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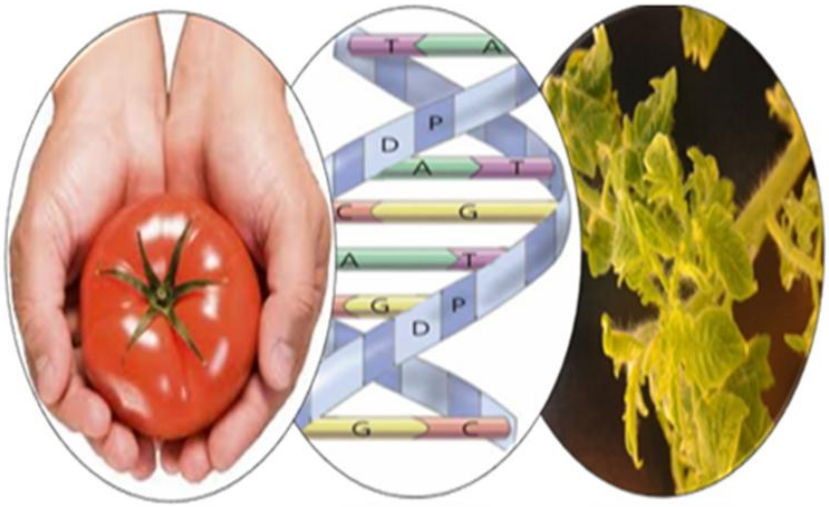
Analysis of Ty-2 region in chromosome 11 of Solanum lycopersicum and Solanum habrochaites



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Analysis of *Ty-2* region in chromosome 11 of *Solanum lycopersicum* and *Solanum habrochaites*

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MSc Major Thesis
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Abstract

Tomato yellow leaf curl virus (TYLCV) is one of the major pathogens of tomato causing huge economic losses around the whole world. Six resistance genes against TYLCV have been identified from different wild tomato accessions. *Ty-2* is one of the major genes that confer resistance to TYLCV, mapped to a region of 300 kb between markers UP8 (51.344 Mb) and M1 (51.645 Mb) on chromosome 11 from *S. habrochaites* accession B6013. Further shortening of this region was not possible as severe recombination suppression was observed in a region of 200 kb between the markers C2_At1g07960 and C2_At3g52090 (called the 'block') in the*Ty-2* region in crosses between *S. habrochaites* and *S. lycopersicum*. In the present study, populations derived from crosses of different accessions of *S. habrochaites* were testedand no recombination suppression was observed in this particular segment in the *Ty-2* region. It was revealed that the block is only observedin interspecific crosses between *S. habrochaites* and *S. lycopersicum*.It was also found that the marker order in the block of *S. habrochaites* and *S. lycopersicum* do not align to each other. An inversion in the region of chromosome 11 between *S. habrochaites* and *S. lycopersicum* is hypothesizedand this inverted region might be responsible of the observed recombination suppression. A second experiment was conducted to study the inheritance of resistance to TYLCV in the progenies carrying the *S. habrochaites*introgression. Molecular marker analysis and disease scoring was done in F_2 and F_4 recombinant lines individuals. Results confirmed the presence of *S. habrochaites* introgression carrying *Ty-2* resistance gene in these individuals. Results also suggested the possibility of the presence of additional genetic factors playing a role on the resistance in these recombinant lines.

Key works: Tomato,TYLCV, recombination suppression, molecular marker

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1 Introduction

1.1 Tomato (*Solanum lycopersicum*)

Tomato (*Solanum lycopersicum*) belongs to the family Solanaceae. Solanaceae is a large and diverse family and contains more than 3000 different species including food crops such as potato, pepper, eggplant, medicinal plants such as *Datura*, as well as ornamental plants such as *Petunia* (Gupta et al. 2009). Tomato is one of the economically important crops grown worldwide with total production of around 100 million metric tons/year (Figure 1). It is originated in the South American Andean region and the most probable region of the domestication is Mexico. Peru has been reported as centre of diversity of wild relatives of tomato (Bai and Lindhout, 2007). Tomato is botanically a fruit (berry), however it is considered as a vegetable. It is one of the widely used vegetables in the world and can be consumed either as fresh or processed. Tomato consumption is beneficial for health as it contain high level of vitamin A, vitamin C, high level of anthocyanin (blue tomato) and lycopene (Isah et al., 2014).It is also reported that tomato has the ability to protect people from certain cancers (Gupta et al. 2009).

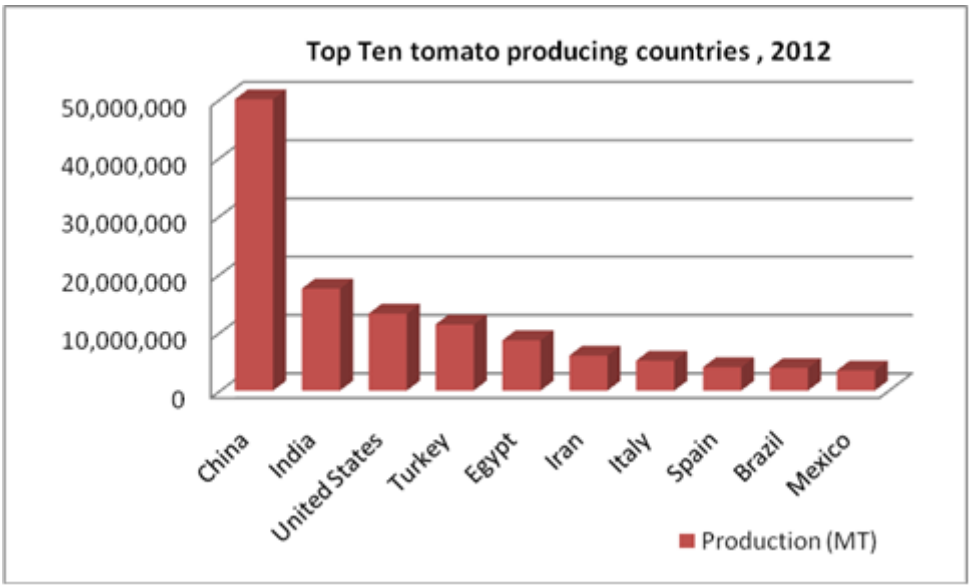

Figure 1Top ten tomato producing countries based on their production in tonnes, (FAOSTAT, 2012). (http://faostat.fao.org/)

Tomato is also a model crop in the study of *Solanaceae*plants. The cultivated tomato and all wild relatives are diploid with 12 chromosomes (2n=2X=24). Crossing of wild relatives with *Solanum lycopersicum* is possible, sometimes with complexity and by using special techniques (Liedl *et al.*, 2013). The tomato genome was sequenced and published in Nature in 2012 (The Tomato Genome Consortium, 2012). The actual genome size of tomato is 950 Mbp with 31,760 genes. The genomic data is available in SOL Genomics database (http://solgenomics.net/). Tomato is a self-pollinated crop, but it can be easily crossed under appropriate conditions. The study of tomato is also easy as it has relatively short generation time compared with many other crops (Gupta et al., 2009). Implementation of new methodological approaches like molecular mapping for important traits, using of backcrossing and advanced introgression lines helps to make tomato crop domesticated and more improved with desired traits (Liedl *et al.*, 2013).

Solanum section Lycopersicon includes the cultivated tomato (*Solanum lycopersicum*) and its 12 wild relatives where *Solanum lycopersicum* is the only domesticated species. The most important wild relatives used for breeding programs are *S. pennelli*, *S. chilense*,*S. habrochaites* and *S. pimpinellifolium* (Table 1). The reproductive biology and mating systems of the wild relatives of the cultivated tomato have a great diversity (Bedinger et al., 2010). Molecular marker technologies have allowed people to know about the large genetic variation of wild relatives. Self-incompatible (SI) species have more genetic variation than accessions of self-compatible (SC) species. Therefore wild species are the important sources of specific traits that have been investigated intensively and used in tomato breeding (Bai and Lindhout, 2007).

Table 1Mating systems in cultivated tomato and its wild relatives

Mating system	Compatibility	Species
Autogamous	All SC	*S. lycopersicum*
		S. cheesmaniae
		S. neorickii
		S. galapagense
Facultative	All SC	*S. pimpinellifolium*
		S. chmielewskii
Facultative	Mostly SI, some SC populations	*S. peruvianum*
		S. habrochaites
		S. pennellii
		S. arcanum
Allogamous	All SI	*S. chilense*
		S. corneliomulleri

(Source: Bedinger et al., 2010)

Different types of biotic and abiotic stresses adversely affect growth and productivity of tomato and significantly cause economic losses. Many improved varieties of tomato are available through domestication, but these are often more vulnerable towards disease and environment changes. Various diseases of tomato are caused by bacteria, fungus, nematodes as well as viruses (Gupta et al., 2009). Many virus species including Tomato yellow leaf curl virus (TYLCV), Pepino mosaic virus (PepMV), Tomato torrado virus (ToTV) are reported that infect tomato plants worldwide (Hanssen et al., 2010).

1.2 Tomato yellow leaf curl virus (TYLCV)

Tomato yellow leaf curl disease (TYLCD) is a widespread devastating disease of tomato.One of the causing agents of TYLCD is Tomato yellow leaf curl viruses (TYLCV) which belong to *Begomovirus* genus under family *Geminiviridae*. It is a major pathogen of tomato grown in tropical and subtropical region of the world (Verlaan et al., 2011; Ji et al., 2009a). It is ranked 3rd among the economically important plant viruses (Verlaan et al., 2013).

1.2.1 Disease Symptoms

TYLCV infection causes leaf curling, yellowing, plant stunting and flower abortion of susceptible plant. In early stages, slight yellowing of the leaflet margins starts in apical leaves with upward curling and cupping appearance of leaflets at later stage (Figure 2). During severe infection stages, plant growth stops completely and flowers may appear but drop before fruit setting. Entire crops are often lost when plants are infected at young growth stage (Ji et al., 2009a). TYLCV has diverse host range including many economically important crops like tomato, potato, eggplants, pepper, tobacco, etc. (Verlaan et al., 2011).

Figure 2Symptoms of TYLCV on susceptible tomato cultivar Moneymaker at 30 DAI
Source: (Verlaan, 2013)

1.2.2 Structure of TYLCV genome

The *Geminiviridae* is the largest virus family which can infect dicot and monocot plants. They consist of circular single-stranded (ss) DNA genome in one or two components and they are transmitted by different species of leafhoppers, treehoppers or the single whitefly species *Bemisia tabaci*. This family is divided into four genera based on their genome organization, host range and vector transmission. The genus Begomovirus is transmitted by whitefly *Bemisia tabaci* and only dicotyledonous plants are infected by them. Most of them contain a bipartite genome with two circular ssDNA particles whereas TYLCV is a true monopartitie virus (Verlaan, 2013).

The TYLCV begomovirus genome is composed of circular and ssDNA genome of 2.7-2.8 kb. They replicate in the nuclei of the host cells by a mechanism known as rolling-circle replication where a double stranded DNA intermediate replicative form is used as a template. The genome of TYLCV contains six partially overlapping open reading frames (ORFs), separated by an intergenic region (IR) of approximately 300 nucleotides. ORFs V1 and V2 are in the viral sense and four ORFs, C1, C2, C3 and C4, are in the complementary sense (Figure 3). The V1 encodes the coat protein (CP) that is the structural protein of virion particle. CP is essential for systemic infection, particle formation and transmission by the insect.V2 encodes a movement protein (MP) and may also function as a

suppressor of host RNA-silencing pathway. The C1 encodes a protein replicase or replication initiator protein (Rep) necessary for initiation of the rolling cycle replication, C2 encodes transcription activator protein (TrAP) that activates transcription and is essential for infectivity. C3 encodes a replication enhancer (REn) protein which acts to increase viral DNA accumulation and enhance infectivity and symptom expression. C4 encodes a small protein embedded within the Rep that induces virus like symptoms (systemic movement) in host plants (Chen et al., 2011).

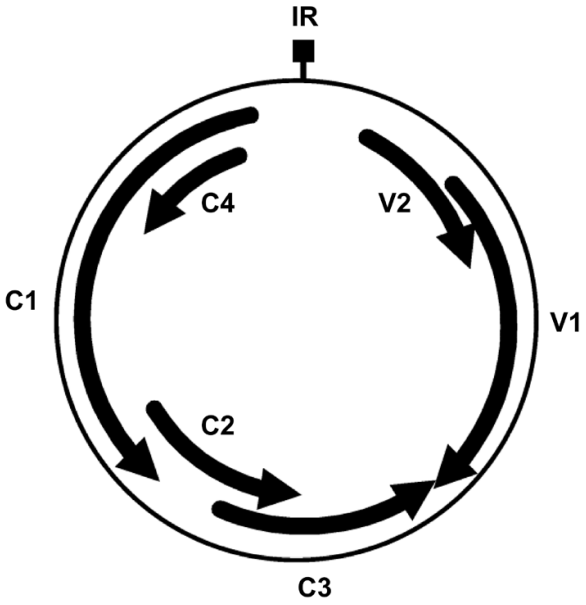

Figure 3Genome organization of a typical monopartite TYLCV
Source: (Verlaan, 2013)

1.2.3 Spread of TYLCV

Adult whitefly (*Bemisia tabaci*, biotype B) is the only known vector of TYLCV, which is also called silver leaf whitefly (SLW). Itis one of the important pestshaving wide host range, rapid propagation and virus transmission ability (Fang et al., 2013). It is capable of feeding on manyagricultural crops including vegetables, ornamental and field crops (Fang et al., 2013, Horowitz and Ishaaya, 2014). It is the vector of different important plant viruses. When whitefly feeds on phloem sap of an infected plant, TYLCV is picked up as TYLCV is phloem limited. Virus persists within the vector but does not replicate within it. It is transmitted from plant to plant by the vector in a circulative manner. But whitefly needs time for translocation of virus from digestive tract to salivary gland for transmitting it during feeding which is called latency period. The latency period takes approximately 8 hours for TYLCV, though some large variations are also reported (Verlaan, 2013).

1.2.4 Management of disease

Pathogens are often controlled by using different kinds of chemical compounds like pesticides. Insecticides and physical barriers such as nets are also used to control whitefly for disease management of TYLCV. Sometimes these methods are not fully effective as well as labour intensive and expensive. Pesticides and insecticides have potential risk to the health of growers and consumers. Using insecticides is not environmental friendly and some whiteflies have been also reported which are also resistant to insecticide (Verlaan et al,. 2011). Therefore breeding for TYLCV resistant tomato cultivars would be an effective alternative to manage TYLCD as wild species of tomato have great sources of resistance (Bai and Lindhout, 2007).

1.3 Sources for TYLCV resistance breeding

All cultivated tomato varieties are susceptible to TYLCV infection. Resistance against TYLCV is reported in different accessions of wild tomato relatives including *S. pimpinellifolium*, *S. chilense*, *S. peruvianum, S. habrochaites, S. cheesmaniae.* Sixresistance genes have been identified from these resistant accessions until now. Therefore, breeding for resistance against TYLCV is mainly based on introgression of resistance genes from wild relatives to cultivated tomatoes (Hutton, 2013; Verlaan, 2013).

From the six mapped resistance loci, 4 genes are dominant (*Ty-1, Ty-2, Ty-3* and *Ty-4*) and *ty-5* gene is recessive one (Table 2). Notmuch information is published about *Ty-6*gene yet, but it is found that it may perhaps a recessive gene.Both *Ty-1* and *Ty-3* are derived from accessions of *S. chilense* and mapped on chromosome 6 (Ji et al., 2009a, Verlaan, 2013). *Ty-2* is reported to derive from *S. habrochaites* accession "B6013" (Ji et al., 2009a; Yang et al., 2014) and mapped to chromosome 11 (Verlaan, 2013; Yang et al., 2014). *Ty-4* is originated from *S. chilense* LA1932 and mapped to the long arm of chromosome 3 (Ji et al., 2009b). Moreover, *ty-5* gene is initially found in tomato breeding line TY172 from *S. peruvianum* where resistance is located in chromosome 4 (Anbinder et al., 2009) and later found in the hybrid cultivar Tyking (Hutton et al., 2012). Resistance gene, *Ty-6* was recently derived from *S. chilense* accession LA2779 on chromosome 10 (Hutton, 2013). The genes *Ty-1* and *Ty-3* have been cloned and found that they are alleles of the same gene. However other genes that confer resistance to TYLCV have not been cloned yet (Verlaan et al., 2013).

Table 2Mapped TYLCV resistance loci that are identified from wild Solanum species

Gene	Genetic source (Accession/line)	Genetic source (Species)	Chromosome
Ty -1	LA1969	*S. chilense*	6
Ty-2	B6013	*S. habrochaites*	11
Ty-3	LA1932, LA2779	*S. chilense*	6
Ty-4	LA1932	*S. chilense*	3
ty-5	TY172	*S. peruvianum*	4
Ty-6	LA2779	*S. chilense*	10

(Source: Verlaan et al., 2011; Hutton, 2013)

The *Ty-2* gene is one of the major sources of breeding program for TYLCV resistance (Ji et al., 2009a). *Ty-2* was previously mapped to the long arm of the chromosome 11 between two RFLP markers TG36 (physical position 51.490 Mb based on version SL2.40 of the tomato genome, 84 cM) and TG393 (103 cM) (Hanson et al, 2006; Ji et al., 2009a; Yang et al., 2014). Later *Ty-2* gene was delimited to a region of 500 kb distance between markers C2_At2g28250 (physical location 51.307Mb) and T0302 (51.878 Mb) (Ji et al., 2009a). To facilitate cloning of resistance gene and eliminate associated linkage drag, further shortening of the *Ty-2* region is important. Yang et al., (2014) mapped *Ty-2* gene between markers UP8 (51.344 Mb) and M1 (51.645 Mb) (approximately 300 kb region interval) by analyzing recombinants in F_4 population (Figure 4). However, further delimiting of this region was not possible because of recombination suppression of the introgression region.

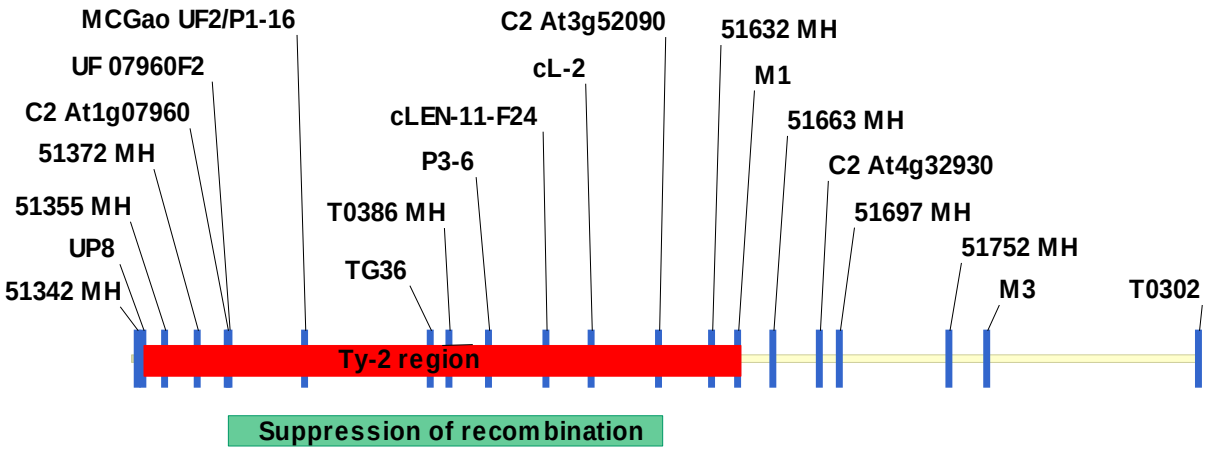

Figure 4The *Ty-2* region on chromosome 11 in *S. lycopersicum*

1.4 Suppression of recombination in the *Ty-2* introgression from *S. habrochaites*

Breeders are interested to introduce desired traits from wild relatives to cultivated species. But crossing barriers or recombination suppression limit the use of wild relatives as donors in introgression breeding. Presence of structural rearrangementssuch as translocations and inversions in the chromosome, is one of the causes of recombination suppression (Peters et al., 2012). Various numbers of chromosomal rearrangements were already observed in tomato, potato, eggplants, peppers and related *Solanum* species (Szinay et al., 2012).

Recombination suppression was also reported in the *Ty-1* introgression region on chromosome 6 of tomato. Verlaan et al., (2011) found suppression of recombination between *S. lycopersicum* and *S. chilense* LA1969 where *Ty-1* was introgressed. They used Fluorescence in situ hybridization(FISH) experiments and large-scale recombinant screening for analyzing the chromosomal structure and recombination behavior in the introgression region. The chromosomal inversions were the cause of the recombination suppression (Verlaan et al., 2011).

