GENETIC CHARACTERIZATION OF CUCUMBER MOSAIC VIRUS (CMV) RESISTANCE IN TOMATO AND PEPPER

A Thesis Submitted to
The Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Biology

by
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October 2005
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ACKNOWLEDGEMENTS

I would like to thank and express my gratitute to my advisor, Assist. Prof. Dr. Sami Doğanlar and my co-advisors Assoc. Prof. Dr. Anne Frary and Assist. Prof. Dr. Mustafa Gümüş for their guidance, understanding, motivation and excellent support during this study and preparation of this thesis.

I want to express my thankfulness to my friends; Dane Rusçuklu, Deniz Göl, Ersin Akıncı, Öyküm Kırsoy and Yeliz Tümbilen. Additionally, special thanks to Mert Sudağıdan and Özgür Apaydın for all kinds of support and help.

Finally, I gratefully thank my family for their excellent support, understanding, patience and encouragement.

ABSTRACT

In this study, tomato and pepper populations were phenotypically and genotypically characterized to identify cucumber mosaic virus (CMV) resistant lines and determine the genetic control of resistance. Populations of both crops and their resistant and susceptible parents were mechanically inoculated with CMV. Plants were evaluated visually and by enzyme-linked immunosorbent assay (ELISA). Since virus was detected only in inoculated leaves but was not usually present in uninoculated leaves of symptomless plants, CMV resistance appeared to be true resistance not immunity. According to phenotypic analysis of F2 tomato population, it was hypothesized that two dominant genes were controlling resistance as the plants fit a 9:7 (resistant:susceptible) segregation ratio as determined by Chi square goodness-of-fit analysis.

In order to perform quantitiative trait locus (QTL) analysis, molecular markers were surveyed for polymorphism using the two parents of the tomato population, *L. esculentum* and a *L. hirsutum* LA1223 F1 hybrid. According to QTL analysis (using 107 polymorphic markers), 11 genomic regions were linked to CMV resistance. For seven loci, resistance alleles were coming from the *L. hirsutum* parent as expected. However, for four loci, resistance was associated with alleles from the CMV-susceptible parent, *L. esculentum*. These results show that the genetic potential for resistance cannot be determined by only looking at the phenotype of the two parents. As a result of this work, developing elite lines or transferring resistance genes into cultivated species by marker assisted selection will be easier.

ÖZET

Bu çalışmada, domates ve biber populasyonları hıyar mozaik virüsüne (CMV) karşı dayanıklı hatları ve dayanıklılığın genetik kontrolünü belirlemek için fenotipik ve genotipik olarak karakterize edilmiştir. Her iki ürüne ait populasyonun dayanıklı ve dayanıksız ebeveyinleri, CMV ile mekanik olarak inokule edilmiştir. Bitkiler görsel olarak ve ELISA (enzyme-linked immunosorbent assay) ile değerlendirilmiştir. Virus sadece simptomsuz bitkilerin inokule edilen yapraklarında belirlenmiş olup inokule edilmeyen yapraklarda rastlanmamıştır. Bu nedenle, CMV dayanıklılığının immunite olmayıp, gerçek dayanıklılık olabileceği sonucuna varılmıştır. F2 domates populasyonunun fenotipik analizlerine göre dayanıklılığın iki dominant gen tarafından kontrol edilmekte olduğu varsayılmaktadır. Bu dağılım (9:7) Ki-kare analizine uymaktadır.

QTL (kantitative karakter lokusu) analizi yapabilmek için moleküler işaretleyiciler domates populasyonunun her iki ebeveynindeki polmorfizm belirlemek için kullanılmıştır. (L. esculentum, L. hirsutum 1223). QTL analizlerine göre (107 polymorfik işaretleyici kullanılarak) 11 genomik bölgenin CMV dayanıklılığı ile bağlantılı olduğu belirlenmiştir. 7 bölgede dayanıklılık allelleri beklenildiği gibi *L. hirsutum* ebeveyninden gelmektedir. Bununla birlikte, 4 bölgede dayanıklılık alleleri CMV dayanıklı olmayan *L. esculentum* ebeveyninden gelmektedir. Bu sonuçlar dayanıklılığın genetik potansiyelinin sadece her iki ebeveynin fenotipine bakılarak belirlenemeyeceğini göstermektedir. Yapılan bu çalışmayla moleküler işaretleyiciye dayalı seleksiyonla ileri hatları geliştirme veya dayanıklılık genlerinin kültür türlerine aktarılmasının daha kolay olacağı sonucuna varılmıştır.

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CHAPTER 1

INTRODUCTION

Plants are very important for the human food chain. According to the Food and Agriculture Organization, the world population will grow to more than eight billion by the year 2030 (WEB_1 2005). Nowadays nearly 1,2 billion people live in poverty, 800 million people do not have food security and 160 million pre-school children suffer from malnutrition. (Pinstrup-Andersen and Cohen 2000). Therefore, feeding these people is becoming very difficult. Plants not only have roles in nutrition, but they are sources of many pharmaceutical drugs and secondary metabolities which can delay important diseases such as cancer.

Since Turkey has a variety of ecologies and a broad range of plants, farmers grow numerous kinds of crops every year. The major ones are wheat, barley, cotton, tobacco, sugarbeets, oilseed, fruits, nuts and vegetables. Among these, cultivation of fruit, nuts and vegetables accounted for nearly 33 % of crop production in the 1990s (WEB_2 2005).

Tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum*) are two of the most important commercially grown vegetables in Turkey. Turkey ranks third in the world for tomato and pepper production. In 2004, farmers produced about 8 million metric tons of tomato and 1,8 million metric tons of pepper per year (WEB_3 2005). Both of these crops are members of the *Solanaceae* family and rich sources of vitamins. Tomato, also contains important minerals and antioxidants. Pepper contains more vitamin C than any other vegetable crop. Tomato and pepper extracts are used in both food and feed industries as well as for producing some pharmaceutical products.

However, fungal, bacterial and viral diseases are major limiting factors for tomato and pepper production. Especially viral diseases affect crop production dramatically. Cucumber mosaic virus (CMV) is one of the important viral diseases which affects both crops and, as with other virus diseases, there is no direct chemical treatment. Therefore, the development of resistant lines is the main method to protect these crops. Resistant lines can be developed using genetic engineering techniques or molecular breeding strategies. In this study the resistance mechanism of tomato and

pepper to cucumber mosaic virus was investigated. In addition, the inheritance of resistance was studied and resistance genes were molecularly mapped in tomato.

CHAPTER 2

PLANT DEFENSE SYSTEM

Plants offer a nutritious environment for phytopathogens including bacteria, fungi, viruses and nematodes. Against these pathogens plants have pre-existing mechanisms for preventing their entrance such as waxy cuticles and antimicrobial compounds. However, many pathogens can break down these physical barriers. Therefore, plants prevent disease by recognizing these invaders and preventing their growth.

When there is an infection, the pathogen releases elicitor molecules that interact with plant receptors which leads to induction of a signalling pathway (Cohn et al. 2001). Thus, physiological and biochemical reactions are triggered in the plant. For example, the phosphorylation state of the cell changes, therefore, Ca^{+2} ion increases in the cytoplasm and activates an oxidative burst. As a result, reactive oxygen species (ROS), superoxide radicals (O_2.) and H_2O_2 are produced. Nitric oxide (NO) collaborates with ROS to trigger transcriptional activation of plant defence genes. These biochemical reactions result in cell wall thickening and cellular damage to both host and pathogen. Additionally, rapid cell death at the site of infection occurs; this is the hypersensitive response (HR). Cell death deprives the pathogen of access to nutrients and prevents its spread. These defence mechanisms are achieved through the interaction of pathogen avirulence (*avr*) gene products and plant resistance (*R*) gene products; gene-for-gene resistance. If either the pathogen or the host lacks the corresponding *avr* or *R* gene , then the plant microbe interaction results in disease. Plant resistance (R) proteins recognise pathogen avirulence (Avr) determinants and in turn trigger signal transduction cascades (Dangl and Jones, 2001).

In addition to defence mechanisms that are activated upon pathogen attack, plants have two distinct systemic defence mechanisms. The first one is systemic acquired resistance. After pathogen attack salicylic acid (SA) accumulation induces this mechanism. As a result, pathogenesis related (PR) proteins are expressed. The other mechanism is induced systemic resistance (ISR). It is independent of SA but instead relies on jasmonic acid and ethylene which induce expression of antimicrobial peptides correlated with systemic resistance. These interactions are shown in Figure 2.1.

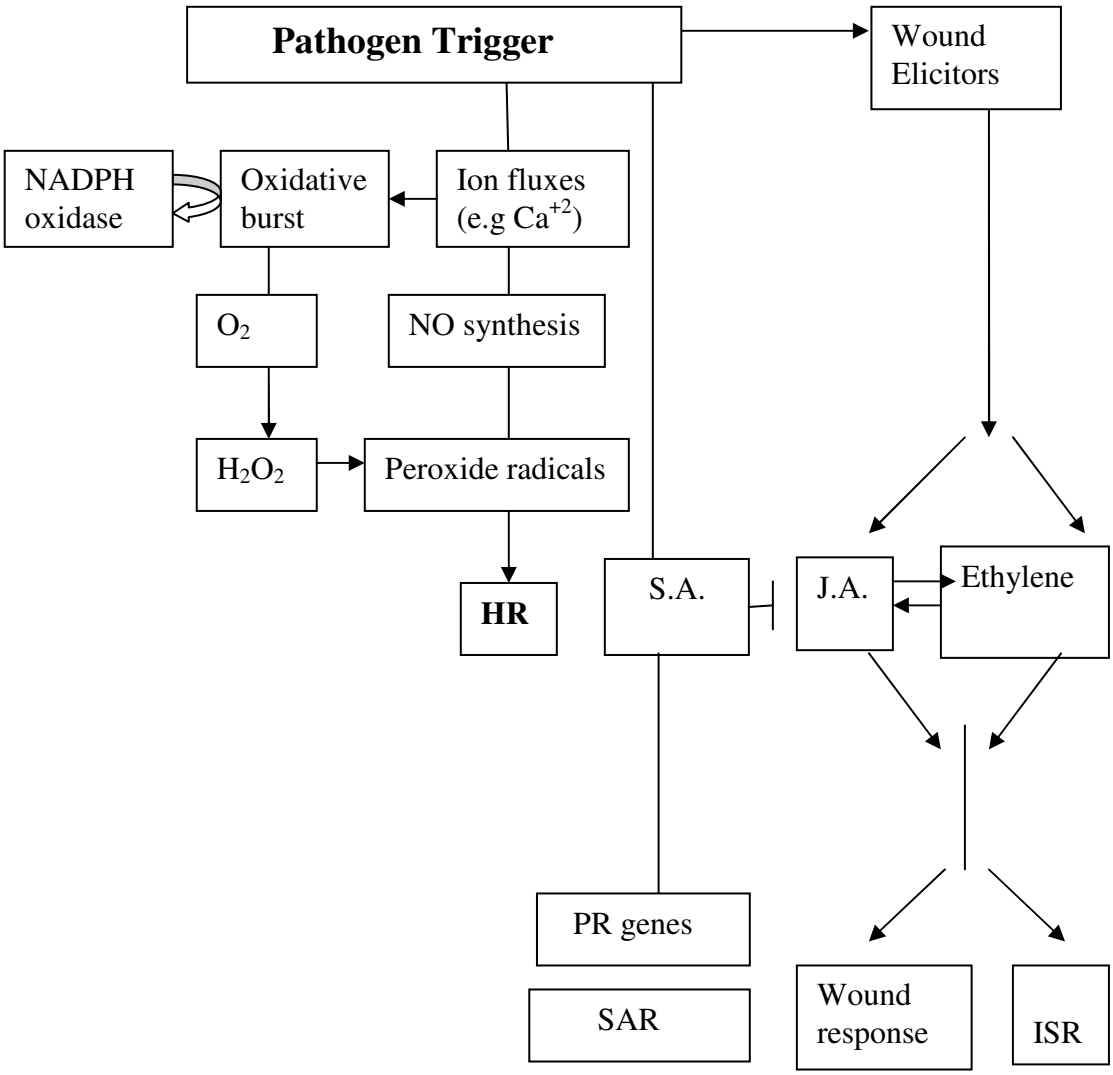

Figure 2.1. Network of resistance response pathways. (JA, jasmonic acid; SA, salicylic acid; ISR, induced systemic resistance; SAR, systemic aquired resistance; HR, hypersensitive response; (PR) genes, pathogenesis related genes) (Adapted from Slater et al. 2003, Deny 2002).

The activation of the signalling pathway leads to the induction of many of the pathogenesis-related proteins. These proteins fit into five basic structural groups: 1. Intracellular protein kinases (PKs); 2. Intracellular proteins having a region of leucine rich repeats (LRRs), a putative nucleotide binding site (NBS), and an N-terminal putative leucine-zipper (LZ) or other coiled-coil (CC) sequence; 3. Intracellular NBS-LRR proteins with a region of similarity to the cytoplasmic domain of mammalian IL-1 receptor (IL-1R) and the Drosophila Toll proteins (i.e. the TIR (Toll/IL-1R) domain); 4.

Extracellular LRR proteins with transmembrane (TM) domains; 5. Receptor-like kinases (RLKs) with an extracellular LRR domain (Martin et al. 2003) (Table 2.1). Since a single mutation in the *R* gene can completely convert a resistant plant to a susceptible host, it is very important to understand how R proteins perceive the signals derived from the *Avr* factors and activate the downstream signalling transduction pathway. For instance LRR domains are involved in mediating interactions between proteins and target proteins, ligands and carbohydrates. NBS domains are critical for ATP/GTP binding in other proteins. Some examples of dominant resistance (*R*) genes against viruses and other pathogens are presented in Table 2.1.

Table 2.1. Selected set of dominant resistance (*R*) genes against viruses and other pathogens.

