CHARACTERIZATION OF PEPPERS FOR ANTIOXIDANT CONTENT AND VIRUS RESISTANCE

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ABSTRACT

CHARACTERIZATION OF PEPPERS FOR ANTIOXIDANT CONTENT AND VIRUS RESISTANCE

In the first part of this study, total water soluble antioxidant activity, phenolic compounds and vitamin C content of 29 Turkish and 14 non-Turkish pepper cultivars were determined. Significant trait diversity was found in the cultivars with the most variation (7.4-fold) seen for total antioxidant capacity which ranged from 2565 to 18957 μmol Trolox/kg. Vitamin C content for the peppers ranged from 522 to 1631 mg/kg, a 3.1-fold difference, while total phenolic content for the pepper cultivars ranged from 607 to 2724 mg/kg, a 4.5-fold difference. A strong correlation between total antioxidant capacity and phenolic content ($r = 0.71$) was also seen. Significant variation for antioxidant content in Turkish germplasm indicates that this material can be used for improvement and genetic mapping of nutritional content in pepper.

In the second part of this study, amplified fragment length polymorphism (AFLP), cleaved amplified polymorphism (CAPs) and simple sequence repeat (SSR) markers were used to map gene(s) for cucumber mosaic virus resistance. The mapping was conducted using a F2 population derived from a cross between *Capsicum annuum* cv. Jupiter (susceptible) X *C. frutescens* (resistant). The F2 population, consisting of 76 individuals, was screened with 13 AFLP primer pairs, 60 CAPs markers and 30 SSR markers to detect markers possibly linked to CMV resistance gene(s). As a result, 7 QTL were found which are related with resistance to CMV in pepper. This will provide pepper breeders an opportunity to use these markers for marker-assisted selection for CMV resistance in pepper.

ÖZET

BİBERLERİN ANTİOXİDANT İÇERİĞİ VE VİRUS DAYANIKLILIĞI BAKIMINDAN KARAKTERİZASYONU

İki bölüm oluşan bu tezin birinci bölümünde 29 Türk ve 14 Türkiye'de yetişen yabancı biber kültür çeşitlerinde toplam suda çözünen antioxidant aktivitesi, toplam fenolik madde içeriği ve C vitamini içerikleri bakımından incelenmiştir. Ayrıca biberler kullanım amaçlarına göre Salçalık, Dolmalık, Sivi, Süs ve Çarliston biberler ve acılık-tatlılık durumlarına göre de Acı ve Tatlı olarak sınıflandırılmıştır. Yapılan çalışmalar sonucunda bu kültür çeşitleri arasında antioxidant içerikleri bakımından gözle görülür bir çeşitlilik olduğu ortaya koyulmuştur. İncelenen özellikler bakımından Sivri biber çeşitlerinin diğerler çeşitlere oranla daha yüksek miktarda antioxidant içeriğine sahip olduğu gözlemlenmiştir. Antioxidant özellikleri bakımından yüksek değerlere sahip biberlerin çoğunluğunun Türk biber hatları olduğu ortaya koyulmuştur. Türk biber hatlarında gözlemlenen bu büyük çeşitlilik, bu hatların antioxidant değerleri düşük olan diğer biber hatlarının geliştirilmesi ve besin içerikleri bakımından haritalama çalışmaları için kullanılabileceğini göstermektedir.

Tezin ikinci bölümünde *C. annuum* cv. Jupiter (CMV'ye hassas ebeveyn) ve *C. frutescens* (CMV'ye dayanıklı ebeveyn) çaprazlamasından elde edilen 76 bireyli F2 populasyonunda 13 AFLP primer çifti, 30 SSR ve 60 COSII markörleri kullanılarak hıyar mozaik virüsüne (CMV) olan daynıklılık karakterize edilmiştir. 76 F2 bireyinden elde edilen 1066 F3 bireyi karakterizasyon için fenotipik olarak incelenmiştir. Yapılan çalışma sonucunda CMV virüsüne karşı dayanıklılığı sağlayan bölgelerle ilişkili 7 adet QTL bulunmuştur. Bu çalışma biber ıslahçıları için virüse dayanıklı biber hatlarının elde edilmesi açısından önem taşımaktadır. Böylelikle bu markörler kullanılarak bulunan CMV dayanıklılığı sağlayan genler, marköre dayalı seçilim yöntemi ile diğer hassas hatlara aktarılabilecektir.

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PART 1

CHAPTER 1

INTRODUCTION

1.1. General Information About Pepper

Capsicum peppers are the fruits of plants belonging to a single genus in the family Solanaceae. *Capsicum sp.* originated in Mexico, Southern Peru and Bolivia and includes five cultivated species and hundreds of varieties (Grubben 1977). All natural populations of pepper are diploid and have the same chromosome number, 2n=24. At least 25 wild species of pepper have been reported. The domesticated species are *C. annuum L., C. frutescens L., C. chinense jacq., C. baccatum var. pendulum L. and C. pubescens* (Pickersgill 1997).

C. annuum L. is the most widely cultivated of these species throughout the world and includes the mild sweet bell peppers as well as many varieties of hot peppers. This species is a small, bushy, herbaceous annual that produces small white flowers similar to those of tomato or potato. The fruits vary considerably in shape, size, and color among the hundreds of varieties. The immature fruits are green. The mature fruits' color can vary between yellow to bright red including white and purple. Their shape also shows differences. Shapes can range from long and narrow to almost spherical. *C. frutescens* is cultivated mainly in the tropics and warm temperate areas and generally has a more fiery taste. *C. chinense*, despite its scientific name, has South American origins. This species includes the habanero, one of the hottest chili peppers known. *C. baccatum* L. is the most widely grown pepper in South America, where it is called *aji*. Grown in the highlands of Central and South America, *C.pubescens* is the least known of the domesticated chilies (Pickersgill 1971, Pickersgill 1997).

The attractive red color of peppers is due to their various carotenoid pigments which are synthesized during ripening. These carotenoids include capsanthin, capsorubin and crypto-capsin (Deepa and others 2007). The biting taste of capsicum peppers is due to a mixture of seven related alkaloids, of which capsaicin is the most

prevalent (Hoffman and others 1983). Capsaicinoids are mainly found in the seeds and placental area (where seeds attach to the ovary wall) (Dong 2000). The capsaicin content is negligible in sweet bell peppers but found in such high concentrations in hot chili or jalapeno peppers that even handling or cutting the peppers can irritate the skin. Capsaicin is so potent that it can be tasted in concentrations as low as 1 part per million. In 1912, a method to record the hotness of various capsicum peppers was developed by Wilbur Scoville (Dong 2000). A panel of subjects tasted extracts from a pepper. The sample was continuously diluted and tested until pungency could no longer be detected. Scoville Heat Units were then assigned. One of the hottest is habanero, with a scoville rating ranging from 100,000 to 300,000 (Table 1). This means that an extract of this pepper is still detectable even in a 100,000:1 dilution. The potency of capsaicin has been utilized in two completely different applications: police use it as a pepper spray to subdue unruly persons, and many people use it in creams that are applied to relieve the pain of arthritis, shingles, cluster headches, and other ailments.

Table 1. Scoville rating of some pepper cultivars.
(Source: Everhart and others 2002)

Pepper	**Scoville Heat Units**
Habanero	100,000-300,000
Scotch bonnet	80,000-260,000
Jamaican hot	100,000-200,000
Chiltepini	50,000-100,000
Thai	50,000-100,000
Aji	30,000-50,000
Cayenne	30,000-50,000
Tabasco	30,000-50,000
Jalapeno	2,500-5,000
Cherry	100-500
Pimento	<1
Banana (sweet)	<1
Bell	<1

Pepper and pepper products are rich in health related food components as they are excellent sources of vitamin C; even one pepper is more than enough to satisfy the daily requirement for this vitamin. The daily Recommended Dietary Allowance (RDA) of vitamin C averages between 60 and 75 mg for adult women and men (Otten and others 2006). The amount of vitamin C is actually higher in peppers than in citrus fruits.

In addition to vitamin C, peppers have vitamins E and A, capsaicin, niacin, riboflavin and thiamine. They also have antioxidant and anti-bacterial properties. There are many benefits of pepper for human health. For example for the digestion system, pepper stimulates taste buds, promotes stomach acid secretion and decreases the formation of intestinal gases. The National Academy of Sciences defines Functional Foods to "encompass potentially healthful products," including "any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Bloch and Thomson 1995). According to this definition we can consider pepper a functional food.

Pepper production in Turkey shows a steady increase. Rate of annual increase in production is about 4 to 10% each year. As a result, pepper production has increased from 220,000 tons in 1980 to 580,000 tons in 1990 to 1,090,000 tons in 2000. A total of 33% of pepper production is grown in the coastal area of the Mediterranean Region. This is followed by the Aegean, Marmara, and Black Sea Regions. Nearly 11% of production is grown in Samsun which is the primary province that grows peppers in the Black Sea Region (Hekimoğlu and Altındeğer 2007). Turkey ranks second worldwide in terms of pepper production with 1,829,000 metric tons (7% of world production) produced in 2005 (FAO 2005) (Figure 1). Only China produces more peppers with 12,531,000 metric tons produced in 2005. Peppers are also an important part of Turkish cuisine and can be consumed either fresh or processed throughout the year. These peppers are consumed pickled, grilled, and stuffed, as well as in salads and as a component of cooked dishes. The remaining 10% is processed. The most important processing forms of pepper are paste and dried red pepper (paprika). Pepper is processed as paste generally in the Marmara and Aegean Regions and as crushed red pepper in the East Mediterranean (Kahramanmaraş) and South-East Anatolia (Şanlıurfa) regions. In addition, especially in the Marmara region a small amount is canned and frozen.

Peppers grown in Turkey can be classified into five groups based on their morphology and/or primary use. These classes are: bell-type (Dolmalık) peppers that are used for stuffing; long, pointed (Sivri) peppers and long, blunt-ended Charleston-type (Çarliston) peppers that are often consumed raw; small-fruited "fancy" (Süs) peppers that are eaten fresh or pickled; and paste (Salçalık) peppers that are processed into paste. Each pepper type is not used exclusively for only one purpose. For that

reason these classes cannot be considered as definitive. For example, paste peppers can also be stuffed. Representative fruits of each type are shown in Figure 2.

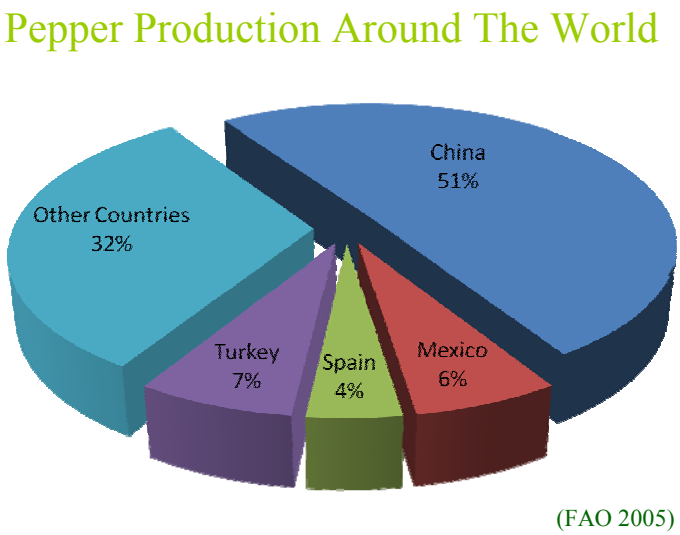

Figure 1. Pepper production around the world.

(Source: FAO 2005)

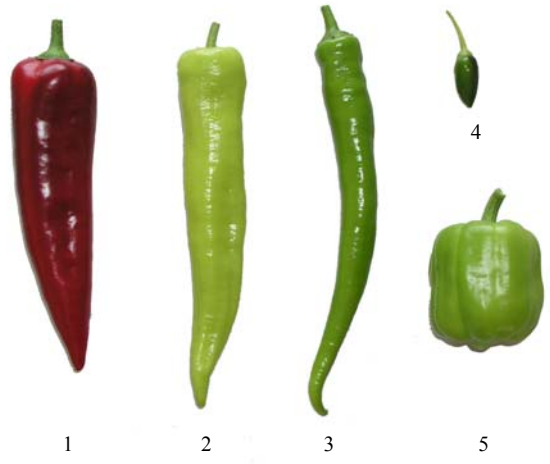

Figure 2. Representative fruits of pepper types. 1. Salçalık type, 2. Çarliston type, 3. Sivri type, 4. Süs type and 5. Dolmalık type.

The most commonly used variety types are ‘Çarliston’, ‘Sivri’ and ‘Dolmalık’. Apart from these, ‘Süs’ and ‘Salçalık’ type local varieties are grown. Bell-type, large-fruited varieties are also grown in small quantities for canning and freezing. Usually, most ‘Salçalık’, ‘Çarliston’ and ‘Dolmalık’ type peppers are sweet whereas ‘Süs’ types are hot peppers. ‘Sivri’ types include both hot and sweet varieties.

1.2. Oxidative Stress and Free Radicals

It is well-known that oxygen is an essential element for human beings and without it, we cannot survive. Although it is such an important element for us, it is also involved in toxic reactions and is therefore a constant threat to all living things (Gilbert 1981). We can tolerate oxygen because during evolution, we developed powerful defence mechanisms which minimize its toxic effects. If we did not have such protection systems, by-products of our metabolism would quickly end our lives. Even though we have evolved such defence mechanisms, they are not sufficient to protect us from all harm caused by oxygen.

Free radicals are molecules that contain one or more unpaired electrons. They are electrically charged molecules. The simplest free radical is atomic hydrogen. Oxygen (O_2) is also a free radical because it has two unpaired electrons. Free radicals are capable of attacking the healthy cells of the body and reacting with lipids (membranes), proteins, nucleic acids and other small molecules causing them to lose their structure and function (Moure and others 2001). Cell damage caused by free radicals appears to be a major reason for aging and degenerative diseases of aging such as cancer (Berneburg and others 1999). Damage in the DNA repair system can lead to mutation and mutation can lead to cancer.

Free radicals include reactive oxygen species (ROS). ROS is a term which covers all highly reactive, oxygen-containing molecules. Types of ROS include the hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, the nitric oxide radical, the hypochlorite radical and various lipid peroxides. Free radicals and other reactive oxygen species in the human body are derived either from normal, essential metabolic processes or from external sources. Examples of both are shown in Table 2. For example cigarette smoke, pollution, exposure to sunlight all cause the formation of free radicals. Also in the human body, free radicals are formed in

mitochondria and peroxisomes. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells.

Table 2. Internal and external sources of free radicals.

Internally generated sources	External sources
- Mitochondria - Phagocytes - Xanthine oxidase - Reactions involving iron and other transition metals - Arachidonate pathways - Peroxisomes - Exercise - Inflammation	- Cigarette smoke - Environmental pollutants - Radiation - Ultraviolet light - Certain drugs - Pesticides - Anaesthetics - Industrial solvents - Ozone

1.3. What are Antioxidants?

Recently antioxidants have been a very popular subject because it is thought that many human diseases have a direct or indirect relation to reactive oxygen species (ROS) which cause oxidative stress in the body (Percival 1998). For example age-related macular degeneration was shown to be associated with light-induced production of O_2^- (Gottsch 1990). More recently, clinical trials were undertaken to evaluate the effects of the antioxidant vitamins A, C, and E in controlling the progression of this disease (Seddon 1994).

An antioxidant is an agent that prevents or inhibits oxidation by donating H_2 or electrons to free radicals, or by reducing the rate of autoxidation of lipids. An antioxidant can be any substance which can delay or inhibit oxidation. Numerous studies suggest that people with higher intakes of fruit and vegetables or increased blood antioxidant concentrations have lower risk of some cancers, coronary heart disease and stroke (Zino and others 1997).

Antioxidants also are important for plant defence mechanisms. When plants are exposed to both biotic and abiotic stresses which generate ROS in the plant, they produce antioxidants (Sakihama and others 2002, Slater and others 2003). In plants, antioxidants perform the same functions as they do in humans. They protect lipids,

membranes, proteins and nucleic acids by reducing free radicals, or stopping the chain reactions caused by them. If these chain reactions are allowed to continue, they can damage cellular components and cause the death of cells or even the whole organism.

Antioxidants can be categorized in many ways. One way is based on their solvent medium resulting in two classes: lipophilic and hydrophilic antioxidants (Rousseaux and others 2005). For example, vitamin C is a hydrophilic, and vitamin E is a lipophilic antioxidant. Another way to classify them is by their origin. According to this classification we can divide antioxidants into exogenous or endogenous antioxidants. If the antioxidant is synthesized in the organism we call it an endogenous antioxidant. But if it is taken from outside then it is called an exogenous antioxidant. Antioxidants can also be classified based on the mode of action of the antioxidant. These are chain breaking and preventive antioxidants. Chain breaking antioxidants prevent the oxidation of lipids by scavenging peroxy radicals. Preventive antioxidants diminish the formation of lipid radicals. Lastly we can divide them into two groups, natural and synthetic antioxidants. Natural antioxidants include various vitamins, carotenoids, amino acids and phytonutrients (Maldavi and others 1996). Examples of synthetic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) (Moure and others 2001). The safety of BHA and BHT, which are used as food additives, is still being examined.

In this study, the total water-soluble antioxidant activity, vitamin C (ascorbic acid) and phenolic content of pepper extracts were measured. Water soluble antioxidant activity includes vitamin C, enzymes and phenolics. Measurement of total water soluble antioxidant capacity is more practical then measuring each component and also takes into account synergistic interactions between compounds. Vitamin C is a water-soluble antioxidant that neutralizes superoxide, hydrogen peroxide and the hydroxyl radical (Podsedek 2007). Vitamin C also reduces α-tocopheryl radicals to regenerate the antioxidant form of vitamin E (Davey and others 2000). Phenolic compounds are the largest category of phytochemicals and include flavonoids, phenolic acids and phenols. Phenolic compounds usually are found in the dermal tissues of the plant body. They have a potential role in protection against ultraviolet radiation. They also have a role as defence chemicals against pathogens and predators (Toor and Savage 2005). Humans cannot produce phenolic compounds. Thus, these compounds must be taken in mainly through the daily diet (Materska and Perucka 2005). These compounds are excellent

antioxidants because their structure allows them to easily donate hydrogens to free radicals (Podsedek 2007). Recent surveys of commonly consumed vegetables have shown that both red and green peppers have high levels of antioxidant activity as compared with other plants. In three separate studies, pepper ranked first with higher total antioxidant activity than vegetables such as broccoli, carrot, spinach and kale (Chu and others 2002, Halvorsen and others 2002, Ou and others 2002) (Table 3). Palevitch and Craker (1995) also found that pepper has the highest antioxidant content among vegetables with very high levels of vitamin C. Other researchers found that only spinach had higher antioxidant activity than pepper (Pellegrini and others 2003). Levels of antioxidants can vary with genotype, stage of maturity, plant part consumed, and conditions during growth and post harvest handling. For example mature green or red peppers' antioxidant contents are higher than those for immature green peppers (Howard and others 2000, Navarro and others 2006, Deepa and others 2007).

Table 3. Antioxidant activity of some vegetables and fruits.
(Source: Chu and others 2002)

Vegetable or Fruit	**Species name**	**Antioxidant level (mmol/100g)**
Chili pepper	*Capsicum annuum*	2.46
Red cabbage	*Brassica oleracea var capitata*	1.88
Grape	*Vitis vinifera*	1.45
Orange	*Citrus sinensis*	1.14
Lemon	*Citrus limon*	1.02
Spinach	*Spinacia oleracea*	0.98
Broccoli	*Brassica oleracea*	0.58
Apricot	*Prunus armeniaca*	0.52
Tomato	*Lycopersicum esculentum*	0.31
Garlic	*Allium sativum*	0.21
Eggplant	*Solanum melongena*	0.17

Genus *Capsicum* is also a rich source of phenolics (Howard and others 2000). When compared with other vegetables, pepper ranked fourth in total phenolics content after broccoli, spinach and onion (Chu and others 2002). In addition to their antioxidant role, phenolic compounds are important in determining pepper color, flavor and pungency (Estrada and others 2002). Marin and others (2004) showed that phenolic

compounds in sweet peppers are mainly located in the peel. Total phenolic content decreases during pepper maturation (Navarro and others 2006).

