum*	BSS-213 F2	Susceptibility
R09096	*Capsicum annuum*	Chilly BSS 141 F2	Susceptibility
R09097	*Capsicum annuum*	Chilly BSS 213 F2	Susceptibility
R09098	*Capsicum annuum*	Unknown34	Susceptibility
R09099	*Capsicum annuum*	Bako Local	Susceptibility
R09100	*Capsicum annuum*	Perennial HDV	Susceptibility
R09101	*Capsicum annuum*	G4	Susceptibility
R09102	*Capsicum annuum*	Wonder Hot	Susceptibility
R09103	*Capsicum annuum*	Festival	Susceptibility
R09104	*Capsicum annuum*	PE02	Susceptibility
R09105	*Capsicum annuum*	Pusa Jwala(Proagrow-PGS)	Susceptibility
R09106	*Capsicum annuum*	JWALA(Navalakha)	Susceptibility
R09107	*Capsicum annuum*	Hyderabad VM	Susceptibility
R09108	*Capsicum annuum*	98HES101CO1806	Susceptibility
R09109	*Capsicum annuum*	98HES102 PBC100-6	Susceptibility
R09110	*Capsicum annuum*	98HES104 HuaRua	Susceptibility
R09111	*Capsicum annuum*	98HES106 PBC30-4	Susceptibility

Accession	Species	Cultivar name	Character
R09112	*Capsicum annuum*	98HES117 B4006-1	Susceptibility
R09113	*Capsicum annuum*	Unknown35	Susceptibility
R09114	*Capsicum annuum*	Hot Pepper AL	Susceptibility
R09115	*Capsicum annuum*	Hot Pepper Novartis F2	Susceptibility
R09116	*Capsicum annuum*	Chilly Long	Susceptibility
R09117	*Capsicum annuum*	Hot Pepper Orissa local-3	Susceptibility
R09119	*Capsicum annuum*	AnKur-228	Susceptibility
R09120	*Capsicum annuum*	PX23595(No.8)	Susceptibility
R09121	*Capsicum annuum*	PX24195(No.10)	Susceptibility
R09122	*Capsicum annuum*	Unknown37	Susceptibility
R09123	*Capsicum annuum*	ChiangRai VM	Susceptibility
R09124	*Capsicum annuum*	PBC59 Bhaskar	Susceptibility
R09125	*Capsicum annuum*	PBC134 LCA-305	Susceptibility
R09126	*Capsicum annuum*	PBC141 X-235	Susceptibility
R09127	*Capsicum annuum*	PBC157 HuaySithon	Susceptibility
R09128	*Capsicum annuum*	PBC455 PBC455	Susceptibility
R09129	*Capsicum annuum*	PBC479 ANK-72	Susceptibility
R09130	*Capsicum annuum*	PBC483 ArunalLu(BL-39)	Susceptibility
R09131	*Capsicum annuum*	PBC580 Chillies Giants	Susceptibility
R09132	*Capsicum annuum*	PBC585 PBC585	Susceptibility
R09133	*Capsicum annuum*	PBC586 PBC586	Susceptibility
R09134	*Capsicum annuum*	PBC636 Galkunda Miris	Susceptibility
R09135	*Capsicum annuum*	99Gonmyeong collection1	Susceptibility
R09136	*Capsicum annuum*	99Gonmyeong collection2	Susceptibility
R09137	*Capsicum annuum*	NP-51	Susceptibility
R09138	*Capsicum annuum*	Tombak-2	Susceptibility
R09139	*Capsicum annuum*	TW99	Susceptibility
R09140	*Capsicum annuum*	G4	Susceptibility
R09141	*Capsicum annuum*	Subicho	Susceptibility
R09142	*Capsicum annuum*	Subicho	Susceptibility
R09143	*Capsicum annuum*	Unknown38	Susceptibility
R09144	*Capsicum annuum*	94PH-21	Susceptibility
R09145	*Capsicum annuum*	95SLU DiRe-9	Susceptibility
R09146	*Capsicum annuum*	Unknown39	Susceptibility
R09147	*Capsicum annuum*	Unknown40	Susceptibility

Accession	Species	Cultivar name	Character
R09148	*Capsicum annuum*	Unknown41	Susceptibility
R09149	*Capsicum annuum*	Subicho	Susceptibility