R gene	Plant	Pathogen	Type
Pto	Tomato	*Pseudomonas syringae* (B)	PK
Mi	Tomato	*Meloidogyne incognita*(N)	LZ-NB-LRR
RPP8	Arabidopsis	*Peronospora parasitica*(O)	LZ-NB-LRR
HRT	Arabidopsis	*Turnip Crinkle Virus*(V)	LZ-NB-LRR
Sw-5	Tomato	*Tomato Spotted Wilt Virus*(V)	LZ-NB-LRR
RPS2	Arabidopsis	*P.syringae p.v. Maculicola* (B)	CC-NB-LRR
Bs2	Pepper	*Xanthomonas campestris*(V)	CC-NB-LRR
Mla1	Barley	*Blumeria graminis*(F)	CC-NB-LRR
RPS4	Arabidopsis	*Pseudomonas syringae*(O)	TIR-NB-LRR
RPP5	Arabidopsis	*Peronospora parasitica(O)*	TIR-NB-LRR
N	Tobacco	*Tobacco Mosaic Virus*(V)	TIR-NB-LRR
Cf-2	Tomato	*Cladosporium fulvum*(F)	LRR-TM
Cf-4	Tomato	*Cladosporium fulvum*(F)	LRR-TM
Cf-9	Tomato	*Cladosporium fulvum*(F)	LRR-TM
Xa-21	Rice	*Xanthomonas oryzae pv.oryzae*(B)	LRR-RLK

Abbreviations: B,bacterium; F, fungus; N, nematode; O, oomycete, V, virus; CC, coiled coil; NB, nucleotide binding site; LRR, leucine-rich repeat; LZ, leucine-zipper motif; PK,serine/threonine protein kinase; RLK, receptor-like kinases; TIR, Toll and interleukin-1 receptor cytosolic domain homology. (Compiled from Hammond-Kosack and Parker 2003, Goldbach et al. 2003, Martin et al. 2003).

2.1. Types of Plant Resistance to Pathogens and Resistance Mechanisms

There are three kinds of resistance that protect plants from pathogens: immunity, true resistance and apparent resistance (Agrios 1988). Immunity is a non-host resistance; in this type, all members of a plant species are resistant to all members of a particular pathogen. Host species are resistant due to mechanisms such as the production of pre-formed toxins or barriers or the lack of essential metabolities or signalling molecules required for the pathogen. There are two types of true resistance, horizontal and vertical resistance. Horizontal resistance is unspecific, polygenic, minor gene resistance which does not distinguish among different races. It can be maintained for long periods. This resistance may be affected by environmental conditions. On the other hand, vertical resistance is specific, monogenic, major gene resistance. This type of resistance is effective against specific races of the pathogen and ineffective against others. Vertical resistance can be overcome due to its single gene inheritance. A single mutation of an *avr* gene that prevents the recognition by the R gene product could convert the avirulent pathogen to a virulent one. Therefore, combinations of major and minor genes for resistance protect plants from various phytopathogens. The third type of resistance is apparent resistance. In this type of resistance, plants are infected but do not exhibit symptoms. Apparent resistance is not heritable and may occur because of unfavourable environment or a non-virulent pathogen. As a result of these factors the plant may be resistant or tolerant. It is a temporary resistance and plants are susceptible to pathogens. Tolerant plants allow the pathogen to develop and multiply but still manage to produce a good crop.

Generally, in the host, genes for resistance are dominant (*R*) while genes for susceptibility are recessive (*r*). Whereas in the pathogen genes for avirulence (inability) to infect are dominant (*A*), while genes for virulence are recessive (*a*). The resistance response only occurs when both the plant and the pathogen carry the dominant alleles for the resistance and avirulence genes, respectively. These gene-for-gene interactions are shown in Table 2.2.

Although resistance is controlled primarily by genetics, physical factors, such as temperature, moisture, light, soil nutrients, soil pH and biotic factors, such as type of crop, age of host plant, and type of pathogen, influence its expression.

Table 2.2. The genetic relationship of the gene-for-gene interaction in the plant

	Resistance or susceptibility genes in the plant	
Virulence or avirulence genes in the pathogen	*R* (resistant) dominant	*r* (susceptible) recessive
A (avirulent) dominant	*AR* (-)	*Ar* (+)
a (virulent) recessive	*aR* (+)	*ar* (+)

(-) ; incompatible (resistant), (+) compatible (susceptible) (Agrios 1988)

2.1.1. Gene for Gene Resistance

The gene for gene interaction is explained by a receptor-ligand model. In this model, an avirulence protein binds to the corresponding R protein and initiates plant defense reactions. For example, the LRR domain of the rice Pi-ta CC-NB-LRR protein directly interact with the rice blast fungus *Magnaporthe grisea* Avr-Pita protein (Jones and Takemato 2004).

However, direct interaction has not been detected in many other systems. An important model for this lack of interaction is explained by the 'Guard hypothesis' (Van der Biezen and Jones 1998). In this model, *Avr-R* interaction is mediated by a second plant protein. The tomato Pto protein is a good example for this model. Pto is a R protein in tomato which confers resistance to Pseudomonas genera carrying the *avrPto* gene. Since Pto does not have a LRR recognition domain it depends on a second plant protein, Prf, which contains this domain. The Pto protein forms a complex with *AvrPto* and Prf. This complex initiates the HR response and activates the signal transduction cascade that induces the expression of various defence proteins (Slater et al. 2003).

2.2. Viral Diseases of Plants

Viruses are submicroscopic infectious particles (virions) consisting of nucleic acids and coat proteins called capsids. Viruses have no energy metabolism of their own. Consequently, they can not perform syntheses and are thus unable to replicate themselves. The genetic information of plant viruses is either encoded by single-stranded (tobamo and cucumoviruses) or double stranded RNA (oryzaviruses) or single-stranded (gemini-viruses) or double-stranded DNA (caulimoviruses) (Agrios 1988).

Viruses can be classified by these different genome types and structures and also by their particle morphology, genome biological and serological properties.

Viruses cause many important plant diseases and are responsible for huge losses in crop production and quality all over the world. The amount of loss in crops can vary depending on the strain of the virus, variety of vegetable, the age of the plant at infection time, climatic factors during disease development, the presence of other diseases, and the extent that viruses have spread in the planting. For example, recent reports show that, in lupin crops not only virus and aphid type but also climatical factors determine yield losses (Thackray et al. 2004). For many viruses, numerous, considerably different strains (wild types) have been isolated. The differences are host range and degree of virulence.

In addition to typical viruses, at least four types of virus-like pathogens are associated with plant disease: 1. Satellite viruses are associated with certain typical viruses and reduce the ability of the typical viruses to multiply and cause disease, they act like parasites of the associated typical viruses. 2. Satellite RNAs are small RNA molecules which completely depend on the viral genome for their replication and spread. SatRNAs generally attenuate the affects of viral infection and may represent a protective response of the host to viral infection. These two classes of virus-like pathogens can be distinguished according to the source of their coat protein and the sizes of their RNAs. In satellite viruses, the RNA encodes its own protein coat whereas satellite RNAs depend on helper virus. Also satellite viruses have larger RNA than satellite RNAs. 3. Viroids are autonomously replicating small, circular RNA molecules that do not encode protein themselves; however, they are capable of causing disease in plants. 4. Virusoids are viroid-like small, single-stranded circular RNAs that are present inside some RNA viruses. Their lives depend on their partner viruses.

Plant viruses cannot easily enter into the host plant cell wall so the infection process often depends on injuries during which the virus enters the plant mechanically through sap. Virus can also be transmitted by seed, pollen, and invertebrates (insects, nematodes, etc.).

For infection of a plant by a virus, the virus must move from one cell to another via plasmodesmata connecting adjacent cells. Once the virus has entered the phloem, the virus spreads, and moves to growing regions (apical meristems) or other regions of food utilization in the plant. Once inside the cell, the viruses uncoat and the viral genetic material is then translated and replicated. The viral proteins are synthesized. Finally,

progeny viral particles are packed into capsids. Infected cells break open (lyse) and thereby progeny virus gain access to nearby cells.

Virus multiplication affects many of the functional systems of the plant directly or indirectly. Viruses cause decreases in photosynthesis because virus infection results in decreases in chlorophyll so photosynthesis is impaired. Virus infection also decreases the amount of growth regulators and nitrogenous compounds. In the plant on the other hand, viruses induce an increase in growth-inhibiting substances and oxidized products of phenolics. These disturbances in the plant are the immediate causes of various types of symptoms such as leaf yellowing, leaf distortion, stunting of the whole plant, and abnormalities in flower or fruit formation.

However, if the host plant has resistance (R) proteins against virus coat protein, replicase and the movement protein that are encoded by virus avirulence gene, avr-R compatible interaction initiates gene-for gene resistance and the virus cannot multiply in the plant.

2.2.1. Cucumber Mosaic Virus (CMV)

Cucumber mosaic virus is an important vegetable disease and causes severe damage in numerous crop species including cucumbers, melons, squash, peppers, tomatoes, crucifers, lilies and many weeds. CMV is the type member of the *Cucumovirus* genus in the family *Bromoviridae* and has the largest host range of any virus througout the temperate regions of the world (Palukaitis et al. 1992). The virus infects more than 800 species in over 70 families of plants, and it is spread naturally by more than 60 aphid species in a nonpersistent manner (Palukaitis et al. 1992).

Cucumber mosaic virus (CMV) is a tripartite polyhedral virus with a diameter of 29 nm. CMV particles are isometric and are composed of a coat protein shell which encapsidates the single-stranded, plus-sense RNA genome. The capsid contains 180 identical protein subunits (icosahedral symmetry). The virions contain 18% RNA and 82% protein. The RNA consists of three genomic RNAs and one or two subgenomic RNAs. The genomic RNAs are designated RNA1 (3,3 kb in length), RNA2 (3,0 kb) and RNA3 (2,2 kb) and are packaged in individual particles. The two subgenomic RNAs are RNA4 (1,0 kb) and possibly RNA4A (682 nucleotides) and are packaged with genomic RNA3. Many strains of CMV have been described and classified into sub-groups IA, IB

and II according to the sequence similarity of the genomic RNAs (Palukaitis et al. 1992) . The nucleotide sequence divergence of the subgroups of CMV is 25% (Roossink et al. 1999).

The three genomic RNAs of CMV encode five proteins. RNA1 is monocistronic and codes for a single product of 110kDa, the 1a protein is required for viral replication and contains methyltransferase and RNA helicase activities (Kadare and Haenni 1997, Rozanov et al. 1992). RNA2 encodes the 2a protein (98 kDa) that contains the conserved amino acid sequence of many viral polymerases (Ishihama and Barbier 1994, O'Reilly and Kao 1998). RNA2 also encodes 2b, which is translated by subgenomic RNA4A that inhibits host transcriptional gene silencing (Beclin et al. 1998, Brigneti et al. 1998). RNA3 encodes the movement protein 3a, (MP) a coat protein (CP) expressed from subgenomic RNA4. Both are required for virus movement (Canto et al. 1997).

CMV can harbour molecular parasities known as satellites (sat RNAs) that modify the symptoms induced by the virus. The CMV satRNAs do not encode any proteins but rely on the RNA for their biological activity (Roossinck 2001). The satellite RNAs (satRNAs) of CMV range from 335 to 405 nucleotides and do not encode any protein. These RNAs can sometimes attenuate disease symptoms induced by the virus (Garcia-Arenal and Palukaitis 1999).

2.2.2. Cucumber Mosaic Virus Replication

CMV replication starts with the entrance of the viral particles into the plant cell via aphid feeding on the host plant (Palukaitis et al. 1992). After virion entry into the host cell, the virus particle is disassembled and the virion RNA is uncoated. Then host ribosomes begin to translate RNA1, RNA2 and RNA3. The translation products are involved in viral RNA replication. The viral replicase generates (-) sense RNA strands from the (+) strand viral templates of each CMV RNAs. These (-) sense RNA strands synthesize progeny virus RNAs and RNA4 with viral replicase. Both host and viral encoded proteins may have functions during this process. Translation of RNA4 produces the coat protein. The plus sense RNAs are encapsidated by the coat protein subunits. Therefore, virions are produced. These virus particles either move into a new

cell with plasmodesmata or move to new host via aphid vectors (Palukaitis et al. 1992). The replication of CMV is shown in Figure 2.2.

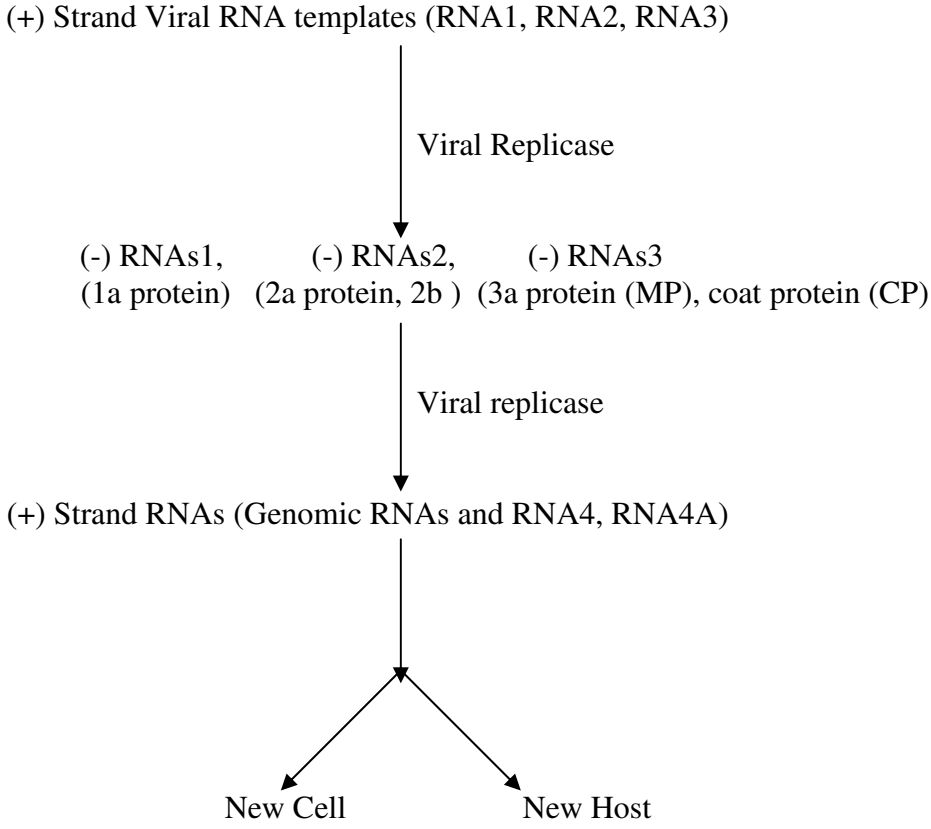

Figure 2.2. Flow of events during the replication of CMV. 1a, 2a protein are involved in viral genome replication, 2b inhibits host post-transcriptional gene silencing, 3a is the movement protein (MP).