1.4. Improvement of Antioxidant Content in Plants

In the past decade, researchers have focused on understanding the link between oxygenated metabolites and human diseases. They have learned that there is a possibility to prevent, postpone or limit the severity of diseases by increasing the body's antioxidant defence systems through improved nutrition (Nuttall and others 1999). As a result, consumers are demanding healthier and more diverse fruits and vegetables. Breeding of phytochemical traits is difficult because of their polygenic nature. However, if the genes controlling the character of interest are identified and localized, molecular breeding techniques and marker-assisted selection can be used for trait improvement. For breeding efforts to be successful, variation for the trait(s) must be present in the species. Thus, a first step toward improving the antioxidant content of a crop like pepper is a screen of germplasm for the trait(s) of interest.

1.5. Previous studies

Numerous studies have examined the total antioxidant, ascorbic acid and phenolic contents of pepper. These studies have commonly used one or a few cultivars and examined the effects of factors such as maturity and growth/environmental conditions (Howard and others 2000, Gnayfeed and others 2001, Marin and others 2004, Chassy and others 2006, Deepa and others 2007). A few researchers have examined these traits in multiple pepper genotypes. In the work of Deepa and others (2007) and Guil-Guerrero and others (2006), ten *C. annuum* cultivars were examined for their nutrient composition including total antioxidant activity and ascorbic acid and carotenoids contents in different maturity stages. They found that these compounds increased during maturity. Antonious and others (2006) examined the concentration of phenols, ascorbic acid and capsaicin in 17 cultivated hot pepper accession from four Capsicum species: *C. annuum*, *C. chinense*, *C. baccatum* and *C. frutescens*. They showed that concentration of these compounds are higher in *C.chinense* and *C.baccatum* than in *C.annuum* and *C. frutescens*. Navarro and others (2006) examined

the effect of salinity and different ripening stage of pepper fruits on antioxidant activity. They found that antioxidant activity increased with fruit maturation. They could not find any positive or negative effect of salinity on antioxidant content (Navarro and others 2006).

1.6. Goals of Our Work

Variability in the presence and concentration of antioxidants in pepper species can be a factor affecting the selection of pepper for breeding programs. The objectives of this study were to survey antioxidant content of Turkish pepper cultivars, determine the concentrations of phenolics, ascorbic acid, and total water soluble antioxidants in 29 Turkish and 14 non-Turkish pepper cultivars and to select candidate accessions of Turkish peppers having high concentrations of these compounds for use as parents in hybridizations in different breeding programs.

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant Materials

For this study, 43 pepper accessions were grown in the greenhouse. Peppers were classified as Turkish or non-Turkish cultivars. We also divided peppers into two groups based on pungency/heat: hot and sweet cultivars. Turkish cultivar seeds were obtained from the Turkish National Germplasm Collection at the Aegean Agriculture Research Institute (AARI, İzmir, Turkey) and from the Atatürk Central Horticultural Research Institute (Yalova, Turkey). In adition to the 29 Turkish cultivars, we also examined 14 non-Turkish cultivars grown in Turkey and/or worldwide. These foreign cultivars included standard, commonly used varieties such as California Wonder and Yolo Wonder. Non-Turkish cultivars' seeds were obtained from the Center for Genetic Resources, the Netherlands, and from seed distributors in Turkey and the U.S.A. Seeds were planted in the greenhouse in April 2006 and three to five replicate plants were grown for each accession. Fruits were harvested at the mature green stage in July and August and samples were stored at -20°C until assays were performed. All assays were completed within one month of harvest (Table 4).

Table 4. Pepper cultivars used for antioxidant trait assays. Type of pepper and heat are also included. na= not applicable, no accession number.

	Accession	Country			
Cultivar (origin)	number (source[z])	of origin	Type	Pungency	Color[y]
333 Biber	na[x] (1)	Turkey	Çarliston	Sweet	Yellow
Acı Biber (Gaziantep)	TR47780 (1)	Turkey	Dolmalık	Hot	Green
Acı Sivri Biber (Bursa)	TR66271 (1)	Turkey	Sivri	Hot	Green
Apollo F1	na (2)	Hungary	Dolmalık	Sweet	Yellow

(cont. on next page)

Table 4. Pepper cultivars used for antioxidant trait assays. Type of pepper and heat are also included. na= not applicable, no accession number (cont.).

Cultivar (origin)	Accession number (source[z])	Country of origin	Type	Pungency	Color[y]
Arnavut Biber	TR66272 (1)	Turkey	Süs	Hot	Green
Arnavut Biber, sivri	TR66299 (1)	Turkey	Süs	Hot	Green
Ayaş	na (1)	Turkey	Sivri	Sweet	Green
California Wonder	na (3)	USA	Dolmalık	Sweet	Green
Çarliston Biber (Bursa)	TR66275 (1)	Turkey	Çarliston	Sweet	Yellow
Carolina Wonder	na (2)	USA	Dolmalık	Sweet	Green
Cecil RZ F1	na (2)	Hungary	Dolmalık	Sweet	Yellow
Charleston Belle	na (2)	USA	Dolmalık	Sweet	Green
Cherry Pick	na (2)	USA	Süs	Sweet	Green
Chile Negro	na (3)	Mexico	Süs	Hot	Dark Green
Cuma Ovası	na (1)	Turkey	Sivri	Hot	Light Green
Dolmalık	TR70630 (1)	Turkey	Dolmalık	Hot	Green
Dolmalık Yeşil (Bursa)	TR66270 (1)	Turkey	Dolmalık	Sweet	Green
Domat Biberi (Bursa)	TR66393 (1)	Turkey	Dolmalık	Hot	Light Green
Düğme Biber (Bursa)	TR66316 (1)	Turkey	Süs	Hot	Green
Edison	na (2)	Netherlands	Dolmalık	Sweet	Green
Ege-91	na (1)	Turkey	Sivri	Sweet	Light Green
Farya	na (2)	USA	Çarliston	Sweet	Yellow
Fiesta	na (2)	Netherlands	Dolmalık	Sweet	Green
Finli Biber	TR66380 (1)	Turkey	Sivri	Hot	Light Green
Kale	na (1)	Turkey	Dolmalık	Hot	Light Green
Kandil Dolma Biber	na (1)	Turkey	Dolmalık	Sweet	Light Green
Menderes	na (1)	Turkey	Sivri	Hot	Light Green
Raspires F1	na (2)	Hungary	Çarliston	Hot	Yellow

(cont. on next page)

Table 4. Pepper cultivars used for antioxidant trait assays. Type of pepper and heat are also included. na= not applicable, no accession number (cont.).

Cultivar (origin)	Accession number (source[z])	Country of origin	Type	Pungency	Color[y]
Şahnalı Biber	na (1)	Turkey	Sivri	Hot	Green
Salçalık Biber	TR66259 (1)	Turkey	Salçalık	Sweet	Red
Salçalık Biber (Bursa)	TR66389 (1)	Turkey	Salçalık	Sweet	Red
Salçalık Biber (Gaziantep)	TR48614 (1)	Turkey	Salçalık	Sweet	Red
Sera Demre	na (1)	Turkey	Sivri	Sweet	Green
Tatlı Kıvırcık Biber	TR66305 (1)	Turkey	Sivri	Sweet	Light Green
Variegated Flash	na (3)	USA	Süs	Hot	Purple
Yağlık Biber	TR66378 (1)	Turkey	Salçalık	Sweet	Red
Yağlık Biber (Bursa)	TR66384 (1)	Turkey	Salçalık	Sweet	Red
Yalova Biber	na (4)	Turkey	Sivri	Sweet	Yellow
Yalova Çarliston 341	na (4)	Turkey	Çarliston	Sweet	Yellow
Yalova Tatlı Sivri Biber	na (4)	Turkey	Sivri	Sweet	Light Green
Yalova Yağlık	na (4)	Turkey	Salçalık	Sweet	Red
Yolo Wonder 31-22	na (3)	USA	Dolmalık	Sweet	Green
Sweet Long Slim Red	na (3)	USA	Sivri	Sweet	Red

[z] 1 = Aegean Agricultural Research Institute, İzmir, Turkey; 2 = purchased from Turkish or U.S.-distributor; 3 = Center for Genetic Resources, The Netherlands; 4 = Atatürk Central Horticultural Research Institute, Yalova, Turkey.

2.2. Phenotypic Characterization

In this study, we analysed 43 pepper cultivars for total water-soluble antioxidant activity, ascorbic acid and phenolic contents. Phenotypic characterization was used to learn how much variability there is for these traits in Turkish pepper cultivars and also to compare Turkish and non-Turkish cultivars.

2.2.1. Determination of Total Water Soluble Antioxidant Activity

To prepare sample extracts for spectrophotometric analysis, 150 g peppers without seeds were homogenized with 150 mL cold distilled water for 2 min at low speed in a Waring blender equipped with a 1L double walled stainless steel jar chilled by circulating water at 4 ^{o}C. For cultivars with small fruits, 50 g sample was homogenized with 50 mL distilled water using a 200 mL jar and the same homogenization conditions. Pepper puree was kept in an ice bath to prevent loss of antioxidant activity. A 20 g sample of fruit pulp was then filtered through 4-layers of cheesecloth. The filtrate was further clarified by centrifugation at 3000 x *g* for 10 min at 4 ^{o}C. The clear supernatant was used for the determination of antioxidant activity according to the method of Re and others (1999). Thus, ABTS radical cation decolorization caused by the test samples was monitored by spectrophotometer (Shimadzu, Model 1700, Japan) at 734 nm. The reaction mixture contained 2 mL potassium persulfate oxidized ABTS solution in phosphate buffered saline (PBS) at pH 7.4 and 2.5, 5 or 7.5 μL of extract (or 20 μL of Trolox [0.0045-0.03 μmol in reaction mixture] to prepare the standard curves). The decrease in absorbance of each sample was monitored for 6 min and tests were conducted three times at each sample volume. The results were calculated as area under the curve (AUC) values and expressed as μmol Trolox/kg fresh weight (fw) of peppers. To calculate the AUC, the percent inhibition/concentration values for the extracts and Trolox were plotted separately against test periods. The ratio of the areas of curves for extracts and Trolox was used to calculate the AUC value.

2.2.2. Determination of Total Phenolic Compounds

Total phenolic compunds were determined with Folin-Ciocalteau reagent using the method of Singleton and Rossi (1965). Gallic acid was used as standard. Homogenates were prepared as described for antioxidant activity determination. After centrifugation, the clear supernatant was collected and total phenolic content was measured spectrophotometrically. The results were expressed as mg gallic acid equivalents/kg fw (fruit weight) of peppers.

2.2.3. Determination of Vitamin C Content

Ascorbic acid was quantitatively determined according to the AOAC 967.21 titrimetric method using 2,6-dichloroindophenol as reactive substance (Augustin 1994). A 100g pepper sample without seeds was homogenized with 115 mL acetic acid-metaphosphoric acid extraction solution for 2 min at low speed in a Waring blender at 4 ^{o}C. Then, 35g sample was taken and diluted to 100 ml with extraction solution. Homogenate was filtered through filter paper. A 15 ml sample was put in a flask and titrated with dye solution. For each pepper extract, the vitamin C content of three replicate samples was measured. The results were expressed as mg ascorbic acid/kg fw of peppers.

2.3. Statistical Analyses

Total water soluble antioxidant activity, ascorbic acid and phenolic contents of the pepper fruits were analyzed using analysis of variance (ANOVA) and Fishers PLSD. Analyses were performed across all cultivars and also across cultivars grouped by morphology/use type as explained in the Results and Discussion.

CHAPTER 3

RESULTS AND DISCUSSION

Forty three Turkish and non-Turkish pepper accessions were examined in this study (Table 4). Twenty five of the 29 Turkish lines were obtained from The National Germplasm Collection at AARI and represent the diversity of pepper accessions grown in Turkey. These lines included varieties which are grown throughout Turkey (Ayaş and Kale) and regional cultivars that are grown only in specific areas (Acı Biber from Gaziantep). Fourteen non-Turkish varieties including F_1 hybrids grown in Turkey (Apollo and Cecil) and standard cultivars grown throughout the world were also included. Most classes included both pungent (hot) and sweet pepper accessions with 15 hot and 28 sweet cultivars (Table 4). Peppers in this study were harvested at the mature green stage of development even though some peppers such as paste (Salçalık) peppers are consumed at the mature red stage. This was done to standardize the results so that comparisons across accessions could be made. Color of mature peppers are included in Table 4.

3.1. Total Water Soluble Antioxidant Activity

Significant variation in total water-soluble antioxidant activity was observed in the pepper cultivars. Activity ranged from 2565 to 18957 μmol Trolox/kg, a 7.4-fold difference (Table 5). Ege-91, Yalova Tatlı Sivri, Domat, Finli and Ayaş, which are all Turkish cultivars, were the five cultivars with highest antioxidant activities. All of these are Sivri types with the exception of Domat, which is a stuffing pepper. Mean antioxidant activity for all lines was 7470 ± 593 (SE) μmol Trolox/kg. Because many different methods are used to determine total antioxidant activity of fruits and vegetables, direct comparison of the results of the present study with those of other researchers is difficult. However, using a similar method, Pellegrini and others (2003) found that green chili peppers had a Trolox equivalent antioxidant capacity of 7.6 mmol/kg, a value which is similar to the mean water-soluble antioxidant activity (7.4 mmol/kg) of the cultivars used in this work.

Table 5. Antioxidant activity, phenolic content and vitamin C content for the pepper cultivars. Cultivars are ordered by total antioxidant activity rank. Rankings for other traits are also included. Values followed by different letters are significantly different at P<0.05 as determined by Fishers PLSD. Vitamin C content for Variegated Flash could not be determined (ND) because the fruit extract was purple.

Cultivar	**Antioxidant activity**		**Phenolic content**		**Vitamin C content**	
(location)	**(µmol trolox /kg) ± SE**	**Rank**	**(mg/kg) ± SE**	**Rank**	**(mg/kg) ± SE**	**Rank**
Ege-91	18957 ± 243 a	1	2724 ± 4.9 a	1	1519 ± 2.7 b	2
Yalova Tatlı Sivri Biber	17651 ± 250 b	2	1220 ± 4.5 no	21	1502 ± 2.7 b	4
Domat Biberi (Bursa)	14603 ± 640 c	3	1796 ± 2.1 f	6	1177 ± 7.6 gh	12
Finli Biber	13396 ± 118 d	4	2239 ± 8.9 c	3	1276 ± 18.4 e	7
Ayaş	12667 ± 101 e	5	1730 ± 3.7 g	9	964 ± 12.4 l	21
Çarliston Biber (Bursa)	12639 ± 108 e	6	1782 ± 19 f	8	1140 ± 48.1 i	15
Duğme Biber (Bursa)	10252 ± 576 f	7	1094 ± 8.6 q	26	1257 ± 4.4 ef	8
Arnavut Biber, sivri	9993 ± 152 fg	8	2185 ± 12.9 d	4	1098 ± 8.5 j	16
Menderes	9883 ± 63 fg	9	1925 ± 8.9 e	5	1164 ± 13.5 ghi	13
Arnavut Biber	9505 ± 180 gh	10	1440 ± 12.4 k	15	1631 ± 9.7 a	1
Sera Demre	9455 ± 107 gh	11	946 ± 1.2 vw	35	926 ± 4.4 mno	25
Variegated Flash	9126 ± 262 hi	12	2311 ± 11.3 b	2	ND	
Cecil RZ F1	8889 ± 60 ij	13	988 ± 7.5 u	33	522 ± 4.7 w	42
Şahnali Biber	8808 ± 156 ij	14	1578 ± 11.9 i	11	778 ± 24.3 s	32
Tatlı Kıvırcık Biber	8784 ± 144 ij	15	1394 ± 4.5 l	17	1198 ± 24.7 g	10
Acı Sivri Biber (Bursa)	8585 ± 141 j	16	1476 ± 7.5 j	13	1376 ± 11.6 d	6
Chile Negro	8515 ± 364 j	17	1790 ± 3.3 f	7	1088 ± 5.0 j	17
Yalova Biber	8369 ± 130 j	18	1691 ± 10.6 h	10	916 ± 8.8 nopq	27
Cherry Pick	7521 ± 101 k	19	1482 ± 9.8 j	12	1436 ± 20.0 c	5
Yolo Wonder 31-22	7338 ± 84 k	20	1232 ± 8.1 n	20	1519 ± 3.0 b	3
Cuma Ovası	6994 ± 77 k	21	1440 ± 13.8 k	14	943 ± 6.1 lmn	23
Charleston Belle	6295 ± 282 l	22	756 ± 9.8 z	41	778 ± 6.1 s	34
Apollo F1	6106 ± 86 lm	23	1110 ± 9.7 pq	25	778 ± 3.0 s	33
California Wonder	6068 ± 107 lm	24	764 ± 12.2 z	40	1153 ± 1.7 hi	14
Kandil Dolma Biber	5623 ± 104 m	25	896 ± 13 x	38	974 ± 5.9 l	20
Dolmalık	5622 ± 42 mn	26	1052 ± 7.7 r	27	627 ± 0.0 u	38
Acı Biber (Gaziantep)	5482 ± 161 no	27	1411 ± 7.4 l	16	1234 ± 22.9 f	9
Dolmalık Yeşil (Bursa)	5425 ± 131 no	28	1014 ± 6.2 st	31	945 ± 11.6 lm	22
Raspires F1	5308 ± 65 no	29	1233 ± 11.9 n	19	939 ± 4.5 lmno	24
Salçalık Biber (Gaziantep)	5033 ± 115 op	30	1204 ± 6.4 o	22	905 ± 4.8 opqr	28
Sweet Long Slim Red	4592 ± 132 pq	31	1202 ± 4.5 o	23	1178 ± 21.8 gh	11
Edison	4439 ± 9 q	32	925 ± 2.5 w	37	766 ± 2.6 s	35
Carolina Wonder	4435 ± 105 q	33	607 ± 3.8 β	43	649 ± 6.9 u	37
Yağlık Biber (Bursa)	4416 ± 17 q	34	1324 ± 6.1 m	18	921 ± 5.8 nop	26
333 Biber	4174 ± 42 qr	35	1024 ± 1.2 st	30	568 ± 10.3 v	39
Farya	3865 ± 132 rs	36	1052 ± 11.3 r	28	561 ± 6.8 v	40
Salçalık Biber	3858 ± 70 rs	37	956 ± 8.6 v	34	1075 ± 13.1 j	18
Yağlık Biber	3665 ± 167 rst	38	1118 ± 9.8 p	24	872 ± 16.1 r	31
Kale	3525 ± 206 st	39	1037 ± 7.7 rs	29	883 ± 2.5 qr	30

(cont. on next page)

Table 5. Antioxidant activity, phenolic content and vitamin C content for the pepper cultivars. Cultivars are ordered by total antioxidant activity rank. Rankings for other traits are also included. Values followed by different letters are significantly different at P<0.05 as determined by Fishers PLSD. Vitamin C content for Variegated Flash could not be determined (ND) because the fruit extract was purple (cont.).

Cultivar	**Antioxidant activity**		**Phenolic content**		**Vitamin C content**	
(location)	**(µmoltrolox /kg) ± SE**	**Rank**	**(mg/kg) ± SE**	**Rank**	**(mg/kg) ± SE**	**Rank**
Fiesta	3143 ± 57 tu	40	649 ± 5.4 α	42	885 ± 3.0 pqr	29
Salçalık Biber (Bursa)	2942 ± 52 uv	41	1011± 4.3 tu	32	714 ± 7.6 t	36
Yalova Yağlık	2720 ± 17 uv	42	926 ± 2.1 w	36	539 ± 4.6 vw	41
Yalova Çarliston 341	2565 ± 89 v	43	852 ± 9.8 y	39	1024 ± 2.8 k	19

When the peppers were grouped by type, it was clear that some types had significantly higher antioxidant activities (Table 6). Sivri types had the highest mean antioxidant activity closely followed by Süs types. Dolmalık and Çarliston types had intermediate levels while Salçalık types had the lowest mean level of antioxidant activity which was 2.8-fold lower than the mean for Sivri types. This result may be because the Salçalık types were not harvested at their consumption stage which is the mature red stage. Some types of peppers showed more variation for antioxidant activity among accessions (Figure 3). Thus, although only five Çarliston type cultivars were tested, this type showed the most variation with a 4.9-fold difference between the cultivars with the highest (Çarliston) and lowest (Yalova Çarliston 341) activities. Similarly, Dolmalık and Sivri types showed 4.6 and 4.1-fold differences in total antioxidant activity, respectively. In contrast, Salçalık and Süs pepper types had only 1.8 and 1.4-fold differences in activity, respectively.