R09150	*Capsicum annuum*	PBC102 Unknown Y13	Susceptibility
R09151	*Capsicum annuum*	B.Wonder	Susceptibility
R09153	*Capsicum annuum*	Unknown43	Susceptibility
R09154	*Capsicum annuum*	Unknown44	Susceptibility
R09155	*Capsicum annuum*	Unknown45	Susceptibility
R09156	*Capsicum annuum*	Unknown46	Susceptibility
R09157	*Capsicum annuum*	Unknown47	Susceptibility
R09158	*Capsicum annuum*	Unknown48	Susceptibility
R09159	*Capsicum annuum*	Unknown49	Susceptibility
R09166	*Capsicum annuum*	Cheongnyongcho	Susceptibility
R09169	*Capsicum annuum*	Ttungttungcho	Susceptibility
R09170	*Capsicum annuum*	Chilseongcho	Susceptibility
AC09012	*Capsicum annuum*	Nitrianska Tenkosteena	Susceptibility
AC09017	*Capsicum annuum*	Wiener Calvill	Susceptibility
AC09020	*Capsicum annuum*	Unknown53	Susceptibility
AC09021	*Capsicum annuum*	Unknown54	Susceptibility
AC09022	*Capsicum annuum*	Unknown55	Susceptibility
AC09032	*Capsicum annuum*	Unknown56	Susceptibility
AC09034	*Capsicum annuum*	Unknown57	Susceptibility
AC09035	*Capsicum annuum*	Unknown58	Susceptibility
AC09043	*Capsicum annuum*	Unknown59	Susceptibility
AC09045	*Capsicum annuum*	Unknown60	Susceptibility
AC09052	*Capsicum annuum*	Cecei	Susceptibility
AC09054	*Capsicum annuum*	Unknown61	Susceptibility
AC09055	*Capsicum annuum*	Ikom II	Susceptibility
AC09059	*Capsicum annuum*	Unknown62	Susceptibility
AC09062	*Capsicum annuum*	Unknown63	Susceptibility
AC09063	*Capsicum annuum*	Unknown64	Susceptibility
AC09068	*Capsicum annuum*	HS-X-3	Susceptibility
AC09071	*Capsicum annuum*	Unknown68	Susceptibility
AC09072	*Capsicum annuum*	Kutansuri	Susceptibility
AC09073	*Capsicum annuum*	Krupnyj Zeltyj 903	Susceptibility
AC092112	*Capsicum annuum*	Numex Sunrise	Susceptibility

Accession	Species	Cultivar name	Character
A11-1	*Capsicum annuum*	Gungyeilhak//Syngenta Korea	Susceptibility
A11-2	*Capsicum annuum*	Geumbit//Syngenta Korea	Susceptibility
A11-4	*Capsicum annuum*	Giribbarksoo//Syngenta Korea	Susceptibility
A11-5	*Capsicum annuum*	Daejangbu//Syngenta Korea	Susceptibility
A11-6	*Capsicum annuum*	Dukyachungchung//Syngenta	Susceptibility
A11-7	*Capsicum annuum*	Mansahyungtong//Syngenta	Susceptibility
A11-8	*Capsicum annuum*	Muhanjilju//Syngenta Korea	Susceptibility
A11-9	*Capsicum annuum*	Bakjangdaso//Syngenta Korea	Susceptibility
A11-10	*Capsicum annuum*	Bulmat//Syngenta Korea	Susceptibility
A11-12	*Capsicum annuum*	Ilsongjung//Syngenta Korea	Susceptibility
A11-13	*Capsicum annuum*	Ilinja//Syngenta Korea	Susceptibility
A11-15	*Capsicum annuum*	Chuljae//Syngenta Korea	Susceptibility
A11-16	*Capsicum annuum*	Hanson//Syngenta Korea	Susceptibility
A11-17	*Capsicum annuum*	Hangun//Syngenta Korea	Susceptibility
A11-26	*Capsicum annuum*	Baerodda//Nongwoo Korea	Susceptibility
A11-27	*Capsicum annuum*	Supermanidda//Nongwoo	Susceptibility
A11-28	*Capsicum annuum*	Youngyangmat//Nongwoo	Susceptibility
A11-29	*Capsicum annuum*	Obok//Nongwoo Korea	Susceptibility
A11-30	*Capsicum annuum*	Jinme//Nongwoo Korea	Susceptibility