2.2.3. Cucumber Mosaic Virus Epidemiology and Control

Most temperate zones, tropical regions and Mediterranean countries are affected by CMV. CMV appears to be the most important virus of some annual crops in Argentina, eastern China, Croatia, France, Egypt, Greece, Israel, Italy, Japan, Poland, Spain, Sweden and, in the north east of US (Tomlinson 1987). CMV symptoms range from no symptoms to severe mosaic, mottling, distortion of the leaves and stunting.

Since CMV has been very successful in rapidly adapting to new hosts and environments it is not easy to control. For a long time plant viruses have been controlled using conventional measures like crop rotation, cross protection, discarding virus

infected plants, using virus free plants and using chemicals against virus vectors such as aphids and leafhoppers (Bos 2000, Hull 2002). Although having a number of advantages, these methods also have some disadvantages. For example, in cross protection plants are inoculated with a mild strain of a virus that causes severe symptoms; however, there may be a reduction in the yield of the crop by the inducing virus and an increased risk of synergistic infection (Palukaitis and Zaitlin 1984). Therefore, only mild CMV strains which contain an ameliorating satRNA should be used for cross protection. Using chemicals also has risks, since they can be toxic to the environment.

In addition to these traditional methods of virus control, there are other techniques including biotechnological approaches. Biotechnology has an important role in food production. It enables the production of human therapeutics, drugs, vaccines and increases yield and quality with lower fertilizer input. Moreover, genetically engineered crop products may be capable of defending against abiotic and biotic stress factors such as drought, cold, salt, insect pests and fungal, viral and bacterial diseases. In the 1980s, it was discovered that plants transformed by nucleotide sequences coding for virus genes showed resistance to the parental viruses (Goldbach et al. 2003). During the last decade, various models for pathogen derived resistance induced by the transgenic virus RNA or protein have been proposed (Baulcombe 1996, Goldbach et al. 2003, Lomonossoff 1995, Palukaitis and Zaitlin 1997, Sanford and Johnston 1985). For example, sat-RNA mediated resistance was successfully used to protect several crops from CMV including tobacco, tomato and petunia (Paek and Hahn 1991, Kim et al. 1992, Lee et al. 1994, Kim et al. 1995). Furthermore, transgenic hot peppers which were transformed with satellite RNA, were shown to be resistant to CMV under greenhouse conditions (Kim et al. 1997). Many reports also show that the coat protein can be used to mediate resistance to CMV (Gonsalves and Slighton 1993, Kaniewski and Lawson 1998). Cp-mediated transgenic tomato lines, which were tested in Italy were reported to be ready for commercialization as a new CMV resistant variety (Tomasolli et al. 1999). The environmental and food safety of these transgenic varieties needs to be documented. The safety of transgenic plant products is an important factor because these genetically modified (GM) crops may also express foreign proteins with unknown allergenicity and effects on the gastrointestinal system of humans. Furthermore, GM crops may posses risks to the environment. Depending on the gene/genes that are introduced into the plant, insecticide/herbicide resistant insects may develop and there is

the possibility that spread of the transgene to other species may reduce biodiversity. In addition some of the metabolities produced by transgenic plants may have toxic effects on soil, water, minerals, plant and insects.

The most efficient and simplest way to fight viral diseases is breeding virus resistant lines by introgression of a gene or genes from a resistant wild species into a commercial variety. The development of disease resistant cultivars can provide a simple and cheap approach to reducing the economic losses caused by plant viruses. In this method resistance is first detected by inoculating accessions from a germplasm collection with a virus and screening the reactions of each accession. Then, virus resistant lines are selected for developing new resistant progenies by sexual crosses between the resistant plant (the donor) and a susceptible individual (the recipient). Selfing of the F1 hybrid then produces an F2 generation. The greatest range of variability can be seen in the F2 generation. If the F1 hybrid is crossed with the recurrent parent, a BC1 generation is produced. The level of heterozygosity in the population decreases with selfing and homozygosity increases. Inheritance studies are generally done using F1, F2 and backcross progenies from these crosses between resistant and susceptible accessions.

2.3. Tomato

Tomato originated in Central and South America. All members of the genus Lycopersicon are annuals or short-lived perennial, herbaceous diploids with a somatic chromosome number of 24. The cultivated tomato is a perennial plant normally grown as an annual. Tomato contains carotenoids (lycopene), ascorbic acid (vitamin C), vitamin E, folate, flavonoids and potassium (Beecher 1998, Leonardi et al. 2000) which are critically important for the human diet. Among them carotenoids, ascorbic acid and phenolic compounds are the main antioxidants in tomatoes (Giovanelli et al. 1999). Regular consumption of tomato is reported to lower the risk of a variety of cancers in particular prostate cancer (Giovannucci 1999).

Virus diseases give serious damage and large economic loss to tomato. The most important viruses which infect tomato are tomato mosaic, tobacco mosaic, cucumber mosaic, tomato ring spot, tomato spotted wilt, tomato aspermy and potato virus Y.

CMV is an important disease on tomatoes in temperate regions and is the most

destructive virus in some areas for instance, in eastern France (Gebre et al. 1990) in southern Italy (Crescenzi et al. 1993), and in Bulgaria (Stamova et al. 1990). In Mediterranean countries CMV and potato virus Y (PVY) are the most two important virus diseases on tomato (Parella et al. 1997).

Several wild tomato species are resistant or tolerant to CMV, including *L. pimpinellifolium, L.peruvianum, L.hirsutum, L. cheesmannii var. minor, L. chilense* and *Solanum lycopersicoides* (Gebre et al. 1990, Nitzany 1992, Parella et al. 1997, Phillis et al.1977, Stamova 1993, Stoimenova et al. 1992).

It was reported that more than 40 genes including both qualitative and quantitative loci that confer resistance to all major classes of plant pathogens have been mapped on the tomato molecular map and/or cloned from solanaceous species (Grube et al. 2000). Since this report, new genes have been added to the tomato map (Bai et al. 2003, Chunwongse et al. 2002, Parrella et al. 2002). Because the tomato genome is so well characterized and so many disease resistance genes have been mapped, marker assisted selection can be used by plant breeders to help in developing cultivars with disease control against pathogens.

2.4. Pepper

Pepper which is originating from Mexico, Southern Peru and Bolivia is one of the important vegetable crops (Eshbaugh 1993). All natural populations of pepper are diploid and have the same chromosome number, 2n=24. The fruits are popular for their nutritional value and contain vitamins C and A, niacin, riboflavin and thiamin. Pepper is a stimulant, decongestant and increases appetite and digestive enzyme secretion. Because it is a powerful circulatory stimulant, it is useful for arthritis and rheumatism and can be helpful for sinus infections and sore throats. Also it improves the absorption of other herbs (Poulos 1991).

At least 25 wild species of pepper have been reported. *C. baccatum, C. chinense, C. frutescens* and *C. pubescens* orginated in South America. The domesticated species are *C. annuum L., C. frutescens L., C. chinense jacq., C. baccatum var. pendulum L. and C. pubescens* Ruiz& Pavo'n (Poulos 1991).

It was reported that (Poulos 1991) about thirty different viruses infect pepper. In Asia, chili venial mottle virus (CVMV), cucumber mosaic virus (CMV), potato virus Y

(PVY), tomato mosaic virus (ToMV), and pepper mild mottle virus (PMMV) are considered the most important.

Among these CMV is one of the most frequent pathogen in mediterranean coastal region of Turkey (Palloix et al. 1994).

It was reported that, CMV tolerance in *C. annuum* was incompletely dominant and associated QTLs have been mapped (Caranta et al. 1997a, Lapidot et al. 1997).

2.5. Molecular Mapping

Resistance can be either a qualitative or quantitative character in plants. A qualitative trait is a monogenic character which segregates according to Mendelian ratios whereas a quantitative trait is a polygenic character and has continuous segregation. A quantitative trait locus (QTL) is an individual locus which controls the quantitative trait. Because disease resistance can be monogenic or polygenic, a QTL strategy was used in this work to detect all possible loci involved in this character.

QTL mapping requires a large, appropriate mapping population and sufficient DNA polymorphism among parents. For analysis there must be significant linkage between the quantitative trait and molecular markers (Edwards and Page 1994, Edwards et al. 1987, Lande and Thompson 1990). F2 populations which contain all possible combinations of parental alleles give maximum genetic information when a codominant marker system is used. With codominant markers, the genotypes of all combinations of parental alleles (i.e; AA, Aa, aa) can be detected.

Different kinds of markers are used in mapping. One type of marker is morphological markers. These markers are assumed to be controlled by a single gene with reproducible expression. For example, anthocyanin production, color of fruit or dwarfism can be used as morphological markers. However, most morphological marker loci segregate as dominant or recessive alleles. Isozymes are differently charged protein molecules that can be separated with electrophoresis. Therefore, independent from phenotypic changes, genes that code for enzymes can be screened for polymorphism. In contrast to isozymes, other molecular markers directly measure DNA variation at chromosomal locations. These types of markers are more abundant and most of them are codominant.

An example of a molecular marker is RFLP (restriction fragment length polymorphism). In this technique, restriction enzymes are used to cut genomic DNA molecules at specific nucleotide sequences. These fragments are separated using electrophoresis. The DNA fragments are transferred to a membrane and are hybridized with labelled probe and the fragment or fragments that have sequence homology will be detected. Polymorphism between individuals is the result of sequence differences at the restriction site or insertions/deletions between adjacent restriction sites.

Currently, instead of RFLP markers, PCR based markers are more commonly used. RFLP analysis requires as much as ten micrograms or more of DNA, whereas, PCR-based markers require only nanogram quantities of DNA with lower cost. There are several types of PCR markers. SSR (simple sequence repeats) or microsatellites are tandemly repeated, short DNA sequences that can be between 2-6 base pairs. Sequences flanking the SSR are used as PCR primers and amplified products are differentiated by gel electrophoresis. For RAPD (random amplified polymorphic DNA) markers, genomic DNA is amplified using single, short oligonucleotide primers (usually 9 to 10 nucleotides). These short primers will hybridize at many sites in the genome. Presence or absence of amplified fragments can be seen after gel electrophoresis. For AFLP (amplified fragment length polymorphism) markers, chromosomal DNA is cleaved with two restriction enzymes. Then specific short DNA sequences (adaptors) are linked to the fragments. The adaptors serve as binding sites for PCR primers. Only fragments with two of the appropriate enzyme adaptors are amplified. Polymorphism can be seen by polyacrylamide gel electrophoresis. For CAPs (cleaved amplified polymorphic sequence), specific primers are used to amplify a sequence which was previously identified as polmorphic by RFLP analysis as explained below. (Konieczyny and Ausubel 1993)

In our experiment we used CAPs markers. CAPs markers are codominant and analogous to RFLP markers in that a region of DNA containing a restriction site unique to an allele is amplified and cleaved. In this technique, genomic DNAs are amplified with specific primers. After amplification, DNA fragments are cut with a restriction enzyme. The products are separated on 2% agarose gel in 1X TAE buffer. As a result of this separation, polymorphism among individuals can be detected. The steps performed in CAPs analysis are shown in Figure 2.3.

Cleaved Amplified Polymorphic Sequence (CAPs) Analysis

1. Amplify specific region in genome

Primer ▶

ATGTTGCTGCCCGTATGCTTAAGCTTCCG**AAAGT**

ATGTTGCTGCCCGTATGCTTAACCTTCCG**AAAGT**

◀ Primer

2. Cut with restriction enzyme that gives different size fragments for in each allele.

TaqI (digestion at a site in parA, no digestion in parB)

↓ ↓

parA:**ATGTT**GCTGCCCGTATGCTTAAGCTTCCG**AAAGT**

parB:**ATGTT**GCTGCCCGTATGCTTAACCTTCCG**AAAGT**

3. Electrophoresis to differentiate alleles: in parA two fragments can be seen on gel whereas in parB only one larger fragment can be seen.

4. If this experiment is done on parental lines and progenies three genotypes can be detected which show the codominant characteristic of this marker.

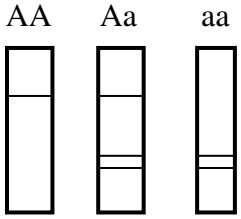

Figure 2.3. Cleaved Amplified Sequence (CAPs) steps. AA homozygote, aa homozygote, Aa heterozygote genotype.

In order to map gene(s) for resistance, statistical analysis is used to establish significant associations between markers (genotype) and resistance (phenotype). The association between phenotypic means and marker genotypes give us the molecular locations of the genes. QTL are detected when a significant difference is observed among the phenotypic means for the genotypic classes of a given marker.