In comparison with F_1 hybrids and standard varieties, some Turkish cultivars showed much higher antioxidant activities. For example, Çarliston Biber (Bursa) had at least 2.4-fold greater antioxidant activity than the other non-Turkish Çarliston types (Figure 3). The three Turkish Süs pepper types also had significantly higher antioxidant activities than the other three Süs cultivars.

Table 6. Mean values for antioxidant traits for pepper cultivars grouped by type and pungency. Within each column and grouping, values followed by a different letter are significantly different at P<0.05 as determined by Fishers PLSD.

Pepper Type	Number of cultivars	Mean antioxidant activity (µmol Trolox/kg) ± SE	Mean phenolics content (mg/kg) ± SE	Mean vitamin C content (mg/kg) ± SE
Sivri	12	10678 ± 1225 a	1630 ± 140 a	1145 ± 70.2 a
Dolmalık	14	6214 ± 755 bc	1017 ± 84 b	921 ± 72.8 ab
Süs	6	9152 ± 413 ab	1717 ± 191 a	1117 ± 204 ab
Çarliston	5	5710 ± 1786 bc	1188 ± 160 b	846 ± 119 ab
Salçalık	6	3773 ± 357 c	1090 ± 63 b	838 ± 76.0 b
Hot	15	8640 ± 769 a	1600 ± 110 a	1044 ± 88 a
Sweet	28	6844 ± 796 a	1163 ± 81 b	962 ± 55 a

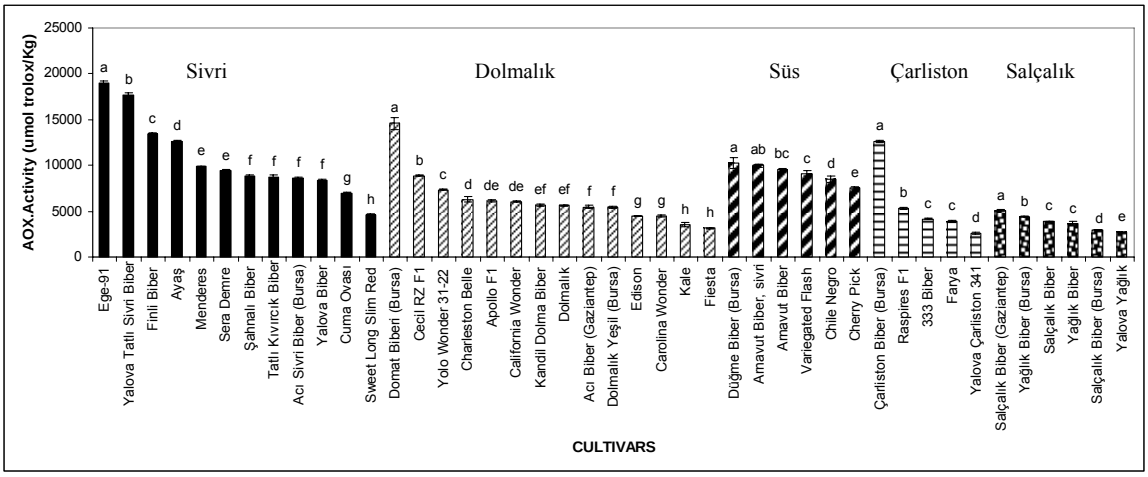

Figure 3. Antioxidant activities of the pepper cultivars grouped by type. Within each type, columns labeled with different letters are significantly different at P<0.05 as determined by Fishers PLSD.

3.2. Total Phenolic Compounds

Total phenolic content for the pepper cultivars ranged from 607 to 2724 mg/kg, a 4.5-fold difference in content (Table 5). This range of phenolic content was similar to that reported by other researchers (Antonious and others 2006, Chassy and others 2006). The five cultivars with the highest phenolic content included four Turkish cultivars and one non-Turkish variety. These cultivars were Ege-91, Variegated Flash, Finli, Arnavut Biber (sivri) and Menderes. These accessions may be useful as parents in breeding programs to produce high phenolics-containing varieties. All of these peppers except for Variegated Flash are Sivri, Turkish types. Variegated Flash is a Süs type and was one of

the two cultivars for which the fruit extract contained seeds. It has been reported that seeds are a source of phenolic compounds in pepper (Velioglu and others 1998). Therefore, the high phenolic content of Variegated Flash may be due to its seeds. However, the other cultivar that had seeds in its extract (Arnavut Biber) did not have a particularly high phenolic content. Mean phenolic content for all lines was 1316 ± 72 (SE) mg/kg. Süs and Sivri types had significantly higher mean phenolic content than the other three types of pepper (Table 6). Dolmalık and Sivri types showed the most variation in phenolic content with approximately 3-fold variation in these cultivars (Figure 4). The least variation was seen in Salçalık types.

As with antioxidant activity, some Turkish lines showed significantly higher phenolic content than the non-Turkish cultivars. For example, the Dolmalık types, Domat and Acı Biber (Gaziantep) had significantly higher phenolic content than Yolo Wonder and Apollo F1 (Figure 4). Çarliston Biber also had significantly higher phenolic content than the F1 hybrid Raspires and cultivar Farya.

The total phenolic content of pepper as measured by the Folin-Ciocalteu assay encompasses a wide diversity of compounds including simple phenols, phenolic acids, flavonoids, lignin precursors, capsaicinoids and reducing sugars (Howard and others 2000). Individual flavonoids that have been measured in pepper include luteolin, quercetin and kaempferol (Howard and others 2000, Chassy and others 2006) with recent studies aimed at detailed qualitative and quantitative characterization of pepper phenolic compounds (Marin and others 2004, Materska and Perucka 2005).

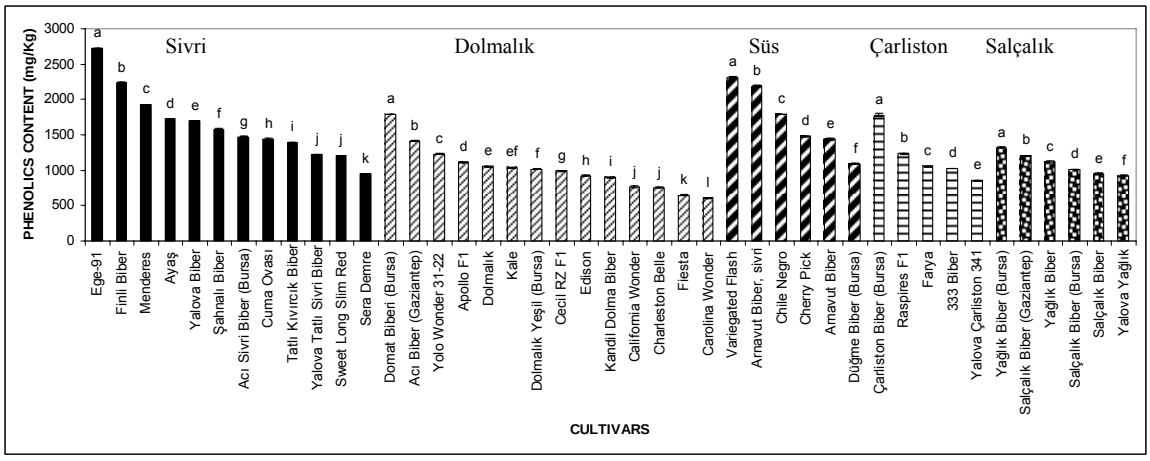

Figure 4. Total phenolic content of the pepper cultivars grouped by type. Within each type, columns labeled with different letters are significantly different at P<0.05 as determined by Fishers PLSD.

3.3. Vitamin C content

Vitamin C content for the peppers ranged from 522 to 1631 mg/kg, a 3.1-fold difference in content (Table 5). This range of vitamin C content was similar to that seen in other studies (Howard and others 2000, Marin and others 2004, Antonious and others 2006, Chassy and others 2006, Deepa and others 2007). A notable exception is the work of Guil-Guerrero and others (2006) which reported vitamin C contents of 100 to 380 mg/100g for ten pepper cultivars grown in Spain. The five cultivars with highest vitamin C content included three Turkish cultivars and two non-Turkish varieties. These lines were Arnavut Biber, Ege-91, Yolo Wonder, Yalova Tatlı Sivri Biber and Cherry Pick. Mean vitamin C content for the lines was 990 ± 47 (SE) mg/kg. Interestingly, 100g serving sizes of all but four of the cultivars assayed in this work supply 100% of the daily Recommended Dietary Allowance (RDA) of vitamin C, 60 mg (Table 5). Similarly, all but seven of the cultivars meet the more recently devised Dietary Reference Intake (DRI) for vitamin C which averages between 60 and 75 mg for adult women and men, respectively (USDA 2005). Sivri and Süs types had the highest mean vitamin C content while Salçalık types had the lowest content (Table 6). Dolmalık types showed the most variation in vitamin C content with a 2.9-fold range in concentration (Figure 5). The other pepper types had 1.5 to 2.0-fold variation in vitamin C content. Turkish Süs and Çarliston type pepper lines had significantly higher vitamin C content than non-Turkish cultivars. However, Yolo Wonder, a non-Turkish cultivar, had the highest vitamin C content of the Dolmalık types (Figure 5). Ascorbic acid content is dependent on the maturity stage and is higher in red pepper as compared to green pepper (Howard and others 2000, Gnayfeed and others 2001, Marin and others 2004, Navarro and others 2006).

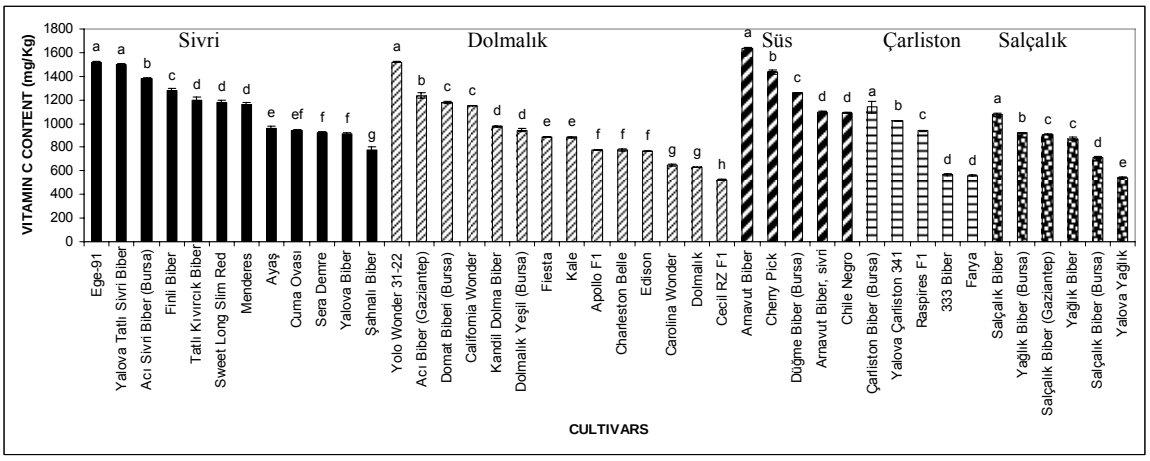

Figure 5. Vitamine C content of the pepper cultivars grouped by type. Within each type, columns labeled with different letters are significantly at P<0.05 as determined by Fishers PLSD.

3.4. Relationship Between Heat and Antioxidant Content

To determine the relationship between heat and antioxidant content, the 43 pepper lines were divided into two groups: hot and sweet peppers. For total antioxidant activity, phenolic and vitamin C content, hot types had higher values, however, this difference was only statistically significant (P<0.05) for phenolic content (Table 6). This result was expected because capsaicin is a capsaicinoid and gives peppers their heat. For example, Materska and others (2005) showed that hot cultivars are rich in capsaicinoids. Because these compounds are phenolic compounds, they affect total antioxdant content.

3.5. Correlation Between Antioxidant Traits

All three antioxidant traits showed statistically significant (P<0.05) correlations between each other. The strongest correlation was between total antioxidant activity and phenolic content (r = 0.71). There were also significant positive but weaker correlations between total antioxidant activity and vitamin C content (r = 0.51) and also between vitamin C and phenolic content (r = 0.31). The correlations between the different traits were also apparent when the pepper cultivars were ranked for each trait (Table 5). Thus, Ege-91, which ranked first for total antioxidant activity, also ranked first for phenolic content and second for vitamin C content. The correlations were especially obvious

when cultivars were ranked within each type (Sivri, Dolmalık, etc., Figures 3-5). Within their type categories, Ege-91 (Sivri) and Çarliston Biber ranked first for all three traits. Domat Biberi ranked first for total antioxidant activity and phenolic content and third for vitamin C content in the Dolmalık types. Similarly Salçalık Biber from Gaziantep ranked first, second and third for total antioxidant activity, phenolic and vitamin C contents, respectively. Such correlations were expected because the total antioxidant activity assay measured the activity of all water-soluble antioxidants including phenolics and vitamin C. Other researchers have also observed significant correlations between antioxidant traits. Significant positive correlations have been seen between total antioxidant activity and phenolic content in pepper (Deepa and others 2007), tomato (Hanson and others 2004), cranberry (Wang and Stretch 2001), and blueberry (Howard and others 2003). Antonious and others (2006) also reported a very strong correlation between phenolics and vitamin C content ($r = 0.97$) in pepper.

3.6. Conclusion

Variation in the chemical composition of a plant or plant part can be due to several factors including genotype, environmental conditions, cultivation practices, plant health, fruit maturity, and storage conditions. However, according to Chassy and others (2006) who examined the antioxidant content of organic and conventionally-grown tomato and pepper cultivars over three years, genotype is the most important factor determining phytochemical content in pepper. Indeed, the results presented here show that genotypic differences between cultivars resulted in significant variation for total water-soluble antioxidant activity, phenolic content and vitamin C content. This genetic diversity can be exploited for the development of populations for identification and genetic mapping of the loci controlling these traits in pepper and for the breeding of cultivars with improved antioxidant content. One strategy for breeding of such cultivars would be to select the best cultivar for all three traits, Ege-91, and to use it as a parent in crosses with different types of peppers. However, recovery of the special morphological characteristics (for example, shape, size, color and pungency) of each type would be difficult as these are multigenic traits. A simpler approach would be to select the best candidate parent within each type. Thus, Domat Biber would be a good candidate for improvement of total antioxidant activity and phenolic content of Dolmalık type

peppers while Ege-91 is the best starting material for alteration of all three traits in Sivri types. This study also showed that when Turkish cultivars were compared with non Turkish cultivars, some Turkish pepper cultivars (Ege-91, Yalova Tatlı Sivi Biber, Domat Biber, Finli Biber, Çarliston Biber) have higher total water soluble antioxidant capacity than the non-Turkish pepper cultivars. This means that Turkish pepper cultivars are good breeding material for these traits. Development and consumption of pepper cultivars with high antioxidant activity may help decrease the incidence of certain types of diseases in humans. It will also be interesting to see if these improved cultivars have increased tolerance to biotic and abiotic stress.

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PART 2

CHAPTER 1

INTRODUCTION

Capsicum pepper refers primarily to *Capsicum annuum* L. and *C. frutescens* L., fruits of these plants are consumed fresh and used in the manufacture of selected commercial products known for their pungency and color. *C. annuum* L. is a herbaceous annual that reaches a height of one meter and has white flowers. Its fruit varies in length, color and pungency depending upon the cultivar. This plant is cultivated primarily in Spain, Eastern Europe, North Africa, California and New Mexico. *C. frutescens* L. is a short-lived perennial with woody stems that reaches a height of two meters. It has two or more greenish-white flowers per node and extremely pungent fruit. This plant is cultivated in the tropics and warmer regions of the world.

Capsicum has been known since the beginning of civilization in the Western Hemisphere. It has been a part of the human diet since about 7500 BC (MacNeish 1964). Capsicum was domesticated at least five times by prehistoric peoples in different parts of South and Middle America. The five domesticated species are *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* R. & P. (IBPGR 1983, Poulos 1991, Pickersgill 1997).

Pepper is one of the most important commercially grown vegetables in Turkey. Pepper is ranked third or fourth among vegetable crops worldwide. There are many types of peppers that are utilized for different purposes, each with different quality requirements and traits required for successful production. The major traits under selection are yield, fruit color and color intensity (Lefebvre and others 1998, Kang and others 2001), size, shape (Ben Chaim and others 2001a, Rao and others 2003), degree of pungency, pericarp thickness, flowering time, fruit set at extreme temperature, growth habit and disease resistance (Caranta and others 1997b, Grube and others 2000c, Ben Chaim and others 2001b).

Plant diseases are significant to humans because they cause damage to plants and plant products. Plant diseases cause economic losses to growers, they result in

increased prices of products to consumers, and they destroy the beauty of the environment by damaging plants around homes, along streets, in parks and in forests. Plants are a primary source of nutrients for human beings and other living things as well. Some of those living things are phytopathogens including bacteria, fungi, viruses and nematodes. Fungal, bacterial and viral diseases are major limiting factors for pepper production. Plants have some physical defence mechanisms against these pathogens: their thick waxy cuticle and antimicrobial compounds. However, even these defenses may not protect plants from diseases as pathogens may break down all these barriers and infect the plant. Therefore, plants developed many other mechanisms to recognize these pathogens.

1.1. Plant Viruses and Plant-Virus Interaction

Recent studies indicate that viruses are among the most serious agents of vegetable diseases. Pepper production is limited by various virus diseases. The most wide-spread virus disease in Turkey is Cucumber Mosaic Virus (CMV), followed by Potato Virus Y (PVY) (Yildiz and others 1990). Tobacco Mosaic Virus (TMV) and Tobacco Etch Virus (TEV) are the other important viruses which cause damage to pepper. Although Pepper Mild Mottle Virus (PMMV) and Tomato Spotted Wilt Virus (TSWV) are seen in Turkey, they do not cause considerable damage.

CMV causes great damage to plants by reducing both the yield and quality. As a result, CMV is responsible for yield losses in many crops such as cucumber, tomato and pepper (Douine and others 1979, Brunt and others 1996, Kearney and others 1990, Daniels and Campbell 1992, Sikora and others 1998, Grube and others 2000b). CMV has a wider range of hosts and attacks a greater variety of vegetables, ornamentals and other plants than any other virus (Palukaitis and others 1992). Some of its hosts are peppers (*C. annuum* L.), cucurbits, tomatoes (*Lycopersicon esculentum* Mill.) and bananas (*Musa* L. spp.).

Plants basically have two different defence mechanisms. These are passive defence and active defence. When there is an infection, the pathogen releases elicitor molecules. These molecules interact with plant receptors. This triggers physiological and biochemical reactions and leads to induction of a signaling pathway (Cohn and others 2001). Reactive oxygen species (ROS), superoxide radicals (O_2) and H_2O_2 are produced because Ca^{+2} ions increase in the cytoplasm and activate an oxidative burst. As a result of this, transcriptional activation of plant defence genes is triggered. Cell wall thickening and cellular damage to

both host and pathogen occur because of these reactions. This is called passive defence. Besides this, plants also have an active defence mechanisms. The hypersensitive response (HR) is the most common mechanism associated with active defence. In this mechanism, cells which surround the primary infection site of the virus die because of a rapidly induced programmed cell death. This causes the formation of a visible necrotic local lesion. This mechanism is also called gene-for-gene resistance because induction of this response starts with specific recognition of the virus. Mostly this is based on matching gene products of the plant (resistance genes, *R* genes) and the virus (avirulence genes, *avr*). If host or pathogen lacks *R* or *avr* gene, the plant-pathogen interaction results in disease because there is no recognition between plant *R* proteins and pathogen *avr* gene products and the signal transduction mechanism that results in resistance is not triggered.

Plants also have two additional distinct systemic defence mechanisms. The first one is systemic acquired resistance (SAR). Salicylic acid (SA) accumulation induces this mechanism as a result of pathogen attack. Then, pathogenesis-related (PR) proteins are expressed (Mysore and Ryu 2004). The second systemic defence mechanism is induced systemic resistance (ISR). This defence mechanism is independent of SA but instead relies on jasmonic acid (JA) and ethylene which induce expression of antimicrobial peptides correlated with systemic resistance (Slater and others 2003). Ethylene, a plant hormone, also has a role in plant defence mechanisms (Mysore and Ryu 2004).

The activation of the signalling pathway leads to the induction of many 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 and others 2003).