In the previous study of fine mapping of the *Ty-2* gene, F_2 and F_4 populations were used that were derived from a commercial hybrid carrying the *Ty-2* gene in the genetic background of *S. lycopersicum* (Yang et al., 2014). *Ty-2* gene was delimited between markers UP8 and M1 to a distance around 300 kb. However, they also found severe recombination suppression between the markers C2_At1g07960 and C2_At3g52090 and this region of 200 kb is called 'the block' (Figure 4). Yang et al., (2014) also used FISH technique to know whether chromosomal rearrangements is the cause of this suppressionbut this technique failed to visualize chromosomal structure of *Ty-2* introgression due to the small interval of this region. The reasons of this suppression are still unknown.

1.5 Objectives

In this study, two experiments were done separately. The first experiment was set to analyzethe *Ty-2* region in populations derived from intraspecific crosses among different accessions of *S. habrochaites.*In the second experiment, phenotypic and marker analysis was done to confirmthe presence of *Ty-2* gene in the region of the introgression lines derived from crosses between *S. habrochaites and S. lycopersicum*.

1.5.1 Objectives of Experiment 1

According to the study of Yang et al., (2014), severe recombination suppression occurred in crosses between *S. habrochaites* and *S. lycopersicum*. Previous studies showed that chromosomal rearrangements might be the cause of suppression of recombination. Inversions are the most reported form of chromosomal rearrangements in *Solanum* species (Szinay D., 2010). Inversions were also found in the *Ty-1* introgressed region derived from *S. chilense*on chromosome 6 (Verlaan et al., 2011).

Ourhypothesis is the potential presence of an inversion in the *Ty-2* region in chromosome 11 in the interspecific cross between*S. habrochaites* and *S. lycopersicum*, this inversion being the reason of the observed recombination suppression. To confirm this hypothesis we analyzed recombination rate in populations derived from crosses between different accessions of *S. habrochaites*.

Therefore the research questions of the present study were:

- Is recombination suppression specific in crosses between *S. habrochaites* and *S. lycopersicum* (interspecific cross)or is it also occurredin crosses among different *S. habrochaites* accessions(intraspecific cross)?

- If recombination occurs in the "block" in intraspecific crosses between *S. habrochaites* accessions, what is the order of the markers? Is this order different from the order in *S. lycopersicum*?

To answer these research questions, markers were picked up from the suppression of recombination region based on tomato Heinz sequenceused in the study byYang et al., (2014). These were tested for the presence of polymorphism between different *S. habrochaites* accessions and polymorphic markers were used for further works of the study.We hypothesized to find recombinationin the block in progeny from crosses between two *S. habrochaites* accessionsand planned to analyze recombinants with several markers to determine whether the order of the markers in the block is the same as in cultivated tomato, or in reverse order. If necessary, we planned to developed more polymorphic markers among different accessions of *S. habrochaites* to conclude result more precisely.

1.5.2 Objective of Experiment 2

*Ty-2*is important in tomato breeding as it possesses resistance against TYLCV. It has been reported as a dominant gene which was introduced from *S. habrochaites* f. *glabratum* accession "B6013" (Banerjee and kallol, 1987; kallol and Banerjee, 1990; Ji et al., 2009a; Yang et al., 2014). In addition to study populations coming from crosses of different accessions of *S. habrochaites*(experiment 1), the second experimentwas conducted on progenies derived from crosses of *S. habrochaites* and *S. lycopersicum* to analyze the *Ty-2* region.

The objective of this experiment was to analyzethe inheritance of resistance to TYLCV in progenies and to confirm the presence of *S. habrochaites* introgression that carries*Ty-2* gene.

To achieve this, different individuals of F_2, F_3 and F_4 recombinant lines reported to contain *Ty-2* introgression region were screened for resistance to TYLCV and genotyped using molecular marker analysis.

2 Material and Methods

2.1 Experiment 1

2.1.1 Plant materials

Two F_2 populations were used from crosses between different accessions of *Solanum habrochaites.* These populations with their parents are mentioned below in Table 3.

Table 3Name of populations and their parents used in the present study

F_2Populations	Parents
PV960357	*S. habrochaites* G1.1560 and *S. habrochaites* G1.1606
PV970303	*S. habrochaites* G1.1560 and *S. habrochaites* G1.1290

Seeds of F_2 populations (PV960357, PV970303) were collected from seed bank of plant breeding and were sown in plastic cell tray and kept into germination chamber for germination. The temperature of this chamber was between 25°C to 27°C with 90% relative humidity. 96 plants were needed for doing PCR using one 96-well PCR plate, but 104 seeds were sown in a tray for ensuring germination for each case. For PV960357, 91 individuals were used for screening as rest of the seeds were failed to germinate and individuals from parents were used as a control. Seeds were sown in 3 trays in case of population PV970303 and 287 seeds were used in total. Simplified procedure of the experiment to meet first research question is mentioned in Figure 5.

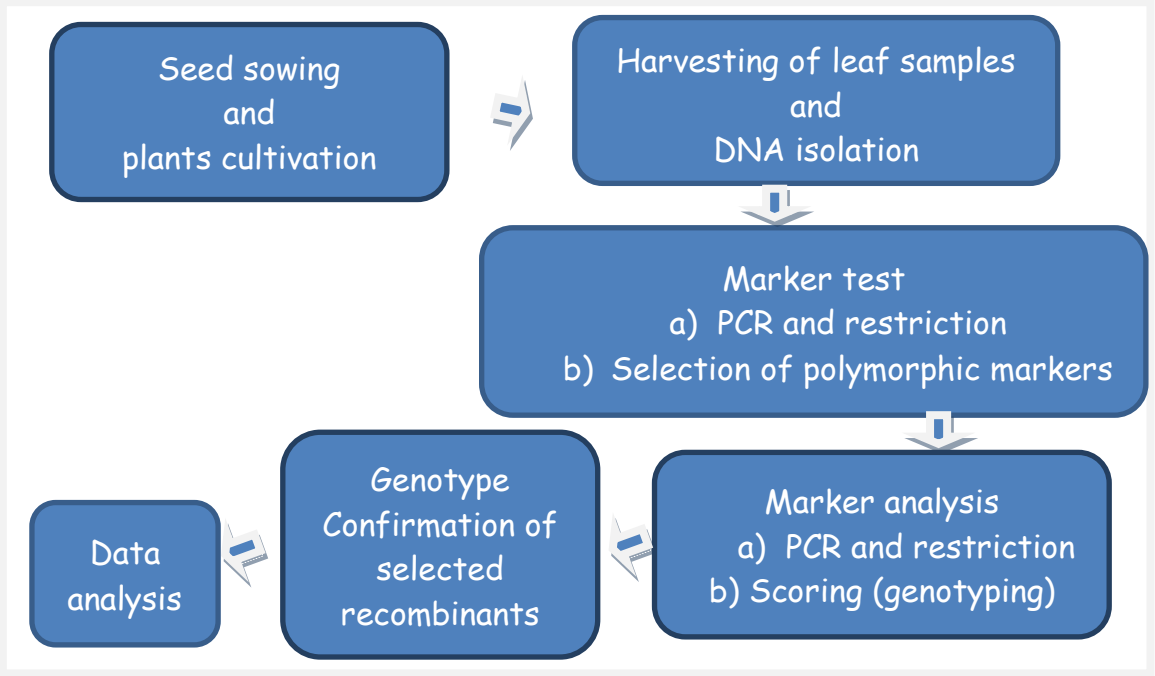

Figure 5Simplified experimental procedure to achieve first research question

2.1.2 DNA isolation

Genomic DNA was extracted from 2-3 weeks old seedlings. Harvesting of leaf samples and extraction of DNA was done by using RETCH protocol 1.4 (Appendix I). In brief, leaf samples were harvested into

1.2 ml 8 strip costar tube filled with 2 steel balls by using forceps in ice. Leaf samples were milled by TissueLyser for 30 sec with 30 r/s frequency in CTAB extraction buffer with RNase. Then chloroform isoamyl alcohol (24:1) was used to remove complex protein and polysaccharides from nucleic acids. Chloroform is more dense than water so that it makes distinct phase after spinning. DNA remains in the upper phase of water whereas protein and polysaccharides bind with chloroform in lower phase. After collecting upper phase of water, Isopropanol was added. DNA is insoluble in isopropanol, DNA aggregate and precipitate after centrifugation. Isopropanol condenses DNA strands and makes DNA more dense and visible. Dry pellets were collected after keeping overnight in the fume hood. Pellets were dissolved in 100µl Milli-Q water to prepare DNA template for PCR and agarose gel electrophoresis was conducted in order to check DNA.

2.1.3 Test for marker selection

2.1.3.1 Molecular markers

In this study, six PCR-based CAPS markers were initially used which were polymorphic for populations of *S. habrochaites* and *S. lycopersicum* (Yang et al., 2014), to check recombination rate in the *Ty-2* region on chromosome 11 (Table 4).

Table 4 Initially used molecular markers to check PCR amplification

Markers	Physical position (Mb)	Restriction enzymes	Primers
UP15	SL2.40ch11: 51.381	*EcoRV*	F-TCTCAAAGCGTTGATCGTTG R- GCTTGCTCTTGTTGGTCTCC
UF_07960F2	SL2.40ch11: 51.388	*BanI*	F-CGTGCCACCCCTTCATAATA R-CCCTTGCGAGGAAAATACAG
C2_At1g07960	SL2.40ch11: 51.387	*RsaI*	F-AAAGCCATTGTTACCGTCTCCGTG R-AGCCATAAGTGGTGTGGAGGACTT
P1-19	SL2.40ch11: 51.432	*AseI*	F-TAACACCAAATCGCGTCTGA R-TTGGGAAAACTATAGCATCG
cLEN-11-F24	SL2.40ch11: 51.549	*RsaI*	F-TTATGGACAGCATGGTCCTCGGAA R-GAAGTCTGGGAGCGATAGTAGTCT
51663_MH	SL2.40ch11: 51.663	*Hin6I*	F-CCCTCTTGCTTAGTGGGTGA R-ACGCTCCAAATCAGAGGTTG

(Source:Yang et al., 2014)

2.1.3.2 Polymerase chain reaction (PCR) and Gel electrophoresis

Initially, only 24 samples from each population were randomly picked up with their parents to do PCR and later to check polymorphism in order to save chemicals. PCR amplifications were done by using all six markers according to standard protocols for 35 cycles and amplification of samples was checked by electrophoresis (Appendix II and III). 1µl loading buffer was mixed with 5µl PCR product of each sample and loaded into the wells in the gel. The machine is run at 100 V for 45 min to 1 hour to let the bands separate.Bands of DNA were checked in the UV chamber. After this, markers were chosen for testing polymorphism.

2.1.3.3 Polymorphism test

Markers which successfully amplified were used for restriction in order to get polymorphic markers for each population. In the study, all 6 markers picked initially were polymorphic for the populations of *S. habrochaites* and *S. lycopersicum*.Therefore, polymorphism test was done to know whether these markersare polymorphic for two *S. habrochaites* accessions.5µl of PCR products were digested overnight at 37°C by 5µl of the appropriate restriction enzyme mixture (Appendix IV) to make a total volume of 10µl. Digested DNA fragments were separated on 1.5% agarose gel in electrophoresis and visualized in UV chamber.

CAPS markers indicate polymorphisms by digesting the PCR products using restriction enzymes. When the amplified segment of one parent has a restriction site for a particular enzyme, two restriction fragments are produced. If the amplified segment of other parent remains uncut as it does not contain any restriction site, then this marker is called polymorphic for both parents. Again amplified segments of one parent can have more than one restriction sites. If one parent have two restriction sites, after restriction it will produce three fragments. However, another parent can produce more fragments for having more than two restriction sites. These two parents also have polymorphism and the number and position of the bands will be different. Each individual can have both alleles from any of the parent or carry one allele from each parent. Alleles coming from different parents produce bands on gel in different position according to their sizes. Therefore when PCR product of a specific marker produced two or more kinds of diagnostic band, it was called polymorphic (Figure 6).

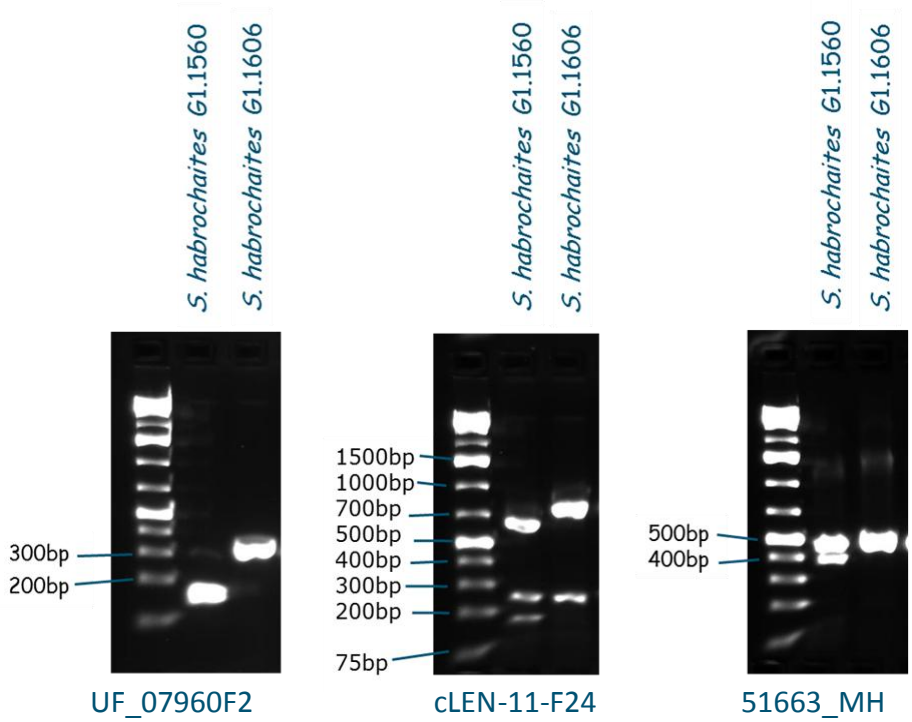

Figure 6Polymorphism of two different accessions of *S. habrochaites* based on different markers

Three markers namely UF_07960F2, cLEN-11-F24 and 51663_MH which were identified to be polymorphic for both populations derived from crosses of different accessions of *S. habrochaites*selected to use in the study.Another reason for selecting markers UF_07960F2 and

cLEN-11-F24 as both are present in the block or the region of the recombination suppression whereas 51663_MH is below the *Ty-2* region (Figure 7). The distance between markers UF_07960F2 and cLEN-11-F24 is 161Kb and the distance is around 115kb between the markers cLEN-11-F24 and 51663_MH, based on the *S. lycopersicum* genome.

UF_07960F2 and cLEN-11-F24 are co-dominant markers whereas 51663_MH is a dominant marker. Co-dominant markersare more informative as they allow to analyse of a single locus so that allelic variation of that locus can be identified. It gives exact genotype of an individual, as it is possible to distinguish heterozygotefrom homozygote. On the contrary, only the absence or presence of a specific allele at a locus is possible to find by using dominant markers.Thus, heterozygous genotype cannot be distinguished from homozygous genotype (Guillot and Carpentier-Skandalis, 2011).

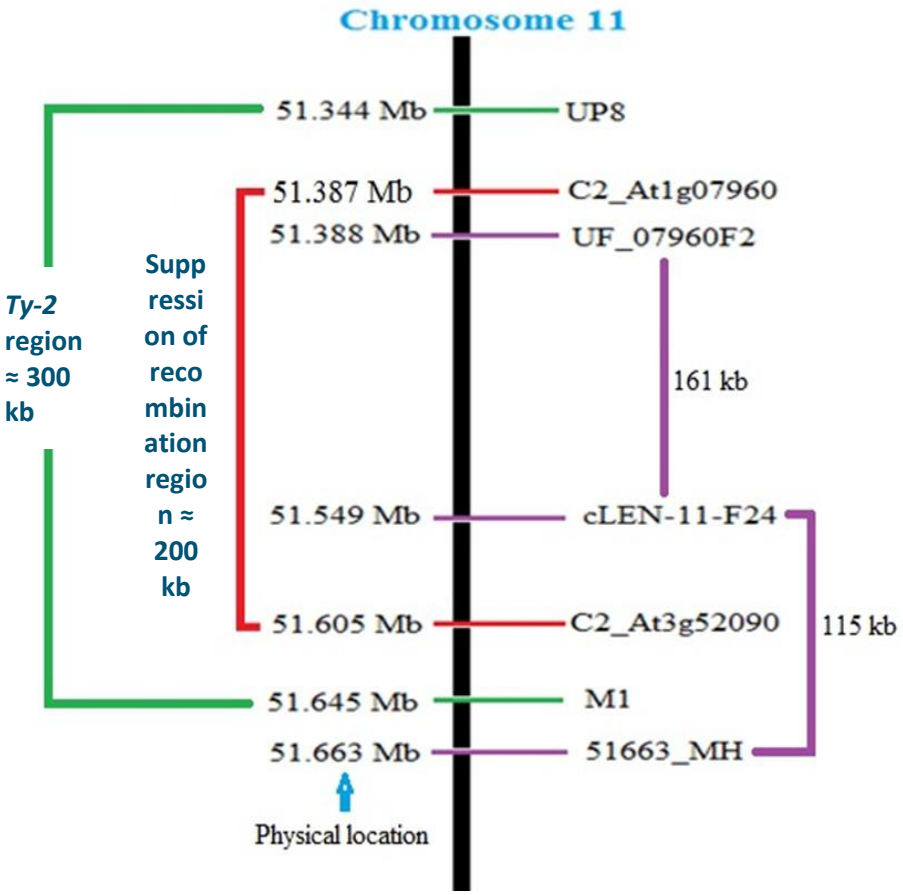

Figure 7Markers located in *Ty-2* region used in the present study with the physical position on chromosome 11 based on tomato Heinz

2.1.4 Final PCR and restriction

Polymorphic markers (UF_07960F2, cLEN-11-F24 and 51663_MH) were used to test all individuals of both populations for recombinant screening in the target region (block of the *Ty-2* region on Chromosome 11). PCR amplifications were checked by gel electrophoresis. PCR products of all samples were digested by specific enzyme at 37°C (Table 4). Bands were checked in UV chamber and imagesof each gel were captured for scoring genotypes of the samples.

2.1.5 Scoring

All data in this study were collected and processed with Excel by scoring individual genotypes. Scoring was done by observing band position after gel electrophoresis and genotypes of the individual plants were recorded in a table. Genotypes of markers UF_07960F2 and cLEN-11-F24 were used to identifypotential recombinants as both are located in the target region (Figure 7).

Progenies from the same parents can have the same combination as any parental chromosome or they have non-parental combination in different loci of a chromosome. Recombination happens between homologous chromosome by the effect of crossing over. When different genotypes were found in two adjacent markers, this individual was considered as a recombinant. After putting all genotypes of different markers in the excel table, the recombinant individualcould be identified clearly within the population.

In case of both co-dominant markers (UF_07960F2 and cLEN-11-F24), the genotype of a plant was scored A when it was same as one of the parental plant (homozygous) and B for other parental plant type (homozygous). Genotype that had alleles from both parents (heterozygous) was scored H. In addition, AH was used for our dominant marker (51663_MH); as homozygote and heterozygote cannot be distinguished.

A chi-square test of homogeneity was done to check similarity of recombination rates in the block in both F2 populations.

2.1.6 Test for confirmation of recombinants

After identifying recombinants in both populations, genotyping was repeated to confirm the results. DNA of recombinant individuals was isolated again and PCRwas done using earlier used markers. After scoring, initially found recombinants were confirmed as recombinant.

2.1.7 Development of CAPS markers

In order to check the order of the markers whether it is same in *S. habrochaites* as *S. lycopersicum*, recombinants from population PV960350 were included from the previous study of Huang, (2014) with the recombinants found in populations PV960357 and PV970303. Population PV960350 was derived from the cross of parents *S. habrochaites* G1.1560 and *S. habrochaites* G1.1257. In the present study, two markers were used that are situated in the block of the *Ty-2* region. Only one marker was used which locates below the block but this was a dominant marker. No polymorphic markersfor different *S. habrochaites* accessionswere available aboveor below the block. Therefore, in order to check the marker order in *S. habrochaites,* it was necessary to develop markers below and above the block.

To developCleaved Amplified Polymorphic Sequence (CAPS) markers below and above the block, parents of all three populations i.e.*S. habrochaites* G1.1257, *S. habrochaites* G1.1606, *S. habrochaites* G1.1290 and *S. habrochaites* G1.1560 were used (*S. habrochaites* G1.1560 was the common parent of all three crosses). All DNA samples were prepared for PCR with selected forward and reverse primer sets. In order to get good results of sequencing, quality of PCR products was checked by gel electrophoresis. Markers that produced clear bands were sequenced directly by GATC sequence service. Few PCR products were purified by QIAquick PCR Purification Kit (QIAGEN) following the

manufacturer's instruction. Finally, samples were prepared with 3 ul of cleaned PCR product, 2.5 ul primer from a 10 uM stock (forward and reverse primer separately for each sample) and 4.5 ul of MQ in a total volume of 10ul. MEGA5.10 program was used to build alignment of all DNA sequences and detect single nucleotide polymorphisms (SNPs) between different parental accessions of *S. habrochaites*. When SNPs were found in a restriction site, developed CAPS markers were used to score genotypes of all recombinants found in populations PV960357, PV970303 and PV960350.