In solanaceous species many single genes and quantitative trait loci which confer resistance have been mapped for different plant pathogens (virus, bacteria, nematode, fungus and insect) (Pillen et al. 1996). Such mapping can help us to understand if these genes confer resistance to the same pathogen in different hosts. For instance, it is known that the genome of tomato is very close to that of pepper. With mapping information, we can determine if the same resistance genes are found in both species.

The use of tightly linked markers for marker assisted selection is another reason to identify QTL for the trait of interest. For example, QTL have been identified and mapped for resistance to *Phytophthora capsisi* (Lefeubre and Palloix 1996), PVY (Caranta et al. 1997b) and CMV (Caranta et al. 1997a) in pepper.

The objectives of this experiment were to evalute and examine the mechanisms and genetic basis of resistance to CMV in tomato and pepper. Once resistance is found and the genes controlling it are identified, it can be transferred to other lines with marker-assisted selection.

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Materials

Tomato and pepper genotypes were tested for their response to CMV infection. For tomato, the parental lines were TA209 (*L. esculentum*) and LA1223 (*L. hirsutum*). Several different progenies from crosses between these two parents were also used including: an *L. hirsutum* CMV F1 (TA496X96T970-13), *L. hirsutum* CMV F2 (TA496XLA1223) and *L. hirsutum* CMV BC1 (TA496X97T914-3). In addition, four different BC2 populations were tested: *L. hirsutum* CMV BC2 (TA496X99T348-3), *L. hirsutum* CMV BC2 (TA496X99T348-10), *L. hirsutum* CMV BC2(TA496X99T348-11) and *L. hirsutum* CMV BC2 (TA496X99T348-13). These lines are summarized in Table 3.2.

For the pepper experiments, the parental lines were 02P35 (*C. annuum* X *C. frutescens* F8 TMV R), 02P3 (*C. annuum* X *C. frutescens* F8 CMV R), 02P19 (*C. annuum* TR0634 Menemen) and Serademre. F1 progenies from these parental lines were tested: 02P35 F1 (*C. annuum* X *C. frutescens* F8 TMV R) X Serademre, and 02P19 F1 (*C. annuum* TR0634 Menemen) X 02P3 (*C. annuum* X *C. frutescens* F8 CMV R) (Table 3.2). The advanced F8 lines were provided by Dr. R. Robinson (Cornell University). These lines were developed by crossing cultivated pepper with a wild species (*C. frutescens*) which carries both CMV and TMV (tobacco mosaic virus) resistance. Each generation, plants were selected for CMV and TMV resistance in the field. The seeds of tomato and pepper were germinated in 2x2 cm trays. Two weeks after germination they were transferred into 10 cm pots in a greenhouse and maintained at 22^0C with 16 h light. The lines were mechanically inoculated with Fny-CMV. Tobacco plants (*Nicotiana tabacum*) were grown from seed, and used as inoculum source.

Table 3.1. Tomato genotypes tested in this work.

Tomato Genotypes	Description
TA 209	*L. esculentum* parent
L HIR LA1223	*L. hirsutum* parent
L HIR CMV(TA496X96T970-13)F1	*L. esculentum* X *L. hirsutum* F1
L HIR CMV(TA496XLA1223)F2	*L. esculentum* X *L. hirsutum* F2
CMV BC1(TA496X97T914-3)	*L. esculentum* X *L. hirsutum* BC1
CMV BC2 (TA496X99T348-10)	*L. esculentum* X *L. hirsutum* BC2
CMV BC2(TA496X99T348-11)	*L. esculentum* X *L. hirsutum* BC2
CMV BC2 (TA496X99T348-13)	*L. esculentum* X *L. hirsutum* BC2
CMV BC2(TA496X99T348-3)	*L. esculentum* X *L. hirsutum* BC2

Table 3.2. Pepper genotypes tested in this work.

Pepper Genotypes	Description
02P35	*C. annuum x C. frutescens* F8 (TMV R)
Serademre	Serademre parent
02P35XSerademre	F1 (02P35 X Serademre)
02P19	*C.annuum* TR0634 Menemen
02P19X02P35	F1 *(C.annuum X* F8 CMV R*)*
02P19X02P3	F1 *(C.annuum X* F8 CMV R*)*
02P3	*C. annuum x C. frutescens* (CMV R)

3.2. Hybridization

In order to transfer CMV resistance into cultivated pepper, crosses were done between resistant pepper lines and susceptible ones. The crossing scheme is shown in Figure 3.1. First of all, hands and forceps were sterilized with alcohol. Then opened flowers were pinched off. Buds which were about to open were chosen. The corolla of these buds were removed with forceps. Then anthers were removed at the base of the filament (emasculation). Pollen grains from the intended male plant were taken with forceps and put on emasculated flower's stigma. To prevent contamination, the pollinated flower was bagged with a piece of cotton. (Opena and Chen 1991)

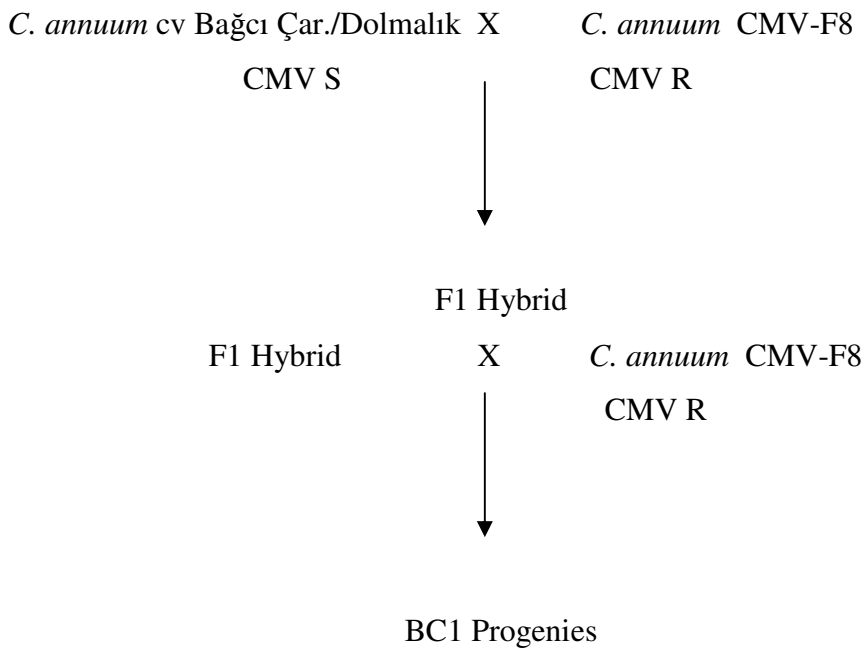

Figure 3.1. Pollination scheme for susceptible and resistant pepper lines and their progeny (F1).

3.3. Virus Isolates

Fny-CMV was obtained from Dr. P. Palukaitis (Dept. Virology, Scottish Crop Research Institute). It was propagated and maintained in tobacco using the mechanical inoculation technique described below.

3.4. Mechanical Inoculation with Fny-CMV

Tomato and pepper seedlings at the 6-7 leaf stage were inoculated with virus infected plant sap by mechanical inoculation. Before inoculation, plants were kept in the dark overnight and after inoculation they stayed in the dark one more day. Mechanical inoculation was done in the afternoon. The inocula were prepared by extracting sap from infected tobacco leaves. The infected leaves were ground with mortal and pestle. Phosphate buffer (1,47 mM KH_2PO_4, 8,1 mM Na_2HPO_4- anhydrous in 1 liter of dH_2O, ph7.4) was used for dilution. The concentration of inoculum was 1:2. After the plant sap was strained through cheesecloth, inocula were put onto ice and used in an hour or less. Before inoculating plants, two holes were punched in the two leaves which were

going to be inoculated. Then these leaves were lightly dusted with carborundum. The leaves were rubbed gently with cotton swab dipped in inoculum. After 30 minutes, the inoculated leaves were rinsed with clean water. For each experiment, two tomato and pepper parent lines and tobacco plants were inoculated with phosphate buffer as negative controls.

3.5. Assessments

CMV infection was detected visually and with DAS-ELISA (double-antibody sandwich enzyme-linked immunosorbent assay). The inoculated leaves of the plants begin to exhibit symptoms 10 days after inoculation. These symptoms were visually scored according to symptom severity. 1: no symptoms, 2: slight chlorosis, 3: some chlorosis or mosaic, 4: severe mosaic and some leaf distortion, 5: severe mosaic and leaf distortion. The symptoms and scoring are shown in Figure 3.2.

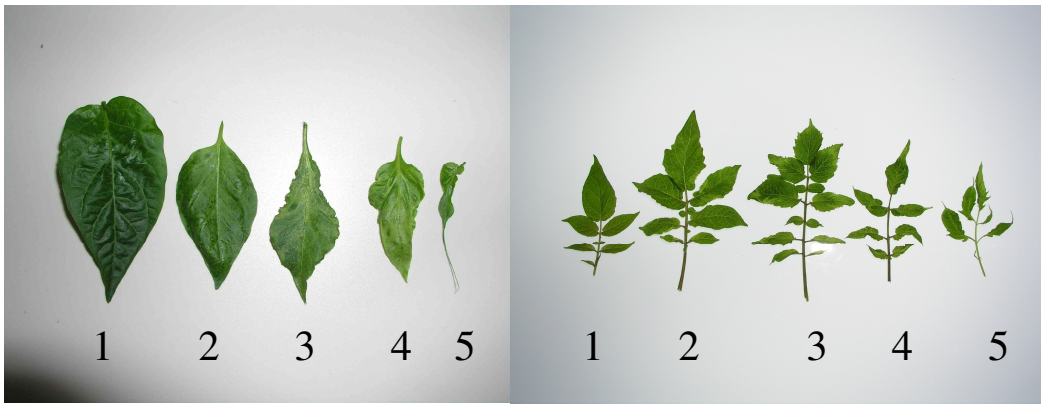

Figure 3.2. Pepper leaves 5 weeks after inoculation and tomato leaves 4 weeks after inoculation. 1: no symptoms, 2: slight chlorosis, 3: some chlorosis or mosaic, 4: severe mosaic and some leaf distortion, 5: severe mosaic and leaf distortion.

3.6. DAS-ELISA (double-antibody sandwich enzyme-linked immunosorbent assay)

In this technique inoculated leaves and infected leaf samples were taken four weeks after inoculation. The uninoculated samples were two leaflets from fourth leaf

position above the inoculated ones. Samples were ground with roller press. Each sample was diluted 1:10 with extraction buffer (two drops of leaf juice put into 450µl extraction buffer; 20 mM Tris buffer (pH 7,4 at 25^0 C) containing 137mM NaCl, 3mM KCl, 2% PVP 24 kD, 0.05 % Tween 20 and 0,02 NaN_3). ELISA plates (Bioreba) were coated with coating buffer (50mM carbonate-bicarbonate buffer pH 9,6 containing 0,02% NaN_3) containing anti-CMV polyclonal antibody. Plates were tightly covered with stretch film and put in a humid box and kept at 4^0C overnight. The next day plates were rinsed four times with washing buffer (10mM phosphate buffer pH 7,4 containing 140 mM NaCl, 3mM KCl, and 0,05 % Tween 20 (PBST)). The diluted plant sap extracts (antigen) were added to the wells (200µl) with two wells (replicates) used for each sample. Again plates were tightly covered with stretch film and put in a humid box and kept at 4^0 C overnight. The next day plates were rinsed four times with washing buffer. Conjugated anti- CMV polyclonal antibody was diluted (1000X) in conjugate buffer (20 mM Tris buffer (pH 7,4 at 25^0 C) containing 137 mM NaCl, 3mM KCI, 1mM $MgCl_2$, 2 % PVY 24 kD, 0,05 % Tween 20, 0,2 % BSA and 0,02 % NaN_3) and 200µl was loaded into each well. The plates were tightly covered with stretch film and put in a humid box and incubated for 4 hours at 30^0C. After rinsing the plates four times with washing buffer, plates were loaded with 200 µl/well p-nitro-phenyl-phosphate substrate solution (1 M diethanolamine pH 9,8, containing 0,02 % NaN_3 and 1mg/ml of pNPP (p-nitro-phenyl-phosphate)). Finally, plates were tightly covered with stretch film and put in a humid box and incubated for 30 to 60 minutes at 22^0C in the dark. The color changes were read visually or photometrically with ELISA Reader at 405 nm. Presence of color in the well indicated that there was virus in the sample. The positive and negative controls provided in Bio-Reba Kit were used. Positive control value for CMV was ≥ 1 at 405 nm.