1.2. Types of Resistance

Resistance to disease of plants can be divided into two major categories: nonhost resistance (NHR) and host resistance (Fraser 1990, Mysore and Ryu 2004). It is known that most plant species are resistant to most plant pathogens. Plants that are resistant to all isolates of a given pathogen species are called non-host plants (Gabriel and Rolfe 1990, Mysore and Ryu 2004). This defence mechanism provides durable protection to plant species against pathogens in the field, whereas the effectiveness of host resistance is characteristically transient.

Nonhost resistance comprises a variety of distinct mechanisms which includes the production of pre-formed toxins or barriers, or the lack of essential metabolites or signaling molecules required by the pathogen. Nonhost resistance is multigenic and the inactivation of any one component may not be sufficient to make a plant susceptible (Heath 1996, Yun and others 2003).

In nonhost resistance, plants are resistant to complete species of microbial invaders (Thordal-Christensen 2003, Jones and Takemoto 2004, Mysore and Ryu 2004). Inducible defence responses in nonhost plants comprise the synthesis and accumulation of antimicrobial reactive oxygen species, phytoalexins, and translation products from pathogenesis-related genes. It also comprise the localized reinforcement of the plant cell wall and hypersensitive, programmed cell death (Mysore and Ryu 2004). Pathogen recognition in non-host plants can occur by pathogen associated molecular patterns (PAMPs), which are also referred to as pathogen surface molecules, general or exogenous elicitors (Gomez-Gomez and Boller 2002, Montesano and others 2003, Nürnberger and others 2004, Mysore and Ryu 2004). Pathogen-associated molecular patterns, are small molecular motifs consistently found on pathogens. They are recognized by toll-like receptors and other pattern recognition receptors (PRRs) in plants and animals. They activate innate immune responses by identifying non-self molecules, protecting the plant from infection (Nürnberger and Lipka 2005). Bacterial Lipopolysaccharide (LPS) is considered to be the prototypical PAMP. Multiple R genes present in nonhost plants may simultaneously recognize their corresponding avr gene-encoded products and, as a result of this, activate the plant defence system (Mysore and Ryu 2004).

Host resistance to plant viruses has been more completely investigated than nonhost resistance. Host resistance, unlike nonhost resistance, is genetically characterized. Host resistance occurs when genetic polymorphism for susceptibility is observed in the plant taxon. For example, some genotypes show heritable resistance to a particular virus whereas other genotypes in the same gene pool are susceptible. In resistant individuals, the virus may or may not multiply to some extent but spread of the pathogen through the plant is restricted relative to susceptible hosts. Generally, disease symptoms are highly localized or are not obvious.

The distinction between resistance to the pathogen and resistance to the disease is important. Resistance to the pathogen typically leads to resistance to the disease. Resistance responses involving necrosis can sometimes be very dramatic, even lethal (Collmer 2000). In tolerance, the virus may move through the host in a manner that is indistinguishable from that in susceptible hosts, but disease symptoms are not observed. If the response is heritable, these plants are said to be tolerant to the disease, although they may be fully susceptible to the pathogen. This host response is very common in nature. It has been used to considerable benefit in some crops. For example, the control of cucumber mosaic virus (CMV) in cucumber. However, the genetic control of this response is typically difficult to study (Fraser 1990).

More recently, another category of host resistance has been identified which was first studied in TMV: systemic acquired resistance (SAR). This results in less susceptibility to later pathogen attack. This response can be activated in many plant species by pathogens that cause necrotic cell death. This can be either as part of the HR or as a symptom of disease (Ross 1961). Another induced defence mechanism to virus disease is virus-induced gene silencing (Baulcombe 2004). The transgenic method is also used in virus resistance. Using this method, transgenic tobacco plants expressing TMV coat protein (CP) were challenged with TMV and shown to be resistant (Ross 1961, Goldbach and others 2003). It is now possible to engineer resistance and tolerance to plant viruses using transgenes derived from a wide range of organisms including plant-derived natural R genes, pathogen-derived transgenes, and even nonplant and nonpathogen-derived transgenes (Dunwell 2000, Tepfer 2002, Nap and others 2003).

1.3. What is Cucumber Mosaic Virus?

Cucumber Mosaic Virus is the type species of the genus Cucumovirus in the family Bromoviridae (Gallitelli 2000, Roossinck 2002). 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. CMV contains three different positive-strand RNA genomes (RNA1, 2 and 3) plus a subgenomic RNA (RNA4) (Roossinck 2001). RNA1 and 2 encode components of viral replicase (Hayes and Buck 1990). RNA3 has two open reading frames (ORF) encoding the coat protein and the 3a protein, which is involved in cell-to-cell movement (Davis and Symons 1988). The coat protein is translated from subgenomic RNA4, which is trancribed from the minus-strand of RNA3 in virus infected cells (Schwinghamer and Symons 1977, Gallitelli 2000). Recently, a 2b protein has been found in plants infected with some strains of CMV. This 2b protein, which was translated from the subgenomic RNA of RNA2, was associated with host-specific long-distance virus movement (Ding and others 1995, Gallitelli 2000) and with suppressing the gene silencing reaction in host plants (Li and others 1999). Therefore, RNA1, 2 and 3 are needed to systemically infect the host plant (Gallitelli 2000). There is also RNA5 present in some CMV strains. It is thought that it may play a role in viral replication (Gallitelli 2000). CMV also has satellite RNAs (satRNA). Satellite RNA decreases accumulation of CMV in the tissue of infected plants (Garcia-Arenal and others 2000).

CMV strains can be classified into three major subgroups based on their sequence similarity and serological relationship. These are subgroup IA, IB and II (Roossinck 2001, Roossinck 2002, Garcia-Arenal and others 2000, Gallitelli 2000). Subgroup IA and IB strains are more frequent than subgroup II strains (Crescenzi and others 1993, Fraile and others 1997).

1.4. Virus Replication

The mechanism of CMV replication (Palukaitis and others 1992) is similar to other plant viruses but there are many specific details of the replication mechanism that are still unknown. When an aphid feeds on a plant, it introduces virus particles into

cells. The virus particles must come apart, releasing the viral RNAs. RNAs 1 and 2 of CMV are translated on cytoplasmic ribosomes (Barnett and Wood 1978) to synthesize protein components of the viral replicase that are involved in viral replication. This complex is bound to membranes (Hayes and Buck 1990). The viral replicase then becomes associated with the viral RNAs to form the viral replication complex. The replicase recognizes and binds to nucleotide sequences in the 3' end noncoding region common to each of the CMV RNAs. The viral replicase synthesizes (-) viral RNA from the (+) viral RNA templates of each of the CMV RNAs. At some point, the synthesis of (-) strands stops and the viral replicase synthesizes (+) strands. It is unknown what controls this switch over or the selection of (-) RNAs 1 and 2 versus (-) RNA 3 as template for (+) strand synthesis. These processes may be regulated by both host factors and the 1a protein.

Synchronized replication studies suggest that the rate of coat protein synthesis is slow during the initial stages of viral replication but becomes predominat later in infection (Gonda and Symons 1979). The regulation of viral protein synthesis probably occurs at the level of viral coat protein mRNA (RNA4) synthesis. The formation of virions is probably a spontaneous process involving the direct interaction between the CMV RNAs and the CMV coat protein (Chen and Francki 1990). It is unknown whether CMV RNA moves from cell to cell within the inoculated leaf as free RNA, as a nucleoprotein complex, or as virus particles. It is assumed that movement from leaf to leaf of the infectious RNA occurs via virus particles. Such virus particles move by way of the phloem to other leaves, where the virus particles somehow enter cells of other tissues. Aphids feeding on infected cells acquire virus particles with the cytoplasm and transfer the virus to other plants, reinitiating the cycle of infection.

1.5. Transmission and Symptoms

Cucumber mosaic virus (CMV) is well-studied. There are many isolates of CMV that infect various plant species and cause different symptoms (Palukaitis and others 1992). CMV is spread by aphids in a nonpersistent manner. The virus can be acquired by aphids from infected plants in less than one minute of feeding and can be instantly transmitted to a susceptible plant, with no latent period. Once a susceptible plant has become infected with CMV, insect vectors and humans during their cultivating and

handling of plants, especially at picking time, spread the virus to many more healthy plants. CMV can be transmitted by more than 80 species of aphids (Gallitelli 2000). The virus does not reproduce in its aphid vector and it is not transmitted to progeny aphids. The most common aphid species found transmitting CMV are *Myzus persicae* (the green peach aphid) and *Aphis gossypii* (the cotton aphid). These are also the species that are used for experimental transmission of CMV. CMV is either not transmitted or is transmitted at a very low frequency in the seed of many CMV-susceptible crops such as peppers, cucurbits and celery. CMV can also be transmitted by the parasitic plant dodder (*Cuscuta* spp.) (Francki and others 1979). Environmental factors are very important for the spread of CMV (Stimmann and Swenson 1967).

Cucumber mosaic virus affects plants by causing mottling or discoloration and distortion of leaves, flowers and fruit. Infected plants may be greatly reduced in size or they may be killed. Crop yields are reduced in quantity and are often lower in quality. Plants are seriously affected in the field as well as the greenhouse.

Young seedlings are seldom attacked in the field during the first few weeks. Most general field infections occur when the plants are about 6 weeks old and growing vigorously. Four or five days after inoculation, the young developing leaves become mottled, distorted and wrinkled. Their edges begin to curl downward. Later growth is drastically reduced. Plants appear dwarfed as a result of shorter stem internodes and petioles. Such plants produce few flowers and fruits. They have a bushy appearance. Fruits produced on the plant after the inoculation show pale green or white areas combined with dark green areas. Infection also causes distortion of the fruit.

1.6. Protection Methods

Control strategies for CMV are the same as for most other viruses. Cucumber mosaic in vegetables and flowers can be controlled primarily through the use of resistant varieties, propagation of virus-free stocks, eradication of virus-infected plants, crop rotation and suppression of vector activity (Goldbach and others 2003). Varieties resistant to CMV have been developed for many crops. In greenhouse, transplant crops should be isolated from other plants which harbor CMV such as cucumbers or lilies. Chemical treatment of insects is another way to protect crops from CMV. However, CMV is not always controlled as effectively as most other viruses because of two

reasons. (1) The broad host range of CMV compounds the problem of control, because so many crop and weed species can act as hosts or virus reservoirs. (2) Genetic resistance to CMV only exists in a few species. Alternative approaches based on the use of cross-protection, coat protein transgene-mediated protection and satellite RNA have also been described (Beachy and others 1990, Tien and Wu 1991).

1.7. Molecular Mapping

Molecular mapping is a promising strategy for studying and understanding traits with complex genetic control. Disease resistance can be difficult to study because of interactions of the genotype with the environment and different degrees of virulence of the pathogen (Young 1996). Using molecular maps to identify genomic regions associated with traits of interest can help the evaluation of these kinds of diseases.

Resistance can be controlled in plants as a qualitative or quantitative character. A qualitative trait is controlled by a single gene with a major phenotypic effect and segregates according to Mendelian ratios. It is a monogenic character. For example, some types of disease resistance in plants, eye color in Drosophila and flower color in pea are monogenic traits. Such traits have discrete phenotypic variation.

Unlike a qualitative trait, a quantitative trait is a polygenic character which is controlled by more than one gene with substantial environmental influence and has continuous segregation. This trait does not segregate according to Mendelian ratios. For instance, plant height, yield and salt tolerance are quantitative traits. Quantitative traits are controlled by quantitative trait loci (QTL). A QTL is a site on a chromosome containing gene(s) affecting a trait with continuous variation.

There are several requirements for QTL mapping. These are a large, appropriate mapping population in which markers and traits are segregating; a complete linkage map with neutral, codominant markers; linkage disequilibrium between marker and trait alleles; a reliable method to measure the quantitative trait; and statistical methods to establish significant associations between markers and traits (Edwards and others 1987, Lande and Thompson 1990, Edwards and Page 1994). Mapping populations are very important in QTL mapping. These populations can be F2 populations, backcross (BC) populations, recombinant inbred lines (RIL) and double haploids (DH) (Figure 1). Development of an F2 population is easy and many recombination events can be

obtained which is an important parameter for mapping. In an F2 population, all genotypes are possible (AA, Aa, aa) so we can estimate gene action: dominant/recessive or additive. Backcross populations are also good for trait transfer and are easy to develop. Therefore, they are good for breeding. However, in these populations we can not detect recessive QTLs because all genotypes are not possible. Individuals that are homozygous for the donor parent are absent in backcross populations. RILs are difficult to make because they need lots of time to produce but one important advantage of such populations is that more recombinations can be seen than in F2 populations. It is very difficult and sometimes impossible to make double haploids in many plants. Disadvantages of DHs are that all genotypes are not possible (no heterozygotes) and, therefore, we can not estimate gene action in these populations.

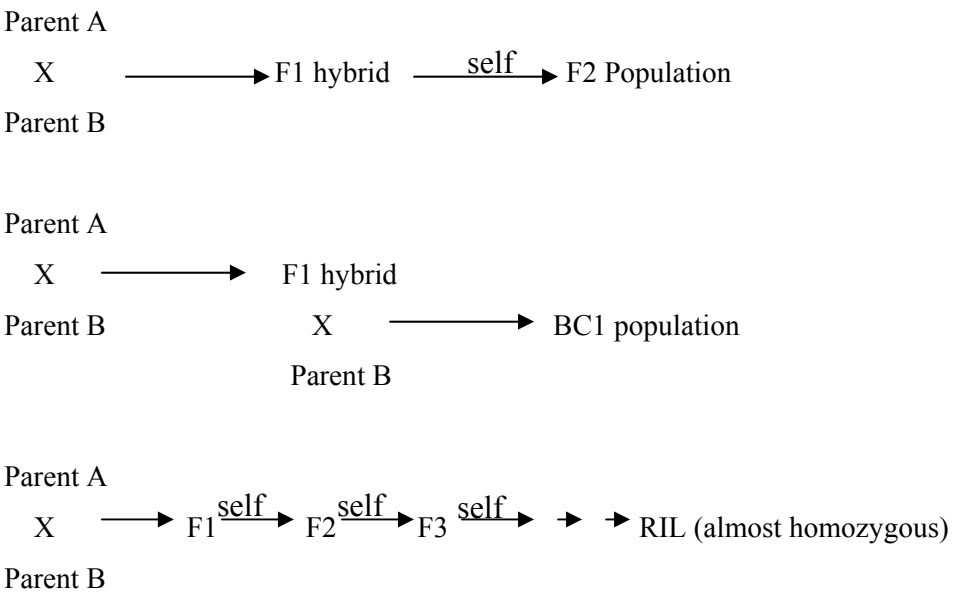

Figure 1. Construction of F2, BC and RIL populations for mapping.

In order to map gene(s) for disease resistance, phenotypic analysis (for example, ELISA values or disease severity ratings), marker analysis and statistical analysis have to be used to establish significant associations between markers (genotype) and resistance (phenotype). The association between phenotypic mean and marker genotype allow us to map genes controlling resistance. After separating the population into classes based on marker genotype (AA, Aa, aa) and determining the phenotypic mean for each genotypic class, a test has to be done (ANOVA) to see if there is a differences

among means. If so, it can be said that a QTL for that trait is linked to the marker. QTL analysis can help us to understand the number of genes controlling the trait of interest, the magnitude of effect of each QTL, gene action (dominant/recessive, additive, overdominance) and environment by QTL interaction.

Markers used in mapping can be classified mainly into two groups. These are morphological markers and molecular markers. Morphological markers are genes that code for a visible phenotypic change. Dwarfism or anthocyanin production are examples of morphological markers. Most morphological marker loci segregate as dominant or recessive alleles. Molecular markers show differences at protein, gene or DNA sequence level. Amplified Fragment Length Polymorphism (AFLPs), Simple Sequence Repeats (SSRs), Cleaved Amplified Polymorphism (CAPs) and Randomly Ampified Length Polymorphism (RFLP) are examples of molecular markers. Among these, AFLPs, SSRs and CAPs are PCR based marker.

1.7.1. Amplified Fragment Length Polymorphism (AFLP)

AFLP markers are one of the most recent innovations in genetic marker technologies (Vos and others 1995). The advantages of using AFLP markers include the fact that no sequence information is needed. They have a high multiplex ratio and thus require fewer primer combinations. They are insensitive to template DNA concentrations and they are highly reproducible (Vos and others 1995, Becker and others 1995, Breyne and others 1999). AFLPs can be modified according to the complexity of the genome and by altering various steps in the process. They have proven to be successful for organisms with very large genomes (Han and others 1999). Applications of AFLP are very versatile and include the construction of linkage maps, marker saturation at specific genomic regions, the analysis of genetic diversity and perhaps most importantly, cultivar identification.

AFLPs are based on fragment length polymorphism after selective PCR. Adapters are ligated to the ends of restriction fragments followed by amplification with adapter-homologous primers. Several hundred fragments can be amplified in this way. To reduce the number of amplification products, primer selectivity can be increased by adding additional arbitrary nucleotides to the 3'-ends of the primers. The amplicons are separated on a denaturing polyacrylamide sequencing gel or using an automated

sequencer which can detect length differences as small as one base-pair. AFLP analysis has the capacity to detect thousands of independent loci with minimal cost and time. AFLP primers can be easily distributed among laboratories by publishing primer sequences. All these unique characteristics make AFLP analysis an excellent method for the detection and study of genetic polymorphism in plant species.

AFLP has been used to make several molecular genetic maps in pepper. Livingstone and others (1999) developed an AFLP/RFLP map for a *C chinense* x *C. annuum* population. The map had more than 1,000 markers and allowed comparisons between tomato and pepper. Using a similar interspecific population, Kang and others (2001) developed another AFLP/RFLP linkage map with nearly 600 markers. AFLP maps for intraspecific populations have also been developed (Ben Chaim and others 2001b, Lefebvre and others 2002). In 2004, Paran and others used the data from six AFLP pepper maps to develop one integrated map.

1.7.2. Simple-Sequence Repeats (SSRs) – Microsatellites

Another promising technique is microsatellite marker analysis, also known as simple-sequence repeats (SSR). They are short, tandemly repeated DNA sequences (ex. AT, CAT, CGG). The number of repeat units can vary between individuals due to replication slippage and unequal cross-over during meiosis. SSRs have been especially useful for molecular genetic analysis because of their great abundance, ability to be "tagged" in the genome, their high level of polymorphism, and their ease of detection via automated systems (Rafalsky and Tingey 1993). SSRs normally occur in non-coding regions of the genome (SOL 2007).

Microsatellites do not have the high multiplex ratios that are found in AFLPs, and prior sequence knowledge is required to design primers. They are codominant and exhibit a much higher degree of polymorphism than do any other markers (Bowers and others 1996). This high degree of polymorphism is because the region of DNA that is being analyzed is a repeat motif and thus susceptible to changes in length due to slippage of DNA polymerase during replication. Since the regions do not contain coding regions, they are generally not under selection. Therefore, modification of these areas of the genome is not detrimental to the organism and thus quite abundant. This makes them a good tool to distinguish between closely related cultivars. Primers are designed

that flank the repeats and variations in lengths of repeat motifs of individuals are revealed by amplification and electrophoresis of the DNA within the regions flanked by the primers.

Like AFLP, SSRs have been used for pepper map construction. Lee and Kim (2003) added 46 SSR markers to the AFLP/RFLP map of Kang and others (2001). Additional pepper SSR markers were developed by Lee and others (2004) and Yi and others (2006). A total of 46 and 180 of these markers were mapped in a *C. annuum-C. chinense* F2 population of pepper, respectively.

1.7.3. Cleaved Amplified Polymorphic Sequences (CAPs)

CAPs are codominant markers which are analogous to RFLP markers. COSII markers are a type of CAPs marker which were developed from a set of single-copy conserved orthologous genes (COSII genes) in Asterid species (SGN 2007). In CAPs marker analysis, a region of DNA containing a restriction site unique to an allele is amplified and cleaved. In this technique genomic DNAs are amplified with sequence specific primers that are identified from Genbank, or constructed from cDNA clones or genomic DNAs. After amplification, DNA fragments are cut with a restriction enzyme and the products are separated on 2-4 % agarose gel in 1X TBE buffer. As a result of this separation, polymorphism among individuals can be detected.

Each marker system has some advantages and disadvantages when compared with others. AFLP, SSR and CAPs are all highly reproducible. Both AFLP and SSR can be very polymorphic. Milbourne and others (1998) found that SSRs were more polymorphic than AFLPs in potato, however, McGregor and others (2000) observed the opposite result. SSRs and COSIIs are codominant markers but AFLP markers are usually scored as dominant. For AFLP and COSII markers high quality DNA is needed. Sequence information is needed for SSRs and COSII markers but not for AFLP. A COSII map for pepper is currently under development (S. Tanksley, personal communication).