2.2 Experiment 2

2.2.1 Plant Materials

Seeds of F_2(PV123002) and F_4recombinant linescontaining*S. habrochaites*introgression carrying the *Ty-2* regionin the genetic background of *Solanum lycopersicum* were collected from the breeding company. F_4 recombinant lines were tagged individuallyby the company as TEP number. 85 F_2individuals, 16 F_4 and 1 F_3(PV143245)recombinants lines (10 individuals from each line) were used to analyze segregation patterns by disease scoring and molecular marker analysis. In both cases, individuals of *Solanum lycopersicum cv.* Moneymaker (MM) were used as a control (susceptible to TYLCV). Plants were grown and kept for whole experiment in greenhouse maintaining 23°C temperature, 60% humidity and 16-8 hours day/night cycle.

2.2.2 TYLCV inoculation and disease evaluation

TYLCV infection was done by Agrobacterium-mediated inoculation method as describe by Verlaan et al. (2011). Inoculation was done after 10 days of transplantation of seedlings and disease scoring was started 30 days after inoculation (DAI). Scoring was done based ondevelopment of disease symptom at 30, 36, 45 and 51 days after inoculation (DAI). Symptom development was evaluated by Symptom Severity Scale (Lapidot and Friedmann, 2002) ranging from 0 to 4 where 0 = no visible symptoms, inoculated plants show same growth and development as non-inoculated plants; 1 = Very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction of size, yet plant continue to develop; 4 = very severe plant stunting and yellowing, pronounced cupping and curling, plants stop growing(Figure 8). Intermediate scores such as 0.5, 1.5 were also taken in account to get more precise disease severity rating.

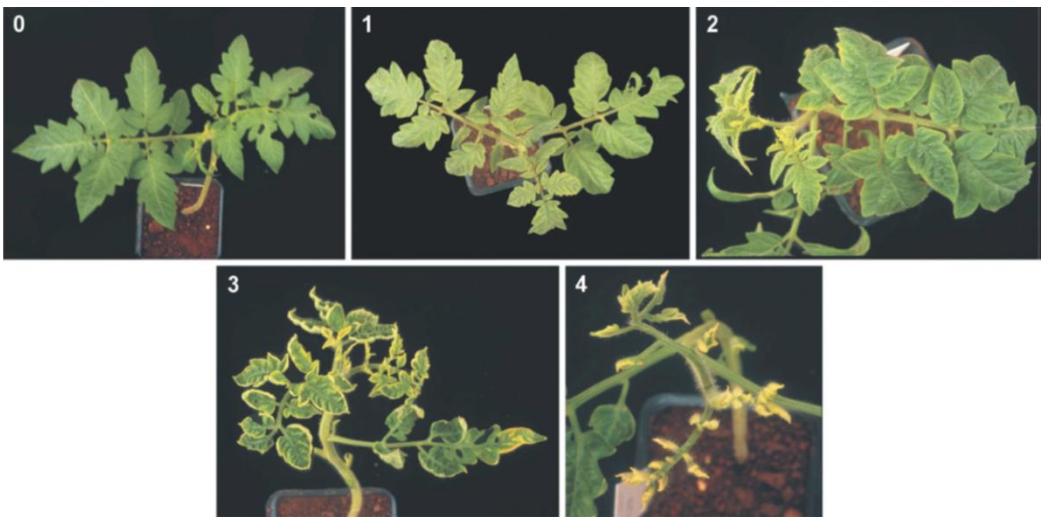

Figure 8TYLCV Symptom Severity Scale. Source: (Lapidot and Friedmann, 2002)

2.2.3 Genotyping by marker analysis

Genomic DNA was isolated from young leaves of the inoculated plants at 1 week after inoculation. In this experiment, PCR based Sequence-Characterized Amplified Region (SCAR) and CAPS markers (Table 5) polymorphic for *Solanum lycopersicum* and *Solanum habrochaites*were used from the study of Yang et al., (2014). DNA isolation, PCR, restriction and gel electrophoresis were done as described earlier in experiment 1.

Table 5Molecular markers used for genotyping

Markers	Physical position (Mb)	Restriction enzymes	Primers
51355_MH	SL2.40ch11: 51.355	*FspBI*	F-GCTAGAGCTTTCAAATCACTCTCAA R-GCTCATTGGCATTCACCTTCT
UF_07960F2	SL2.40ch11: 51.388	*BanI*	F-CGTGCCACCCCTTCATAATA R-CCCTTGCGAGGAAAATACAG
MCGao UF2 / P1-16	SL2.40ch11: 51.426	None	F-CACACATATCCTCTATCCTATTAGCTG R-CGGAGCTGAATTGTATAAACACG
M1	SL2.40ch11: 51.645	*BstUI*	F-CGCTCGGGCAAATAGTTCGTAATGG R-TTCATGGTCTAGAAATGTCCCCTGT
T0302	SL2.40ch11: 51.878	None	F-TGGCTCATCCTGAAGCTGATAGCGC R-TGATKTGATGTTCTCWTCTCTMGCCTG

(Source:Yang et al., 2014)

Three CAPS markers; 51355_MH, UF_07960F2 and M1 and two SCAR markers; MCGaoUF2 and T0302 were used for marker analysis. Markers 51355_MH, UF_07960F2, MCGao UF2 and M1 are located in the *Ty-2* region and marker T0302 locates below the *Ty-2* region in chromosome 11 of *S. lycopersicum*. Markers UF_07960F2 and MCGaoUF2 (P1-16) locate in the suppression of recombination region (the block).Initially, all markers except M1 were used for screening to save experimental cost. Marker M1 was used later to find the exact location of recombinationonly in those lines where recombination between markers was observed. However, in order to check the presence of any other known TYLCV resistance gene in these recombinant lines, markers of those genes were included for screening(Table 6).

Table 6Markers of other TYLCV resistant genes used in present study

Gene	Markers	Annealing temperature	Primers	References
Ty -1	MSc05732-4	55°C	F- ACGAGATGGAGCGGTCTTCAAGCT R- GACAGATCTCCCGGTAGGAGAGCA	(Verlaan et al., 2011)
Ty-4	C2_At5g60160	55°C	F-ACACAATGCTAATCAACGTTATGC R- TCATCCACCGCGCACATTTC	(Ji et al., 2009)
ty-5	SlNAC1	58°C	F-TGCCTGGTTTCTGCTGTCA R-TAAAGCTGAAGAAGGACTTACCCT	(Anbinder et al., 2009)
Ty-6	ch10-60348-1 (SNP marker)	58°C	F-CCAGCCAACCTCTCATCAAT R- CCAGCCAACCTCTCATCAAT	Developed by our group

CAPS markers were used to identify *Ty-1, Ty-4* and *ty-5* gene in F_3 and F_4 recombinant lines individuals (Table 6). Sequencing was done for the identification of *Ty-6* gene where one SNP is present in marker ch10-60348-1 which can differentiate *Solanum lycopersicum* (MM) and *Ty-6* introgressed region (Figure 9). PCR and restriction was done using earlier described procedures (Appendix II and IV).

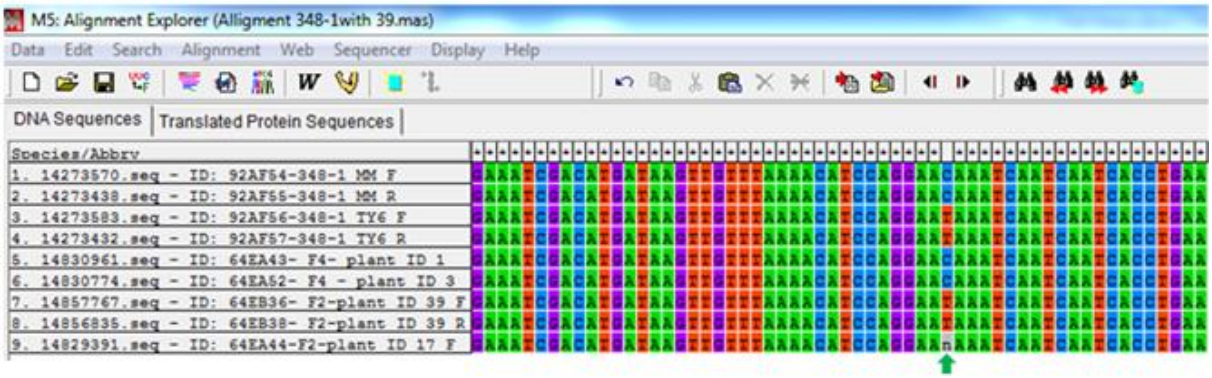

Figure 9Alignment of the sequences of the marker ch10-60348-1
SNP is indicated by green arrow, which can differentiate *Solanum lycopersicum* (MM) and *Ty-6* introgressed region.

2.2.4 Statistical Analysis

A chi-square goodness-of-fit test was done to test fitness of segregation ratio of F_2in disease scoring and molecular markers analysis.

3 Results

3.1 Result of Experiment 1

3.1.1 Scoring genotypes and analysis of the recombination

91 individuals of population PV960357 and 287 individuals of population PV970303 were used in this study. Three polymorphic markers UF_07960F2, cLEN-11-F24 and 51663_MH were used to score genotypes. Scoring results of all individuals for both populations are included in Appendix V and VI. Both UF_07960F2 and cLEN-11-F24 are in the region of recombination suppression while51663_MH is outside the *Ty-2* region (Figure 7). Therefore, markers UF_07960F2 and cLEN-11-F24 were taken into consideration for scoring genotypes to find recombinants within the block. Examples of pictures of gel of 96 individuals after restriction of PCR products using 3 CAPS markers are included in Appendix VII, VIII and IX.

3.1.1.1 F_2Population PV960357 (*S. habrochaites* G1.1560 × *S. habrochaites* G1.1606)

Analysis of recombination events in the target region was done to evaluate recombination rate in populations. In order to find potential recombinants in the population from cross between *S. habrochaites* G1.1560 and *S. habrochaites* G1.1606, markers UF_07960F2 and cLEN-11-F24 were used to score genotypes in the target region. The hypothesis of the study isthe presence of an inversion in the block of *Ty-2* region in chromosome 11 derived from *S. habrochaites*. Markers were arranged considering inversion in the target region for recombination analysis (Table 7). Eight recombinants were recordedamong91 individuals in population PV960357. Among these, seven had recombination events in the block, between markerscLEN-11-F24 and UF_07960F2 and the genetic distance of the markers UF_07960F2 and cLEN-11-F24 was 7.69 cM. However another one had recombination between markers UF_07960F2 and 51663_MH.

Table 7Recombinants in F_2population PV960357

Serial Number	Individual Number	Markers with physical position in chromosome 11		
		cLEN-11-F24	UF_07960F2	51663_MH
		51.549	51.388	51.663
1	7	A	H	AH
2	8	A	H	AH
3	26	A	H	AH
4	33	H	B	B
5	44	H	B	B
6	64	H	B	B
7	73	H	H	B
8	90	H	A	AH

A : Genotype of *S. habrochaites* G1.1560 (homozygous)
B : Genotype of *S. habrochaites* G1.1606 (homozygous)
H : Genotype of plantshaving alleles from both parents (heterozygous)

Red coloured numbers are indicating recombination events in the block and blue coloured for individuals with recombination outside the block. Among seven recombinants in the block, three individuals belonged to the recombination type A-H (serial no 1-3), threeindividuals have

recombination type H-B (serial no 4-6) and the last individual belongs to the recombination type H-A (serial no 8).

3.1.1.2 F_2Population PV970303 (*S. habrochaites* G1.1560 × *S. habrochaites* G1.1290)

An intraspecific cross between *S. habrochaites* G1.1560 and *S. habrochaites* G1.1290, six recombinants were identified in population PV970303 among 287 individuals,with recombination events between markers cLEN-11-F24 and UF_07960F2 (Table 8). The genetic distance of the markers UF_07960F2 and cLEN-11-F24 was 2.09 cM in the population PV970303.Three individuals were recombinant type H-A (serial no 2 to 4), one was recombinant type H-B (serial no 1) and another two belonged to the recombinant type A-H (serial 5 and 6). No recombinant was recorded between markers UF_07960F2 and 51663_MH.

Table 8Recombinants in F_2population PV970303

Serial Number	Individual Number	Markers with physical position in chromosome 11		
		cLEN-11-F24	UF-07960F2	51663-MH
		51.549	51.388	51.663
1	38	H	B	B
2	76	H	A	AH
3	108	H	A	AH
4	161	H	A	AH
5	191	A	H	AH
6	201	A	H	AH

A : Genotype of *S. habrochaites* G1.1560(homozygous)
B : Genotype of *S. habrochaites* G1.1290(homozygous)
H : Genotype of plants having alleles from both parents (heterozygous)

Some recombination events were observed in the target region in chromosome 11 in F_2 populations used in the study derived from crosses of different accessions of *S. habrochaites*.Therefore, this result indicated that no block of suppression present in intraspecific crosses among *S. habrochaites* accessions.

3.1.1.3 Comparison of recombination rates in the block

In population PV960357, 91 individuals were used and seven recombinants were found in the block between markerscLEN-11-F24 and UF_07960F2.On the other hand, six recombinants out of 287 individuals were observed in population PV970303 in the same physical distance. To check whether recombination rateswere similar in both F_2 populations, chi-square test of homogeneity was conducted. Recombination rates were significantly different in both populations as the *P* value (0.011) was smaller than the critical value (0.05) (Table 9).

Table 9A Chi-square (χ2) test of homogeneity to check recombination rates in both F_2 populations

Populations	Number of total individuals	Number of recombinants	Chi-square	*P*
PV960357	91	7	6.529	0.011
PV970303	287	6		

The critical value of probability level = 0.05

3.1.2 Marker development and analysis of the order of markers in *S. habrochaites*

Another research question of experiment 1 was to know the marker order in *S. habrochaites,*whether it follows the same order asin *S. lycopersicum*. To achieve the answer of this question, two CAPS markers were developed below the block. Details of these markers are presented in table 10. Developed marker M1 locates below the block ofthe *Ty-2* region and C2_At4g32930 locatesbelow the *Ty-2* region.

Table 10Developed molecular markers located below the block in chromosome 11

Name	Primer	Annealing temperature	Restriction enzyme	Buffer	Digestion temperature
M1	F-CGCTCGGGCAAATAGTTCGTAATGG R-TTCATGGTCTAGAAATGTCCCCTGT	58°C	*Ssi I*	Orange	37°C
C2_At4g32930	F-TCCTCTTCCTATTGGCAAGGGC R-TGGACACTCCCCCTTTTCATCATAC		*Ssp I*	Green	

Development of CAPS markers above the block was not possible, but two SNPs were found in the sequences of marker C2_At2g28250in *S. habrochaites* G1.1257, *S. habrochaites* G1.1606, *S. habrochaites* G1.1290 and *S. habrochaites* G1.1560 (Figure 10). Common parent *S. habrochaites* G1.1560 was differentiated from all other three parental accessions by these two SNPs. Marker C2_At2g28250 was amplified by PCR using forward primer AGACTTCATCATCGTCATGTGGTTCCG and reverse primer TTTGGAGGTGCTTTGCCATACCAAG, the PCR products were sequenced.

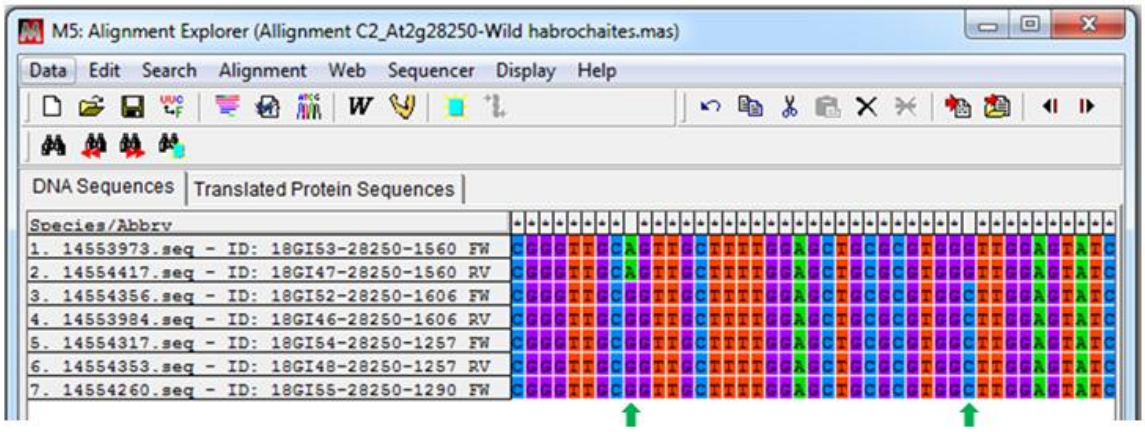

Figure 10Alignment of sequences of parental accessions for marker C2_At2g28250. Two SNPs (indicated by green arrows) were found which can differentiate *S. habrochaites* G1.1560 from all other three parental accessions.

Two developed CAPS markers were co-dominant so that it was easy to get differences in band patterns and these were used to score genotype of recombinants in populations PV960357, PV970303 and PV960350. For the region above the block, sequencing of marker C2_At2g28250 ofthe recombinants was done and genotype was scored based on the sequence alignment with the parents. Results of marker scoring of all recombinants are shown in Table 11 and 12 (for population PV960357); 13 and 14 (for population PV970303); 15 and 16 (for population PV960350). Here, markers were arranged for scoring based on tomato Heinzas well as considering inversion in the block of *S. habrochaites*. Marker C2_At1g07960 used in population PV960350, also locates in the

block of the *Ty-2* region. Genotype of *S. habrochaites* G1.1560 is indicated by A for all three populations and genotypesof *S. habrochaites* G1.1606(for population PV960357), *S. habrochaites* G1.1290(for population PV970303) and *S. habrochaites* G1.1257(for population PV960350) were indicated by B where H means heterozygous.

Comparing both tables of each population, it is clearly showed that the result is more likely if inversion is considered in the block. Therefore, the order of the markers in the block in *S. habrochaites* is not alike as in *S. lycopersicum*.

Table 11Markers scoring of recombinants in population F_2PV960357 (*)

Serial Number	Individual Number	Markers with physical position in chromosome 11					
		C2_At2g28250	UF_07960F2	cLEN-11-F24	M1	51663_MH	C2_At4g32930
		51.307	51.388	51.549	51.645	51.663	51.688
1	7	A	H	A	H	AH	H
2	8	A	H	A	H	AH	H
3	26	A	H	A	H	AH	H
4	33	H	B	H	B	B	B
5	44	H	B	H	B	B	B
6	64	H	B	H	B	B	B
7	73	H	H	H	B	B	B
8	90	H	A	H	A	AH	A

*Marker order based on tomato Heinz

Table 12Markers scoring of recombinants in population F_2PV960357 (**)

Serial Number	Individual Number	Markers with physical position in chromosome 11					
		C2_At2g28250	cLEN-11-F24	UF_07960F2	M1	51663_MH	C2_At4g32930
		51.307	51.549	51.388	51.645	51.663	51.688
1	7	A	A	H	H	AH	H
2	8	A	A	H	H	AH	H
3	26	A	A	H	H	AH	H
4	33	H	H	B	B	B	B
5	44	H	H	B	B	B	B
6	64	H	H	B	B	B	B
7	73	H	H	H	B	B	B
8	90	H	H	A	A	AH	A

**Marker order based on considering inversion of the block in chromosome 11 *of S. habrochaites*

Table 13Markers scoring of recombinants in population F_2 PV970303 (*)

Serial Number	Individual Number	Markers with physical position in chromosome 11					
		C2_At2g28250	UF_07960F2	cLEN-11-F24	M1	51663_MH	C2_At4g32930
		51.307	51.388	51.549	51.645	51.663	51.688
1	38	H	B	H	B	B	B
2	76	H	A	H	A	AH	A
3	108	H	A	H	A	AH	A
4	161	H	A	H	A	AH	A
5	191	A	H	A	H	AH	H
6	201	A	H	A	H	AH	H

*Marker order based on tomato Heinz

Table 14Markers scoring of recombinants in population F_2 PV970303 (**)

Serial Number	Individual Number	Markers with physical position in chromosome 11					
		C2_At2g28250	cLEN-11-F24	UF_07960F2	M1	51663_MH	C2_At4g32930
		51.307	51.549	51.388	51.645	51.663	51.688
1	38	H	H	B	B	B	B
2	76	H	H	A	A	AH	A
3	108	H	H	A	A	AH	A
4	161	H	H	A	A	AH	A
5	191	A	A	H	H	AH	H
6	201	A	A	H	H	AH	H

**Marker order based on considering inversion of the block in chromosome 11 *of S. habrochaites*

Table 15Markers scoring of recombinants in population F_2 PV960350 (*)

Serial Number	Individual Number	Markers with physical position in chromosome 11					
		C2_At2g28250	C2_At1g07960	cLEN-11-F24	M1	51663_MH	C2_At4g32930
		51.307	51.387	51.549	51.645	51.663	51.688
1	32	H	B	H	B	B	B
2	41	B	A	H	A	AH	A
3	49	H	A	H	A	AH	A
4	70	H	A	H	A	AH	A
5	75	B	H	B	H	AH	H
6	84	H	A	H	A	AH	A
7	87	H	B	H	B	B	B
8	92	A	H	A	H	AH	H

*Marker order based on tomato Heinz

Table 16Markers scoring of recombinants in population F_2 PV960350 (**)

Serial Number	Individual Number	Markers with physical position in chromosome 11					
		C2_At2g28250	cLEN-11-F24	C2_At1g07960	M1	51663_MH	C2_At4g32930
		51.307	51.549	51.387	51.645	51.663	51.688
1	32	H	H	B	B	B	B
2	41	B	H	A	A	AH	A
3	49	H	H	A	A	AH	A
4	70	H	H	A	A	AH	A
5	75	B	B	H	H	AH	H
6	84	H	H	A	A	AH	A
7	87	H	H	B	B	B	B
8	92	A	A	H	H	AH	H

**Marker order based on considering inversion of the block in chromosome 11 *of S. habrochaites*

3.2 Result of Experiment 2

3.2.1 F_2(PV123002) progenies analysis

85 F_2individuals were used for inheritance study of *Ty-2*, here *S. lycopersicum* cv. Moneymaker (MM) was used as a control.