3. 7. Molecular Marker Analysis

For gene mapping, a previously phenotyped *L. esculentum* TA209 X *L. hirsutum* LA1223 F2 population was used. The 81 F2 plants had been phenotyped for CMV response by ELISA (4 weeks after inoculation) and by visual rating (4, 6, 8, 12 weeks after inoculation) as described in the Materials and Methods section (data from S. Doğanlar). In addition, the population was genotyped with 48 RFLP markers (data from

S. Doğanlar). To obtain beter genome coverage, CAPs (Cleaved Amplified Polymorphic Sequence) markers were used in the current work. DNA from all plants was extracted as described by Fulton et al. (1995). CAPs (Cleaved Amplified Polymorphic Sequence) markers were tested on parental DNAs. (LA1223 F1 hybrid and TA209) to identify polymorphic markers. For PCR, 50 µl reaction mixtures containing 1 µl DNA (100ng/µl), 5 µl 10X PCR buffer(50m*M* KCI, 10m*M* Tris-HCI, pH 8,3, 1,5 m*M* $MgCl_2$), 0,2 m*M* dNTP, 10pmol of each forward and reverse primer, 0,5 U *Taq* and 40,5µl dH_2O were prepared. PCR Reactions were performed in a thermocycler, GeneAmp® PCR System 9700 (Applied Biosystems) using the following programme:

Step1: 94^0C for 5 min
Step2: 94^0C for 30 sec (denaturation)
Step3: 50^0C for 45 sec (annealing) → (30 cycles)
Step4: 72^0C for 45 sec (elongation)
Step5: 72^0C for 5 min (final extension)
Step 6: hold at 4^0C

Then amplified DNAs were digested with one of fourteen different enzymes: *Taq*I ,*Dpn*II, *Hinf*I, *Rsa*I, *Alu*I, *Apa*I, *Ava*II, *Dra*I, *Hae*III, *Cfo*I, *Mbo*I, *Eco*RI, *Eco*RV or *Hind* III and electrophoresed through 2% TAE agarose to detect polymorphism. (Only *Taq*I was incubated at 65^0C,others were incubated at 37^0C for at least 3 hrs). Markers that exhibited polymorphism between the two parents with at least one of these enzymes were then assayed on the mapping population. The MAPMAKER computer program (Lander et al. 1987) was used for linkage analysis of both RFLP and CAPs markers. A minimum LOD score of 3.0 was used. The ripple command was used to test the most probable order of markers within a linkage group. The Kosambi mapping function (Kosambi 1944) was used to estimate distances between markers in centiMorgans (cM). Correlation coefficients were calculated by QGENE (Nelson 1997). QTL mapping was also performed by QGENE using simple linear regression. A significance threshold of $P \leq 0.05$ was used for QTL declaration. Estimates of magnitudes of effect (R^2 from QGENE) and trait means were determined for the most significant marker for each QTL.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Phenotypic Analysis

4.1.1. Tomato Lines

A total of 282 tomato lines were evaluated visually and by ELISA 4 and 8 weeks after inoculation. The parental lines of tomato were TA209 (*L. esculentum*) and LA1223 (*L. hirsutum*) and progenies were ; F1: *L.hirsutum* CMV (TA496X96T970-13), F2: *L.hirsutum* CMV (TA496XLA1223), *L.hirsutum* CMV BC1: (TA496X97T914-3), *L.hirsutum* CMV BC2: (TA496X99T348-3), *L.hirsutum* CMV *L.hirsutum* BC2: (TA496X99T348-10), *L.hirsutum* CMV BC2: (TA496X99T348-11) and *L.hirsutum* CMV BC2: (TA496X99T348-13). The cut off value for ELISA was <0,5. Therefore, plants that had an ELISA value less than 0.5 were considered to be resistant to the virus. For visual rating the cut off was 1. Table 4.1 gives the ELISA and visual rating mean values for the tested plants. Table 4.2. summarizes the numbers of resistant and susceptible plants identified in each line/population. After inoculation, all of the TA209 lines showed susceptibility except for one plant that may have escaped disease inoculation. On the other hand, LA1223 had both resistant and susceptible individuals. Based on the 4 WE (4 week ELISA), 50% of the LA1223 plants were resistant. However, by 8WE (8 week ELISA), 60% of the plants were classified as resistant based on their virus accumulation. This indicates that line LA1223 was not homogeneous. As expected, the F1, F2, BC1, BC2-3, BC2-10, BC2-11 and BC2-13 showed segregation of disease resistance. Some of the plants were resistant and others were susceptible to the virus. For example, in the F2, 64% of the plants were resistant at 4WE with no significant change by 8WE (65%).

Table 4.1. ELISA and visual rating mean values for the tomato population. Standard errors (SE) and ranges are also given. (C) indicates buffer-inoculated control plants.

Pedigree	Treatment	ELISA ±SE (range) 4 wks Inoc.Leaf	ELISA ±SE (range) 4 wks Uninoc.Leaf	Virus Rating 4 wks after inoc. ±SE (range)	ELISA ±SE (range) 8wks Inoc.Leaf	Virus Rating 8 wks after inoc. ±SE (range)	# Plants
TA 209(C)	Buffer inoc.	0,06±0,02 (0,2-0,09)	0,14±0,05 (0,03-0,25)	1±0 (1-1)	0,34±0,03 (0,3-0,4)	1±0 (1-1)	4
TA 209	Virus inoc.	0,97±0,28 (0,14-1,64)	2,08±0,4 (0,99-2,8)	4±0,45 (3-5)	1,51±0,5 (0,4-2,6)	5±0 (5-5)	5
L HIR LA1223(C)	Buffer inoc.	0,01±0,01 (0,01-0,04)	0,04±0,03 (0,01-0,09)	1±0 (1-1)	0,41±0,12 (0,2-0,6)	1±0,6 (1-1)	4
L HIR LA1223	Virus inoc.	0,83±0,39 (0,001-1,94)	0,89±0,56 (0,18-3,63)	1,5±0,5 (1-4)	1,56±0,8 (0,3-3,7)	2±0,5 (1-4)	6
L HIR CMVF1	Virus inoc.	0,70±0,37 (0,04-3,06)	1,8±0,48 (0,09-3,43)	2±0,4 (1-5)	1,07±0,4 (0,16-3,6)	2,4±0,5 (1-5)	10
L HIR CMVF2	Virus inoc.	0,39±0,05 (0,01-3,71)	0,99±0,114 (0,014-3,7)	1,7±0,1 (1-5)	0,59±0,08 (0,04-3,7)	2,1±0,12 (1-5)	119
CMV BC1	Virus inoc.	0,16±0,03 (0,01-0,48)	0,39±0,09 (0,037-2,1)	1,2±0,1(1-2)	0,47±0,13 (0,04-3,3)	1,9±0,3 (1-5)	32
CMV BC2-3	Virus inoc.	0,15±0,05 (0,02-1,218)	0,54±0,101 (0,08-1,98)	1,5±0,1(1-3)	0,61±0,17 (0,03-3,3)	2,1±0,4 (1-5)	27
CMV BC2-10	Virus inoc.	0,1±0,02 (0,003-0,39)	0,38±0,097 (0,01-1,65)	1,18±0,08(1-2)	0,44±0,23 (0,04-3,6)	3,1±0,4 (1-5)	23
CMV BC2-11	Virus inoc.	0,27±0,06 (0,07-1,05)	0,6±0,12 (0,08-2,66)	1,57±0,2(1-4)	0,67±0,25 (0,15-3,5)	2,5±0,3 (1-5)	26
CMV BC2-13	Virus inoc.	0,04±0,01 (0,01-0,225)	0,16±0,07 (0,002-1,28)	1,5±0,11(1-3)	0,63±0,3 (0,03-3,6)	2,1±0,4 (1-4)	26

Table 4.2. Number of resistant and susceptible tomato plants in the different populations tested. Both ELISA (E) and visual rating (VR) results are given for 4 and 8 weeks after inoculation. R; resistant, S; susceptible. For ELISA, R<0,5; resistant to virus and S>0,5; susceptible to virus infection.

Population	Uninoc. 4 WE	4 WVR	Uninoc. 8 WE	8 WVR
TA 209	0R, 5S	0R, 5S	1R, 3S	0R, 3S
L. hir. LA1223	3R, 3S	4R, 1S	3R,2S	2R, 3S
L. hir. CMV(TA496X96T970-13)F1	3R, 7S	4R, 5S	4R, 6S	3R, 5S
L. hir. CMV(TA496XLA1223)F2	76R, 42S	63R,44S	66R, 35S	45R, 57S
CMV BC1(TA496X97T914-3)	24R, 8S	24R,7S	19R,6S	16R,10S
CMV BC2(TA496X99T348-3)	18R, 8S	15R, 10S	17R,6S	9R, 8S
CMV BC2 (TA496X99T348-10)	17R, 5S	18R, 4S	14R, 1S	3R, 12S
CMV BC2(TA496X99T348-11)	15R, 11S	18R, 7S	8R, 5S	5R, 10S
CMV BC2 (TA496X99T348-13)	20R, 8S	15R, 13S	15R, 3S	4R, 4S

4.1.2. Inheritance

In order to understand the genetic basis for resistance, F2 lines segregating for response to CMV were analyzed. ELISA data for 118 and 101 plants were evaluated with ChiSquare goodness-of-fit analysis four and eight weeks after inoculation, respectively. Four weeks after inoculation, 76 plants were resistant and 42 plants were susceptible and eight weeks after inoculation 66 plants were resistant and 35 plants were susceptible. The data fit a 9:7 (resistant:susceptible) ratio with χ^2=3,3 and χ^2=3,6, for four and eight weeks, respectively (P>0,05). These results suggest that resistance is encoded by two dominant genes. This result is different from that of Stamova et al. (1998). According to their experiment with *L.chilense,* a single dominant gene (*Cmr)* controls resistance.

4.1.3. Evaluation of Visual Ratings

The degree of resistance in two parental lines and F2 hybrids was evaluated by visual rating 4 weeks and 8 weeks after inoculation. It was observed that the degree of resistance decreased over time. This result was as expected since virus multiplication

and movement increase during the time since inoculation. This increase in susceptibility is shown in Figure 4.1. Additionally, when the parental line scores are compared, it can be seen that *L. hirsutum,* as the resistant parent, had a lower virus rating than the susceptible *L. esculentum* parent. Furthermore, the viral disease in *L. esculentum* was much more severe than in *L. hirsutum*.

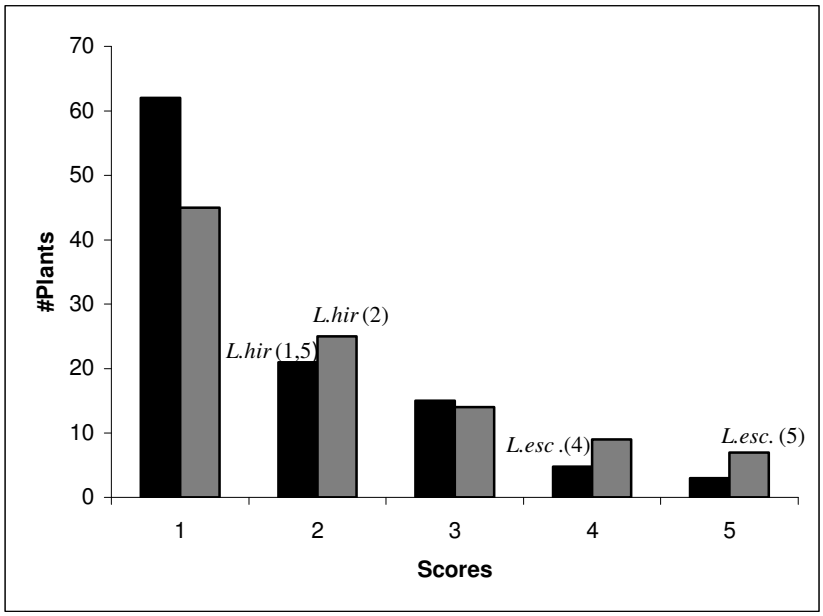

Figure 4.1. F2 tomato lines visual scores 4 and 8 weeks after inoculation with CMV. Black columns are for 4 weeks data and grey ones are for 8 weeks data *L. hirsutum* and *L. esculentum* parental line results are given above the appropriate columns.

4.1.4. Trait Correlations

The correlation between 4 and 8 week ELISA values and visual ratings were high in F2 lines (P<0,05) (Table 4.3). This result indicates that there is good agreement between visual ratings and ELISA values. There is also a positive correlation in F1 and F2 lines between 8 week visual ratings and ELISA values. Additionally, BC1 and BC2 lines showed similar correlations between traits. This result was expected because virus multiplication reflects symptom development and this event can be detected serologically by ELISA. This is different from Stamova and Chetelat (2000). According to their statement there is no direct correlation between the appearance of resistance and absence of the virus.

Table 4.3. Correlations between traits. Only r values for statistically significant correlations are given (P<0,05). P-values are in parenthesis.

L HIR CMV(TA496X96T970-13)F1	4 Wk ELISA	4 Wk Score	8 Wk ELISA
4 Wk Score	-		
8 Wk ELISA	-	-	
8Wk Score	0.790 (0.020)	-	0.803 (0.016)

L HIR CMV(TA496XLA1223)F2	4 Wk ELISA	4 Wk Score	8 Wk ELISA
4 Wk Score	0.742 (0.000)		
8 Wk ELISA	0.266 (0.007)	-	
8Wk Score	0.553 (0.000)	0.333 (0.001)	0.407 (0.000)

CMV BC1(TA496X97T914-3)	4 Wk ELISA	4 Wk Score	8 Wk ELISA
4 Wk Score	0.399 (0.026)		
8 Wk ELISA	-	-	
8Wk Score	0.726 (0.000)	0.489 (0.011)	-

CMV BC2(TA496X99T348-11)	4 Wk ELISA	4 Wk Score	8 Wk ELISA
4 Wk Score	0.772 (0.000)		
8 Wk ELISA	-	-	
8Wk Score	0.701 (0.004)	0.698 (0.012)	-

4.1.5. Pepper Lines

A total of 105 pepper lines were used to observe CMV segregation. For the pepper experiment, the parental lines were 02P35 (*C. annuum*X*C. frutescens* F8 TMV R), 02P3 (*C. annuum*X*C. frutescens* F8 CMV R), 02P19 (*C. annuum* TR0634 Menemen) and Serademre. The F1 progenies were: 02P35 (*C. annuum*X*C. frutescens* F8 TMV R) x Serademre, and 02P19 (*C. annuum* TR0634 Menemen) x 02P3 (*C. annuum*X*C. frutescens* F8 CMV R). Both parental lines and their F1 showed heterogeneity. Table 4.4 gives the ELISA and visual rating mean values for the tested plants. Table 4.5 summarizes the numbers of resistant and susceptible plants identified in each line/population. Heterogenity in this lines was possible because the reportedly resistant F8 lines had only been selected for resistance based on field tests. Therefore, escapes may have been possible and the lines were not completely genetically fixed (stable, homozygous) for resistance. Some individuals of the susceptible parent line (Serademre) also appeared to be resistant. This resistance may have been the result of disease escape or because of environment and physiological factors. It was reported by Parrela (1997) that environment and physiological factors like temperature and plant age highly affect CMV resistance.