1.8. Previous Studies About Mapping Virus Resistance in Pepper

Many single genes and quantitative trait loci (QTL) which confer resistance to various plant pathogens (virus, bacteria, nematode, fungus and insect) have been mapped in solanaceous species (Pillen and others 1996). Mapping in related species allows an understanding of whether the same genes confer resistance to the same pathogen in different hosts.

The identification and mapping of disease resistance loci is a first step toward map-based cloning of these loci and can be used to identify tightly linked markers for use in marker-assisted selection. For example, QTL have been identified and mapped for resistance to *Phytophthora capsici* (Lefevbre and Palloix 1996), PVY (Caranta and others 1997b) and CMV (Caranta and others 1997a). Caranta and others (1997a) mapped 138 markers and detected three genomic regions affecting CMV resistance on pepper chromosomes Noir, Pourple and linkage group 3. Grube and others (2000a) studied the genomic position of disease resistance genes and homologues of these genes in tomato, potato and pepper. Caranta and others (2002) analyzed 101 doubled-haploid lines derived from F1 hybrids of *C. annuum* "Vania" (resistant to CMV) and *C. annuum* "H3" (susceptible to CMV) for both CMV resistance and molecular markers. They mapped 184 markers (93 AFLP, 51 RFLP, 38 RAPD and 2 phenotypic markers) on 20 linkage groups (LGs).

1.9. Goals of Our Work

Molecular breeding techniques are methods that are used to develop disease resistant cultivars and thereby control plant diseases caused by viruses. The objectives of this experiment were to evalute and examine the mechanisms and genetic basis of resistance to CMV in pepper using molecular breeding techniques. In addition, a molecular markers were used to identify CMV resistance gene(s). Resistance genes can then be transferred to other lines with marker-assisted selection.

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant Materials

In this study, 1066 plants belonging to 76 F3 families from a cross of *C. annuum* cv. Jupiter (CMV-S) and *C. frutescens* (CMV-R) (Figure 2) were tested for their response to CMV infection. The seeds of peppers were germinated in 2x2 cm trays. Two weeks after germination they were transferred into 10 cm pots, put in a growth chamber and maintained at 22^0 C with 16 h light. Plants were mechanically inoculated at the 4-leaf stage with Fny-CMV which belongs to CMV subgroup I (Celebi-Toprak and others 2003). Frozen tissue of CMV inoculated tobacco was used as an inoculum source. Fny-CMV was obtained from Dr. P. Palukaitis (Dept. Virology, Scottish Crop Research Institute). The virus was propagated and maintained in tobacco using the mechanical inoculation technique.

Inocula were prepared by extracting sap from infected tobacco leaves using a mechanical grinder and diluting the sap 1:10 with phosphate buffer (1.47 mM KH_2PO_4; 8.1 mM Na_2HPO_4-anhydrous in 1 L of dH_2O, pH 7.4). Inocula were kept on ice and used within 1 hour. Pepper seedlings were inoculated by first lightly dusting plants with carborundum and then applying inoculum with a cotton swab on the upper side of two leaves per plant. Two tobacco plants and two CMV-susceptible pepper cultivars were also inoculated during the experiment to act as positive controls. Two pepper plants and two tobacco plants were mock inoculated with phosphate buffer only to serve as negative controls. Two small holes were punched in each inoculated leaf to identify inoculated leaves. All plants were shaded for one day before and after mechanical inoculation. Then plants were kept in a growth chamber under the conditions described above. One week after this proccess, plants were re-inoculated with the same method.

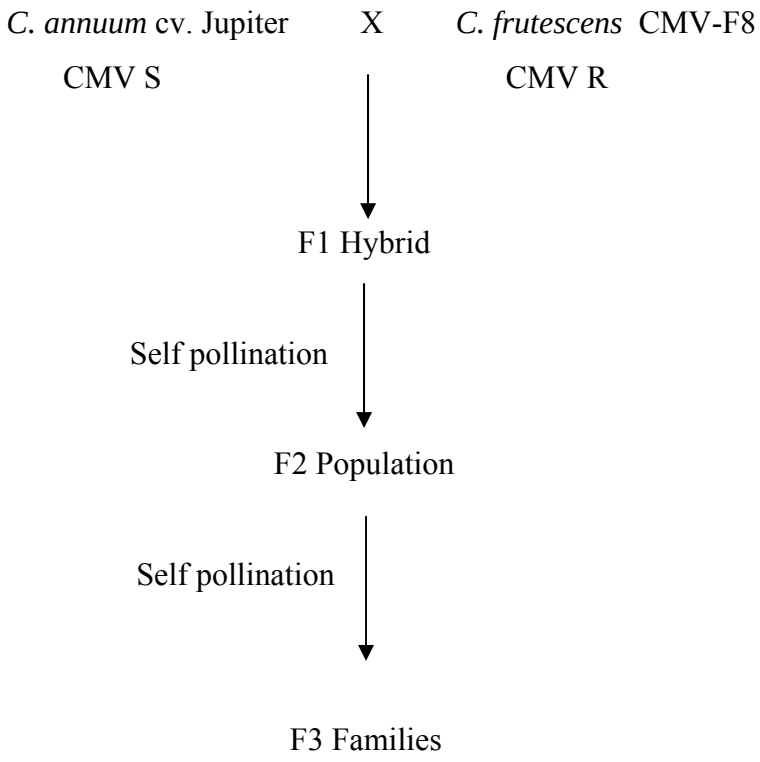

Figure 2. Cross of *C. annuum cv.* Jupiter (CMV-S) and *C. frutescens* (CMV-R) and developement of F3 families.

2.2. Phenotypic Characterization

Plants were scored visually for symptoms and samples were tested for virus by using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) of Clark and Adams (1977). Two weeks after inoculation, ELISA of inoculated leaves was done to detect whether inoculation was successful or not. Four and eight weeks after inoculation, ELISA of uninoculated leaves was done to see whether virus had moved to other parts of the plant.

2.2.1. Visual Score

Eight weeks after inoculation with virus, each plant was scored visually for symptoms. The first visual symptoms started to appear between 7 and 10 days after inoculation. Symptoms were scored according to their severity from 1 to 5 where 1 was no symptoms, 2 was slightly chlorosis, 3 was some chlorosis or mosaic, 4 was severe

mosaic and some leaf distortion and 5 was severe mosaic and leaf-fruit distortion (Figure 3).

Figure 3. CMV symptoms ranked from 1 to 5. 1: no symptoms, 2: slight chlorosis, 3: some chlorosis or mosaic, 4: severe mosaic and some leaf distortion, 5: severe mosaic and leaf distortion.

2.2.2. DAS-ELISA

Viral antigen was detected by using direct double-antibody sandwich ELISA, essentially according to Clark and Adams (1977). Microtiter plates (Nunc-Immuno Plates MaxiSorp F96, Bioreba) were coated with anti-CMV antibodies (Agdia peroxidase label) diluted 1:200 in coating buffer (1.59 g $NaCO_3$, 2.93 g $NaHCO_3$, 0.2 g NaN_3), tightly covered with stretch film and incubated for 12 to 16 h at 4^{o}C or for 4 h at room temperature in a humid box. Plates were washed 3 times with washing buffer (PBST buffer) using an ELISA washer (ASYS Hitech GmbH-Atlantis). Samples were taken from the upper 2 or 3 leaves and ground with roller press. Each sample was diluted 1:10 with extraction buffer (900 µl buffer + 2 drops plant sap extract) containing 20 g Tween 20, 20 g polyvinylpyrrolidone (PVP), 1.3 g Na_2SO_3-anhydrous, 0.2 g $NaNO_3$ and 2 g powdered egg (chicken) albumin-garade II, pH 7,4. Diluted plant sap extracts were added to each well (200 µl). For each plate, 4 wells were loaded with extraction buffer as buffer controls, 2 wells were loaded with positive control and 2 wells were loaded with negative controls. Positive and negative controls were provided in the Agdia Kit. Plates were tightly covered with stretch film and put in a humid box for 2 h at room temperature, then washed 3 times with washing buffer. Plates were then

loaded with conjugated anti-CMV polyclonal antibody (200 µl) diluted 1:200 in conjugate buffer. Plates were covered tightly and put in a humid box and incubated 2 h at room temperature. Plates were rinsed 3 times and loaded with p-nitro-phenyl-phosphate substrate solution (0.2 NaN_3, 97 ml Diethanolamine, 0.1 g $MgCl_2$ $6H_2O$) with 100 µl put into each well. Plates were covered with stretch film and incubated in humid box for 30 to 60 min and color change was evaluated both visually and photometrically with ELISA Reader (Thermo Labsystems-MultiskanEX) at 405 nm. Yellow color in the plate indicated infected samples.

2.3. Molecular Markers and Genotypic Characterization

Each plant's DNA was extracted using the Promega Wizard® Genomic DNA Purification Kit. In this study, AFLP, SSR (microsatellites) and CAPs markers were used to identify pepper resistance gene(s) for CMV. All of these methods are PCR based. Each marker type was first tested on parental DNAs to determine which markers were polymorphic. Then polymorphic markers were assayed on the mapping population using the appropriate enzyme for CAPs.

AFLP marker analysis was done using 13 primer combinations (Table 1). For AFLP, the first step was restriction digestion of genomic DNA: 5 µl DNA (~250 ng), 5 µl 5x reaction buffer, 2 µl *Eco*R I/*Mse* I and 13 µl sterile dH_2O were put in a 1.5 µl microcentrifuge tube, mixed and incubated 2 h at 37°C. Then to inactivate the restriction endonucleases, this mixture was incubated 15 min at 70°C. In the second step, 24 µl adapter ligation solution and 1 µl T4 DNA ligase were added to the DNA mixture and incubated 2 h at 20°C. This ligation mixture was diluted at a 1:10 ratio with TE buffer (10 µl mixture + 90 µl TE buffer). The third step was preamplification reaction. In this step, 40 µl pre-amp primer mix, 5 µl diluted template DNA from the previous step, 5 µl 10x PCR buffer plus Mg and 1 µl *Taq* DNA polymerase were added to a 0.5 ml microcentrifuge tube. After mixing and centrifugation, this mixture was amplified using the following profile: 94°C/30 s, 56°C/1 min, 72°C/1 min for 20 cycles; hold at 4°C. From this PCR reaction, 3 µl of product was taken and diluted with 147 µl TE buffer. The last step was selective AFLP amplification. In this step, two mixtures were prepared. The first mixture (mix 1) contained 2.5 µl labeled *EcoR* I primer, 1.5 µl *Mse* I primer (contains dNTP) and 1 µl sterile dH_2O. The second mixture (mix 2) contained 2

µl 10x PCR buffer plus Mg, 0.1 µl *Taq* DNA polymerase and 79 µl sterile dH_2O. To set up the PCR reaction, 5 µl diluted PCR product from the third step, 5 µl mix 1 and 10 µl mix 2 were put into a PCR tube and amplified using the following touchdown profile: 94°C/30 s, 65°C/30 s, 72°C/60s; then 12 cycles during which the annealing temperature was reduced 0.7°C for each cycle; finally, 23 cycles at 94°C/30 s, 56°C/30 s, 72°C/1 min.

Table 1. AFLP primer combinations used for mapping F2 population of *C. annuum* X *C. frutescens* population.

EcoRI primer	MseI primer
E AAC	M CAC
E AAC	M CTG
E ACC	M CAA
E ACC	M CAC
E ACC	M CTA
E ACT	M CAG
E ACT	M CTA
E ACT	M CTG
E AGC	M CAA
E AGC	M CAT
E AGC	M CTC
E AGG	M CAA
E AGG	M CTA

After the PCR, two dilutions were done. The first one was a 1:3 PCR product/dH_2O (7 µl PCR + 14 µl dH_2O) dilution. The second dilution was 1:10 using 27 µl Sample Loading Solution (SLS), 0.5 µl size standard 600 and 3 µl from the first dilution. Samples were loaded into a sequencer plate and covered with 1 drop of mineral oil. Then plate were put in a Beckman-Coulter Genetic Analysis System CEQ^{TM}8800 to run the samples. Frag-4 method was used for AFLP analysis (denaturation 90 °C, 120 sec. ; capillary 50 °C; injection 2.0 kV, 30 sec.; separation 4.8 kV, 60 min.). After this step the results were filtered using the parameters in Table 2 to eliminateunwanted or low quality samples.

Table 2. Filtering parameters for AFLP analysis.

Name	Operator	Value(s)
Analysis outcome	Not equal	Pass
Avg current	>	13
Avg current	<	6
Low D1 SNR	=	Yes
# of peaks D4	<	10
Current change	>	5

For SSR markers, Hpms markers were used in this study. For Hpms markers, PCR reactions were done as follows: 25 µl reaction mixtures contained 1 µl DNA (40-60 ng/µl); 2.5 µl 10X PCR buffer (1x); 0.5 µl dNTP (0.2 mM); 0.5 µl of each forward (F) and reverse (R) primer (10 pmol); 0.25 µl Taq polymerase (0.25 U) and 19.75 µl sterile dH_2O. PCR reactions were performed in a thermocycler, GeneAmp® PCR System 9700 (Applied Biosystems). The Hpms markers were amplified using the following profile: 94°C/5 min; 94°C/30 sec, 50 or 55°C/45 sec, 72°C/45 sec for 35 cycles; 72°C/5 min; hold at 4°C. Finally, all of the samples were run on 2-4% agarose gels in 1x TBE buffer for separation of fragments.

COSII markers are CAPs markers. For COSII (CAPs) markers, PCR reactions were done as follows: 25 µl reaction mixtures contained 1 µl DNA (40-60 ng/µl); 2.5 µl 10X PCR buffer (1x); 0.5 µl dNTP (0.2 mM); 0.5 µl of each forward (F) and reverse (R) primer (10 pmol); 0.25 µl Taq polymerase (0.25 U) and 19.75 µl sterile dH_2O. PCR reactions were performed in a thermocycler, GeneAmp® PCR System 9700 (Applied Biosystems). The COSII markers were amplified using the following profile: 94°C/5 min; 94°C/30 sec, 50°C/45 sec, 72°C/45 sec for 35 cycles; 72°C/5 min; hold at 4°C. After thermalcycling, the PCR products were checked for amplification by electrophoresis. Digestion with enzyme was done as follows: 15 µl DNA amplification product, 1.5 µl 10x digestion buffer (1x), 0.2 µl (100x) BSA (1x) (if required for the enzyme), 0.5 enzyme, 2.8 µl sterile dH_2O. Reactions were incubated at appropriate temperature depending on enzyme type at least 3-4 hours or overnight. Finally, all of the samples were run on 2-4% agarose gels in 1x TBE buffer for separation of fragments.

QTL mapping was done using simple linear regression and the QGENE computer program (Nelson 1997). A significance threshold of $P \leq 0.01$ was used for QTL identification.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. CMV Screening Results

F3 families derived from 76 F2 individuals from the cross *C. annuum* cv. Jupiter x *C. frutescens* were inoculated with CMV at the 4-leaf stage. *C. annuum* cv. Jupiter was inoculated as a positive control and the *C. frutescens* parent was also inoculated. In this way, 1066 plants were screened for their response to CMV. ELISA analysis was performed on inoculated leaves 2 weeks after inoculation and on uninoculated leaves 4 and 8 weeks after inoculation. Plants were also scored for disease symptoms 8 weeks after inoculation. Results of these tests are summarized in Table 3. Testing of the *C. frutescens* parent showed that this line was segregating for CMV resistance with only two of the lines showing complete resistance throughout the testing period (data not shown).

In general, ELISA of the inoculated leaves showed that these tissues contained the virus and that inoculation was successful. In some cases, however, the inoculated leaves did not contain significant titers of the virus 2 weeks after inoculation. F3 families 48-7 and 48-16 are examples of this (Table 3). These results may be explained in two different ways. First, it is possible that inoculation of all of the plants in these families was unsuccessful and that these plants were escapes. If the plants are escapes, we would expect that all of the plants in these families would appear resistant in later ELISA tests of uninoculated leaves. This was not the case as can be seen for the 4 and 8 week ELISA results for families 48-7 and 48-16 which show that plants in these families did contain virus (Table 3). A second explanation is that inoculation was successful but that virus was no longer present in the inoculated leaves or could not be detected. This seems to be the more likely explanation because the inoculated leaves often died and sometimes fell off of the plant within 2 weeks. As a result, very little sap was collected for some samples.

Table 3. Average ELISA results for inoculated leaves 2 weeks after inoculation, uninoculated leaves 4 and 8 weeks after inoculation and average visual scores of plants.

LINES	2 weeks ELISA (± SE)	4 weeks ELISA (± SE)	#R:#S Plants	8 weeks ELISA (± SE)	#R:#S Plants	visual score (± SE)	#R:#S Plants
48-1	0,223 ± 0,03	0,716 ± 0,27	12R:6S	0,589 ± 0,25	12R:6S	2,722 ± 0,28	2R:16S
48-2	0,933 ± 0,32	2,587 ± 0,34	0R:9S	2,601 ± 0,40	1R:8S	4 ± 0,33	0R:9S
48-4	0,826 ± 0,21	1,80 ± 0,29	3R:15S	1,245 ± 0,32	5R:13S	2,611 ± 0,28	3R:15S
48-5	0,412 ± 0,17	2,947 ± 0,20	1R:17S	2,980 ± 0,25	1R:17S	4,705 ± 0,14	0R:18S
48-6	0,331 ± 0,09	0,600 ± 0,16	11R:6S	0,483 ± 0,16	11R:6S	3,823 ± 0,23	0R:17S
48-7	0,089 ± 0,03	0,995 ± 0,24	1R:6S	0,896 ± 0,28	1R:6S	4,571 ± 0,20	0R:7S
48-8	0,366 ± 0,06	2,008 ± 0,28	2R:12S	1,685 ± 0,31	2R:12S	3,285 ± 0,28	0R:14S
48-10	0,263 ± 0,02	0,688 ± 0,25	10R:8S	0,628 ± 0,26	12R:6S	3,777 ± 0,13	0R:18S
48-11	0,402 ± 0,08	2,325 ± 0,33	0R:7S	1,481 ± 0,24	0R:7S	3,142 ± 0,70	2R:5S
48-14	0,393 ± 0,14	1,428 ± 0,30	4R:12S	1,133 ± 0,28	4R:12S	2,875 ± 0,27	1R:15S
48-15	0,197 ± 0,03	0,710 ± 0,25	11R:7S	0,644 ± 0,27	14R:4S	2,722 ± 0,32	3R:15S
48-16	0,053 ± 0,01	0,956 ± 0,36	10R:4S	0,913 ± 0,37	10R:4S	3,285 ± 0,32	0R:14S
48-17	0,047 ± 0,01	0,542 ± 0,41	6R:1S	0,487 ± 0,42	6R:1S	2,428 ± 0,57	2R:5S
48-19	0,163 ± 0,02	3,252 ± 0,06	0R:2S	3,103 ± 0,01	0R:2S	3 ± 0,01	0R:2S
48-20	1,083 ± 0,48	2,349 ± 0,30	0R:6S	1,493 ± 0,38	0R:6S	4 ± 0,25	0R:6S
48-21	0,329 ± 0,13	1,498 ± 0,33	7R:11S	1,374 ± 0,33	8R:11S	4,111 ± 0,17	0R:18S
48-22	0,386 ± 0,08	1,323 ± 0,48	2R:6S	1,014 ± 0,44	3R:5S	3,571 ± 0,36	0R:8S
48-23	0,283 ± 0,10	1,464 ± 0,31	4R:12S	1,397 ± 0,32	4R:12S	3,5 ± 0,31	1R:15S
48-24	0,347 ± 0,15	1,473 ± 0,21	4R:14S	1,473 ± 0,21	4R:14S	4,111 ± 0,25	0R:18S
48-25	0,177 ± 0,03	0,809 ± 0,28	11R:7S	0,809 ± 0,28	11R:7S	3,277 ± 0,26	1R:17S
48-27	0,130 ± 0,01	0,281 ± 0,17	14R:3S	0,281 ± 0,17	14R:3S	2,411 ± 0,31	4R:13S
48-29	0,120 ± 0,01	0,431 ± 0,22	13R:5S	0,431 ± 0,22	13R:5S	2,666 ± 0,24	1R:17S
48-30	0,161 ± 0,03	0,019 ± 0,02	2R:0S	0,019 ± 0,02	2R:0S	3 ± 0,01	0R:2S
48-31	0,177 ± 0,02	0,412 ± 0,21	12R:4S	0,412 ± 0,21	12R:4S	2,75 ± 0,23	1R:15S
48-32	0,642 ± 0,17	1,264 ± 0,28	4R:14S	0,828 ± 0,21	4R:14S	2,444 ± 0,31	6R:12S

(cont. on next page)

Table 3. Average ELISA results for inoculated leaves 2 weeks after inoculation, uninoculated leaves 4 and 8 weeks after inoculation and average visual scores of plants (cont.)