3.2.1.1 Molecular marker analysis of F_2 plants

Molecular marker analysis was done by using three CAPS markers 51355_ MH, UF_07960F2 and M1 located in the *Ty-2* region on chromosome 11 in *S. lycopersicum* (Appendix X). These markers were used to identify and locate the introgression of *Ty-2* region in F_2individuals.

Molecular marker analysis of F_2individuals showed that 10 among 85 individualsare homozygous for *S. lycopersicum* allele (A plants) for the whole region (markers 51355_ MH, UF_07960F2 and M1). 30 individuals were homozygous for *S. habrochaites* allele (B plants) and 45 individualswere heterozygous (H plants)indicating the presence of *S. habrochaites* introgressioncarryingthe*Ty-2* region. A chi-square test was done to assess the goodness of fit between observed plants number in each genotype and expected number in ratio of 1:2:1 where *P* value was 0.0078 that was smaller than critical value (0.05). Therefore, it did not indicate a reasonable good fit to the expected segregation ratio (Table 17). A skewed ratio of genotypes (less homozygous A plants) was observed.

Table 17A Chi-square (χ^2) test for the segregation of TYLCV resistance (expected ratio: 1:2:1) among the F_2 plants that possess *Ty-2* gene

Types of Genotype	Number of plants (observed)	Number of plants (expected)	Chi-square	*P*
Homozygous, A	10	21.25	9.7059	0.0078
Heterozygous, H	45	42.5		
Homozygous, B	30	21.25		
Total	85	85		

The critical value of probability level = 0.05

3.2.1.2 Evaluation of TYLCV disease severity in F_2individuals

Disease symptoms wereobserved on MM plants 3 weeks after inoculation. Disease scoring was done 30 and 36 DAI (Appendix X). Among 85 individuals, 77 (45 H, 30 B and 2 A plants) did not show any symptom of TYLCV and were scored resistant (R). Disease symptom were observed in 8 individuals(Figure 11). 7 individuals showed severe symptoms (disease severity rating 4) including severe stunting, leaf yellowing and curling while only oneindividual showedslight symptom of TYLCV such as yellowing of apical leaves. These individuals were scored susceptible(S).

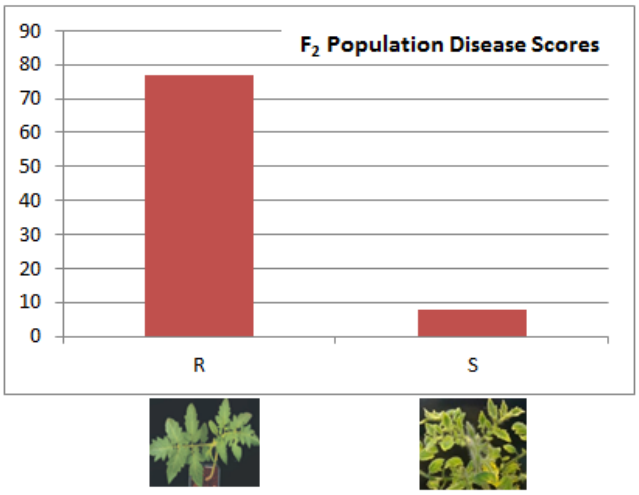

Figure 11Number of F_2 plants with different disease severity rates

A chi-square (χ^2) goodness-of-fit test was done for conformity with expected ratio (3:1, 13:3, 15:1, Table 18). The *P* value was 0.2285 (> 0.05), when expected ratio was 15:1, while for other two ratios, *P* value were smaller than 0.05 (0.0009 for expected ratio 3:1 and 0.0274 for expected ratio 13:3). The segregation of F_2 was found to reasonable good fit to a ratio of 15:1 (Table 18).

Table 18A Chi-square (X^2) test for the segregation of TYLCV resistance (based on phenotypic data) among the F_2 plants that possess *Ty-2* introgression

Types	Number of plants (observed)	Expected ratio: 3:1			Expected ratio: 13:3			Expected ratio: 15:1		
		Number of plants (expected)	X^2	*P*	Number of plants (expected)	X^2	*P*	Number of plants (expected)	X^2	*P*
R	77	63.75	11.0157	0.0009	69.06	4.8655	0.0274	79.68	1.4502	0.2285
S	8	21.25			15.94			5.32		
Total	85	85			85			85		

R = Resistant and S = Susceptible
The critical value of probability level = 0.05

Results showed that all individuals which were homozygous or heterozygous for *S. habrochaites* allele(total number of 75 individuals) in *Ty-2* region were completely resistant. 8 individuals having homozygous genotypes for *S. lycopersicum* allele were susceptible. But exceptional result was found in twoindividuals(plant ID 17 and 39) which were carrying homozygous *S. lycopersicum* allelewithout havingany symptom of TYLCV.

The result of the chi-square test of the phenotypic data showed good fit in the ratio 15:1.This result could be possible for the skewed segregation of*Ty-2* region.However, a test was done to checkpossible other sources of resistance (*Ty-6*) in these 2 individuals(plant ID 17 and 39) where *Ty-2* gene was not responsible for resistance. As this could be possible for escaping of virus, so test for other genes *Ty-1*, *Ty-4* and *ty-5*were not conducted for these two individuals.

PCR was done for three samples (individual plant ID 14, 17 and 39) using the forward primer CCAGCCAACCTCTCATCAAT and reverse primer CCAGCCAACCTCTCATCAAT to amplify the region on chromosome 10 to detect *Ty-6*introgression (Table 6). PCR products were sequenced and their

sequences were aligned to the sequences of *S. lycopersicum* cv. Moneymaker (MM) and *Ty-6* introgressed region to obtain their genotypes.Results of sequencing showed that these two individuals (plant ID 17 and 39) contained heterozygous and homozygous alleles of *Ty-6* introgression respectively (Table 19).

Table 19Scoring of F_2 plants to detect *Ty-6* introgression

Individual plant ID	Genotype (*Ty-2* region)	Score of disease	Phenotype	Genotype (*Ty-6* region)
14	A	4	S	A
17	A	0	R	H*
39	A	0	R	B *

A = Homozygous *S. lycopersicum* cv. Moneymaker allele
B* = Homozygous alleleof *Ty-6* introgression
H* = Heterozygous

3.2.2 Analysis of F_3(PV143245)progenies

CAPS markers 51355_MH, UF_07960F2 andM1 and SCAR markers MCGaoUF2 and T0302 were used for marker analysis. Tenindividuals of F_3 family PV143245 which were derived from a recombinant individual of F_2PV123002 (Code PV123002-45)were used inthe present study.Seeds of F_2PV123002 were collected from the company having *Ty-2* introgression. Nineindividualshad genotype heterozygous or homozygous for *S. habrochaites* allele except one (plant Id 6 where data was not complete)formarkers 51355_MH, UF_07960F2, MCGaoUF2 and M1.Evaluation of disease severity showed that all individuals were completely resistant showing no symptom of TYLCV up to 51 DAI (Appendix XI). Results indicate that all resistant individuals were either homozygous or heterozygous for *S. habrochaites*introgression(except plant ID 6). No recombination occurred between markers 51355_MH andM1.

3.2.3 F_4 progenies analysis

16 F_4 recombinant lines (10 individuals for each line) from the breeding company tagged by TEP number were used in this study where MM plants were used as control. Results of disease severity scoring and marker analysis of each line are presented in the Appendix XIand pictures of gel of different markers are included in the Appendix XII.

3.2.3.1 Evaluation of TYLCV disease severity in F_4individuals

After 3 weeks of inoculation, disease symptoms wereobservedin MM plants. Individuals showing disease symptoms such as yellowing and curling of leaves at 30 DAI were scored susceptible (S), but some individuals remained symptomless until 51 DAI; these individuals were scored as resistant (R). Disease scoring was done 4 times and severe disease symptom (severity rating 4) i.e. yellowing and curling of leaves as well as growth reduction were clearly visible on the susceptible individuals at 51 DAI (Figure 12).

Figure 12TYLCV symptoms (yellowing and curly leaves); picture was taken at 45 DAI. Here, (A) MM (Susceptible); (B) 12TEP070121 (Susceptible); (C) 12TEP070056 (Susceptible); (D) 12TEP070117 (Resistant)

3.2.3.2 Molecular marker analysis in F_4individuals

Molecular marker analysis showed that the introgression of *S. habrochaites* was absent in all individuals of F_4 recombinant lines; 12TEP070049, 12TEP070050, 12TEP070051, 12TEP070054, 12TEP070056, 12TEP070116, 12TEP070119 and 12TEP070121. These individuals carried homozygous genotypes for *S. lycopersicum* allele in all markers position. Disease symptoms were very severe (rating 4) for all individuals. Only 2 or 3 plants in each line showed no TYLCV symptoms (Figure 13).

However, markers 51355_MH, UF_07960F2, MCGaoUF2 and M1 showed homozygous *S. lycopersicum*genotype for all individuals of recombinant lines 12TEP070123, 12TEP070124, 12TEP070138, 12TEP070139. These individuals carried homozygous genotype for *S. habrochaites* introgression in marker T0302. The individuals also showed severe susceptibility against TYLCV (Figure 13).

All individuals of recombinant line 12TEP070117 were homozygous for *S. habrochaites* introgression carrying the *Ty-2* gene in markers51355_ MH, UF_07960F2 , MCGaoUF2 and M1.These individualswere homozygous for *S. lycopersicum* allelein the position of marker T0302 which was located outsidethe region of *Ty-2*.All plants of this line showed high level of resistance without showing any disease symptom (Figure 13).

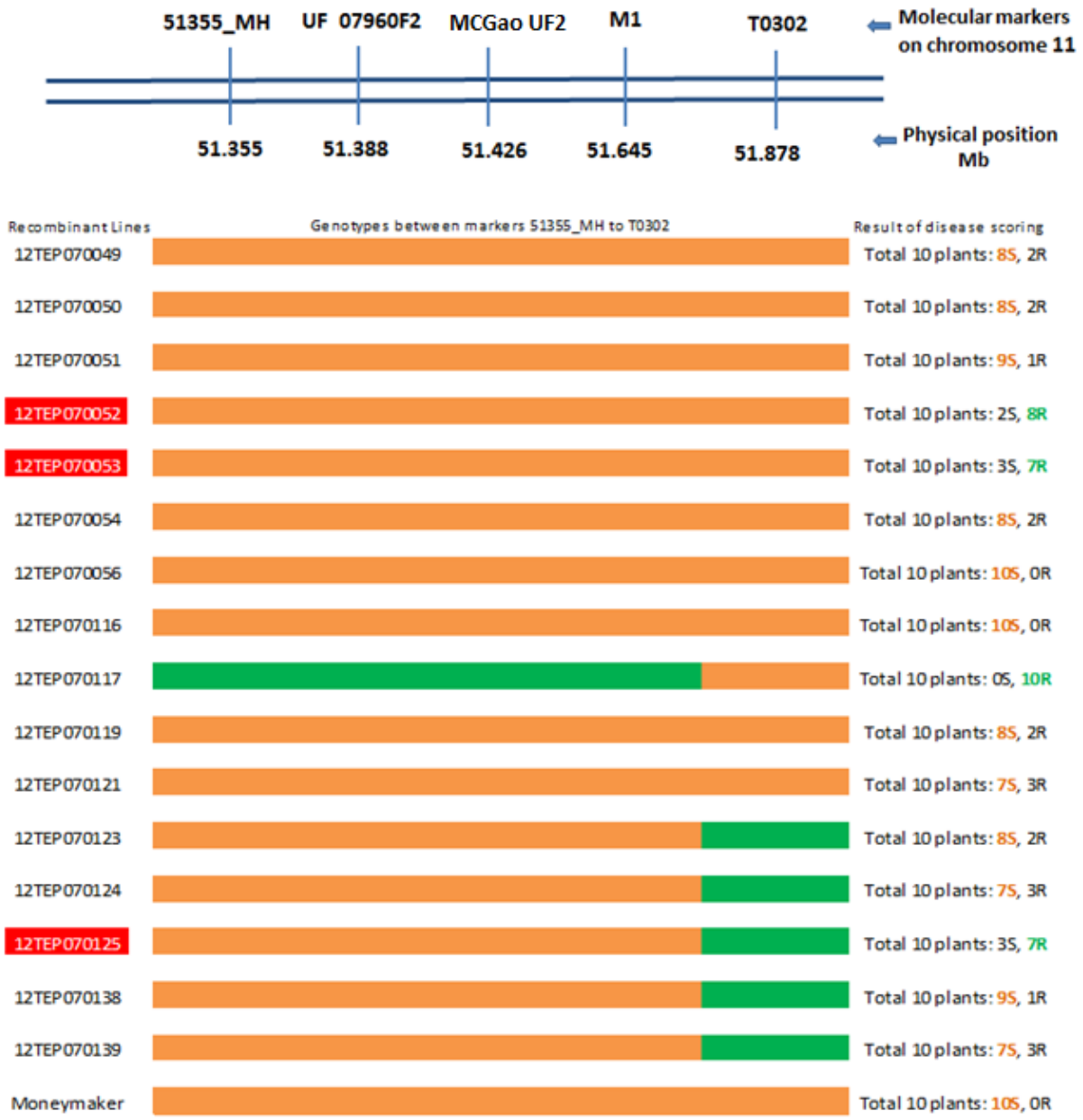

Figure 13Genotypes of F4 recombinant lines with the result of disease scoring
Genotype of each line shaded orange colour indicating *S. lycopersicum* genome and the introgressed segments of *S. habrochaites* genome are shaded as green colour. Lines showed non-corresponding results are marked as red shade.

The recombinant lines were categorized into three types according to the results of the analysis of phenotype and genotypesof the*Ty-2* region (Table 20). Results indicated that all susceptible individuals were devoid of the introgression of *S. habrochaites* carrying *Ty-2* region in *S. lycopersicum* background (Category 1). All resistant individuals of line 12TEP070117 were homozygous for *S. habrochaites* introgressionsupporting the inheritance of *Ty-2* gene (Category 3).

However, lines 12TEP070052, 12TEP070053 and 12TEP070125 showed non corresponding results and considered as category 2 (Table 20). Most of the individuals of these lines showed resistance against TYLCV. But these individualswere homozygous for *S. lycopersicum* allelein the *Ty-2* region(Figure 13). These results indicate that resistance of these individualsdid not come from *Ty-2*

gene as no introgression was present. Therefore a test was done to identify the possible sources of resistance.

Table 20Categorization of F_4 recombinant lines based on the phenotype and genotype

Category	Recombinant lines	Genotype	Phenotype
Category 1	12TEP070049	A	S
	12TEP070050		
	12TEP070051		
	12TEP070054		
	12TEP070056		
	12TEP070116		
	12TEP070119		
	12TEP070121		
	12TEP070123		
	12TEP070124		
	12TEP070138		
	12TEP070139		
	Moneymaker		
Category 2	12TEP070052	A	R
	12TEP070053		
	12TEP070125		
Category 3	12TEP070117	B	R

Genotypes were measured between markers 51355_MH and M1

3.2.1 Marker analysis for other TYLCV resistance genes in F_3 and F_4 lines

In order to reveal the presence of other known TYLCV resistant genes in these lines, the CAPS markers of *Ty-1*, *Ty-4* and *ty-5* were used to screen all F_3 and F_4individuals (Table 6). The result was presented in the Appendix XI. All markers showed only *S. lycopersicum* genotype for all individuals of each line. Therefore, the resistance against TYLCV of category 2 was not for the presence of *Ty-1* , *Ty-4* and *ty-5* gene.

Moreover, in order to identify the existence of *Ty-6* gene in these lines, sequencing was done for 3 individuals from each line 12TEP070052 and 12TEP070053 containing homozygous *S. lycopersicum* alleles in the *Ty-2* region of chromosome 11without carrying *Ty-2*. The results of sequencing indicate absence of *Ty-6* gene in both resistant or susceptible individuals (Table 21).

Table 21Scoring of F_4 plants to detect *Ty-6* introgression

Recombinant lines	Individual plant ID	Genotype (*Ty-2* region)	Score of disease	Phenotype	Genotype (*Ty-6* region)
12TEP070052	1	A	4	S	A
	3		0	R	
	4		0	R	
12TEP070053	10		4	S	
	4		0	R	
	8		0	R	

4 Discussion

4.1 Discussion on experiment 1

4.1.1 Recombination analysis

Introduction of valuable traits from different parents into a new elite variety through introgressive hybridization is one of the important goals of the plant breeders. The success of breeding depends on the combination of desired alleles on chromosomes in a hybrid. Therefore recombination is very crucial for exchanging genetic information of homologous chromosome segments during meiosis (Wijnker and Jong, 2008). Structural chromosomal rearrangements like inversions, translocations, duplications and deletions have major influences on the recombination process. The wild relatives of solanaceous crops have potential resources that have been utilized for crop improvement. But chromosomal rearrangements suppress recombination in interspecific crosses that impact on the success of breeding programs (Szinay D., 2010).

However suppression of recombination is not rare in introgressed region from wild relatives into tomato. This suppression of recombination is likely to be observed in the introgressed region of*Ty-2* gene (Yang et al., 2014). *Ty-2* gene was limited in an introgressed part of 500kb between markers C2_At2g28250 (physical location 51.307Mb) and T0302 (51.878 Mb) in previous work (Ji et al., 2009a). Yang et al., (2014) shortened*Ty-2* regionto a distance of 300 kb between markersUP8 (51.344 Mb) and M1 (51.645 Mb). They screened 11,000 F_4 progeny of H9205, a tomato hybrid with resistance from H24 line derived from interspecific cross of *S. habrochaites* accession B6013(resistant to TYLCV, *Ty-2* gene) and *S. lycopersicum*and found 157 recombinants between the markers C2_At1g07960 (physical distance 51.387 Mb, genetic distance 82.5 cM) and T0302 (51.878 Mb, 89 cM). As the genetic distance between the markers C2_At1g07960 and T0302 is 6.5 cM, so that expected number of recombinants between these markers was 715. But, they observed only 157 recombinants, where29 recombinants were found above the marker M1 and 27 recombinants were in the distance of 40 Kb region between markers C2_At3g52090 (51.605 Mb) and M1 (51.645 Mb).Only 2 recombinants were found between markers P1-16/MCGaoUF2 (51.426 Mb) and TG36 (51.490 Mb) (Appendix XIII), an approximately 60Kb region whereas no recombination occurred between markers TG36 and C2_At3g52090.To clarify these results, another 1900 plants of F_2 population derived from a commercial hybrid carrying *Ty-2* gene were tested and no recombinantswere found between markers C2_At1g07960 and C2_At3g52090, confirming recombination suppression in this region. Therefore further delimiting of *Ty-2* region was not possible. They tried to find the causes of this suppression of recombination by visualizing the chromosome structure of the *Ty-2* introgression by FISH analysis. They failed to find any potential chromosomal rearrangement as 300 Kb region was not so big for FISH resolution.

In the present study, two F_2 populations PV960357 and PV970303 were used derived from intraspecific crosses among different accessions of *S. habrochaites*. If the marker order is arranged considering an inversion between *S. habrochaites and S. lycopersicum* in the block, the distance between markers cLEN-11-F24 and 51663_MH would be approximate 230 Kb. Eight recombinants were found in population PV960357 where only one had recombination between markers UF_07960F2 and 51663_MH. There were no recombination events found between these two markers in population PV970303. More recombinants might exist between markers UF_07960F2 and

51663_MH but it is difficult to precisely analyse recombinant between these two because 51663_MH is a dominant marker.