Table 4.4. The pepper population ELISA and visual rating mean values. Standard errors (SE) and ranges are also given.

Pedigree	Treatment	ELISA ±SE (range) 4 wks Inoc.Leaf	ELISA ±SE (range) 4 wks Uninoc.Leaf	Virus Rating 4 wks after inoc. ±SE (range)	# Plants
*C.annuum*X*C.frutescens* F8(02P35)	Virus inoc.	1,03±0,12 (0,4-2,6)	0,45±0,08 (0,06-1,08)	2,8±0,3 (1-5)	25
Seradem re	Virus inoc.	1,34±0,14 (0,42-3,1)	0,50±0,05 (0,06-1,02)	4,1±0,3 (1-5)	23
F1 (02P35XSeradem re)	Virus inoc.	1,36±0,12 (0,3-2,01)	0,46±0,06 (0,08-0,8)	3,8±0,3 (1-5)	15
C.annuum TR0634 Menemen	Virus inoc.	1,05±0,21 (0,4-2,15)	0,48±0,14 (0,06-1,5)	2,8±0,5 (1-5)	16
F1 *C.annuum*X F8 CMV R (02P19X02P35)	Virus inoc.	1,6±0,2 (0,5-2,3)	0,39±0,09(0,007-0,98)	2,8±0,5 (1-5)	12
F1 *C.annuum*X F8 CMV R (02P19X02P3)	Virus inoc.	1,47±0,22 (0,38-2,3)	0,33±0,07 (0,006-1,6)	2,2±0,3 (1-5)	14
*C.annuum*X*C.frutescens* CMVR(02P3)	Virus inoc.	1,51±0 (1,5-1,5)	0,91±0,194 (0,6-1,3)	3,5±1,5 (2-5)	12

Table 4.5. Number of resistant and susceptible pepper plants in the different populations tested. Both ELISA (E) and visual rating (VR) results are given for 5 weeks after inoculation. R; resistant, S; susceptible. For ELISA, R<0,5; resistant to virus and S>0,5; susceptible to virus infection.

Population	Uninoc. 5 WE	5 WVR
*C.annuum*X*C.frutescens* F8 (02P35)	19R, 6S	5R, 18S
Serademre	10R, 11S	2R, 19S
F1 (02P35XSerademre)	8R, 6S	2R, 13S
C.annuum TR0634 Menemen	7R, 3S	4R, 7S
F1 (*C.annuum*X F8 CMV R) (02P19X02P35)	8R, 4S	4R, 8S
F1 (*C.annuum*X F8 CMV R) (02P19X02P3)	19R, 6S	12R, 10S
*C. annuum*X*C. frutescens* (CMV R) (02P3)	0R,3S	0R,2S

Five weeks after inoculation, it was observed that virus had replicated in all inoculated leaves of the pepper lines. However, uninoculated leaves did not contain the same amount of virus, the level of virus content was much lower. This result indicates that long distance movement of the virus was restricted in some plants. A similar phenomenon was also seen by Caranta et al. (2002) in pepper.

4.1.6. Testing Reliability of the Visual Scores

In order to test the reliability of visual scores, plants were classified based on their visual scores and the mean ELISA value for each class was calculated for both tomato and pepper (Tables 4.6 and 4.7). Examination of this data shows that visual scores did indeed reflect virus multiplication as plants that were visually scored as resistant (a score of 1) usually had ELISA values less than 0,5 (the maximum value used for declaring resistance). The only exception was one CMV BC2 population, (TA496X99T348-11) which had a mean 8 week ELISA value of 0.9.

Table 4.6. Mean ELISA values of tomato plants classified by visual rating. Bold letters are used to highlight classes that were considered to be resistant.

Population	Visual Rating	ELISA 4 Wks Uninoc.(±SE)	ELISA 8 Wks Uninoc.(±SE)
L HIR CMV(TA496X96T970-13)F1	**1**	**0,46±(0,18)**	**0,25±(0,06)**
	2	3,33±(0,06)	0,56±(0,04)
	3	3,35±(0)	0,81±(0)
	4	-	0,64±(0)
	5	2,89±(0)	3,59±(0)
L HIR CMV(TA496XLA1223)F2	**1**	**0,36±(0,08)**	**0,23±(0,02)**
	2	1,23±(0,3)	0,85±(0,2)
	3	2,46±(0,3)	0,67±(0,09)
	4	2,9±(0,5)	0,74±(0,185)
	5	3,6±(0,097)	1,46±(0,4)
CMV BC1(TA496X97T914-3)	**1**	**0,29±(0,09)**	**0,27±(0,06)**
	2	3,33±(0,06)	0,56±(0,04)
	3	3,35±(0)	0,81±(0)
	4	-	0,18±(0)
	5	-	0,58±(0,19)
CMV BC2(TA496X99T348-3)	**1**	**0,46±(0,1)**	**0,25±(0,05)**
	2	0,48±(0,17)	0,18±(0,06)
	3	1,2±(0,5)	1,02±(0,8)
	4	-	-
	5	-	0,58±(0,19)
CMV BC2 (TA496X99T348-10)	1	**0,18±(0,03)**	**0,22±(0,2)**
	2	1,27±(0,13)	0,36±(0,05)
	3	-	0,16±(0,04)
	4	-	-
	5	-	0,17±(0,1717)
CMV BC2(TA496X99T348-11)	1	**0,31±(0,06)**	**0,9±(0,7)**
	2	1,28±(0,5)	0,15±(0)
	3	0,9±(0,2)	0,6±(0,2)
	4	2,66±(0)	-
	5	-	-
CMV BC2 (TA496X99T348-13)	1	**0,14±(0,08)**	**0,28±(0,1)**
	2	0,09±(0,06)	0,3±(0)
	3	1,28±(0)	0,22±(0,18)
	4	-	0,09±(0)
	5	-	-

Table 4.7. Mean ELISA values of pepper plants classified by visual rating. Bold letters are used to highlight classes that were considered to be resistant.

Population	Visual Rating	ELISA 5 Wks Uninoc.(±SE)
F1(02P35XSerademre)	**1**	**0,09±(0,01)**
	2	-
	3	0,54±(0,11)
	4	0,55±(0,005)
	5	0,49±(0,09)
F1(C.annuumX F8 CMV R)02P19X02P35	**1**	**0,07±(0,04)**
	2	0,48±(0,13)
	3	0,72±(0)
	4	0,41±(0,07)
	5	0,72±(0,27)
F1(C.annuumX F8 CMV R)(02P19X02P3)	**1**	**0,1±(0,04)**
	2	-
	3	1,57±(0)
	4	0,6±(0)
	5	-

4.1.7. Type of Resistance

A total of 282 tomato lines were evaluated according to ELISA and visual ratings. In general, virus multiplication was detected in the inoculated leaves of the tomato lines. In addition, individuals that did not show any symptoms usually had low virus multiplication in uninoculated leaves. Only 8% of the F2 tomato lines had no visual symptoms but had high titers of virus in inoculated leaves. Thus, it appears that the plants carried true resistance rather than immunity. In true resistance, the virus may be able to multiplicate in the inoculated leaves but it does not travel to other parts of the plant. A few of the plants (the 8% that were symptomless but had high levels of virus) appeared to be tolerant. In tolerance, the virus can multiply and travel throughout the plant and the plant can still survive and may even be symptomless.

Similar results were obtained in pepper. Five weeks after inoculation the inoculated leaves had high titers of virus whereas uninoculated leaves did not. Thus, the peppers also exhibited true resistance. For both tomato and pepper, the resistance

appeared to be due to restriction of long-distance systemic movement of CMV. Similar results were observed in CMV-resistant potato lines (Celebi et al. 1998).

4.2. Genotypic Analysis

4.2.1. Marker Segregation

For CMV resistance gene mapping, 81 F2 tomato lines were used. These lines were from a cross between *L. esculentum* TA496 and *L. hirsutum* LA1223 and were previously phenotyped for CMV disease response (S. Doganlar, personal communication). A total of 107 CAPs markers were tested for polymorphism between the *L. esculentum* parent and an *L. esculentum* x *L. hirsutum* F1 hybrid. An F1 hybrid was used because *L. hirsutum* LA1223 DNA was not available. For each marker, up to 14 different enzymes were used to cut PCR products. Of the tested markers, 64% showed apparent polymorphism after digestion with restriction enzyme. Table 4.8 lists these CAPs markers and the enzymes for which polymorphism was detected. Figure 4.2. shows an example of a gel with different markers digested with different enzymes. When 40 of these markers were assayed on F2 progenies, 30 of them showed true polymorphism. On the other hand, 10 of the markers were found to be not polymorphic. Usually this difference in results was because the initial polymorphism assay had given misleading results and the marker was dominant instead of codominant. Figure 4.3. shows an example of a CAPs assay marker T801 tested on the F2 population.

It was expected that the markers tested on the F2 individuals would fit Mendelian segregation with a ratio of 1:2:1. Based on the Chi-square goodness-of-fit test, 73% (22) of the markers fit the expected ratio for a codominant marker. Five of the markers were skewed away from the *L. hirsutum* homozygous genotype while three were skewed away from the *L. esculentum* homozygous genotype (Table 4.9). This skewed segregation of molecular markers has also been observed in other interspecific tomato populations (Doganlar et al. 2002).

Table 4.8. List of CAPs markers that were found to be polymorphic between *L. esculentum* TA209 and *L. hirsutum* LA1223. Enzyme(s) that gave polymorphism for each marker are also listed as well as sizes of amplification and restriction products.

Marker	Product Size	Polymorphic Enzymes	LA1223 Size	TA209 Size
CT16	1650	*Taq*I	1000	850
CT20	850	*Rsa*I,*Hinf*I,*Taq*I	650	400
CT59	400	*Taq*I,*Dpn*II,*Hinf*I	500	400
CT64	650	*Alu*I	450	600
CT99	850	*Hinf*I	350	300
CT112	700	*Taq*I	500	600
CT118	600	*Hinf*I	850	500
CT138	800	*Dra*I,*Apa* II	750	650
CT143	1200	*Rsa*I	1400	850
CT147	800	*Rsa*I	500	650
CT167	400	*Taq*I	300	400
CT183	1400	*Dpn*II	1650	1000
CT197	700	*Rsa*I	500	700
CT198	700	*Hind*III	650	400
CT206	1000	*Rsa*I	1500	1000
CT228	1700	*Dpn*II	1000	850
CT269	1500	*Taq*I	1650	1000
CT276	2000	*Hinf*I	1000	650
CD35	1650	*Hinf*I	900	550
CD174	800	*Rsa*I	500	600
CT883	1000	*Hinf*I	700	900
T266	750	*Hinf*I	650	300
T307	1000	*Rsa*I	1000	850
T347	500	*Rsa*I	300	400
T408	2000	*Rsa*I	1400	1100
T463	900	*Dpn*II,*Taq*I	900	500
T564	1600	Already Polymorphic	1600	2000
T650	1800	*Ava* II	1650	2000
T668	500	*Hinf*I	400	200
T671	800	*Hae*III	850	400
T766	3000	*Hae*III	1400	1000
T801	1650	*Hae*III	1650	1350
T989	2000	*Rsa*I,*Hinf*I	900	1400
T1012	1800	*Rsa*I,*Hinf*I,*Taq*I	650	1000
T1106	1400	*Rsa*I,*Hinf*I,*Taq*I	850	1000
T1131	1500	*Mbo*I	300	600
T1143	1100	*Hinf*I,*Dpn*II,*Taq*I	900	400
T1171	1800	*Hae*III	850	1200
T1277	600	*Dpn*II, *Rsa*I	500	550
T1283	800	*Taq*I	300	500
T1328	2200	*Rsa*I,*Hinf*I,*Taq*I	1650	1200

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Table 4.8 (cont.)

Marker	Product Size	Polymorphic Enzymes	LA1223 Size	TA209 Size
T1413	1800	*Rsa*I,*Hinf*I,*Taq*I	850	500
T1422	750	*Ava* II	700	500
T1483	1000	*Rsa*I,*Dpn*II	600	900
T1584	1650	*Apa*II	700	800
T1768	1400	*Rsa*I,*Hinf*I,*Taq*I	850	700
Cler17N-11	900	*Hae*III	400	600
TG36	600	*Rsa*I	650	500
TG46	1600	*Hinf*I	500	900
TG65	1600	*Taq*I	1650	2000
TG114	1200	*Hind*III	900	1000
TG143	400	*Hinf*I	400	300
TG147	450	*Mbo*I	300	450
TG174	1800	*Hind*III	400	850
TG180	1000	*Rsa*I	650	1000
TG232	1100	*Eco*RV	1400	400
TG237	850	*Hinf*I	650	500
TG253	1000	*Hae*III	850	650
TG307	1000	*Rsa*I,*Taq*I	1000	850
TG318	1000/1200	Already Polymorphic	1000	850
TG339	1400	*Dra*II,*Hinf*I	1000	600
TG408	1000	*Rsa*I	1200	1000
TG443	1300	Already Polymorphic	1300	1500
TG498	500	*Dpn*II	400	300
TG503	1400	*Dra*II	1200	1000
TG510	1300	*Rsa*I,*Hinf*I,*Taq*I	1650	1400
TG517	1600	*Taq*I	1600	400
TG566	450	*Rsa*I	300	200
TG608	1400	*Hinf*I	1200	1650
TG703	1200	*Rsa*I	1000	500

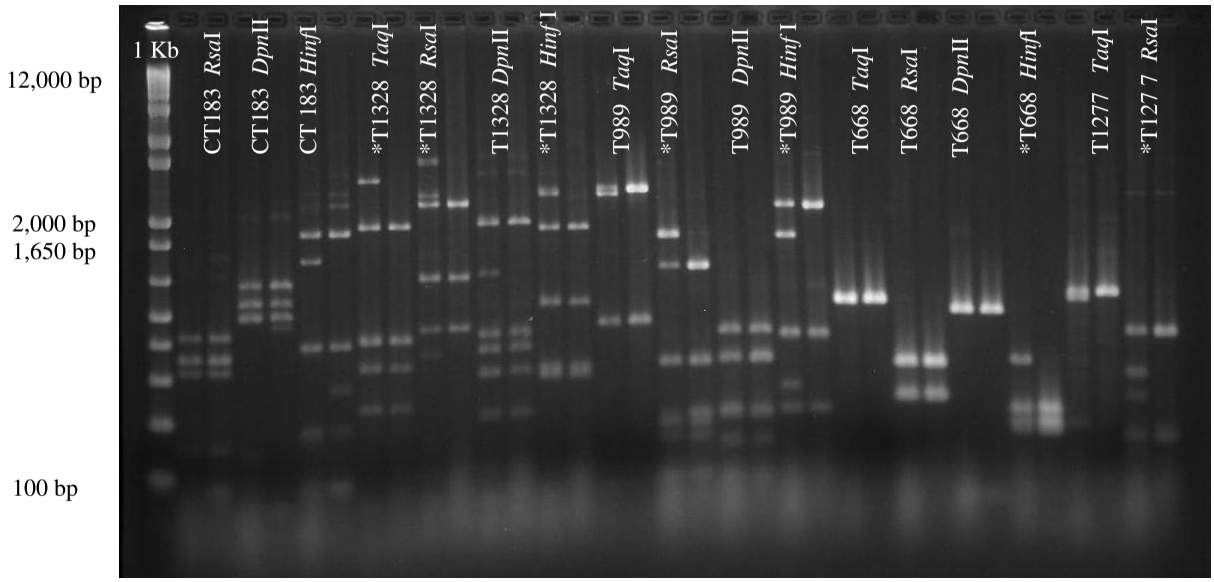

Figure 4.2. Survey for marker polymorphism. In each pair of lanes (TA496, F1 hybrid), the PCR product for the given marker was digested with the listed enzyme. 1 Kb=1 kb size standard DNA ladder. Samples with an * are apparently polymorphic.