LINES	2 weeks ELISA (± SE)	4 weeks ELISA (± SE)	#R:#S Plants	8 weeks ELISA (± SE)	#R:#S Plants	visual score (± SE)	#R:#S Plants
48-34	0,694 ± 0,20	1,498 ± 0,30	3R:12S	1,145 ± 0,31	5R:10S	3,266 ± 0,34	1R:14S
48-35	1,191 ± 0,43	2,943 ± 0,15	0R:5S	2,443 ± 0,52	0R:5S	4,6 ± 0,24	0R:5S
48-36	0,480 ± 0,14	0,900 ± 0,23	6R:12S	0,469 ± 0,18	10R:8S	2,166 ± 0,37	6R:12S
48-37	0,271 ± 0,05	0,886 ± 0,40	8R:3S	0,839 ± 0,41	8R:3S	3,909 ± 0,31	0R:11S
48-38	0,202 ± 0,02	0,328 ± 0,16	14R:4S	0,237 ± 0,16	16R:2S	2,277 ± 0,22	3R:15S
48-40	0,283 ± 0,08	0,422 ± 0,22	15R:3S	0,350 ± 0,22	16R:2S	2,833 ± 0,23	1R:17S
48-41	0,124 ± 0,02	0,531 ± 0,16	1R:17S	0,247 ± 0,16	16R:2S	3,833 ± 0,12	0R:18S
48-42	0,321 ± 0,08	0,391 ± 0,10	0R:18S	0,159 ± 0,11	17R:1S	2,555 ± 0,21	3R:15S
48-43	0,153 ± 0,04	1,771 ± 32	0R:18S	1,673 ± 0,35	10R:8S	4,444 ± 0,16	0R:18S
48-45	0,803 ± 0,22	1,960 ± 0,35	0R:18S	1,163 ± 0,27	7R:11S	1,944 ± 0,24	6R:12S
48-46	0,194 ± 0,05	1,518 ± 0,26	0R:18S	1,347 ± 0,30	8R:10S	3,888 ± 0,29	0R:18S
48-47	0,567 ± 0,19	1,919 ± 0,27	0R:17S	1,185 ± 0,27	3R:14S	3,058 ± 0,38	2R:15S
48-48	0,365 ± 0,12	1,693 ± 0,35	1R:12S	1,347 ± 0,34	2R:11S	3,153 ± 0,33	0R:13S
48-49	0,170 ± 0,05	0,557 ± 0,28	12R:4S	0,557 ± 0,28	12R:4S	2,625 ± 0,27	2R:14S
48-50	0,119 ± 0,06	0,797 ± 0,37	8R:4S	0,797 ± 0,37	8R:4S	3,083 ± 0,33	0R:12S
48-51	0,132 ± 0,03	0,555 ± 0,29	9R:3S	0,555 ± 0,29	9R:3S	3 ± 0,40	2R:10S
48-52	0,197 ± 0,04	1 ± 0,24	8R:10S	1 ± 0,24	8R:10S	3,277 ± 0,33	1R:17S
48-55	0,179 ± 0,07	0,292 ± 0,17	13R:3S	0,292 ± 0,17	13R:3S	2,75 ± 0,23	1R:17S
48-56	0,442 ± 0,15	1,909 ± 0,19	1R:17S	1,909 ± 0,19	1R:17S	3,888 ± 0,42	3R:15S
48-57	0,908 ± 0,40	1,560 ± 0,32	2R:7S	1,560 ± 0,32	2R:7S	3,777 ± 0,40	0R:9S
48-59	0,254 ± 0,08	1,289 ± 0,28	3R:15S	1,133 ± 0,27	5R:13S	2,888 ± 0,22	1R:17S
48-60	0,101 ± 0,01	0,481 ± 0,18	11R:4S	0,314 ± 0,17	12R:3S	3,533 ± 0,21	0R:15S
48-61	0,108 ± 0,01	1 ± 0,32	4R:9S	0,803 ± 0,32	9R:4S	2,384 ± 0,43	5R:8S
48-65	0,346 ± 0,27	0,381 ± 0,20	6R:3S	0,162 ± 0,07	6R:3S	2,444 ± 0,24	1R:8S

(cont. on next page)

Table 3. Average ELISA results for inoculated leaves 2 weeks after inoculation, uninoculated leaves 4 and 8 weeks after inoculation and average visual scores of plants (cont.).

LINES	2 weeks ELISA (± SE)	4 weeks ELISA (± SE)	#R:#S Plants	8 weeks ELISA (± SE)	#R:#S Plants	visual score (± SE)	#R:#S Plants
48-66	0,614 ± 0,21	1,178 ± 0,28	5R:13S	0,816 ± 0,28	10R:8S	3,777 ± 0,15	0R:18S
48-67	0,270 ± 0,11	1,838 ± 0,50	2R:5S	1,701 ± 0,55	2R:5S	4,285 ± 0,36	0R:7S
48-68	0,992 ± 0,24	1,914 ± 0,28	3R:15S	1,554 ± 0,26	3R:15S	2,944 ± 0,31	2R:16S
48-70	0,120 ± 0,01	0,606 ± 0,29	13R:3S	0,559 ± 0,28	13R:3S	3,25 ± 0,28	1R:15S
48-73	0,475 ± 0,20	0,425 ± 0,21	8R:6S	0,328 ± 0,21	11R:3S	2,714 ± 0,28	1R:13S
48-74	0,499 ± 0,16	0,382 ± 0,19	14R:4S	0,303 ± 0,15	14R:4S	3,555 ± 0,16	0R:18S
48-81	0,329 ± 0,10	0,909 ± 0,29	8R:8S	0,469 ± 0,19	9R:7S	3,25 ± 0,21	1R:16S
48-83	0,215 ± 0,10	0,478 ± 0,24	14R:3S	0,277 ± 0,16	15R:2S	3,294 ± 0,16	0R:17S
48-84	0,438 ± 0,20	0,627 ± 0,21	11R:7S	0,573 ± 0,22	13R:5S	3,611 ± 0,20	0R:18S
48-87	0,280 ± 0,10	1,707 ± 0,19	1R:13S	1,693 ± 0,20	2R:12S	4,642 ± 0,19	0R:14S
48-88	0,436 ± 0,17	0,605 ± 0,19	12R:6S	0,506 ± 0,20	12R:6S	3,888 ± 0,17	0R:18S
48-89	0,518 ± 0,18	1,487 ± 0,36	2R:5S	1,470 ± 0,37	2R:5S	4,428 ± 0,36	0R:7S
48-90	0,382 ± 0,35	0,912 ± 0,40	4R:3S	0,899 ± 0,41	4R:3S	4,285 ± 0,28	0R:7S
48-92	0,093 ± 0,06	1,114 ± 0,25	7R:8S	1,032 ± 0,26	7R:8S	4,133 ± 0,23	0R:15S
48-93	0,027 ± 0,01	0,318 ± 0,24	7R:1S	0,28 ± 0,25	7R:1S	1,625 ± 0,53	5R:3S
48-100	0,050 ± 0,03	1,062 ± 0,24	9R:9S	1,048 ± 0,24	9R:9S	4,5 ± 0,12	0R:18S
48-102	0,019 ± 0,01	0,062 ± 0,01	3R:0S	0,029 ± 0,01	3R:0S	3,666 ± 0,33	0R:3S
48-103	0,355 ± 0,16	1,818 ± 0,32	3R:12S	1,065 ± 0,25	12R:3S	4,066 ± 0,11	0R:15S
48-104	0,049 ± 0,03	1,300 ± 0,66	2R:3S	0,929 ± 0,57	3R:2S	4 ± 0,31	0R:5S
48-105	0,076 ± 0,01	0,550 ± 0,47	5R:1S	0,521 ± 0,47	5R:1S	3,666 ± 0,42	0R:6S
48-111	0,134 ± 0,08	0,586 ± 0,26	13R:4S	0,546 ± 0,27	13R:4S	3,529 ± 0,21	0R:17S
48-115	0,031 ± 0,01	0,023 ± 0,01	4R:0S	0,041 ± 0,01	4R:0S	2,5 ± 0,64	1R:3S
48-116	0,105 ± 0,06	1,462 ± 0,31	7R:10S	1,390 ± 0,30	7R:10S	4,352 ± 0,17	0R:17S
48-118	0,434 ± 0,22	1,311 ± 0,31	4R:8S	1,200 ± 0,34	6R:6S	4,083 ± 0,35	1R:11S
48-119	0,216 ± 0,10	1,245 ± 0,30	5R:9S	0,762 ± 0,26	7R:7S	3,714 ± 0,26	0R:14S
48-123	0,259 ± 0,11	1,079 ± 0,31	7R:10S	0,900 ± 0,32	9R:8S	3,235 ± 0,27	0R:17S
48-128	0,019 ± 0,02	0,705 ± 0,30	11R:4S	0,618 ± 0,29	11R:4S	3,2 ± 0,31	0R:15S

By 4 weeks after inoculation, virus had multiplied in the plant and moved to uninoculated leaves. ELISA analysis of the F3 individuals at this stage showed that some plants were completely resistant and free of virus while others had very high titers of CMV with ELISA values greater than 3 in uninoculated leaves (data not shown). Average ELISA values were calculated for each F3 family and individual plants were characterized as resistant or susceptible based on their individual ELISA values (>0.2 indicated susceptibility) (Table 3). In general, F3 families showed segregation for virus resistance with both resistant and susceptible plants in each family. However, there were several examples of families (for example, 48-42 and 48-43) which contained only susceptible plants at 4 weeks after inoculation. A distribution histogram of the average ELISA values at this stage showed that the ELISA values were not normally distributed and that 10 of the familes (13%) had values near or less than the *C. frutescens* resistant individuals (Figure 4).

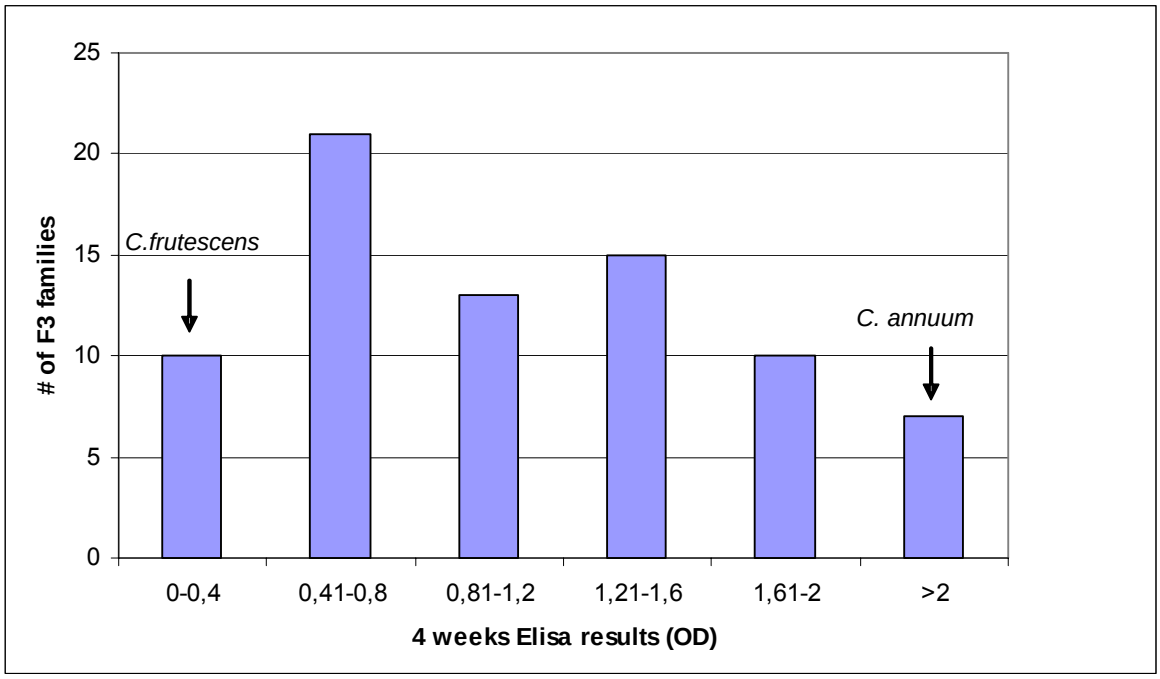

Figure 4. ELISA results for uninoculated pepper leaves 4 weeks after inoculation. Arrows show average ELISA values for the two parents.

ELISA values for the uninoculated leaves 8 weeks after inoculation with CMV were very similar to those obtained at 4 weeks as can be seen in Table 3. This is also seen in the significant positive correlation between the 4 and 8 week ELISA values (r =

0.94, p<0.0001). Numbers of resistant and susceptible plants were also similar but, in some cases, more resistant plants were observed at 8 weeks after inoculation. This result suggests that the plants' resistance mechanism may have been activated more slowly in these plants or that the plants were somehow able to outgrow the virus. In other words, that plant growth occurred more rapidly than virus multiplication and movement. This second explanation seems unlikely given what is known about the speed of these two processes and also the fact that pepper is not a fast-growing plant. The distribution histogram for the plants at the 8 week stage also showed skewing toward resistance with 15 (20%) of the families having ELISA values similar to the resistant parent individuals (Figure 5).

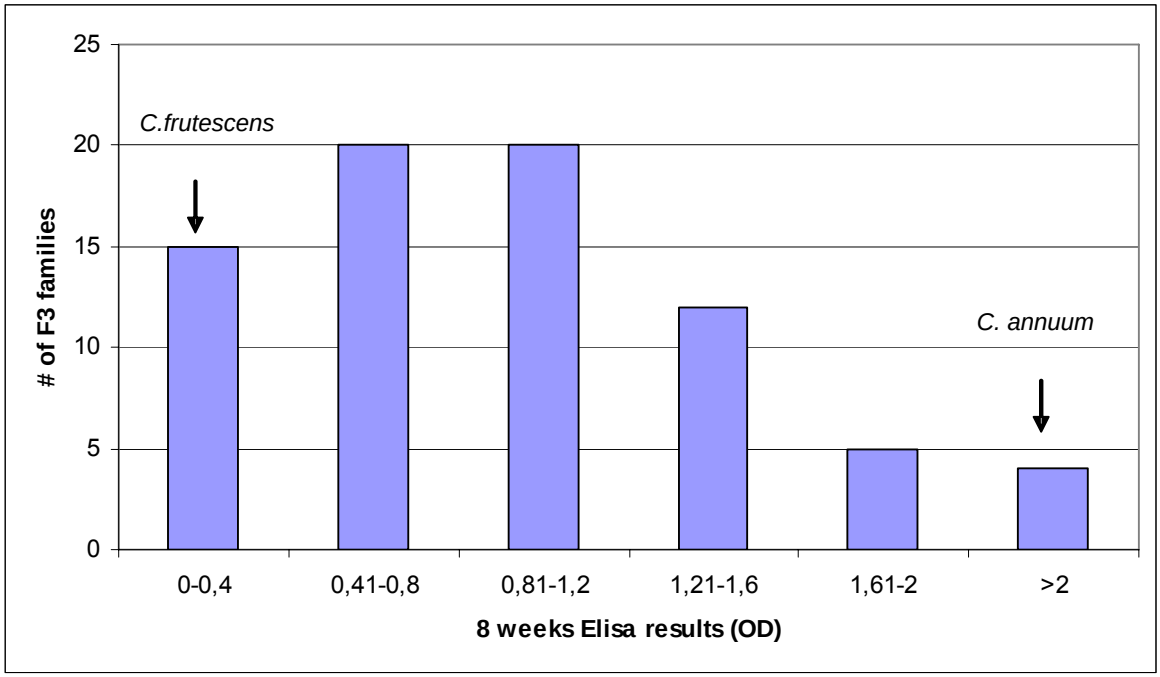

Figure 5. ELISA results for uninoculated pepper leaves 8 weeks after inoculation. Arrows show average ELISA values for the two parents.

Plants were assessed for virus symptoms 8 weeks after inoculation and were given a visual score. Many more plants were classified as susceptible based on these scores as compared with the results when ELISA values were used (Table 3). This is probably because nutrient stress and other physiological conditions can cause symptoms that can be confused with the symptoms of virus disease. As a result, there was a relatively poor correlation between visual scores and ELISA values (r=0.48, p<0.0001)

and the distribution histogram for the visual scores looked very different from those for ELISA values (Figure 6).

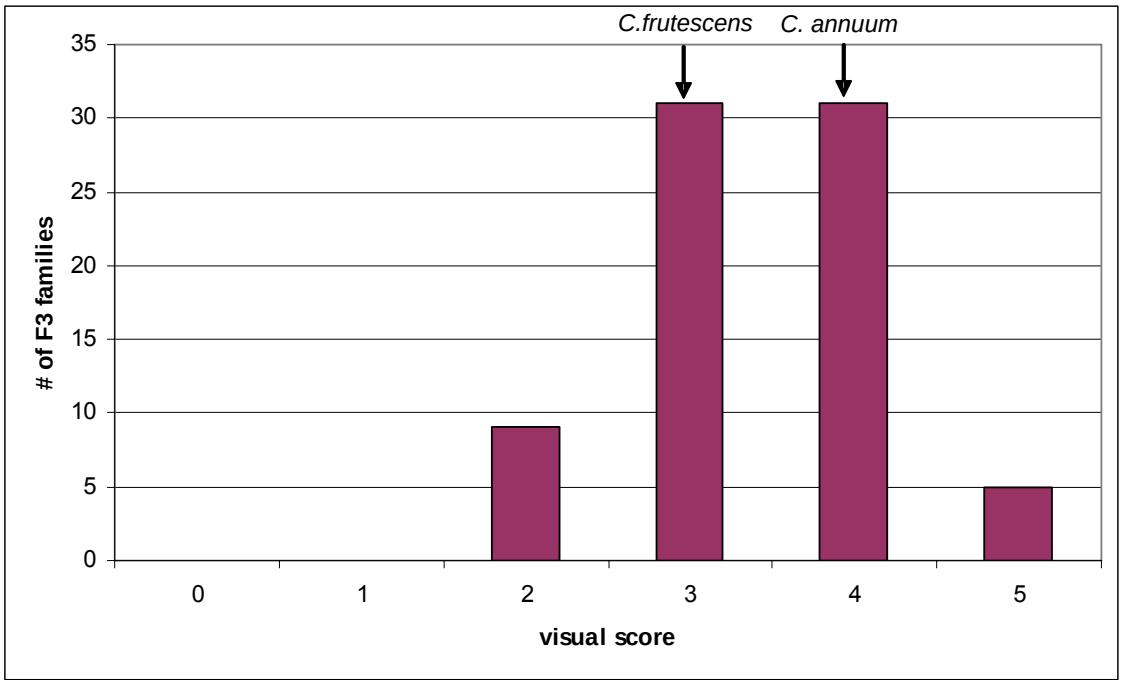

Figure 6. Distribution histogram of visual scores for F3 plants.

3.2. Inheritance

The results of virus testing of the F3 individuals suggest that CMV resistance in pepper is a quantitative trait as every type of response to the disease was seen from complete resistance to complete susceptibility. This is seen in the ELISA values for uninoculated leaves which ranged from 0 to more than 3. Another characteristic of quantitative traits is normal distribution. In this study, response to CMV was similar to a normal distribution (Figures 4-5). When examined at the family level, it appears that there may be one or two major genes involved in resistance as there were more resistant plants than expected (Figures 4-5). Therefore, these results suggest that QTL mapping is the best approach for the identification of CMV resistance genes in pepper.

3.3. Molecular Analysis

For CMV resistance gene identification, 76 F2 pepper lines were used. These lines were obtained from a cross between *C. annuum* cv. Jupiter and *C. frutescens.* A total of 13 AFLP primer combinations, 144 SSR and 287 COSII CAPs markers were tested for polymorphism.