Markers UF_07960F2 (51.388 Mb) and cLEN-11-F24 (51.549 Mb) were especially interesting to obtain potential recombinants as both are present inside the block of *Ty-2* region and the physical distance between these markers is approximately 160kb region. The genetic distance of the markers UF_07960F2 and cLEN-11-F24 was 7.69 cM and 2.09 cM in the population PV960357 and PV970303 respectively. Seven recombinants were found in population PV960357 and six in population PV970303 between these two markers(Appendix XIII). However, Yang et al., (2014) screened 11,000 plants derived from interspecific cross of *S. habrochaites* and *S. lycopersicum*and found only two recombinants in a region of approximate 217 Kb between markers C2_At1g07960 and C2_At3g52090 (Appendix XIII). Two recombinants among 11,000 progeny were negligible and indicated severe recombination suppression in this region. The probabilities of crossing over is less near the centromere than in regions away from the centromere. The*Ty-2* gene is located in the long arm of chromosome 11 so that possibilities of recombination should be higher. According to Canady et al., (2006) recombination rate has positive correlation with the length of introgressed region. Therefore it was expected to find more recombinants in a 217 Kb region than in 160 Kb.Similar results were also found in BSc thesis by Huang, (2014) on analysis of chromosome 11 of *S. habrochaites*. 88 individuals of the F_2 population PV95279 derived from interspecific cross between *S. habrochaites* (susceptible to TYLCV) and *S. lycopersicum* cv. Moneymaker were used in that study and no recombinant was found in the target region of *Ty-2* (Appendix XIII).

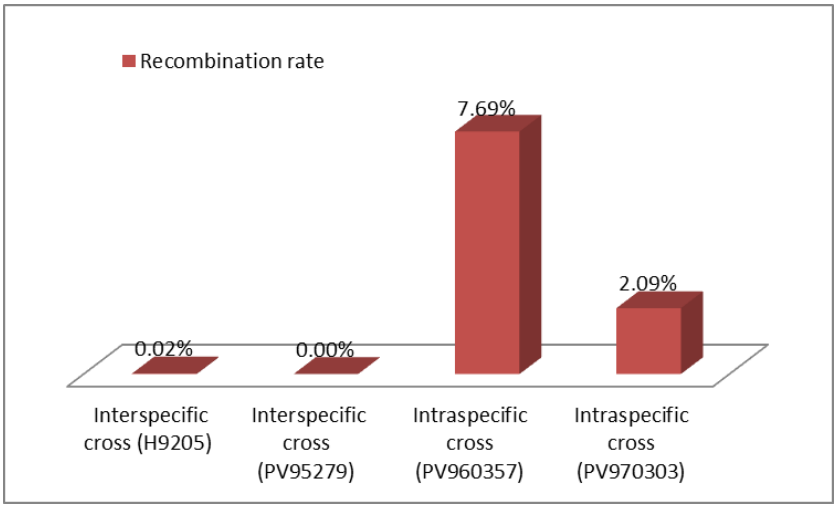

Figure 14Comparison of recombination rates of different crosses

Comparing results of the study of both interspecific and intraspecific crosses, it was proved that no block occurred in the *Ty-2* region of chromosome 11 of tomato when two *S. habrochaites* were crossed (intraspecific) (Figure 14). Therefore suppression of recombination only happened in interspecific crosses between *S. habrochaites* and *S. lycopersicum*. Moreover, these resultssupport the hypothesis about the marker positions in *S. habrochaites* genome. All tested accessions of *S. habrochaites* mighthave a similar structure in the target region of chromosome 11 without any recombination barrier. The chromosomal structure of *S. habrochaites* and *S. lycopersicum* might be different in the target 'block'.

4.1.2 Comparison of recombination rates in both populations

In this study, both F_2 populations PV960357 and PV970303 were derived from one same parent, *S. habrochaites* G1.1560. The other parent was *S. habrochaites* G1.1606 or*S habrochaites* G1.1290 for the population PV960357 and populationPV970303 respectively. But recombination rate was more than 3.5 times higher in population PV960357 compared to the population PV970303 in the same region (Figure 14).

*S. habrochaites*is predominantly self-incompatible (SI) with some self-compatible populations. Different levels of compatibility of different accessions and wider range of geographic distribution cause variability within a species. It was found that more genetic variability was present in accessions of *S. habrochaites* collected from different origins. Moreover, an outcrosser form of *S. habrochaites* have more intraspecific genetic diversity than self-compatible one (Ercolano et al., 2005). In the present study, information about the origin or characteristics of *S. habrochaites*accessions was not available. Therefore the reasons of the variation of recombination rates in this particular region between two accessions *S. habrochaites* G1.1606 and *S habrochaites* G1.1290 are unclear.

Identification of genotypes which are carrying alleles of higher recombination rate would be good crossing parents for successful breeding schemes. In map based cloning, marker assisted selection strategies and in crossing program, parents with high recombination rate with a region of high markers density would be a potential choice (Bauer et al. 2013). Therefore, selection of the appropriate parent would be important for further study of the*Ty-2* gene on chromosome 11 of tomato.

4.1.3 The markers order in *S. habrochaites*

To study the marker order in the block in *S. habrochaites,* development of CAPS marker in both above and below the *Ty-2*region was attempted as more polymorphic markers were required to study different accessions of *S. habrochaites*. But development of markers above the block was not possible for some reasons. The PCR products of some regions did not give good results of sequencing. Sometimes the results were very good but no SNP was found which can show polymorphism between *S. habrochaites* accessions. Again, in some regions, the presence of SNP was detected but the restriction enzyme that could be used to produce CAPS markers was not available.

Two developed CAPS markers were used below the block and genotyping of marker above the block was done by sequencing in population PV960357, PV970303 and PV960350. If the genotype of each individual was arranged according to the marker order as Heinz genome, it indicates the presence of double or even more recombination events between markers C2_At2g28250 and M1, approximately 338kb region,which was unlikely. When markers were placed by considering inversion of the block on chromosome 11 *of S. habrochaites* and the marker positions of cLEN-11-F24 and UF_07960F2 was reversed, the result are more likely. Aninversion might be present in the block of the *Ty-2* region in *S. habrochaites* compared to cultivated tomato. The inverted segment in *Ty-2* introgressed region on chromosome 11 derived from *S. habrochaites* might be the reason of recombination suppression in the block in crosses with the cultivated tomato.

Considering results of the experiment 1, it could be stated that the reason of recombination suppression might be inversion in the block on chromosome 11. When two *S. habrochaites* were crossed, they align to each other in this region, so no suppression of recombination would occur.

However, when *S. habrochaites* were crossed with *S. lycopersicum*, this block produced might be because ofthe inverted region. Inversions were already reported in *Solanum* genera (Szinay D., 2010). Moreover, Verlaan et al. (2011) analysed chromosomal rearrangement in the *Ty-1* introgressed region derived from *Solanum chilense* LA1969. They found two chromosomal inversions in chromosome 6 between *S chilense* LA1969 and *S. lycopersicum*, and the occurrence of the suppression of recombination was observed in the region where both inversions were located.

Inversions can interrupt chromosomal pairing during meiosis. These can cause failure of accurate mapping of the *Ty-2* gene when interspecific crosses are made. The introduction of larger segment from wild relatives would make problem in introgression breeding. But occurrence of linkage drag linked to *Ty-2* have not been reported yet (Yang et al., 2014). Because of the absence of recombination suppression in intraspecific crosses, using intraspecific crosses among different *S. habrochaites* accessionswith contrasting phenotypes of the same traits would be a solution of this situation to fine map and clone the *Ty-2*genes. Moreover, crossing between *Ty-2* line (*Ty-2* region is introgressed in *S. lycopersicum*) and susceptible *S. habrochaites* would be a good alternative to facilitate the cloning ofthe*Ty-2* gene, as the region of the block would be align in both *Ty-2* line and *S. habrochaites* due to the inversion.

4.2 Discussion on experiment 2

4.2.1 Analysis of F_2 plants

Molecular marker analysis was done by using three CAPS markers 51355_MH, UF_07960F2 and M1 located in the *Ty-2* region on chromosome 11 in *S. lycopersicum.* 51355_MH is located above the block in the *Ty-2* region, UF_07960F2 in the block and M1 below the block. Markers from 51355_MH to M1 showed that *S. habrochaites* introgressioncarrying the *Ty-2* gene was present in 75 among 85 F_2individuals. These individualswere scored as resistant and resistant individuals were either homozygous or heterozygous for *S. habrochaites* alleles at all tested markers indicating dominant effect of the *Ty-2* gene.

There was shortage of plants homozygous for *S. lycopersicum* allele. In this experiment, usually the large sized seeds were selected to sow and seeds were sown in a plot together. After germination, the seedlings which were relatively larger in size were selected to transplant in separate pots. Selection of larger seeds and fast germinating seedlings may be the cause of this skewed ratio of genotypes. Plants homozygous for *S. lycopersicum* allele in the *Ty-2* region could have small seed size and slower rate of germination compared to the plants homozygous or heterozygous for *S. habrochaites* allele. Similar types of results of skewed ratio of genotypes was observed in F_3 and F_4 population of *Arabidopsis thaliana* in screening of novel types of resistance to tomato powdery mildew (Gao et al., 2014).

According to the Mendel's laws of inheritance, the phenotypic ratio of F_2 is 3:1 if resistance come from single dominant gene and 15:1 when two independent dominant genes are involved. However, the segregation ratio could be 13:3 by epistatic interaction of two genes whereresistance comes from one dominant and one recessive gene. Banerjee and kallol (1987) found the resistance derived from *S. habrochaites* accession B6013 was segregated in the F_2 based on 13:3 ratio.But the phenotypic resultsof present study did not show good fit to 3:1 and 13:3 ratio, thus indicated that

the resistance might not be from one dominant gene as well as not from the effect of two epistatic genes as found in the study of Banerjee and kallol (1987). However, resultsshowed good fit to 15:1 segregation ratio indicating presence of the involvement of two dominant genes in F_2. But in this case, 15:1 ratio might be caused from skewed segregation of genotypes, not from two dominant genes.

Besides these results, two individuals having genotypeshomozygous for *S. lycopersicum* alleleswere resistant to TYLCV. This resistance might come from other sources or it could be due to escape. The results indicated the presence of the introgression of the *Ty-6* gene in these two individuals. These individuals were homozygous or heterozygous for *Ty-6* introgressed region. The resistance of all R scored individuals might be come from two genes (*Ty-2* and *Ty-6*). But any specific interpretation of these results was not possible as only three individuals were used to check *Ty-6* introgression by sequencing. No CAPS markers of *Ty-6*is available to check all the individualsandthe sequencing is expensive. Again, the marker of *Ty-6*is not the best one as it based on only one SNP. Moreover, it is reported in some speech and news that *Ty-6* may perhaps a recessive gene though no published articles are available on it yet.Theskewed segregation of genotypes was also another problem to clarify the results.These resistance might be possible to come from a gene located in any other chromosome, even not from reported resistant gene against TYLCV.Furthermore, these two individuals could not be infected by chance of escape. Therefore, further work using larger numbers of individuals would be necessary to conduct and selection of seedlings should not be done to get more precise results.

4.2.2 Analysis of F_3 and F_4 plants

Markers such as 51355_MH, UF_07960F2, MCGaoUF2, M1 and T0302 were used for analysis. All are located in the *Ty-2* region whileT0302 is further. Both markers UF_07960F2 and MCGaoUF2 are in the block, so that there was very low possibilities to find recombination between these markers. But to confirm accurate genotypes in the block, both markers were used in the study.

In a previous study of our group, it was found that recombination occurred between markers 51697_MH and 51752_MH(both are outside the block, between markers M1and T0302) in the F_2 parent (PV123002-45) of the F_3 familyPV143245. Then a small scale experiment was done using 5 individuals of F_3 family PV143245. One individual among 5 showed recombination between the markers 51355_MH (homozygous *S. lycopersicum*genotype) and UF_07960F2(heterozygous genotype) where the phenotype was resistant. To check that result, 10 individuals of F_3 family PV143245 were included in the present study. But no recombination was found between these markers. In all individualsof F_3 family PV143245, recombination took place between markers M1 and T0302.

Nine among 10 individuals of F_3 family showed severe TYLCV symptom. One individual (plant ID 6) did not show any TYLCV symptom although having homozygous genotype for *S. lycopersicum*allele in UF_07960F2, MCGaoUF2 and M1. Genotype of this individual was missed for51355_MHmarker, therefore it is not possible to interpret the result. However, all resistant F_3individuals were either homozygous or heterozygous for the introgression of *S. habrochaites,*therefore it supported the dominant inheritance of the *Ty-2* gene.

In case of F_4 recombinant lines, the results of the study indicated that almost all individuals of 12 lines produces symptoms similar to the susceptible control, MM and these all were homozygous for the susceptible allele from markers 51355_MH to M1. Only few individualsdid not produce any TYLCV symptom and these results could be caused by escape. All individuals of one line homozygous for *S. habrochaites* allele from markers 51355_MH to M1, were resistant. In the both cases, recombination events only happened between markers M1 and T0302 and T0302 is outside the *Ty-2* gene region. These results indicate that the *Ty-2* gene was responsible for the resistant to TYLCV and the *Ty-2* gene locates between markers 51355_MH to M1.

On the other hand, three F_4 lines displayed different results. These lines were devoid of the introgression of *S. habrochaites* carrying *Ty-2* region but showed resistance against TYLCV.So that the test for other TYLCV resistant genes was done to confirm whether resistance is mediated by only one single gene or more than one gene. Results showed that the resistance was not mediated by other known TYLCV resistant gene such as, *Ty-1*, *Ty-4*, *ty-5* and *Ty-6*. There might be additional factors playing a roleon the resistance in these lines. Banerjee and kallol (1987) identified the resistance to TYLCV coming from wild *S. habrochaites* was based on two epistatic genes. Again, according to Hassan et al., (1984) resistance in Tomato againstTYLCV which was derived from *S. habrochaites* is dominant and this is mediated by more than one gene.

4.2.3 Impact of the result of F_2 and F_4 analysis in breeding

Lines resistant to TYLCV could be used in TYLCV infested areas. Resistant lines also can be used as donor parents to develop hybrids carrying the *Ty-2* gene. As *Ty-2* is a dominant gene, it is easy to develop hybrids which would be resistant to TYLCV by crossing resistant homozygous line for the *Ty-2* gene with susceptible line with desired characteristics. But resistance mediated by single gene may not always behave wellunder high inoculation pressure. Therefore, combination of more resistance genes in a single cultivar is necessary to show more durable resistance against TYLCV(Ji et al., 2009). If the resistance of F_2 populations used in the present study is controlled by two genes (*Ty-2* and *Ty-6* or other gene),these resistance would be stronger than that of by a single gene. Similarly, the resistant F_4 linescould be used for further study to locate the existence of additional new gene. Therefore, the resistant individuals of F_2, F_3 and F_4 could be contribute in the process of mapping for later on pyramiding with other resistance genes from different sources to make a single cultivar with wider range of resistance.

5 Conclusion

5.1 Conclusion of Experiment 1

- No recombination suppression was observed in the target region in chromosome 11 in intraspecific crosses among *S. habrochaites* accessions.
- The order of markers in the target region of chromosome 11 might evidence an inversion in the block in S*. habrochaites* compare to*S. lycopersicum.*
- Selection of appropriate parents might be taken in account for further analysis of *Ty-2* region on chromosome 11 as the recombination rate varies among different accessions.
- Inverted region present in the block of *Ty-2* introgressed region on chromosome 11 derived from *S. habrochaites* might be the cause of recombination suppression in interspecific crosses.

5.2 Conclusion of Experiment 2

- *Ty-2* gene is responsible for resistance against TYLCV in our study population and it acts aa a dominant gene.
- *Ty-2* gene is located between markers 51355_MH to M1.
- In F_2 population, *Ty-6* gene might be present which was also contributed to resistance.
- In F_4population, there might be another new major or minor gene present which might be responsible for the TYLCV resistance in the absence of the *Ty-2* gene.

6 Recommendations for further study

- Study on sequencing of *S. habrochaites* compared to Heinz to check the region in the block.
- Fine mapping of *Ty-2* gene using cross between *Ty-2* line and *S. habrochaites* accession.
- Development of additional polymorphic markers to locate the exact position of recombination for precisely characterizing the introgression region and to locate the position of gene.
- To confirm the presence of *Ty-6*or any other gene in F_2 population, a new study could be conducted using larger plant numbers by random selection of seeds and seedlings.
- Screening of recombinant lines 12TEP070052, 12TEP070053 and 12TEP070125 should be repeated to confirm the results of the present study.

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8 Appendices

Appendix I. RETCH protocol 1.4 (May 2007)

This essentially is a CTAB based method up scaled to multiple 96 racks with tubes.
We make use of titer plate centrifuges and we use multichannel pipets for transferring samples and dispensers for adding liquids to tubes.

Harvesting of leaf material

1. Put the 96 micronic tubes in blue holder. Mark position of holder or try to put tubes asymmetrically on the holder so that you will be never puzzled about the original order of the tubes containing samples.

2. Put in every tube of a micronic titre plate two steel balls and put 2 fresh leaf disks (size eppendorf tube lid) in the tubes. While harvesting put your samples on ice.

DO NOT CLOSE THE TUBES UNTIL GRINDING BECAUSE 'CLOSING, OPENING AND RECLOSING' WILL LOOSEN THE CAPS AND CAUSE LEAKAGE DURING MILLING.
(Harvesting and filling the tubes with balls will take you about 45 min per 96 samples.
Using dehydrated material or N_2 frozen (instead of fresh) followed by dry milling will provide excellent material. Material from the –80°C keep in liquid N_2.

DNA extraction

3. Add 2x 250µl CTAB extraction buffer with RNase (per 1 ml CTAB 1 µl RNase (2 mg/ml) using a dispenser and close caps

4. Put the blue holders on the white (bottom) adapter from the RETSCH apparatus put the black adapter on top of your tubes. The protrusion on both the white and black adapter should be in the same orientation. ALWAYS MILL TWO, THUS A BALANCED SET OF PLATES!

5. Mill for 30 secs on 30 r/s speed then change the orientation of the plate and mill another time.

6. Place the tubes and holder in the brown press and tight the nuts (vleugelmoertjes) to prevent popping off the caps during incubation at 65ºC for 60 minutes.

7. Cool tubes on ice (water) to prevent popping off.

8. Work in fumehood. Add 250 µl chloroform isoamyl alcohol (24:1) and mix by inversion for 5 min. (If your samples are still warm the caps will pop off)

9. Separate phases by centrifuging at 3500 RPM for 15 min.

10. Meanwhile take new COSTAR tube holder and fill new tubes with 200µl of Isopropanol.

11. Pipette 200µl water phase to the isopropanol using the multichannel pipet. Cover tubes with 8 strips caps and mix briefly by inversion.

12. Pellet the DNA by centrifuging for 15 min at 3500 RPM

13. Dry pellets for minimally one hour by putting it in the fume hood

14. Dissolve DNA in TE buffer. In most cases the pellet will be dissolved in 100µl.

15. Measure the concentration with the Eppendorf Bio-Photometer or Nanodrop

16. Adjust your DNA at a concentration of 100 ng/µl

17. To check the DNA quality and concentration put 2 µl on agarosegel

If you have -80°C stored material:
Cool down the pins of the Tissue Striker in liquid N_2 and also the tubes. Grind the leaf material and then add 200 µl of CTAB buffer and go on

Composition of the CTAB buffer

100 ml 1 M TRIS pH 7.5
140 ml 5 M NaCl
20 ml 0.5 M EDTA pH 8.0
740 ml MiliQ H_2O
Add 2% CTAB (dissolve in a 65°C waterbath)

Appendix II. PCR Procedure

A total volume of 20µl were used (1µl DNA templatewith 19µl of the reaction mix) for each PCR.

PCR master mix preparation (19µl)

Chemical	Concentration
DreamTaq buffer (10x)	2µl
dNTPs	1µl
Forward primer	1µl
Reverse primer	1µl
Milli-Q water	13.9µl
DreamTaq DNA Polymerase (5u/µl)	0.1µl
Total volume	**19µl**

Thermal cycling condition

Step	Temperature	Time	Number of cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	30 second	35
Annealing	58°C	30 second	
Extension	72°C	1 min	
Final extension	72°C	5 min	1

Appendix III. Preparation of 150 ml 1.5 % agarose gel

150 ml 1.5% agarose gel was made by adding 2.25g Agarose powder in 150ml TBE buffer, followed by heating in microwave oven until the liquid looks transparent. 150 ml 1.5% agarose gel were poured into the tray and combs were inserted to make holes in the gel. The gel was kept at room temperature to solidify. After solidification, gel was put into Horizontal cell.