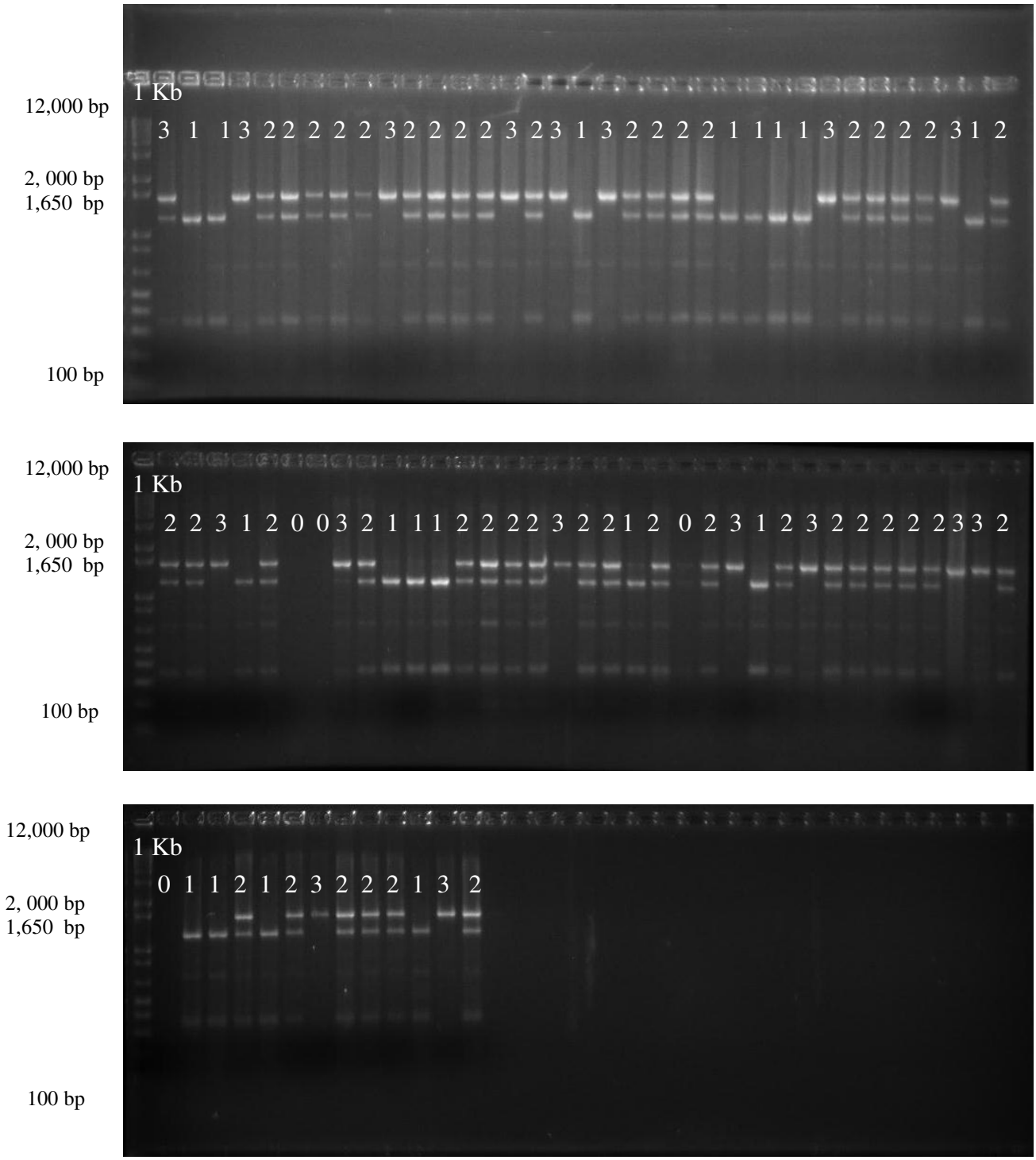

Figure 4.3. Example of a CAPs assay tested on the tomato F2 population. F1 (lane 2), *L.esculentum* (lane 3) and F2 progenies (lanes 4 to end) were amplified with marker T801 and and *Hae*III was used as restriction enzyme. Lane 1 is 1 kb ladder size standard. Samples are scored as: 1 for homozygous *L. esculentum*, 3 for homozygous *L. hirsutum* and 2 for heterozygous progenies.

Table 4.9. The ChiSquare and *P*- value results from a Chi-square goodness-of-fit test for F2 progenies. * marks the statistically significant values (P<0.05). Numbers of individuals in each genotypic class (AA, Aa, aa) are also given.

Marker	Chromosome	ChiSq	P	AA	Aa	aa	N
T1413	1	8,45	0,0146*	15	49	10	74
TG237	1	6,72	0,0347*	21	48	10	79
CD35	2	1,33	0,5143	18	32	22	72
CT59	2	0,87	0,6473	23	36	19	78
T266	2	2,1	0,3499	17	36	25	78
TG608	2	4,4	0,1108	13	38	26	77
T668	3	0,88	0,3482	22	36	12	74
T1143	3	4,51	0,0337*	28	31	10	71
T1283	3	7,22	0,0271*	26	35	10	71
CT118	5	2,92	0,2322	18	32	25	75
CT167	5	1,32	0,5169	20	39	14	73
TG318	5	0,34	0,5598	20	36	22	79
TG503	5	0,4	0,8187	15	32	13	62
TG253	6	2,6	0,2725	16	45	15	76
T463	7	4,94	0,0262*	11	46	20	78
T671	7	3,63	0,1628	20	41	11	72
T1328	7	7,04	0,0296*	13	30	4	47
CT64	8	3,92	0,1409	21	45	12	78
CT228	8	2,39	0,3027	16	31	9	56
TG307	8	4,06	0,1313	12	41	24	77
TG510	8	2,49	0,2879	20	39	12	71
CT183	9	9,97	0,0068*	7	47	20	74
CT198	9	2,34	0,1261	7	25	13	46
CT16	10	1,89	0,3887	14	40	22	76
CT20	10	5,1	0,0781	12	39	26	77
CT112	10	2,61	0,2712	14	39	24	77
CT269	11	0,24	0,8869	18	39	17	74
T1012	11	1,34	0,5117	17	43	16	76
TG36	11	6,07	0,0481*	14	37	29	80
T801	12	1,59	0,4516	17	44	16	77

4.2.2. QTL Analysis

QTL analysis was performed with the genotyped F2 population. For this analysis, a total of 78 (30 CAPs and 48 RFLPs) markers were used. In all, 11 genomic regions linked to CMV resistance were detected on chromosomes 1, 2, 3, 5, 6, 7, 9, and 12 (Table 4.10, Figure 4.4). For 64% of the loci, resistance was coming from *L. hirsutum*. *cmv*9.1 had the most significant value among the 11 QTL and explained 30%

of the variance for the trait. This locus had resistance (18 WVR) coming from the *L. esculentum* allele. The *cmv*1.1 locus explained 18% of variance for the trait (4 WE). In this case the resistant allele was coming from *L. hirsutum*. Additionally *cmv*3.2 (4 WE) and *cmv* 5.1 (18 WVR) each explained 14% of variance and resistance was coming from *L. hirsutum.* Similarly, *cmv6.1* (4WE) explained 14% of phenotypic variation, however, the resistance was coming from the *L.esculentum* parent. The remaining 6 QTLs each explained less than 12% of the variance for disease resistance.

According to the inheritance test, resistance appeared to be encoded by two dominant genes; whereas, as a result of QTL analysis, it was observed that 11 QTL encode resistance. This difference in results highlights the inadequacy of using simple Mendelian ratios to study quantitative traits. More powerful statistical methods, such as QTL mapping analysis, are needed to identify loci with minor effects. In addition, using QTL analysis it was possible to identify alleles for CMV resistance from *L. esculentum*, the CMV-susceptible parent. Such genes would not normally be detected using Mendelian analysis.

When the results of QTL mapping were compared with previously published work, it appeared that *cmv12.2* (linked to CT211) matches with a putative monogenic CMV resistance gene (*Cmr)* also mapped to chromosome 12 of tomato (Stamova and Chetelat 2000). None of the other QTL identified in the current study appeared to match other CMV resistance loci identified in tomato and pepper (Caranta et al. 1997, Chaim et al. 2001).

Table 4.10. QTL detected in the F2 population. The most significant markers are shown under Marker column. Effect column indicates whether the *L. hirsutum* alleles were associated with resistance (R) or susceptibility (S). RSq is the percent of phenotypic variatiance explained. Traits column lists the traits for which each QTL was identified.

QTL	Chromosome	Marker	P-value	RSq	Traits	Effect
cmv1.1	1	T1619	0,0002	18%	**4 WE**, 8 WE, 12 WVR, 18 WVR	R
cmv2.1	2	T266	0,012	11%	**18 WVR**	S
cmv2.2	2	T706	0,0157	9%	**4 WE,** 4 WVR, 6 WVR, 12 WVR	R
cmv3.1	3	CT31	0,0292	8%	8 WVR**, 12 WVR**	S
cmv3.2	3	T1143	0,0053	14%	**4 WE,** 4 WVR, 6 WVR, 18 WVR	R
cmv5.1	5	TG318	0,0031	14%	**18 WVR**	R
cmv6.1	6	TG253	0,0037	14%	**4 WE,** 4 WVR, 6 WVR, 12 WVR	S
cmv7.1	7	TG639	0,0358	8%	8 WVR, **12 WVR**	R
cmv9.1	9	CT183	0,0001	30%	4 WVR, 6 WVR, 8 WVR, 12 WVR, **18 WVR**	S
cmv12.1	12	CT19	0,0252	9%	**8 WVR**	R
cmv12.2	12	CT211	0,0459	7%	**12 WVR**	R

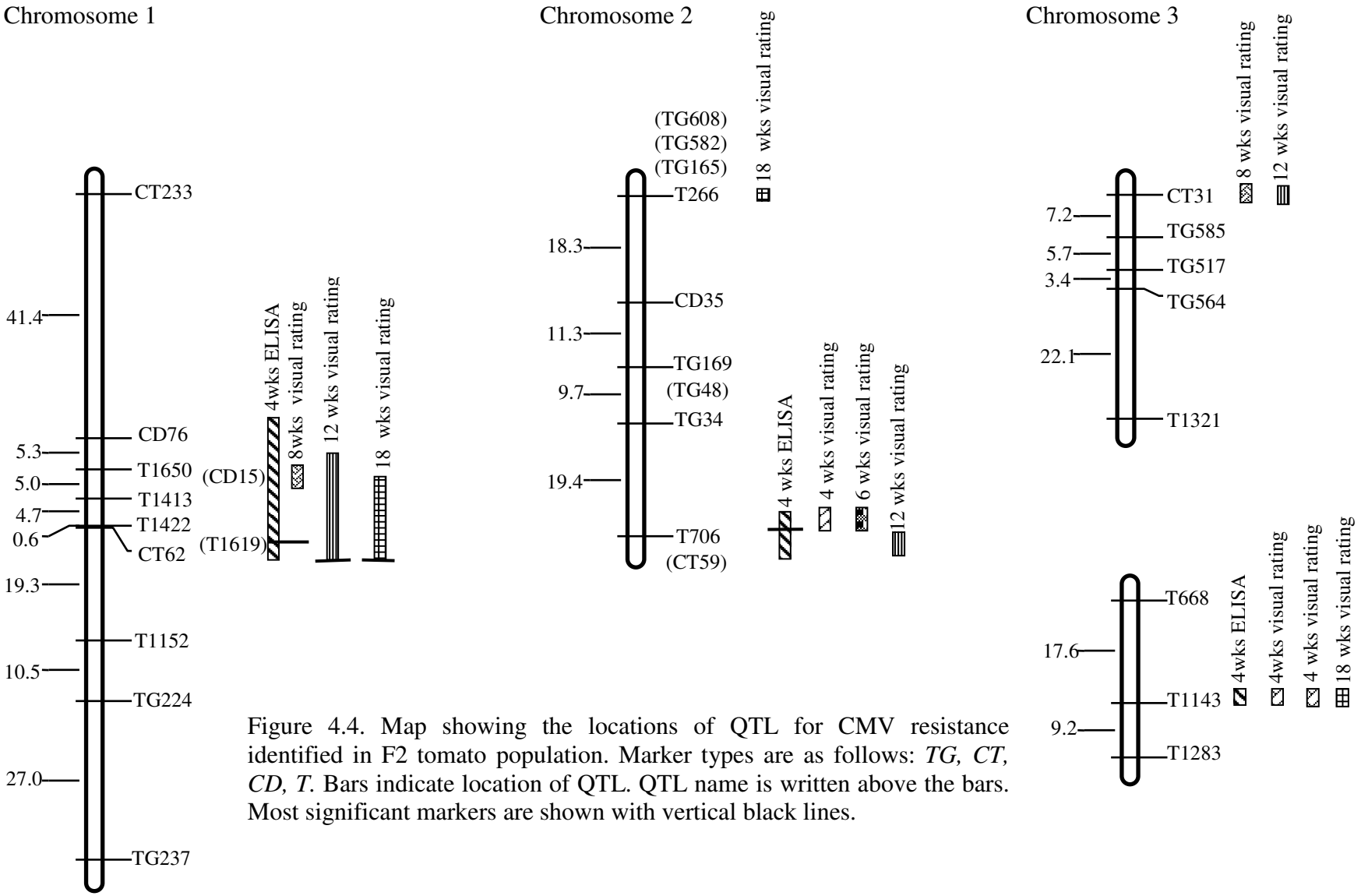

Figure 4.4. Map showing the locations of QTL for CMV resistance identified in F2 tomato population. Marker types are as follows: *TG, CT, CD, T*. Bars indicate location of QTL. QTL name is written above the bars. Most significant markers are shown with vertical black lines.