3.3.1. AFLP Markers

In this study 13 AFLP primer combinations were used (Table 4). A representative picture of some of these fragments are shown in Figures 7-9. To check the system reproducibility, overlay graphs for the size standard were made and the samples that had fragments that did not overlap with the expected size fragments were excluded (Figure 8). A total of 2,095 bands were amplified for the 13 primer combinations. Primer combination E-ACC/M-CAC gave the fewest bands, 78, while combination E-ACT/M-CTA gave the most bands, 298. Of the total number of bands, 480 were polymorphic (Table 4). The number of polymorphic bands for primer combination ranged from 10 (for E-ACC/M-CAC) to 62 (for E-ACT/M-CTA). Primer combination E-ACC/M-CTA had the lowest rate of polymorphism, 8.5%, while combinations E-ACT/M-CAG and E-AAC/M-CTG had the highest rates, 37.7 and 35.0%, respectively. The average poymorphism rate for all primer combinations was 24.4%. For 12 of the 13 primer combinations that were tested, the majority of bands were specific for *C. annuum*. Overall, 297 *C. annuum*-specific and 183 *C. frutescens*-specific bands were identified with an average of 64.3 and 35.7% *C. annuum* and *C. frutescens*-specific bands, respectively. Sixteen bands were polymorphic for the parents but were not segregating in the F2 population and were removed from further analyses. Thus, 464 AFLP markers were used for QTL analysis. Chi square goodness-of-fit analysis indicated that 33.4% (155) of the markers fit the 3:1 segregation ratio expected for a dominant marker. The percentage of skewed markers is higher than that seen in other AFLP studies in pepper (Kang and others 2001, Lee and Kim 2003).

Table 4. Number of amplified and polymorphic bands for each AFLP primer combination. Numbers of polymorphic bands that were specific for each parent are also given.

EcoRI primer	MseI primer	Total # bands	# Polymorphic bands (%)	# C. a. specific bands (%)	# C. f. specific bands (%)
E AAC	M CAC	185	49 (26.5%)	28 (57.1%)	21 (42.9%)
E AAC	M CTG	140	49 (35.0%)	46 (93.9%)	3 (6.1%)
E ACC	M CAA	149	47 (31.5%)	15 (31.9%)	32 (68.1%)
E ACC	M CAC	78	10 (12.8%)	8 (80.0%)	2 (20.0%)
E ACC	M CTA	259	22 (8.5%)	13 (59.1%)	9 (40.9%)
E ACT	M CAG	130	49 (37.7%)	27 (55.1%)	22 (44.9%)
E ACT	M CTA	298	62 (20.8%)	41 (66.1%)	21 (33.9%)
E ACT	M CTG	154	44 (28.6%)	24 (54.5%)	20 (45.5%)
E AGC	M CAA	160	33 (20.6%)	18 (54.5%)	15 (45.5%)
E AGC	M CAT	134	34 (25.4%)	27 (79.4%)	7 (20.6%)
E AGC	M CTC	177	47 (26.5%)	24 (51.1%)	23 (48.9%)
E AGG	M CAA	97	17 (17.5%)	15 (88.2%)	2 (11.8%)
E AGG	M CTA	134	17 (12.7%)	11 (64.7%)	6 (35.3%)

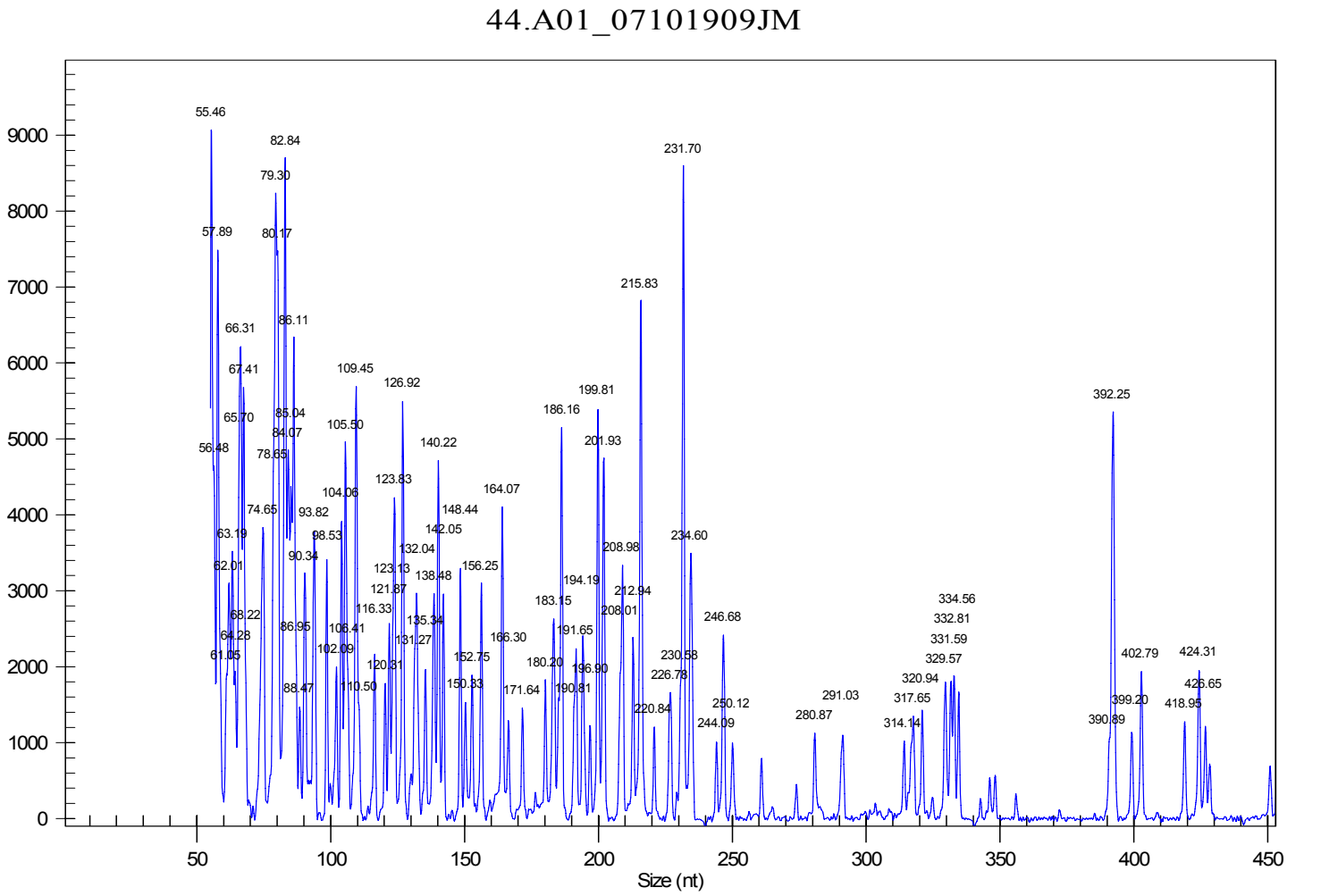

Figure 7. Example of an AFLP graph for primer combination E-ACT/M-CAG. Each peak represents an amplified fragment from the sample, *C. annuum* cv. Jupiter. Fragment sizes are given above each peak.

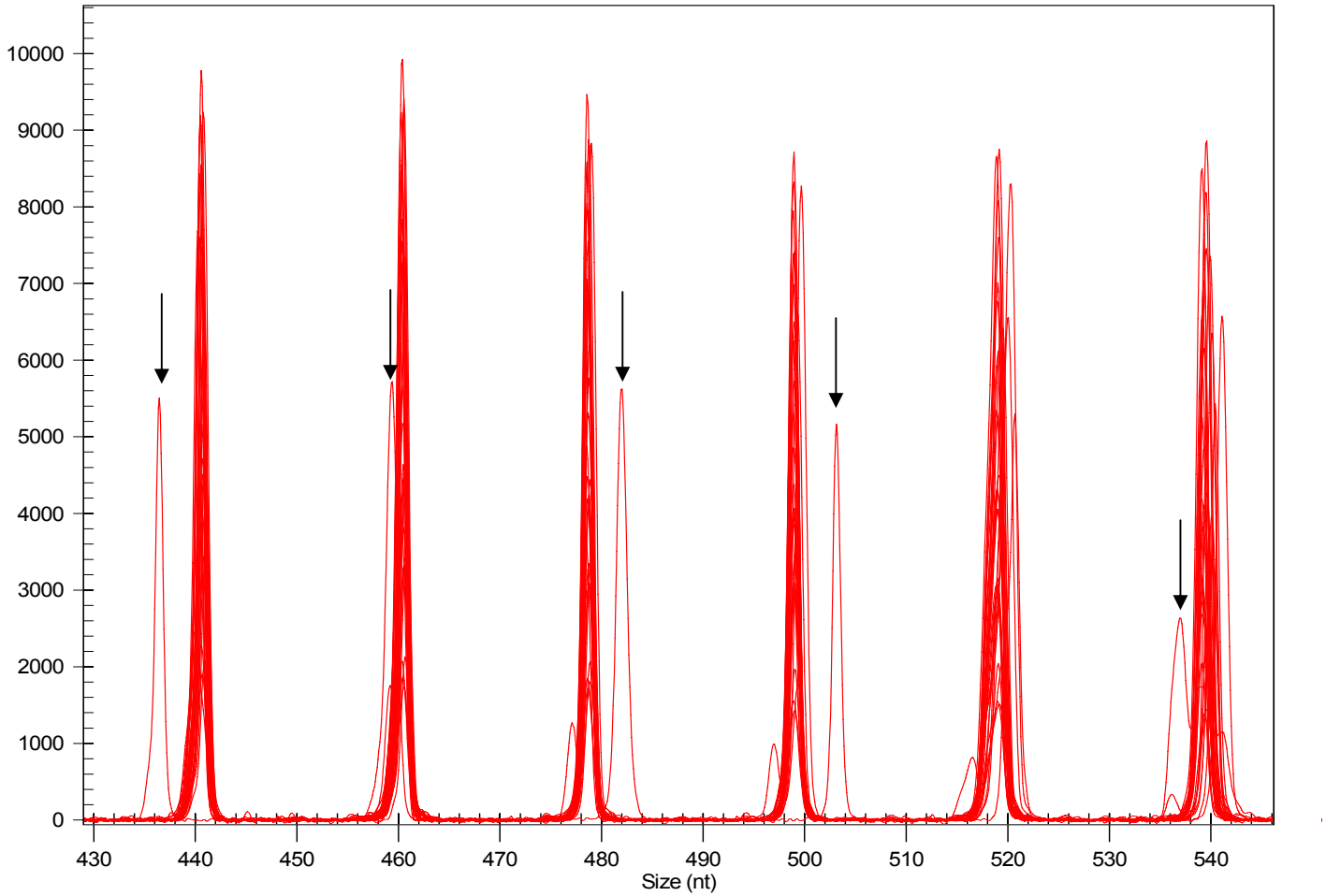

Figure 8. Overlay graph showing size standart traces for 65 samples using one primer combination. Arrows show fragments that do not overlap with the expected sized fragments. Samples with such fragments were eliminated from analysis.

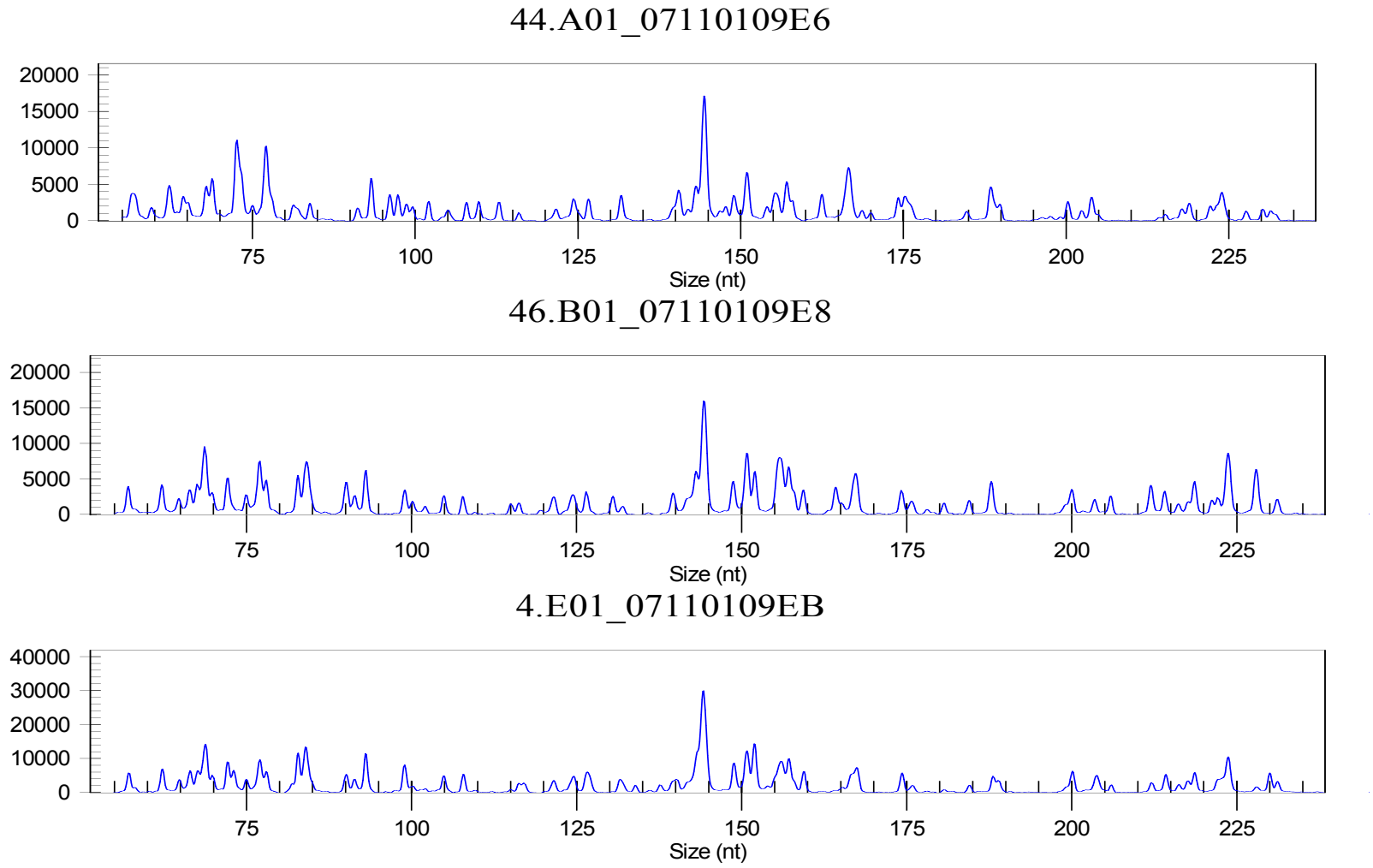

Figure 9. Stacked graph view showing AFLP results for three samples (44-*C.annuum* cv. Jupiter; 46-*C.frutescens*; 4-F2 individual) using primer combination E-AGG/M-CTA.

3.3.2. Pepper Microsatellite Markers

A total of 144 pepper Hpms SSR markers (Lee and others 2004) and 29 TC markers (TIGR) were surveyed for polymorphism using the parents of the mapping population, *C. annuum* cv. Jupiter and *C. frutescens* CMV R F8. Of these markers, 30 (21%) of them gave polymorphisms that could be detected on 2-4% agarose gels and were mapped in the population. Table 5 lists these markers and gives the expected sizes of amplification products for each parent. Figure 10 shows an example of an Hpms markers tested on the mapping population. Of the 30 Hpms markers that were mapped, 13 (43%) did not fit the 1:2:1 segregation ratio expected for codominant markers (Table 7). Approximately equal numbers of markers were skewed toward the *C. annuum* homozygous and heterozygous genotypes (six and five markers, respectively). Only two markers were skewed toward the *C. frutescens* homozygous genotype.

Table 5. List of Hpms markers found to be polymorphic between *C. annuum* cv. Jupiter and *C. frutescens*. Sizes of amplified fragments after PCR are also listed.

Primer Name	Size for C.frutescens	Size for C.annuum
Hpms1-5	280	420+300
Hpms1-281	150	120
Hpms1-3	200	210
Hpms1-41	210	200
Hpms1-155	160	180
Hpms1-43	190	220+200
Hpms2-24	200	210
HpmsAT2-20	200	180
Hpms2-21	250	300
Hpms2-23	300	350
HpmsE002	300	350
HpmsE005	450	400
HpmsE003	350	330
HpmsE049	500	450
HpmsE116	400	390
HpmsE129	480	500
HpmsE013	510	500
HpmsE130	500+490	470
HpmsE006	480	500
HpmsE149	180	200
HpmsE014	100	110

(cont. on next page)

Table 5. List of Hpms markers found to be polymorphic between *C. annuum* cv. Jupiter and *C. frutescens*. Sizes of amplified fragments after PCR are also listed (cont.).

Primer Name	Size for C.frutescens	Size for C.annuum
HpmsE017	190	200
HpmsE033	250	240
HpmsE016	180	170
HpmsE082	240	230
HpmsE126	210	190
HpmsE074	240	230
Hpms1-214	200	190
HpmsE144	320	350
HpmsE137	200	190

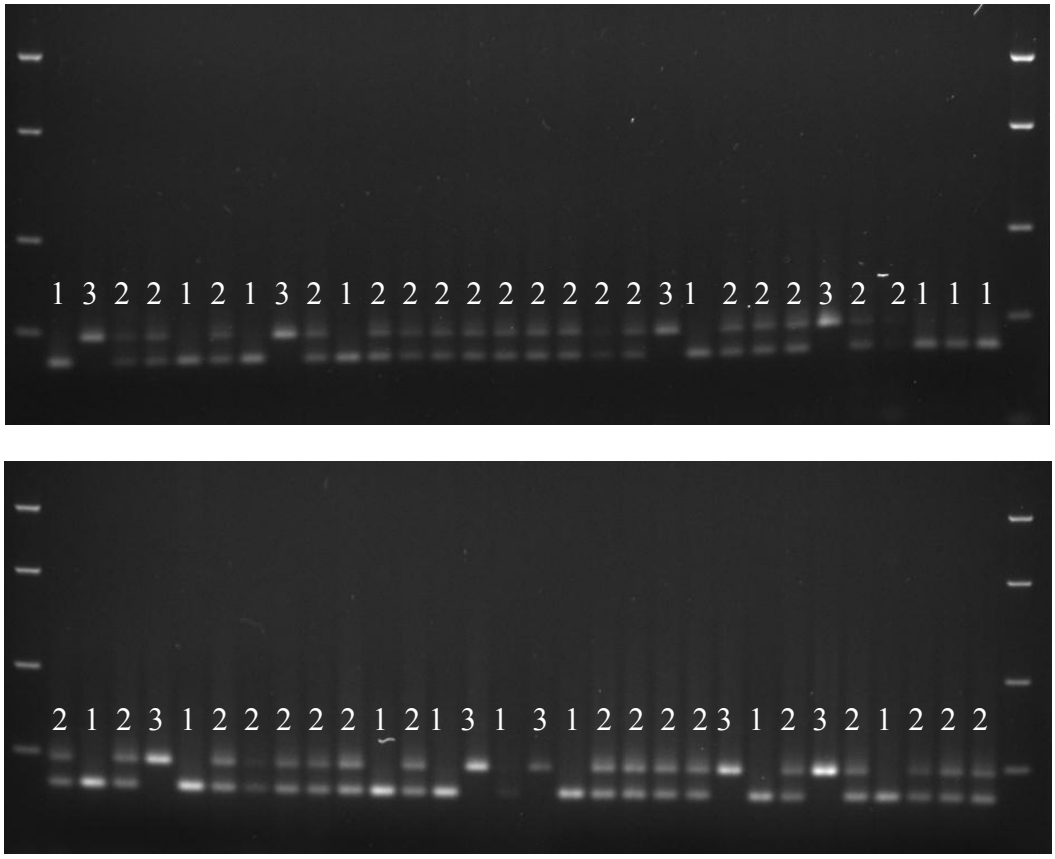

Figure 10. Example of a Hpms assay tested on the pepper F2 population. *C. annuum* (lane 2), *C. frutescens* (lane 3) and F2 progenies (lane 4 to end) were amplified with marker Hpms. First and last lanes are 100 bp ladder size standard. Samples are scored: 1 for homozygous *C. annuum*, 3 for homozygous *C. frutescens* and 2 for heterozygous progenies.