Appendix IV. Preparation of restriction enzyme

The PCR products of CAPS markerswere digested by using specific enzyme in specific buffer condition. The enzyme mixture mix was prepared by using following composition.

Enzyme mixture mix

Chemical	Concentration
Buffer	1µl
Milli-Q water	3.9µl
Enzyme	0.1µl
Total volume	**5µl**

List of enzymes used for CAPS marker digestion

Gene	Markers	Restriction enzymes	Buffer	Digestion temperature
Ty-2	51355_MH	*FspBI*	Tango	37°C
	UF_07960F2	*BanI*	Orange	
	cLEN-11-F24	*RsaI*	Tango	
	M1	*BstUI*	Red	
	51663_MH	*Hin6I*	Tango	
Ty -1	MSc05732-4	*DdeI*	Tango	
Ty-4	C2_At5g60160	*Hin1II*	Green	
ty-5	SlNAC1	*TaqI*	Unique	65°C

PCR product of each marker wasdigested overnight except for marker SlNAC1 (only 1.30 hour).

Appendix V. Marker scoring for population PV960357

Marker names and physical position of markers on chromosome 11 are included in the upper rows. Recombinants were analysed between two markers, cLEN-11-F24 and UF_07960F2 considering inversion of *Ty-2* region in *S. habrochaites* X *S. lycopersicum*.

P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
1	A	A	AH		1	A	A	AH
2	H	H	AH		2	H	H	AH
3	H	H	H		3	H	H	H
4	A	A	AH		4	A	A	AH
5	H	H	AH		5	H	H	AH
6	H	H	AH		6	H	H	AH
7	H	A	AH		7	A	H	AH
8	H	A	AH		8	A	H	AH
9	A	A	AH		9	A	A	AH
10	A	A	AH		10	A	A	AH
11	H	H	AH		11	**H**	H	AH
12	B	B	B		12	B	B	B
13	H	H	AH		13	H	H	AH
14	H	H	AH		14	H	H	AH
15	B	B	B		15	B	B	B
16	B	B	B		16	B	B	B
17	A	A	AH		17	A	A	AH
18	H	H	AH		18	H	H	AH
19	H	H	AH		19	H	H	AH
20	A	A	AH		20	A	A	AH
21	H	H	AH		21	H	H	AH
22	A	A	AH		22	A	A	AH
23	H	H	AH		23	H	H	AH
24	H	H	AH		24	H	H	AH
25	H	H	AH		25	H	H	AH
26	H	A	AH		26	A	H	AH
27	B	B	B		27	B	B	B
28	H	H	AH		28	H	H	AH
29	B	B	B		29	B	B	B
30	H	H	AH		30	H	H	AH
31	H	H	AH		31	H	H	AH
32	B	B	B		32	B	B	B
33	B	H	B		33	H	B	B
34	H	H	AH		34	H	H	AH
35	A	A	AH		35	A	A	AH
36	A	A	AH		36	A	A	AH
37	H	H	AH		37	H	H	AH
38	B	B	B		38	B	B	B
39	-	H	AH		39	H	-	AH
40	A	A	AH		40	A	A	AH
41	A	A	AH		41	A	A	AH
42	A	A	AH		42	A	A	AH
43	B	B	B		43	B	B	B
44	B	H	B		44	H	B	B
45	H	H	AH		45	H	H	AH
46	H	H	AH		46	H	H	AH
47	A	A	AH		47	A	A	AH
48	H	H	AH		48	H	H	AH

P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
49	H	H	AH		49	H	H	AH
50	A	A	AH		50	A	A	AH
51	H	H	AH		51	H	H	AH
52	H	H	AH		52	H	H	AH
53	H	H	AH		53	H	H	AH
54	A	A	AH		54	A	A	AH
55	B	B	B		55	B	B	B
56	H	H	AH		56	H	H	AH
57	H	H	AH		57	H	H	AH
58	A	A	AH		58	A	A	AH
59	H	H	AH		59	H	H	AH
60	B	B	B		60	B	B	B
61	A	A	AH		61	A	A	AH
62	B	B	B		62	B	B	B
63	H	H	AH		63	H	H	AH
64	B	H	B		64	H	B	B
65	A	A	AH		65	A	A	AH
66	A	A	AH		66	A	A	AH
67	B	B	B		67	B	B	B
68	H	H	AH		68	H	H	AH
69	H	H	AH		69	H	H	AH
70	H	H	AH		70	H	H	AH
71	H	H	AH		71	H	H	AH
72	H	H	AH		72	H	H	AH
73	H	H	B		73	H	H	B
74	B	B	B		74	B	B	B
75	B	B	B		75	B	B	B
76	B	B	B		76	B	B	B
77	A	A	AH		77	A	A	AH
78	H	H	AH		78	H	H	AH
79	H	H	AH		79	H	H	AH
80	H	H	AH		80	H	H	AH
81	H	H	AH		81	H	H	AH
82	H	H	AH		82	H	H	AH
83	H	H	AH		83	H	H	AH
84	H	H	AH		84	H	H	AH
85	H	H	AH		85	H	H	AH
86	H	H	-		86	H	H	-
87	B	B	B		87	B	B	B
88	A	A	AH		88	A	A	AH
89	H	H	AH		89	H	H	AH
90	A	H	AH		90	H	A	AH
91	A	A	AH		91	A	A	AH

Number in left column: Number of the plants
A : Genotype of *S. habrochaites* G1.1560 (homozygous)
B : Genotype of *S. habrochaites* G1.1606 (homozygous)
H: Genotype of plants having alleles from both parents (heterozygous)
AH: Genotype of heterozygous shows same as homozygous genotype of *S. habrochaites* G1.1560 (dominant marker)
P : Markers arrangement according to *Ty-2* region in *S. lycopersicum*
P*: Markers arrangement considering inversion of *Ty-2* region in *S. habrochaites*
Recombinant events are marked as red colour (in the block) and blue colour in outside the block

Appendix VI. Marker scoring for population PV970303

Markers names and physical position of markers on chromosome 11 are included in the upper rows. Recombinants were analysed between two markers, cLEN-11-F24 and UF_07960F2 considering inversion of *Ty-2* region in *S. habrochaites X S. lycopersicum.*

P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
1	H	H	AH		1	H	H	AH
2	H	H	AH		2	H	H	AH
3	B	B	B		3	B	B	B
4	B	B	B		4	B	B	B
5	B	B	B		5	B	B	B
6	A	A	AH		6	A	A	AH
7	B	B	B		7	B	B	B
8	H	H	AH		8	H	H	AH
9	B	B	B		9	B	B	B
10	H	H	AH		10	H	H	AH
11	B	B	B		11	B	B	B
12	H	H	AH		12	H	H	AH
13	H	H	AH		13	H	H	AH
14	B	B	B		14	B	B	B
15	B	B	B		15	B	B	B
16	H	H	AH		16	H	H	AH
17	H	H	AH		17	H	H	AH
18	H	H	AH		18	H	H	AH
19	B	B	B		19	B	B	B
20	H	H	AH		20	H	H	AH
21	A	A	AH		21	A	A	AH
22	H	H	AH		22	H	H	AH
23	H	H	AH		23	H	H	AH
24	H	H	AH		24	H	H	AH
25	H	H	AH		25	H	H	AH
26	H	H	AH		26	H	H	AH
27	B	B	B		27	B	B	B
28	H	H	AH		28	H	H	AH
29	B	B	B		29	B	B	B
30	H	H	AH		30	H	H	AH
31	H	H	AH		31	H	H	AH
32	A	A	AH		32	A	A	AH
33	H	H	AH		33	H	H	AH
34	H	H	AH		34	H	H	AH
35	H	H	AH		35	H	H	AH
36	A	A	AH		36	A	A	AH
37	H	H	AH		37	H	H	AH
38	B	H	B		38	H	B	B
39	A	A	AH		39	A	A	AH
40	A	A	AH		40	A	A	AH
41	B	B	B		41	B	B	B
42	A	-	AH		42	-	A	AH
43	B	B	B		43	B	B	B
44	A	A	AH		44	A	A	AH
45	B	B	B		45	B	B	B
46	H	H	AH		46	H	H	AH
47	B	B	B		47	B	B	B
48	H	H	AH		48	H	H	AH

P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
49	H	H	AH		49	H	H	AH
50	B	B	B		50	B	B	B
51	A	A	AH		51	A	A	AH
52	A	A	AH		52	A	A	AH
53	B	B	B		53	B	B	B
54	A	A	AH		54	A	A	AH
55	B	B	B		55	B	B	B
56	H	H	AH		56	H	H	AH
57	-	-	-		57	-	-	-
58	H	H	AH		58	H	H	AH
59	H	H	AH		59	H	H	AH
60	H	H	AH		60	H	H	AH
61	A	A	AH		61	A	A	AH
62	H	H	AH		62	H	H	AH
63	B	B	B		63	B	B	B
64	A	A	AH		64	A	A	AH
65	A	A	AH		65	A	A	AH
66	A	A	AH		66	A	A	AH
67	A	A	AH		67	A	A	AH
68	H	H	AH		68	H	H	AH
69	H	H	AH		69	H	H	AH
70	A	A	AH		70	A	A	AH
71	A	A	AH		71	A	A	AH
72	H	H	AH		72	H	H	AH
73	H	H	AH		73	H	H	AH
74	A	A	AH		74	A	A	AH
75	A	A	AH		75	A	A	AH
76	A	H	AH		76	H	A	AH
77	B	B	B		77	B	B	B
78	H	H	AH		78	H	H	AH
79	B	B	B		79	B	B	B
80	H	H	AH		80	H	H	AH
81	A	A	AH		81	A	A	AH
82	H	H	AH		82	H	H	AH
83	H	H	AH		83	H	H	AH
84	H	H	AH		84	H	H	AH
85	H	H	AH		85	H	H	AH
86	B	B	B		86	B	B	B
87	H	H	AH		87	H	H	AH
88	H	H	AH		88	H	H	AH
89	A	A	AH		89	A	A	AH
90	A	A	AH		90	A	A	AH
91	B	B	B		91	B	B	B
92	B	B	B		92	B	B	B
93	A	A	AH		93	A	A	AH
94	B	B	B		94	B	B	B
95	H	H	AH		95	H	H	AH
96	A	A	AH		96	A	A	AH
97	A	A	AH		97	A	A	AH
98	A	A	AH		98	A	A	AH
99	H	H	AH		99	H	H	AH
100	A	A	AH		100	A	A	AH

P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
101	H	H	AH		101	H	H	AH
102	H	H	AH		102	H	H	AH
103	B	B	B		103	B	B	B
104	A	A	AH		104	A	A	AH
105	H	H	AH		105	H	H	AH
106	A	A	AH		106	A	A	AH
107	H	H	AH		107	H	H	AH
108	A	H	AH		108	H	A	AH
109	H	H	AH		109	H	H	AH
110	H	H	AH		110	H	H	AH
111	B	B	B		111	B	B	B
112	H	H	AH		112	H	H	AH
113	B	B	B		113	B	B	B
114	B	B	B		114	B	B	B
115	B	B	B		115	B	B	B
116	H	H	AH		116	H	H	AH
117	A	A	AH		117	A	A	AH
118	H	H	AH		118	H	H	AH
119	B	B	B		119	B	B	B
120	A	A	AH		120	A	A	AH
121	H	H	AH		121	H	H	AH
122	H	H	AH		122	H	H	AH
123	H	H	AH		123	H	H	AH
124	B	B	B		124	B	B	B
125	B	B	B		125	B	B	B
126	H	H	AH		126	H	H	AH
127	H	H	AH		127	H	H	AH
128	H	H	AH		128	H	H	AH
129	H	H	AH		129	H	H	AH
130	H	H	AH		130	H	H	AH
131	B	B	B		131	B	B	B
132	A	A	AH		132	A	A	AH
133	H	H	AH		133	H	H	AH
134	A	A	AH		134	A	A	AH
135	H	H	AH		135	H	H	AH
136	H	H	AH		136	H	H	AH
137	A	A	AH		137	A	A	AH
138	B	B	B		138	B	B	B
139	A	A	AH		139	A	A	AH
140	H	H	AH		140	H	H	AH
141	B	B	B		141	B	B	B
142	A	A	AH		142	A	A	AH
143	H	-	AH		143	-	H	AH
144	H	H	AH		144	H	H	AH
145	H	H	AH		145	H	H	AH
146	H	H	AH		146	H	H	AH
147	B	B	B		147	B	B	B
148	-	-	-		148	-	-	-
149	A	A	AH		149	A	A	AH
150	H	H	AH		150	H	H	AH
151	H	H	AH		151	H	H	AH
152	H	H	AH		152	H	H	AH
153	A	A	AH		153	A	A	AH
154	H	H	AH		154	H	H	AH
155	H	H	AH		155	H	H	AH

P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
156	H	H	AH		156	H	H	AH
157	B	B	B		157	B	B	B
158	A	A	AH		158	A	A	AH
159	B	B	B		159	B	B	B
160	B	B	B		160	B	B	B
161	A	H	AH		161	H	A	AH
162	H	H	AH		162	H	H	AH
163	H	H	AH		163	H	H	AH
164	A	A	AH		164	A	A	AH
165	B	B	B		165	B	B	B
166	A	A	AH		166	A	A	AH
167	B	B	B		167	B	B	B
168	H	H	AH		168	H	H	AH
169	B	B	B		169	B	B	B
170	A	A	AH		170	A	A	AH
171	A	A	AH		171	A	A	AH
172	A	A	AH		172	A	A	AH
173	H	H	AH		173	H	H	AH
174	A	A	AH		174	A	A	AH
175	H	H	AH		175	H	H	AH
176	H	H	AH		176	H	H	AH
177	H	H	AH		177	H	H	AH
178	B	B	B		178	B	B	B
179	A	A	AH		179	A	A	AH
180	H	H	AH		180	H	H	AH
181	H	H	AH		181	H	H	AH
182	B	B	B?		182	B	B	B?
183	H	H	AH		183	H	H	AH
184	A	A	AH		184	A	A	AH
185	A	A	AH		185	A	A	AH
186	A	A	AH		186	A	A	AH
187	B	B	B		187	B	B	B
188	H	H	AH		188	H	H	AH
189	H	H	AH		189	H	H	AH
190	H	H	AH		190	H	H	AH
191	H	A	AH		191	A	H	AH
192	H	H	AH		192	H	H	AH
193	A	A	AH		193	A	A	AH
194	A	A	AH		194	A	A	AH
195	B	B	B		195	B	B	B
196	B	B	B		196	B	B	B
197	B	B	B		197	B	B	B
198	A	A	AH		198	A	A	AH
199	A	A	AH		199	A	A	AH
200	B	B	B		200	B	B	B
201	H	A	AH		201	A	H	AH
202	B	B	B		202	B	B	B
203	A	A	AH		203	A	A	AH
204	H	H	AH		204	H	H	AH
205	B	B	B		205	B	B	B
206	H	H	AH		206	H	H	AH
207	H	H	AH		207	H	H	AH
208	H	H	AH		208	H	H	AH
209	H	H	AH		209	H	H	AH
210	A	A	AH		210	A	A	AH

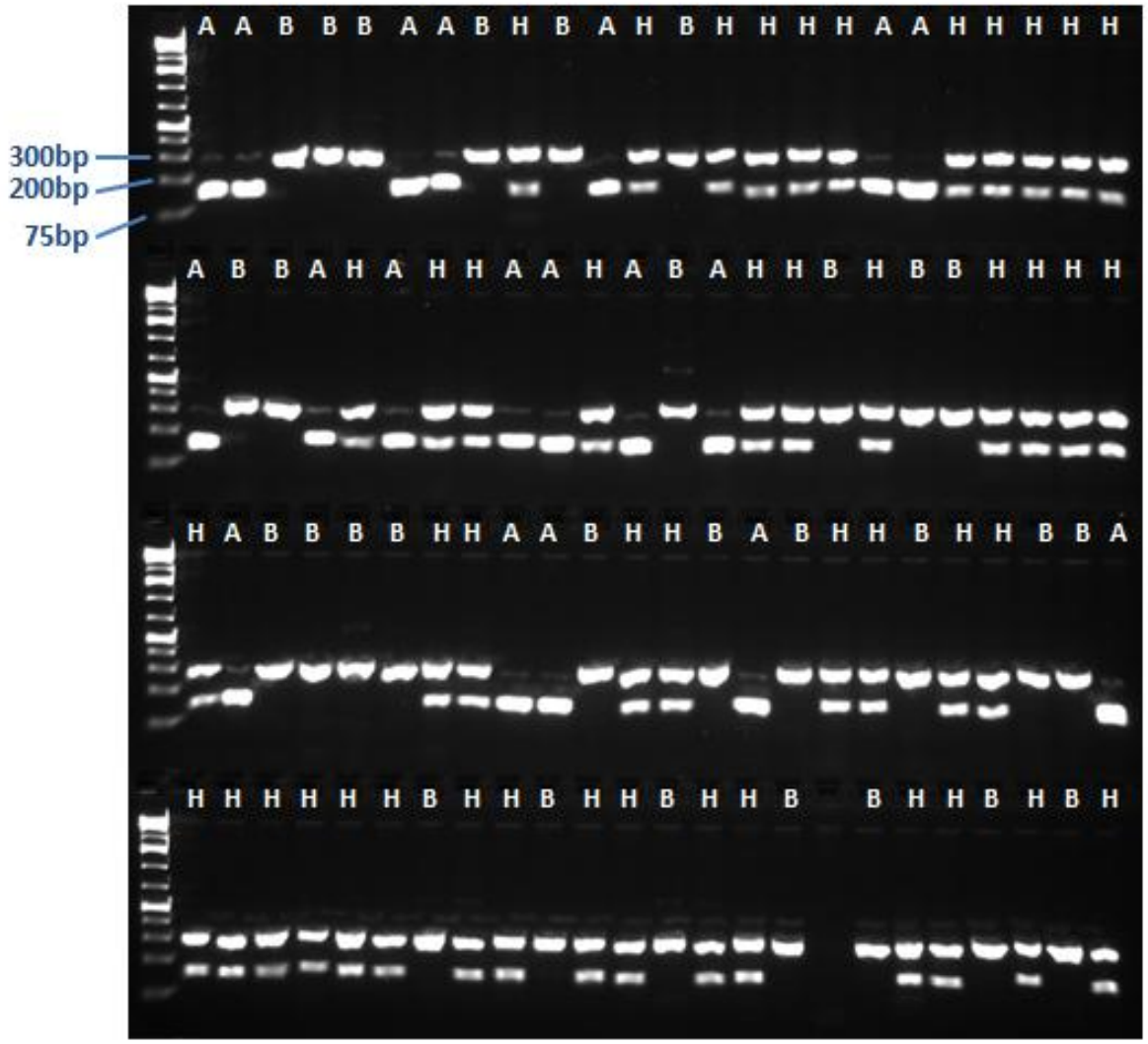
P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
211	A	A	AH		211	A	A	AH
212	H	H	AH		212	H	H	AH
213	H	H	AH		213	H	H	AH
214	H	H	AH		214	H	H	AH
215	H	H	AH		215	H	H	AH
216	H	H	AH		216	H	H	AH
217	A	A	AH		217	A	A	AH
218	B	B	B		218	B	B	B
219	B	B	B		219	B	B	B
220	A	A	AH		220	A	A	AH
221	H	H	AH		221	H	H	AH
222	A	A	AH		222	A	A	AH
223	H	H	AH		223	H	H	AH
224	H	H	AH		224	H	H	AH
225	A	A	AH		225	A	A	AH
226	A	A	AH		226	A	A	AH
227	H	H	AH		227	H	H	AH
228	A	A	AH		228	A	A	AH
229	B	B	B		229	B	B	B
230	A	A	AH		230	A	A	AH
231	H	H	AH		231	H	H	AH
232	H	H	AH		232	H	H	AH
233	B	B	B		233	B	B	B
234	H	H	AH		234	H	H	AH
235	B	B	B		235	B	B	B
236	B	B	B		236	B	B	B
237	H	H	AH		237	H	H	AH
238	H	H	AH		238	H	H	AH
239	H	H	AH		239	H	H	AH
240	H	H	AH		240	H	H	AH
241	H	H	AH		241	H	H	AH
242	A	A	AH		242	A	A	AH
243	B	B	B		243	B	B	B
244	B	B	B		244	B	B	B
245	B	B	B		245	B	B	B
246	B	B	B		246	B	B	B
247	H	H	AH		247	H	H	AH
248	H	H	AH		248	H	H	AH
249	A	A	AH		249	A	A	AH
250	A	A	AH		250	A	A	AH
251	B	B	B		251	B	B	B
252	H	H	AH		252	H	H	AH
253	H	H	AH		253	H	H	AH
254	B	B	B		254	B	B	B
255	A	A	AH		255	A	A	AH
256	B	B	B		256	B	B	B
257	H	H	AH		257	H	H	AH
258	H	H	AH		258	H	H	AH
259	B	B	B		259	B	B	B
260	H	H	AH		260	H	H	AH
261	H	H	AH		261	H	H	AH
262	B	B	B		262	B	B	B
263	B	B	B		263	B	B	B
264	A	A	AH		264	A	A	AH
265	H	H	AH		265	H	H	AH