A11-34	*Capsicum annuum*	Hongmiin//Nongwoo Korea	Susceptibility
A11-35	*Capsicum annuum*	Hongjinju//Nongwoo Korea	Susceptibility
A11-37	*Capsicum annuum*	PR Geummak//Nongwoo	Susceptibility
A11-38	*Capsicum annuum*	PR Mansae//Nongwoo Korea	Susceptibility
A11-39	*Capsicum annuum*	PR Sangsang//Nongwoo Korea	Susceptibility
A11-40	*Capsicum annuum*	PR Aeulim//Nongwoo Korea	Susceptibility
A11-41	*Capsicum annuum*	PR Yauljung//Nongwoo Korea	Susceptibility
A11-49	*Capsicum annuum*	Daedeulbo//Takii korea Korea	Susceptibility
A11-50	*Capsicum annuum*	PR Hwanhosung//Takii korea	Susceptibility
A11-54	*Capsicum annuum*	Bulseachul//Sakata Korea	Susceptibility
A11-57	*Capsicum annuum*	Anjeonbelt//Sakata Korea	Susceptibility
AC03001	*Capsicum frutescens*	Kathmandu-1//Nepal	Susceptibility
AC061241	*Capsicum frutescens*	Tabasco	Susceptibility
AC064141	*Capsicum frutescens*	Onamental1	Susceptibility
AC02142	*Capsicum baccatum*	Dedo de moca//Brazil	Susceptibility
AC07277	*Capsicum baccatum*	unknown//Argentine	Susceptibility

Accession	Species	Cultivar name	Character
AC09213	*Capsicum baccatum*	Aji Limon	Susceptibility
AC08003	*Capsicum frutescens*	AVRDC//C00050	Susceptibility
AC080062	*Capsicum frutescens*	AVRDC//C00065	Susceptibility
AC080075	*Capsicum frutescens*	AVRDC//C00086	Susceptibility
AC08008	*Capsicum frutescens*	AVRDC//C00087	Susceptibility
AC080101	*Capsicum frutescens*	AVRDC//C00090	Susceptibility
AC080111	*Capsicum frutescens*	AVRDC//C00098	Susceptibility
AC08012	*Capsicum frutescens*	AVRDC//C00276	Susceptibility
AC080152	*Capsicum frutescens*	AVRDC//C00309	Susceptibility
AC08024	*Capsicum frutescens*	AVRDC//C00644	Susceptibility
AC080281	*Capsicum frutescens*	AVRDC//C00657	Susceptibility
AC080291	*Capsicum frutescens*	AVRDC//C00669	Susceptibility
AC08031	*Capsicum frutescens*	AVRDC//C00737	Susceptibility
AC08038	*Capsicum frutescens*	AVRDC//C00874	Susceptibility
AC08040	*Capsicum frutescens*	AVRDC//C00897	Susceptibility
AC08045	*Capsicum frutescens*	AVRDC//C00966	Susceptibility
AC080011	*Capsicum baccatum*	AVRDC//C00044	Susceptibility
AC080161	*Capsicum baccatum*	AVRDC//C00313	Susceptibility
AC08033	*Capsicum baccatum*	AVRDC//C00739	Susceptibility
AC08034	*Capsicum baccatum*	AVRDC//C00753	Susceptibility
AC08042	*Capsicum baccatum*	AVRDC//C00941	Susceptibility
AC08046	*Capsicum baccatum*	AVRDC//C01172	Susceptibility
AC08057	*Capsicum baccatum*	AVRDC//C01557	Susceptibility
AC080601	*Capsicum baccatum*	AVRDC//C01686	Susceptibility
AC08067	*Capsicum baccatum*	AVRDC//C02667	Susceptibility
AC080691	*Capsicum baccatum*	AVRDC//C03946	Susceptibility
AC080711	*Capsicum baccatum*	AVRDC//C04066	Susceptibility
AC080731	*Capsicum baccatum*	AVRDC//C04068	Susceptibility
AC08054	*Capsicum pubescens*	AVRDC//C01374	Susceptibility
AC08085	*Capsicum pubescens*	AVRDC//C04895	Susceptibility
AC08062	*Capsicum chacoense*	AVRDC//C01728	Susceptibility
AC08075	*Capsicum chacoense*	AVRDC//C04389	Susceptibility
AC080812	*Capsicum chacoense*	AVRDC//C04398-A	Susceptibility