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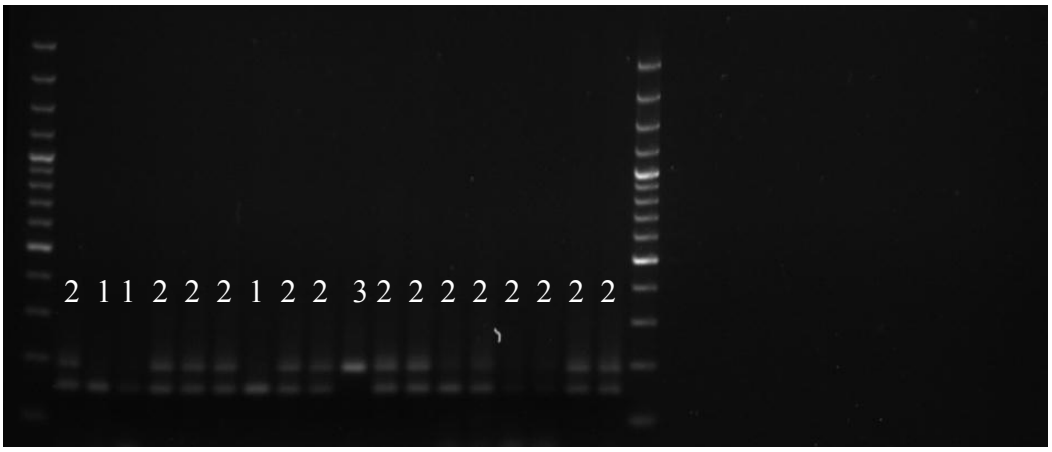

Figure 10. Example of a Hpms assay tested on the pepper F2 population. *C. annuum* (lane 2), *C. frutescens* (lane 3) and F2 progenies (lane 4 to end) were amplified with marker Hpms. First and last lanes are 100 bp ladder size standard. Samples are scored: 1 for homozygous *C. annuum*, 3 for homozygous *C. frutescens* and 2 for heterozygous progenies (cont.).

3.3.3. Tomato Microsatellite Markers

In addition to using markers designed for pepper microsatellites, tomato SSR markers were surveyed for polymorphism in the parental lines. A total of 26 tomato SSR markers (Frary and others 2005) were surveyed. However, because only one of these markers was polymorphic, the tomato SSRs were not mapped in the pepper population.

3.3.4. COSII CAPs Markers

COSII markers are CAPs markers designed based on consensus sequences shared by tomato and its relatives (SGN 2007). A total of 287 COSII markers were tested for polymorphism. Figure 11 shows an example of a parental survey for some COSII markers. A total of 60 markers (21%) were mapped in the population. Figure 12 shows an example of mapping results for one of the COSII markers. Table 6 lists these markers and gives the restriction enzyme the revealed polymorphism and the digestion product sizes. Of the 60 COSII markers that were mapped, 21 (35%) did not fit the 1:2:1 segregation ratio expected for codominant markers (Table 7). Seventeen of the markers were skewed toward the *C. annuum* homozygous genotypes and three of markers were skewed toward heterozygous genotypes. Only one marker was skewed toward the *C. frutescens* homozygous genotype.

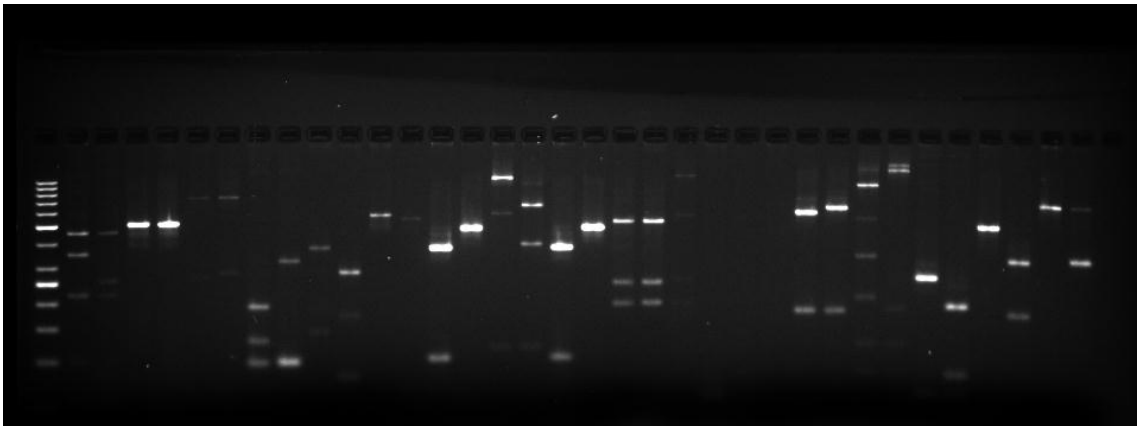

Figure 11. Survey of COSII markers for polymorphism. In each pair of lanes (*C. annuum* and *C. frutescens*), the PCR product for the given marker was digested with the listed enzyme. First lane is 100 bp size standard DNA ladder. Samples with an * are polymorphic.

Table 6. List of COSII markers and sizes of restriction products after cutting with indicated enzyme.

Primer Name	**Enzyme**	**Size for C.frutescens**	**Size for C.annuum**
1g07080	EcoRV	650	310
4g33250b	Hinf I	50	80
3g63190b	EcoRV	380+120	210+150+120
1g27385	Hinf I	250	190
5g13450	Cfo I	750	640
1g71810	Afl II	1100	620+500
1g55880	Dra I	580	400+200
2g37240	Taq I	500	300+200
5g60990	Taq I	650	700
3g23400	PCR	820	850
2g28490	Rsa I	900	840
1g46480	EcoRI	1000	1100
2g45910	Apo I	900	1100
3g13700	PCR	920	800
4g16580	Hind III	550+200	350+200
1g17410	Rsa I	480+250+210	480+350+210
1g30360	PCR	850+700	850+750
4g18593	Alu I	750	500
1g18270	Dra I	380	490
2g28250	Hinf I	500	300+180
3g06580	Rsa I	200	300

(cont. on next page)

Table 6. List of COSII markers and sizes of restriction products after cutting with indicated enzyme (cont.).

Primer Name	Enzyme	Size for C.frutescens	Size for C.annuum
1g67730	PCR	700	920
5g59960	Rsa I	550+250	450+250
3g56040	Acc I	520+210	800
4g37130	Rsa I	220	210
5g49970	EcoRV	750	500
5g38530	Rsa I	400	390
3g17040	Ase I	400	380
1g14790	Cfo I	1100	680+390
2g38020	Alu I	390+300+250	700+250
3g27200	Dpn II	280+220	350+220
1g78230	Rsa I	290+170	170+150
5g19690	PCR	850	920
5g27390	EcoRV	480	470
1g51160	Dra I	1000	600+380
1g78690	Hinc II	1050	550+500
5g52820	Ase I	800	400
3g62940	Cfo I	750+320	1100
4g26680	Nsi I	400	300
1g20575	Taq I	400	500
1g18660	Taq I	180	280
1g18640	Cfo I	280+140	280+120
1g65720	Cfo I	390	400
2g39690	Ssp I	580	480
2g46580	Taq I	400+310	400+350
5g60540	PCR	850	1000
4g16710	Taq I	550+350	350
4g30580	Rsa I	210+190	400
1g24360	Hae III	1050	650+400
4g35560	ScrF I	280+80	280+110
3g63190	EcoRV	350+120	210+150+120
5g01990	Sty I	250	120
5g07960	Rsa I	350+200+180	400+200+180
3g13180	Rsa I	350	320
5g57970	Dra I	830	700
3g09920	Hae III	180+110	140+110
3g02220	Rsa I	800+280	800+290
2g18050	Xba I	220	250
3g58790	Hae III	510	500
4g01880	ScrF I	400	380

Table 7. Chi Squareand *P*- valuesresults from a chi-square goodness-of-fit test for F2 progenies. *marks the statistically significant values (P<0.05).

Marker	**ChiSq**	**P**	**AA**	**Aa**	**aa**	**N**
Hpms1-3	9,02	0,011*	29	32	12	73
Hpms1-5	2,31	0,3151	18	44	14	76
Hpms1-41	4,52	0,1044	27	32	17	76
Hpms1-43	9,32	0,0095*	30	32	13	75
Hpms1-155	39,65	<0.0001*	2	65	9	76
Hpms1-214	0,29	0,865	15	32	18	65
Hpms1-281	2,21	0,3312	24	34	16	74
Hpms2-21	0,25	0,8825	20	38	17	75
Hpms2-23	4,74	0,0295*	26	40	5	72
Hpms2-24	0,71	0,7012	19	41	16	76
HpmsAT 2-20	8,5	0,0143*	18	49	9	76
HpmsE002	1,12	0,2899	15	42	18	76
HpmsE003	1,18	0,5543	22	33	20	75
HpmsE005	13,97	0,0009*	16	53	7	76
HpmsE006	2,84	0,2417	15	36	25	76
HpmsE013	0,63	0,4274	22	24	29	76
HpmsE014	4,31	0,0379*	17	38	9	65
HpmsE016	6,84	0,0089*	23	24	5	53
HpmsE017	7,29	0,0069*	19	39	7	66
HpmsE033	0,1	0,7518	12	26	12	52
HpmsE049	17,97	0,0001*	14	27	35	76
HpmsE074	7,06	0,0293*	25	31	10	66
HpmsE082	5,34	0,0693	23	31	10	64
HpmsE116	2,21	0,3312	12	37	20	69
HpmsE126	12,44	0,002*	27	31	7	65
HpmsE129	7,65	0,0218*	10	39	27	76
HpmsE130	0,01	0,995	18	35	18	71
HpmsE137	3,09	0,2133	19	35	10	64
HpmsE144	0,12	0,729	17	31	15	65
HpmsE149	1,59	0,4516	21	32	14	67
1g07080	3,12	0,2101	21	32	11	64
1g14790	3,55	0,1695	16	27	7	50
1g17410	9,92	0,007*	5	16	18	49
1g18270	1,48	0,4771	20	33	13	66
1g18640	3	0,2231	18	27	9	54
1g18660	2,79	0,2478	20	36	11	67
1g20575	11,32	0,0008*	28	22	12	65
1g24360	0,49	0,7827	12	26	10	48
1g27385	7,33	0,0256*	25	38	9	72

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Table 7. Chi Squareand *P*- valuesresults from a chi-square goodness-of-fit test for F2 progenies. *marks the statistically significant values (P<0.05) (cont.).

Marker	**ChiSq**	**P**	**AA**	**Aa**	**aa**	**N**
1g30360	1,09	0,5798	16	34	12	62
1g46480	10,13	0,0063*	25	22	11	58
1g51160	1,74	0,1871	20	35	6	62
1g55880	3,41	0,1818	24	35	13	72
1g65720	1	0,6065	15	25	10	50
1g67730	2,71	0,2579	18	22	16	56
1g71810	0,41	0,522	25	25	14	65
1g78230	7,52	0,0233*	25	23	17	65
1g78690	6,91	0,0316*	19	16	14	49
2g18050	1,57	0,2102	20	29	11	61
2g28250	0,69	0,4062	12	14	11	39
2g28490	5,21	0,0739	10	32	23	65
2g37240	1,35	0,2453	22	36	11	71
2g38020	3	0,0833	20	33	10	64
2g39690	3,31	0,1911	20	33	10	63
2g45910	6,44	0,04*	26	29	13	68
2g46580	19,8	0,0001*	24	16	5	45
3g02220	1,43	0,4892	16	25	10	51
3g06580	6,94	0,0311*	25	24	19	68
3g09920	0,72	0,6977	15	32	12	59
3g13180	6,74	0,0344*	17	39	7	63
3g13700	14,14	0,0009*	27	30	6	63
3g17040	0,17	0,9185	12	27	12	51
3g23400	8,5	0,0143*	25	36	8	69
3g27200	4,75	0,0293*	22	24	11	59
3g56040	0,02	0,8875	16	41	3	62
3g58790	3,67	0,1596	17	36	9	62
3g62940	9,95	0,0069*	10	44	9	63
3g63190	10,71	0,0011*	27	24	11	63
3g63190b	9	0,0111*	27	27	12	66
4g01880	2,96	0,2276	20	24	14	58
4g16580	16,52	0,0003*	25	20	6	51
4g16710	1,93	0,1648	15	0	0	44
4g18593	1,28	0,5273	18	33	12	63
4g26680	4,79	0,0912	19	39	9	67
4g30580	0,65	0,7225	17	33	13	63
4g33250b	48	<0.0001*	42	12	18	74
4g35560	4,75	0,093	23	30	11	64
4g37130	2,91	0,2334	20	37	11	68
5g01990	0,53	0,4666	8	35	18	62

(cont. on next page)

Table 7. Chi Squareand *P*- valuesresults from a chi-square goodness-of-fit test for F2 progenies. *marks the statistically significant values (P<0.05) (cont.).

Marker	**ChiSq**	**P**	**AA**	**Aa**	**aa**	**N**
5g07960	3,88	0,0489*	16	33	8	58
5g13450	8,93	0,0028*	28	27	13	69
5g19690	0,04	0,9802	13	25	12	50
5g27390	4,39	0,1114	20	27	9	56
5g38530	4,39	0,1114	22	26	12	60
5g49970	3,6	0,1653	15	36	9	60
5g52820	0,12	0,729	18	39	9	67
5g57970	4,36	0,113	22	25	13	60
5g59960	2,35	0,3088	17	37	11	65
5g60540	3,92	0,1409	15	17	7	41
5g60990	18,31	0,0001*	33	25	12	70

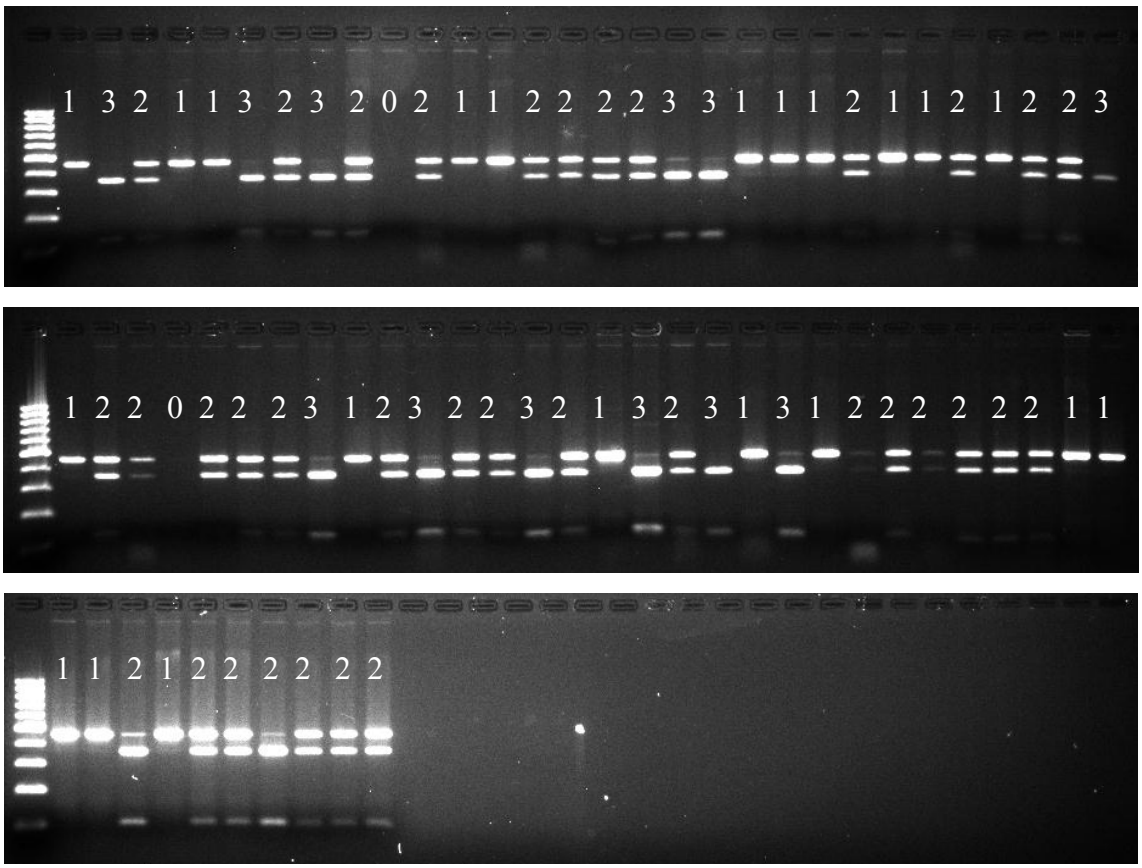

Figure 12. Example of a COSII assay tested on the pepper F2 population. *C. annuum* (lane 2), *C. frutescens* (lane 3) and F2 progenies (lane 4 to end) were amplified with marker At_1g 18270 and *Dra* I was used as restriction enzyme. First lane is 100 bp ladder size standard. Samples are scored: 1 for homozygous *C. annuum*, 3 for homozygous *C. frutescens* and 2 for heterozygous progenies.

3.4. QTL Analysis

QTL analysis was performed with the genotyped pepper F2 population and the phenotypic data from the F3 families. For this analysis a total of 570 markers (30 SSR, 60 COSII CAPS and 480 AFLP) were used. In all, 7 genomic regions linked to CMV resistance were detected (Table 8). For 57% of the loci, resistance was coming from *C. frutescens*. The *cmv*1 locus explained 18% of variance fot the trait and with the resistance allele coming from *C. frutescens*. The *cmv*2 locus explained 16% of variance for the trait. In this case, the resistance allele was coming from *C. annuum*. Additionaly *cmv*3 and *cmv*5 each explained 16% of variance with resistance coming from *C. frutescens*. *cmv*4 explained 14% of variance, however, resistance was coming from *C. annuum* parent. The remaining 2 QTLs explained 12 and 11% of the variance for the trait with resistance coming from *C. annuum* and *C. frutescens*, respectively.

Overall, the results confirm what has been seen in previous studies in pepper: resistance to CMV is a quantitative trait. In previously published works, Caranta and others (1997a) found 2 QTL regions explaining 24% and 19% of the phenotypic variation. Ben Chaim and others (2001b) detected 4 main QTLs explaining between 16% to 33% of the phenotypic variation. Caranta and others (2002) detected 4 QTLs explaining between 45% to 63% of the phenotypic variation. It was also seen that, as expected, the majority of resistance alleles and the were derived from the CMV-resistant *C. frutescens* parent. These alleles also had higher magnitudes of effect than the *C. annuum*-derived resistance alleles.Unfortunately, no CMV resistance QTL with a major phenotypic effect was identified in this study.

Table 8. QTLs detected in the pepper F2 population. The most significant markers are shown under Marker column. R allele column indicates from which parent resistance comes. *R*Sq is the percent of phenotypic variatiance explained. Traits column lists the traits for which each QTL was identified.

QTL	Marker	P-value	*R*Sq	Traits	R Allele
cmv1	AB120	0,0008	18%	**4WE,** 8WE, AVE	Cf
cmv2	CD110	0,0015	16%	4WE, 8WE, **AVE**	Ca
cmv3	GH73	0,0025	16%	**4WE,** 8WE, AVE	Cf
cmv4	FG63	0,004	14%	**4WE,** 8WE, AVE	Ca
cmv5	Hpms 1-214	0,0055	16%	**4WE,** AVE	Cf
cmv6	AB70	0,0059	12%	4WE, **8WE,** AVE	Ca
cmv7	CD120	0,0082	11%	**4WE,** 8WE, AVE	Cf

3.5. Conclusion

CMV is one of the most important viruses infecting pepper worldwide. It gives great damages to plant by reducing yield and quality. The objective of this study was characterization of peppers for CMV resistance. To this end, pepper populations were phenotypically and genotypically characterized. Pepper plants were mechanically inoculated with CMV and evaluated visually and serologically by ELISA. According to the results, CMV resistance was true resistance. A total of 480 AFLP fragments, 30 SSR and 60 COSII markers were found to be polymorphic and genotyped on the population. Genotypic characterization showed that CMV resistance in pepper has a multigenic character and is controlled by multiple Quantitative Trait Loci (QTL). As a result of genotypic characterization, 7 QTLs related to CMV resistance were found. For 57% of the loci, resistance was coming from *C. frutescens* allele. The results also showed that alleles for individual resistance genes can even be found in susceptible plants.

The next step for this study should be construction of a map for these QTLs, use of markers linked to resistance genes for marker assisted selection to transfer resistance to other susceptible varieties of pepper and determination of the molecular mechanism of resistance.

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