P →	UF_07960F2	cLEN-11-F24	51663_MH		P*→	cLEN-11-F24	UF_07960F2	51663_MH
	51.388	51.549	51.663			51.549	51.388	51.663
266	H	H	AH		266	H	H	AH
267	H	H	AH		267	H	H	AH
268	H	H	AH		268	H	H	AH
269	H	H	AH		269	H	H	AH
270	H	H	AH		270	H	H	AH
271	B	B	B		271	B	B	B
272	H	H	AH		272	H	H	AH
273	H	H	AH		273	H	H	AH
274	B	B	B		274	B	B	B
275	H	H	AH		275	H	H	AH
276	H	H	AH		276	H	H	AH
277	B	B	B		277	B	B	B
278	H	H	AH		278	H	H	AH
279	H	H	AH		279	H	H	AH
280	B	B	B		280	B	B	B
281	-	B	B		281	B	-	B
282	B	B	B		282	B	B	B
283	H	H	AH		283	H	H	AH
284	H	H	AH		284	H	H	AH
285	B	B	B		285	B	B	B
286	H	H	AH		286	H	H	AH
287	B	B	B		287	B	B	B

Number in left column: Number of the plants
A : Genotype of *S. habrochaites* G1.1560 (homozygous)
B : Genotype of *S. habrochaites* G1.1290 (homozygous)
H: Genotype of plantshaving alleles from both parents (heterozygous)
AH: Genotype of heterozygous shows same as homozygous genotype of *S. habrochaites* G1.1560 (dominant marker)
P : Markers arrangement according to *Ty-2* region in *S. lycopersicum*
P*: Markers arrangement considering inversion of *Ty-2* region in *S. habrochaites*
Recombinant events are marked as red colour

Appendix VII: Picture of gel of CAPS marker UF_07960F2

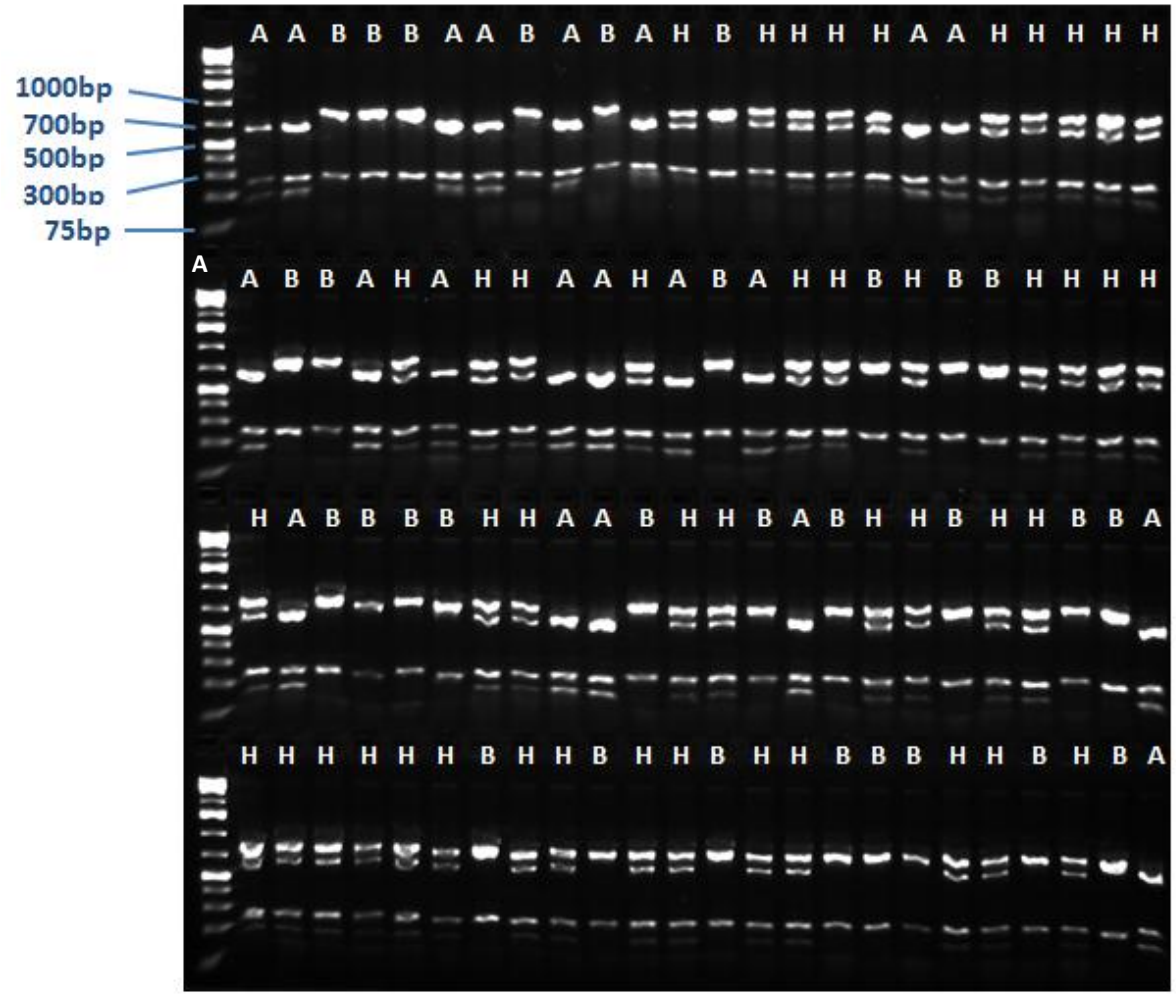
1kb plus DNA ladder (first left column) was included with 96 F_2 individuals of population PV970303 to determine the molecular weight of each band in a gel.



A : Genotype of *S. habrochaites* G1.1560(homozygous)
B : Genotype of *S. habrochaites* G1.1290(homozygous)
H : Genotype of plantshaving alleles from both parents (heterozygous)

Appendix VIII: Picture of gel of CAPS marker cLEN-11-F24

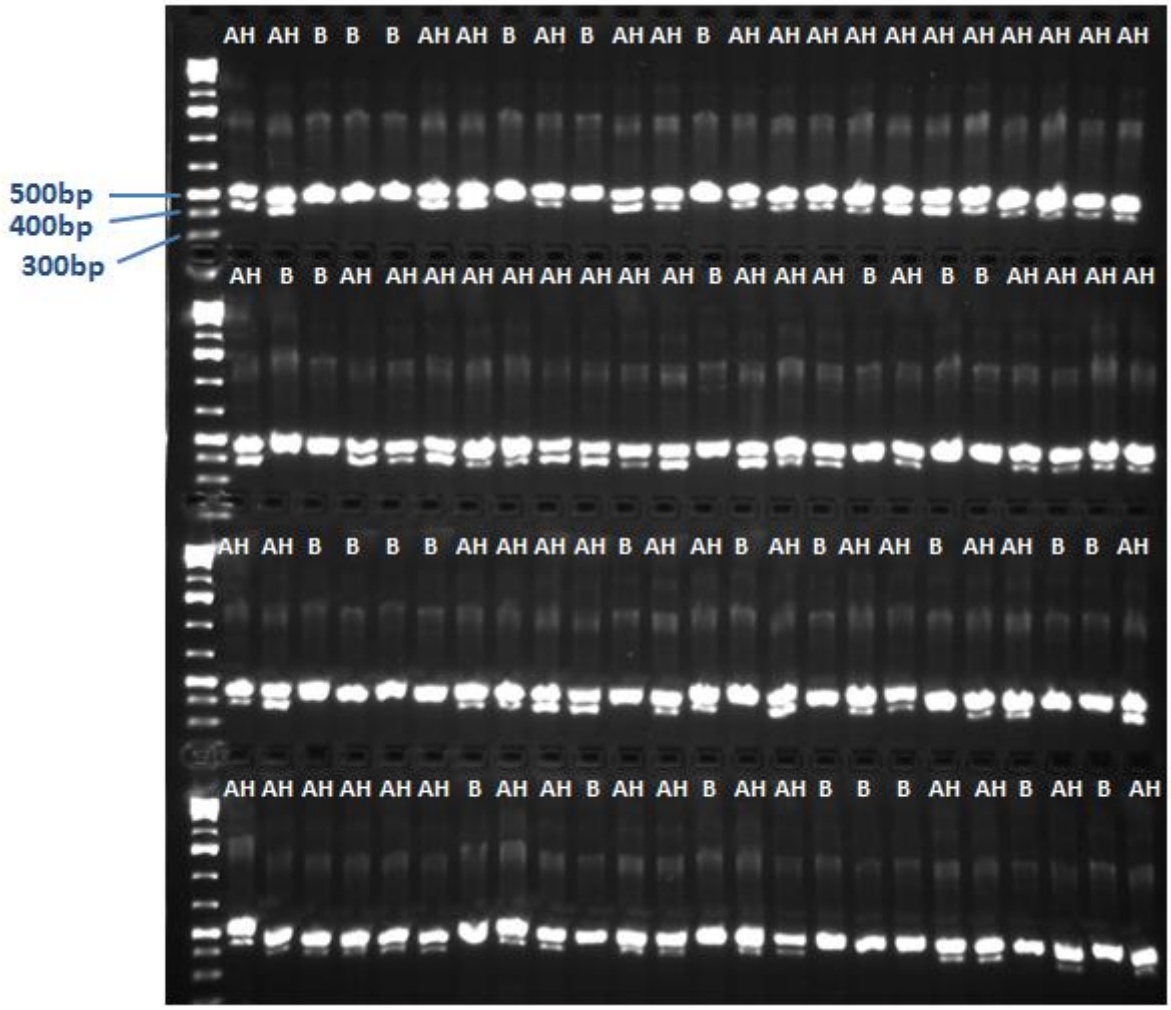
1kb plus DNA ladder (first left column) was included with 96 F_2 individuals of population PV970303 to determine the molecular weight of each band in a gel.



A : Genotype of *S. habrochaites* G1.1560 (homozygous)
B : Genotype of *S. habrochaites* G1.1290 (homozygous)
H : Genotype of plantshaving alleles from both parents (heterozygous)

Appendix IX: Picture of gel of dominant CAPS marker 51663_MH

1kb plus DNA ladder(first left column) was included with 96 F_2 individuals of population PV970303 to determine the molecular weight of each band in a gel.



A : Genotype of *S. habrochaites* G1.1560 (homozygous)
B : Genotype of *S. habrochaites* G1.1290 (homozygous)
H : Genotype of the plant have alleles from both parents (heterozygous)
AH: Genotype of heterozygous shows same as genotype of *S. habrochaites* G1.1560 (dominant marker)

Appendix X: Genotyping and disease scoring data of F_2

Individual	Markers with physical position in chromosome 11			Score of disease severity		Phenotype
Plant	51355_MH	UF_07960F2	M1	30 DAI	36 DAI	
ID	51.355	51.388	51.645			
1	BH	B	B	0	0	R
2	BH	H	H	0	0	R
3	BH	H	H	0	0	R
4	BH	B	B	0	0	R
5	BH	H	H	0	0	R
6	BH	B	B	0	0	R
7	BH	H	H	0	0	R
8	A	A	A	3	4	S
9	BH	B	B	0	0	R
10	BH	B	B	0	0	R
11	BH	H	H	0	0	R
12	BH	H	H	0	0	R
13	BH	B	B	0	0	R
14	A	A	A	4	4	S
15	A	A	A	4	4	S
16	BH	B	B	0	0	R
17	A	A	A	0	0	R
18	BH	B	B	0	0	R
19	BH	H	H	0	0	R
20	BH	B	B	0	0	R
21	BH	H	H	0	0	R
22	BH	B	B	0	0	R
23	BH	B	B	0	0	R
24	BH	H	H	0	0	R
25	BH	H	H	0	0	R
26	BH	B	B	0	0	R
27	BH	H	H	0	0	R
28	A	A	A	3	4	S
29	-	B	B	0	0	R
30	A	A	A	3	4	S
31	BH	B	B	0	0	R
32	BH	B	B	0	0	R
33	BH	H	H	0	0	R
34	BH	H	H	0	0	R
35	BH	H	H	0	0	R
36	BH	B	B	0	0	R
37	BH	H	H	0	0	R
38	BH	B	B	0	0	R
39	A	A	A	0.5	0	R
40	BH	H	H	0	0	R
41	BH	H	H	0	0	R
42	BH	H	H	0	0	R
43	BH	B	B	0.5	0	R
44	BH	B	B	0	0	R
45	BH	H	H	0	0	R
46	BH	H	H	0	0	R
47	BH	H	H	0	0	R
48	A	A	A	0	1.5	S

Individual	Markers with physical position in chromosome 11			Score of disease severity		Phenotype
Plant	51355_ MH	UF_07960F2	M1	30 DAI	36 DAI	
ID	51.355	51.388	51.645			
49	BH	H	H	0	0	R
50	A	A	A	3.5	4	S
51	BH	B	B	0.5	0	R
52	BH	H	H	0	0	R
53	BH	H	H	0	0	R
54	BH	B	B	0	0	R
55	BH	H	H	0	0	R
56	BH	H	H	0	0	R
57	BH	H	H	0	0	R
58	BH	H	-	0	0	R
59	BH	B	B	0	0	R
60	BH	H	H	0	0	R
61	BH	B	B	0	0	R
62	BH	B	B	0	0	R
63	BH	H	H	0.5	0	R
64	BH	B	-	0.5	0	R
65	BH	H	H	0	0	R
66	BH	H	H	0	0	R
67	BH	H	H	0	0	R
68	BH	B	B	0	0	R
69	BH	H	H	0	0	R
70	BH	B	B	0	0	R
71	BH	H	H	0.5	0	R
72	BH	H	H	0	0	R
73	BH	B	B	0	0	R
74	BH	H	H	0	0	R
75	BH	H	H	0	0	R
76	BH	H	H	0	0	R
77	BH	H	H	0	0	R
78	BH	H	H	0	0	R
79	A	A	A	3	4	S
80	BH	B	B	0	0	R
81	BH	B	B	0	0	R
82	BH	H	H	0	0	R
83	BH	H	H	0	0	R
84	BH	H	H	0	0	R
85	BH	H	H	0	0	R
MM -1	A	A	A	4	4	S
MM-2	-	-	-	3.5	4	S
MM-3	-	-	-	3.5	4	S
MM-4	-	-	-	4	4	S
MM-5	-	-	-	4	4	S
MM-6	-	-	-	3.5	4	S
MM-7	-	-	-	4	4	S
MM-8	-	-	-	4	4	S
MM-9	-	-	-	3	4	S
MM-10	-	-	-	4	4	S

A - Homozygous for *S. lycopersicum* allele; B - Homozygous for *S. habrochaites* allele
H – Heterozygous; BH: Genotype of heterozygous show same as homozygousB plants (dominant marker); Plants showed non-corresponding results are marked as red shade

Appendix XI: Genotyping and disease scoring data of F_3 (PV143254) and F_4 recombinants lines

Recombinants	Individual	Markers located in *Ty-2* region with physical position in chromosome 11					Score of disease severity				Phenotype	Marker of	Marker of	Marker of
Lines	Plants	51355_ MH	UF_07960F2	MCGao UF2	M1	T0302	30 DAI	36 DAI	45 DAI	51 DAI		*Ty-1*	*Ty-4*	*ty-5*
	ID	51.355	51.388	51.426	51.645	51.878						MSC05732-4	C2_At5g60160	SINAC 1
12TEP070049	1	A	A	A	-	A	4	4	4	4	S	A	A	A
	2	A	A	A	-	A	0	0	0	0	R	A	A	A
	3	A	A	A	-	A	4	4	4	4	S	A	A	A
	4	A	A	A	-	A	2.5	4	4	4	S	A	A	A
	5	A	A	A	-	A	3	4	4	4	S	A	A	A
	6	A	A	A	-	A	3	4	4	4	S	A	A	A
	7	A	A	A	-	A	3	4	4	4	S	A	A	A
	8	A	A	A	-	A	0	0	0	0	R	A	A	A
	9	A	A	A	-	A	4	4	4	4	S	A	A	A
	10	A	A	A	-	A	0	0	4	4	S	A	A	A
12TEP070050	1	A	A	A	-	A	2.5	4	4	4	S	A	A	A
	2	A	A	A	-	A	2	4	4	4	S	A	A	A
	3	A	A	A	-	A	4	4	4	4	S	A	A	A
	4	A	A	A	-	A	2	4	4	4	S	A	A	A
	5	A	A	A	-	A	2	4	4	4	S	A	A	A
	6	A	A	A	-	A	4	4	4	4	S	A	A	A
	7	A	A	A	-	A	0	0	0	0	R	A	A	A
	8	A	A	A	-	A	4	4	4	4	S	A	A	A
	9	A	A	A	-	A	0	0	0	0	R	A	A	A
	10	A	A	A	-	A	1	4	4	4	S	A	A	A
12TEP070051	1	A	A	A	-	A	1	4	4	4	S	A	A	A
	2	A	A	A	-	A	4	4	4	4	S	A	A	A
	3	A	A	A	-	A	0	0	0	0	R	A	A	A
	4	A	A	A	-	A	4	4	4	4	S	A	A	A
	5	A	A	A	-	A	4	4	4	4	S	A	A	A
	6	A	A	A	-	A	4	4	4	4	S	A	A	A
	7	A	A	A	-	A	1	4	4	4	S	A	A	A
	8	A	A	A	-	A	4	4	4	4	S	A	A	A
	9	A	A	A	-	A	4	4	4	4	S	A	A	A
	10	A	A	A	-	A	3	4	4	4	S	A	A	A

Recombinants	Individual	Markers located in *Ty-2* region with physical position in chromosome 11					Score of disease severity				Phenotype	Marker of	Marker of	Marker of
Lines	Plants	51355_MH	UF_07960F2	MCGao UF2	M1	T0302	30 DAI	36 DAI	45 DAI	51 DAI		*Ty-1*	*Ty-4*	*ty-5*
	ID	51.355	51.388	51.426	51.645	51.878						MSC05732-4	C2_At5g60160	SINAC 1
12TEP070052	1	A	A	A	-	A	2.5	4	4	4	S	A	A	A
	2	A	A	A	-	A	2	4	4	4	S	A	A	A
	3	A	A	A	-	A	0	0	0	0	R	A	A	A
	4	A	A	A	-	A	0	0	0	0	R	A	A	A
	5	A	A	A	-	A	0	0	0	0	R	A	A	A
	6	A	A	A	-	A	0	0	0.5	0	R	A	A	A
	7	A	A	A	-	A	0	0	0.5	0	R	A	A	A
	8	A	A	A	-	A	0	0	0	0	R	A	A	A
	9	A	A	A	-	A	0	0	0	0	R	A	A	A
	10	A	A	A	-	A	0	0	0	0	R	A	A	A
12TEP070053	1	A	A	A	-	A	0	0.5	4	4	S	A	A	A
	2	A	A	A	-	A	3	3	4	4	S	A	A	A
	3	A	A	A	-	A	0	0	0	0	R	A	A	A
	4	A	A	A	-	A	0	0	0	0	R	A	A	A
	5	A	A	A	-	A	0	0.5	0.5	0	R	A	A	A
	6	A	A	A	-	A	0	0	0	0	R	A	A	A
	7	A	A	A	-	A	0	0	0	0	R	A	A	A
	8	A	A	A	-	A	0	0	0	0	R	A	A	A
	9	A	A	A	-	A	0	0	0	0	R	A	A	A
	10	A	A	A	-	A	4	4	4	4	S	A	A	A
12TEP070054	1	A	A	A	-	A	4	4	4	4	S	A	A	A
	2	A	A	A	-	A	0	0	0	0	R	A	A	A
	3	A	A	A	-	A	1	3	4	4	S	A	A	A
	4	A	A	A	-	A	1	3	4	4	S	A	A	A
	5	A	A	A	-	A	3.5	4	4	4	S	A	A	A
	6	A	A	A	-	A	4	4	4	4	S	A	A	A
	7	A	A	A	-	A	4	4	4	4	S	A	A	A
	8	A	A	A	-	A	1	2	4	4	S	A	A	A
	9	A	A	A	-	A	0	0	0	0	R	A	A	A
	10	A	A	A	-	A	4	4	4	4	S	A	A	A