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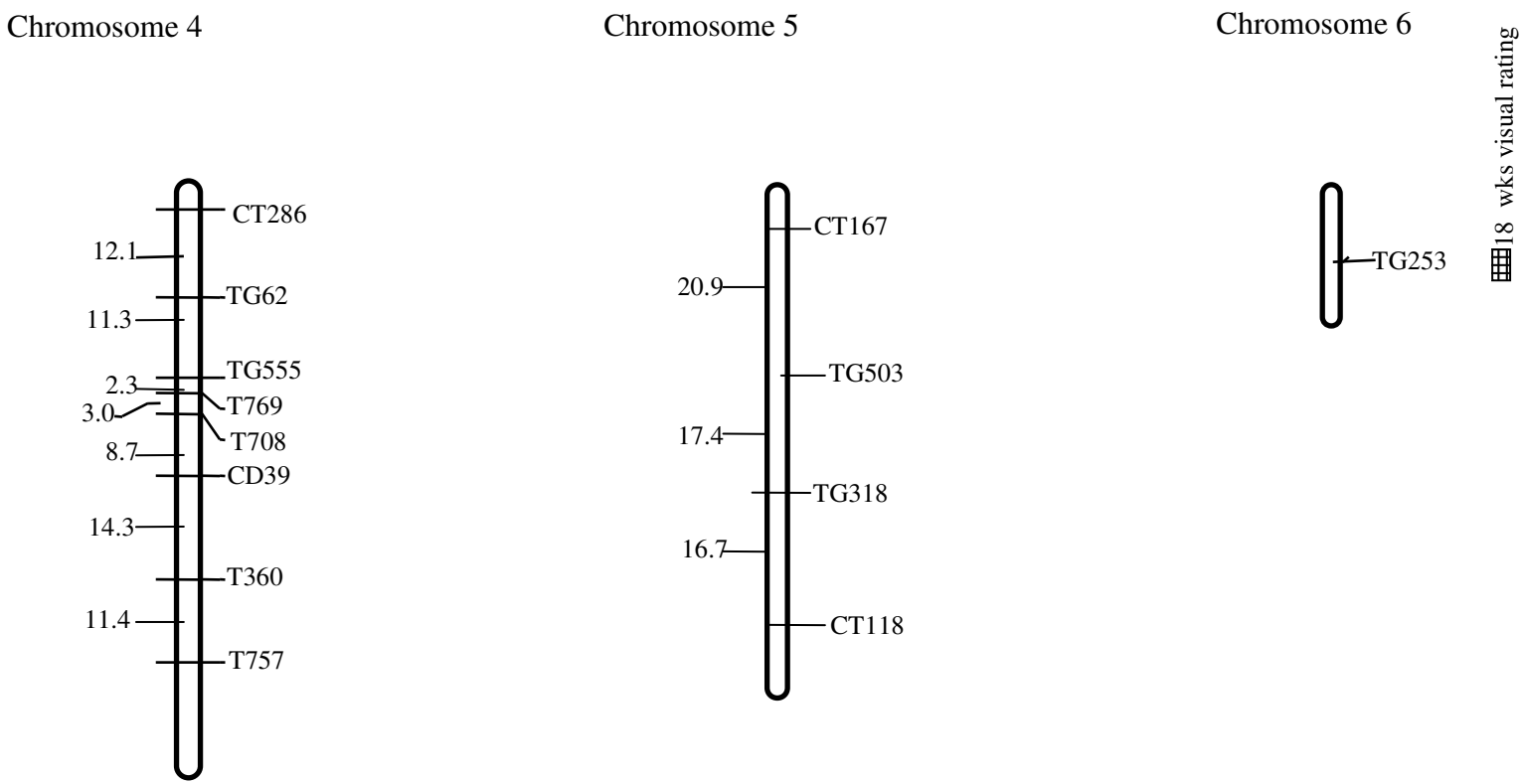

Figure 4.4 (cont.)

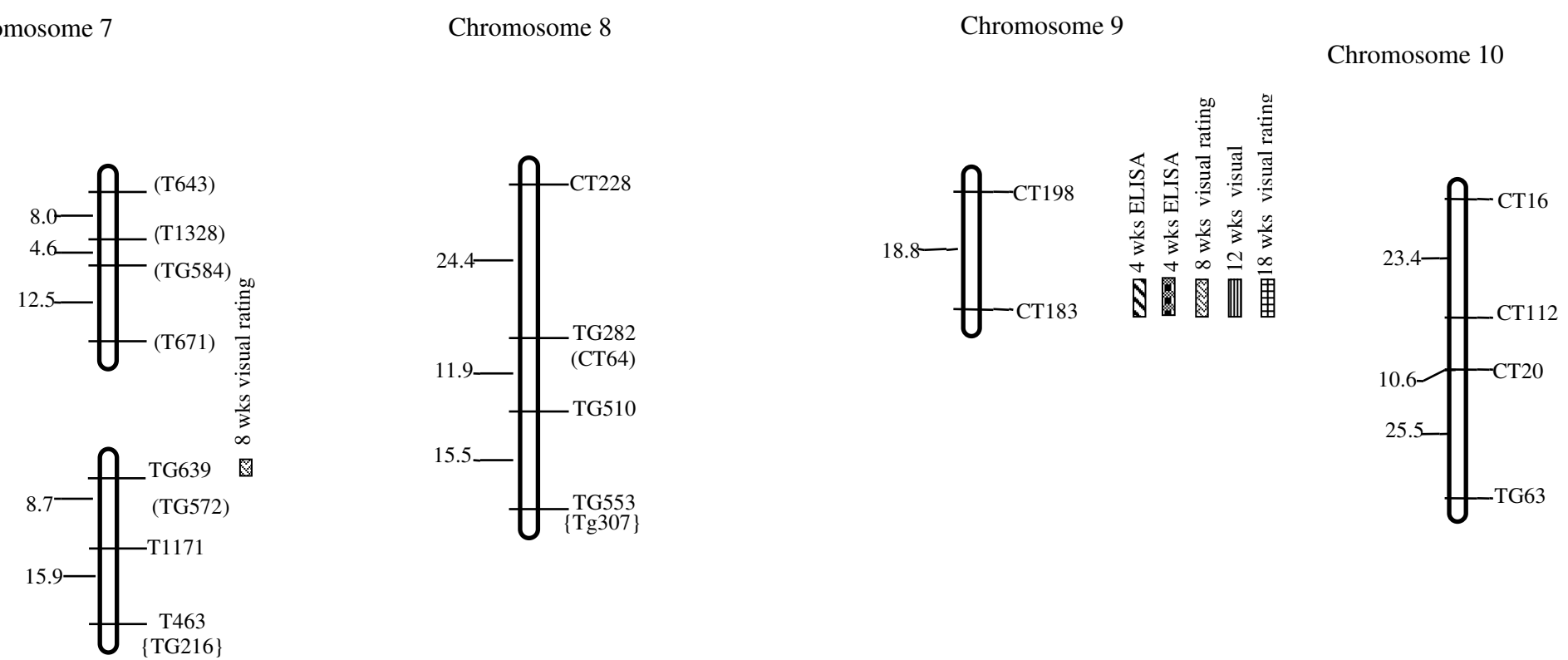

Figure 4.4 (cont.)

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4.3. Hybridization

Hybridization was done between resistant and susceptible parent pepper lines. As a result, progenies which may carry resistance genes were bred. The parental fruits and progenies are shown in Figure 4.5. As can be seen in the figure, the progenies' fruit shapes are intermediate between the two parental lines. In future work, these lines will be tested for CMV resistance.

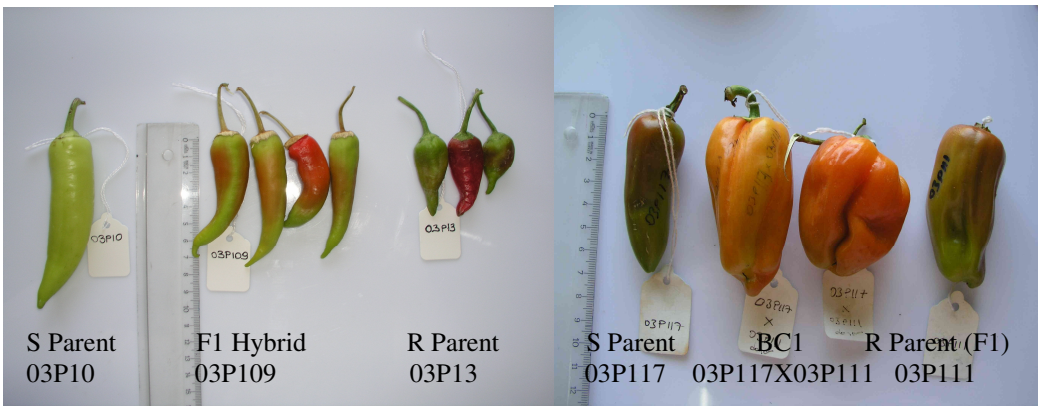

Figure 4.5. Pepper fruit of parental lines and hybrids.

03P10:*C.annuum* TR 70634 MENEMEN

F1 Hybrid : 03P109:*C.annuum* TR70634 MENEMEN X *C. annuum* x *C. frutescens* 98-8- C-1

03P13:*C.annuum*x*C.frutescens* 98-8-C-1

BC1 : 03P117: *C.annuum* TR 70630 MENEMEN

03P117X03P111:*C.annuum* TR70630 MENEMEN X *C.annuum* TR 70630 MENEMEN X *C. annuum* x *C. frutescens* 98-8-C-1

F1 Hybrid : 03P111: *C.annuum* TR 70630 MENEMEN X *C. annuum* x *C. frutescens* 98-8-C-1

CHAPTER 5

CONCLUSION

The objectives of this study were genetic and molecular characterization of cucumber mosaic virus (CMV) resistance in tomato and pepper. For this purpose, tomato and pepper populations were phenotypically and genotypically characterized. Additionally, a molecular map of tomato F2 lines was constructed and resistance genes were located on this map.

In this study, resistance derived from wild tomato and pepper plants was characterized. Parental lines and progenies were mechanically inoculated and evaluated visually and serologically by ELISA. It was observed that some plants had virus only in inoculated leaves while others had virus both in inoculated and uninoculated leaves. In general, the plants which lacked virus in uninoculated leaves lacked symptoms while those which had virus in inoculated leaves had disease symptoms. Thus, CMV resistance was true resistance rather than immunity. A few plants also seemed to display tolerance as they had virus throughout the plant but did not have any disease symptoms. Resistance appeared to be the result of restriction of the long distance movement of the virus in the plant.

According to inheritance tests in tomato, the segregation of F2 tomato lines was 9:7 (resistant:susceptible). This results suggests that resistance is encoded by two dominant genes. In order to study control of resistance in more detail, molecular mapping of tomato population was done with CAPs, a type of PCR-based molecular marker. As a result of this study, 11 genomic regions (QTL) linked to CMV resistance were detected. The difference between inheritance test results and QTL analysis were expected because many natural disease resistances do not fit the gene-for-gene hypothesis and instead are controlled by multiple genes or QTLs. For seven (64%) of the QTL, resistance was associated with the *L. hirsutum* allele. Thus, the results show that alleles for individual resistance genes can even be found in susceptible plants like *L. esculentum* TA209. It was also found that *cmv12.2* (linked to CT211) matches with a putative monogenic CMV resistance gene (*Cmr)* also mapped to chromosome 12 of tomato (Stamova and Chetelat 2000). This result suggests that this R gene has been

conserved over time in wild germplasm and indicates that this gene may be a good target for marker assisted selection. If the resistance is detected in other wild types or exotic germplasm, these lines can be used as donor parents for new populations.

In conclusion, identification and molecular mapping of genes/QTL for disease resistance will be useful for marker assisted selection. In future, mapping of other resistance genes for new pathogens can be done and elite lines with resistance genes can be constructed.

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