Recombinants	Individual	Markers located in *Ty-2* region with physical position in chromosome 11					Score of disease severity				Phenotype	Marker of	Marker of	Marker of
Lines	Plants	51355_MH	UF_07960F2	MCGao UF2	M1	T0302	30 DAI	36 DAI	45 DAI	51 DAI		*Ty-1*	*Ty-4*	*ty-5*
	ID	51.355	51.388	51.426	51.645	51.878						MSC05732-4	C2_At5g60160	SINAC 1
12TEP070056	1	A	A	A	-	A	4	4	4	4	S	A	A	A
	2	A	A	A	-	A	4	4	4	4	S	A	A	A
	3	A	A	A	-	A	4	4	4	4	S	A	A	A
	4	A	A	A	-	A	4	4	4	4	S	A	A	A
	5	A	A	A	-	A	4	4	4	4	S	A	A	A
	6	A	A	A	-	A	4	4	4	4	S	A	A	A
	7	A	A	A	-	A	4	4	4	4	S	A	A	A
	8	A	A	A	-	A	3	4	4	4	S	A	A	A
	9	A	A	A	-	A	2	4	4	4	S	A	A	A
	10	A	A	A	-	A	4	4	4	4	S	A	A	A
12TEP070116	1	A	A	A	-	A	3.5	4	4	4	S	A	A	A
	2	A	A	A	-	A	3.5	4	4	4	S	A	A	A
	3	A	A	A	-	A	4	4	4	4	S	A	A	-
	4	A	A	A	-	A	3.5	4	4	4	S	A	A	A
	5	A	A	A	-	A	4	4	4	4	S	A	A	A
	6	A	A	A	-	A	4	4	4	4	S	A	A	A
	7	A	A	A	-	A	3.5	4	4	4	S	A	A	A
	8	A	A	A	-	A	0	1	4	4	S	A	A	A
	9	A	A	A	-	A	0	0	3	4	S	A	A	A
	10	A	A	A	-	A	2	3	4	4	S	A	A	A
12TEP070117	1	BH	B	B	B	A	0	0	0	0	R	A	A	-
	2	BH	B	B	B	A	0	0	0	0	R	A	A	A
	3	BH	B	B	B	A	0	0	0	0	R	A	A	A
	4	BH	B	B	B	A	0	0	0	0	R	A	A	A
	5	BH	B	B	B	A	0	0	0	0	R	A	A	A
	6	BH	B	B	B	A	0	0	0	0	R	A	A	A
	7	BH	B	B	B	A	0	0	0	0	R	A	A	A
	8	BH	B	B	B	A	0	0	0	0	R	A	A	A
	9	BH	B	B	B	A	0	0	0	0	R	A	A	A
	10	BH	B	B	B	A	0	0	0	0	R	A	A	A

Recombinants	Individual	Markers located in *Ty-2* region with physical position in chromosome 11					Score of disease severity				Phenotype	Marker of	Marker of	Marker of
Lines	Plants	51355_MH	UF_07960F2	MCGao UF2	M1	T0302	30 DAI	36 DAI	45 DAI	51 DAI		*Ty-1*	*Ty-4*	*ty-5*
	ID	51.355	51.388	51.426	51.645	51.878						MSC05732-4	C2_At5g60160	SINAC 1
12TEP070119	1	A	A	A	-	A	2	4	4	4	S	A	A	A
	2	A	A	A	-	A	0	0	0	0	R	A	A	A
	3	A	A	A	-	A	0.5	1.5	4	4	S	A	A	A
	4	A	A	A	-	A	0.5	1.5	4	4	S	A	A	A
	5	A	A	A	-	A	3	4	4	4	S	A	A	A
	6	A	A	A	-	A	3	4	4	4	S	A	A	A
	7	A	A	A	-	A	3	4	4	4	S	A	A	A
	8	A	A	A	-	A	3	4	4	4	S	A	A	A
	9	A	A	A	-	A	0	0	0	0	R	A	A	A
	10	A	A	A	-	A	0	0.5	3	4	S	A	A	A
12TEP070121	1	A	A	A	-	A	3.5	4	4	4	S	A	A	A
	2	A	A	A	-	A	0	0	0	0	R	A	A	A
	3	A	A	A	-	A	3	4	4	4	S	A	A	A
	4	A	A	A	-	A	0	0	0	0	R	A	A	A
	5	A	A	A	-	A	1.5	4	4	4	S	A	A	A
	6	A	A	A	-	A	3	4	4	4	S	A	A	A
	7	A	A	A	-	A	3	4	4	4	S	A	A	A
	8	A	A	A	-	A	0	0	0	0	R	A	A	A
	9	A	A	A	-	A	4	4	4	4	S	A	A	A
	10	A	A	A	-	A	4	4	4	4	S	A	A	A
12TEP070123	1	A	A	A	A	B	0.5	4	4	4	S	A	A	A
	2	A	A	A	A	B	0	4	4	4	S	A	A	A
	3	A	A	A	A	B	0.5	0	0	0	R	A	A	A
	4	A	A	A	A	B	0	4	4	4	S	A	A	A
	5	A	A	A	A	B	3	4	4	4	S	A	A	A
	6	A	A	A	A	B	0	0.5	3	4	S	A	A	A
	7	A	A	A	A	B	1.5	3	4	4	S	A	A	A
	8	A	A	A	A	B	1.5	2.5	4	4	S	A	A	A
	9	A	A	A	A	B	1	1	3	4	S	A	A	A
	10	A	A	A	A	B	0	0	0	0	R	A	A	A

Recombinants	Individual	Markers located in *Ty-2* region with physical position in chromosome 11					Score of disease severity				Phenotype	Marker of	Marker of	Marker of
Lines	Plants	51355_MH	UF_07960F2	MCGao UF2	M1	T0302	30 DAI	36 DAI	45 DAI	51 DAI		*Ty-1*	*Ty-4*	*ty-5*
	ID	51.355	51.388	51.426	51.645	51.878						MSC05732-4	C2_At5g60160	SINAC 1
12TEP070124	1	A	A	A	A	B	0.5	1	3	4	S	A	A	A
	2	A	A	A	A	B	1.5	2	4	4	S	A	A	A
	3	A	A	A	A	B	0	0	0	0	R	A	A	A
	4	A	A	A	A	B	0	0	4	4	S	A	A	A
	5	A	A	A	A	B	0	0	3	4	S	A	A	A
	6	A	A	A	A	B	2.5	3	4	4	S	A	A	A
	7	A	A	A	A	B	0	0	0	0	R	A	A	A
	8	A	A	A	A	B	2	2.5	4	4	S	A	A	A
	9	A	A	A	A	B	4	4	4	4	S	A	A	A
	10	A	A	A	A	B	0	0	0	0	R	A	A	A
12TEP070125	1	A	A	A	A	B	3.5	4	4	4	S	A	A	A
	2	A	A	A	A	B	4	4	4	4	S	A	A	A
	3	A	A	A	A	B	0	0	0	0	R	A	A	A
	4	A	A	A	A	B	0	0	0	0	R	A	A	A
	5	A	A	A	A	B	0	0	0	0	R	A	A	A
	6	A	A	A	A	B	3	4	4	4	S	A	A	A
	7	A	A	A	A	B	0.5	0.5	0	0	R	A	A	A
	8	A	A	A	A	B	0	0	0	0	R	A	A	A
	9	A	A	A	A	B	0	0	0	0	R	A	A	A
	10	A	A	A	A	B	0	0	0	0	R	A	A	A
12TEP070138	1	A	A	A	A	B	0	0	0	0	R	A	A	A
	2	A	A	A	A	B	1.5	3	4	4	S	A	A	A
	3	A	A	A	A	B	2	4	4	4	S	A	A	A
	4	A	A	A	A	B	2	2.5	4	4	S	A	A	A
	5	A	A	A	A	B	1	1	3	4	S	A	A	A
	6	A	A	A	A	B	3.5	4	4	4	S	A	A	A
	7	A	A	A	A	B	2.5	4	4	4	S	A	A	A
	8	A	A	A	A	B	2.5	4	4	4	S	A	A	A
	9	A	A	A	A	B	3	3.5	4	4	S	A	A	A
	10	A	A	A	A	B	3	3.5	4	4	S	A	A	A

Recombinants	Individual	Markers located in *Ty-2* region with physical position in chromosome 11					Score of disease severity				Phenotype	Marker of	Marker of	Marker of
Lines	Plants	51355_MH	UF_07960F2	MCGao UF2	M1	T0302	30 DAI	36 DAI	45 DAI	51 DAI		*Ty-1*	*Ty-4*	*ty-5*
	ID	51.355	51.388	51.426	51.645	51.878						MSC05732-4	C2_At5g60160	SINAC 1
12TEP070139	1	A	A	A	A	B	0	0	0	0	R	A	A	A
	2	A	A	A	A	B	2	4	4	4	S	A	A	A
	3	A	A	A	A	B	2	3.5	4	4	S	A	A	A
	4	A	A	A	A	B	0	0	0	0	R	A	A	A
	5	A	A	A	A	B	1	3	4	4	S	A	A	A
	6	A	A	A	A	B	3	3	4	4	S	A	A	A
	7	A	A	A	A	B	0.5	0.5	2	4	S	A	A	A
	8	A	A	A	A	B	0	0	0	0	R	A	A	A
	9	A	A	A	A	B	1	2	4	4	S	A	A	A
	10	A	A	A	A	B	1.5	3	4	4	S	A	A	A
PV143245	1	BH	H	H	H	B	1?	0.5?	0	0	R	-	A	A
	2	BH	H	H	H	B	0	0	0	0	R	A	A	A
	3	BH	H	H	H	B	0	0	0	0	R	A	A	A
	4	BH	B	B	B	B	0	0	0	0	R	A	A	A
	5	BH	H	H	H	B	0	0	0	0	R	A	A	A
	6	-	A	A	A	B	0	0	0	0	R	A	A	A
	7	BH	H	H	H	B	0	0	0	0	R	A	A	A
	8	BH	H	H	H	B	0	0	0	0	R	A	A	A
	9	BH	B	B	B	B	0	0.5	0	0	R	A	A	A
	10	BH	H	H	H	A	0	0	0	0	R	A	A	A
MM	1	A	A	A	-	A	4	4	4	4	S	A	A	A
	2	A	A	A	-	A	4	4	4	4	S	A	A	A
	3	A	A	A	-	A	4	4	4	4	S	A	A	A
	4	A	A	A	-	A	4	4	4	4	S	A	A	A
	5	A	A	A	-	A	4	4	4	4	S	A	A	A
	6	A	A	A	-	A	4	4	4	4	S	A	A	A
	7	A	A	A	-	A	4	4	4	4	S	A	A	A
	8	A	A	A	-	A	4	4	4	4	S	A	A	A
	9	A	A	A	-	A	4	4	4	4	S	A	A	A
	10	A	A	A	-	A	4	4	4	4	S	A	A	A

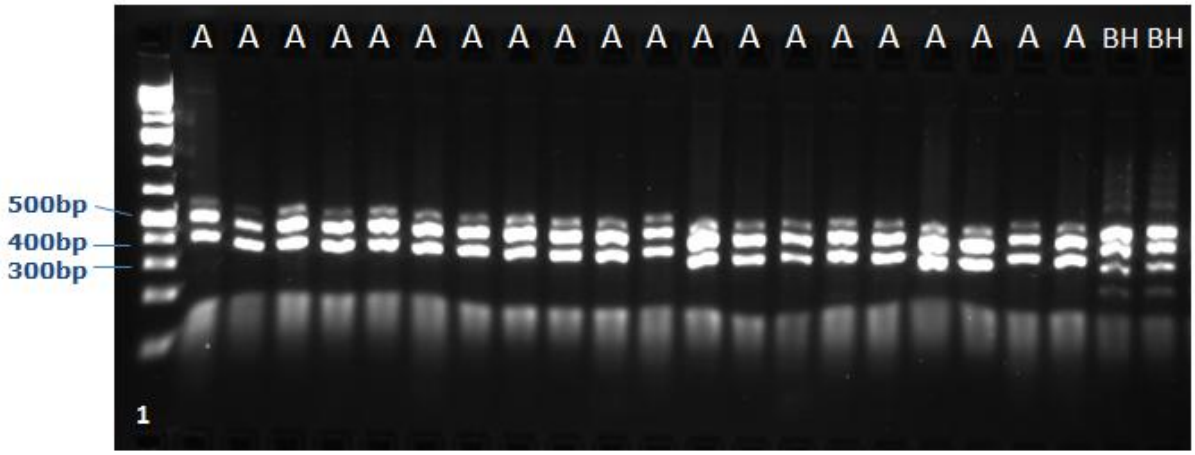
A - Homozygous for *S. lycopersicum* allele; B - Homozygous for *S. habrochaites* allele; F_3 family PV143245 are indicated by blue shad

Appendix XII: Picture of gel of different markers

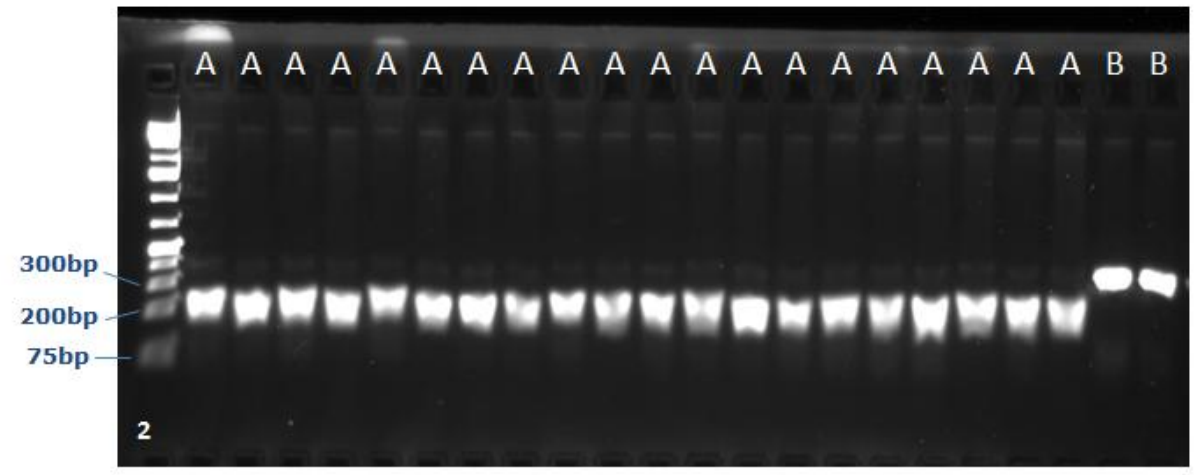
Five markers were used for scoring of genotypes of F_4recombinants lines. 1kb plus DNA ladder (first left column) was put to determine the molecular weight of each band in a gel.

CAPS markers : 51355_MH (Dominant), UF_07960F2 andM1 are CAPS markers
SCAR markers : MCGao UF2 and T0302

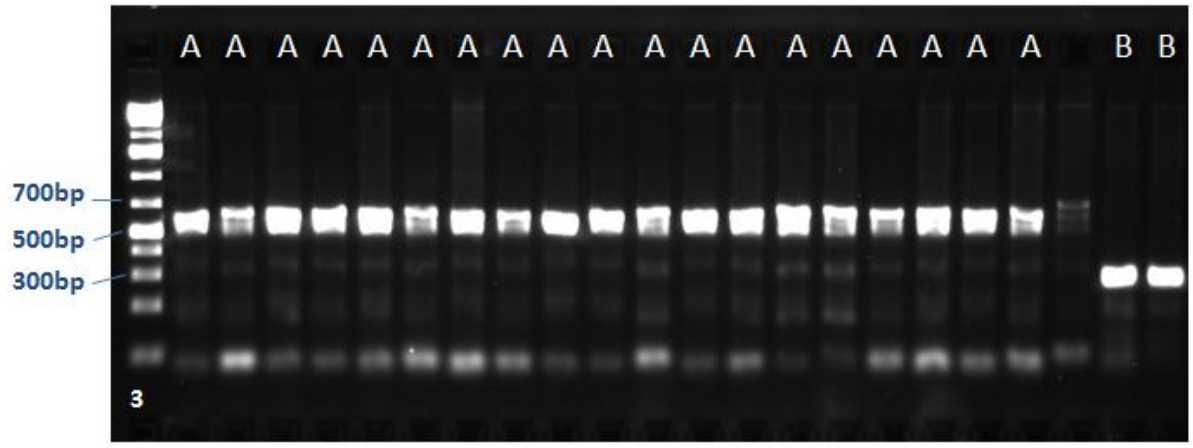
A = Genotypes of *S. lycopersicum* allele
B = Genotypes of *S. habrochaites* allele



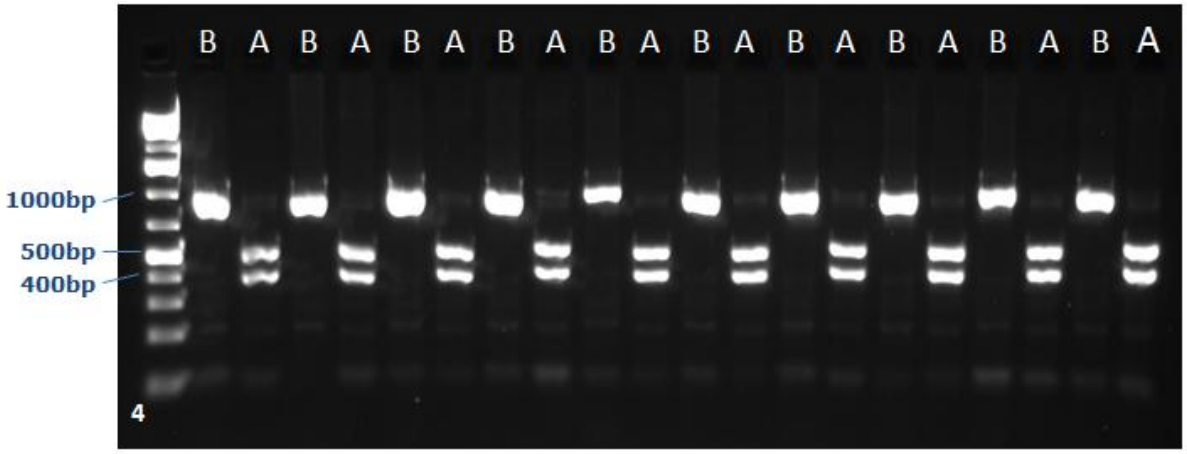
(1) Gel picture of marker 51355_MH (CAPS)



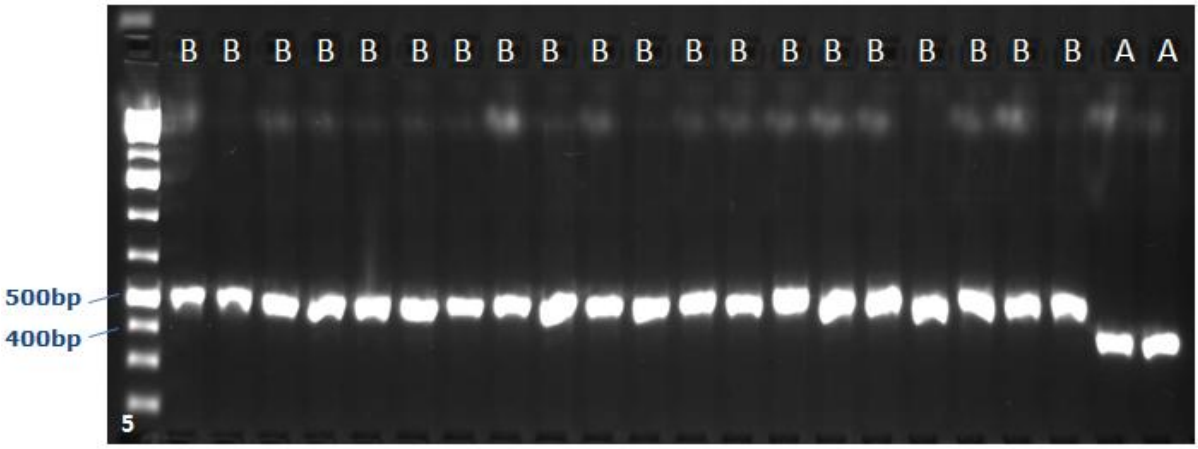
(2) Gel picture of marker UF_07960F2 (CAPS)



(3) Gel picture of marker MCGao UF2(SCAR)



(4) Gel picture of marker M1 (CAPS)



(5) Gel picture of marker T0302 (SCAR)

Appendix XIII. Recombination rates in F_2 and F_4 populations in present and previous study

Populations	Parents	Number of total individuals	Number of recombinants	Genetic distance
H9205 (Yang et al., 2014)	*S. habrochaites* (resistant to TYLCV, *Ty-2* gene) × *S. lycopersicum* cv. Moneymaker	11000	2 (Between the markers P1-16 and TG36)	0.02 cM
PV95279 (Huang, 2014)	*S. habrochaites* G1.1257(susceptible to TYLCV) × *S. lycopersicum* cv. Moneymaker	88	0 (Between the markers UF_07960F2 and cLEN-11-F24)	0 cM
PV960357	*S. habrochaites* G1.1560 × *S. habrochaites* G1.1606	91	7 (Between the markers UF_07960F2 and cLEN-11-F24)	7.69 cM
PV970303	*S. habrochaites* G1.1560 × *S. habrochaites* G1.1290	287	6 (Between the markers UF_07960F2 and cLEN-11-F24)	2.09 cM