Tomato Breeders' Round Table
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The search for resistance to tomato leaf curl disease in Guatemala. Luis Mejia. University of Guatemala.

Breeding for resistance to whitefly-transmitted Geminiviruses with focus on South America. Francisco Morales. CIAT, Cali, Colombia.

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Distribution and genetic diversity of tomato-infecting geminiviruses in Brazil. Murillo Zerbini Dep. de Fitopatologia, Universidade Federal de Vicsa, Vicsa, MG, Brazil


Biotechnology and its regulations in Guatemala. Dr. Carlos Orozco, .

Development of the tomato Geminivirus problem and work done by ICTA in Guatemala. Porfirio Masaya/Luis Calderon. ICTA, Guatemala.

Attempts to elucidate the components of tomato flavor for improved breeding efficiency. Jay Scott. University of Florida, Gulf Coast Research and Education Center, Bradenton, FL.

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The PCR-based marker REX-1, linked to the gene Mi, can be used as a marker to TYLCV tolerance. Judith Milo. Hebrew University of Jerusalem, Rehovot, Israel.

In search of "Breeder Friendly" polymorphic PCR Markers Tightly linked to Frl. Ryan Walker. Brigham Young University, Provo, Utah.

Breeding for resistance to bacterial spot: prospects for marker assisted selection. David Francis, The Ohio State University, OARDC, Wooster, OH.


Visit to Sanarate (Douglas Maxwell and Luis Mejia field trial, we also will stop at a tomato commercial field).

Salama Station

Bitopo del Quetzal.
Molecular Characterization of Tomato-Infecting Geminiviruses in Central America and an Overview of the Tomato Crisis in the Dominican Republic. Douglas P. Maxwell, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, USA, 53706. (email:dum@plantpath.wisc.edu)

Tomato-infecting geminiviruses became a major constraint for tomato production in Central America in the late 1980’s. Many different management strategies have been evaluated, but in Central America, most tomatoes still become infected with geminiviruses. In order to assist in the development of management strategies, the tomato-infecting geminiviruses were molecularly characterized. A 1.3-kb PCR fragment, which included part of the rep gene, the intergenic region and part of the cp gene, was obtained for samples from Costa Rica, Nicaragua, Honduras, Guatemala, and Jamaica. These PCR fragments were cloned and sequenced. Seven begomoviruses were identified: Tomato yellow mottle virus and Tomato leaf curl Sinaloa virus in Costa Rica, Tomato severe leaf curl virus in Nicaragua; Tomato severe leaf curl virus, Tomato mosaic Havana virus, Tomato mild mottle virus in Honduras; Tomato severe leaf curl virus, Tomato golden mosaic virus, Tomato mosaic Havana virus, Tomato leaf curl Sinaloa virus, and Pepper golden mosaic virus in Guatemala; and Tomato mosaic Havana virus-[JM1] in Jamaica. Additionally, specific PCR primers were used to detect Tomato yellow leaf curl virus in Jamaica and Dominican Republic. Other research teams have detected Pepper Hausteco virus and Tomato leaf crumple virus in Central America. General PCR primer pairs and a general DNA probe of the cp gene are available for detection of begomoviruses. Specific PCR primer pairs and specific DNA probes were developed for detection for Tomato yellow mottle virus, Tomato leaf curl Sinaloa virus, Tomato severe leaf curl virus, Tomato mild mottle virus, Tomato mosaic Havana virus, Tomato golden mottle virus, Pepper golden mosaic virus, and Tomato yellow leaf curl virus. Evidence was presented that recombination is an important mechanism in geminiviral evolution.

Tomato yellow leaf curl virus (TYLCV) is a monopartite Eastern Hemisphere begomovirus, whereas all Western Hemisphere tomato-infecting geminiviruses are bipartite. In 1994, two groups showed that TYLCV was present in the Dominican Republic and this introduced virus caused losses of 65% in the processing tomato production. With the introduction of a host-free period in the Dominican Republic, tomato production returned to the levels occurring before the introduction of TYLCV. Other management strategies such as virus-free transplants, new insecticidal chemistries, and slightly resistant cultivars also contributed to this yield increase. As in the Dominican Republic, TYLCV caused major losses in tomato production in Jamaica. Interestingly, both in the Dominican Republic and Jamaica, TYLCV replaced the indigenous bipartite tomato-infecting geminiviruses. TYLCV has also become more important in Florida than Tomato mottle virus.

In conclusion, geminiviruses continue to be a major constraint to tomato production in Central America and efforts should be increased to develop cultivars for Central America, which have resistance to the diverse bipartite geminiviruses. Even though TYLCV has not been detected in Central America, as yet, these cultivars should also have resistance to TYLCV.
THE SEARCH FOR RESISTANCE TO TOMATO LEAF CURL DISEASE IN GUATEMALA

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The evaluation of tomato germplasm with tolerance to TYLCV has been conducted in eastern Guatemala, under heavy pressure from bipartite geminiviruses and no chemical protection against the whitefly vector, since 1998. This germplasm was obtained through the introgression of genes for resistance from different wild species. The process of selection during several consecutive growth cycles has allowed the production of several lines which show high levels of tolerance to the local geminiviruses. These lines are currently showing decreased symptoms of infection and greater yield than the susceptible commercial hybrid used as control. On average, the lines derived from the hybrid FAVI-9 (obtained from The Hebrew University of Jerusalem, Israel) with L. hirsutum as source of resistance, have larger fruit size; those derived from breeding lines TY-197 and TY-198 (obtained from The Volcani Center, Israel), with L. peruvianum as source of resistance have intermediate fruit size; and those derived from the population Pimper J-13 (obtained from INRA, France), with resistance originating from L. pimpinellifolium and L. peruvianum, have smaller fruits. Selected lines with resistance from different origins were screened with general and specific PCR primers and hybridization probes. These lines showed differential levels of infection with three geminiviruses known to be present in the area of the trial, tomato severe leaf curl virus (TSLCV), tomato golden mottle virus (TGMoV) and pepper golden mosaic virus (PGMV).
Breeding for resistance to whitefly-transmitted geminiviruses

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Introduction

The whitefly Bemisia tabaci Genn. (Homoptera:Aleyrodidae) has caused millions of dollars worth of crop losses in tropical and subtropical agricultural regions in the five continents of the world (Brown, 1994). Besides the direct damage caused by B. tabaci (plant nutrient loss; physiological disorders; honey dew excretions, etc), the sweet potato or cotton whitefly is an efficient vector of numerous geminiviruses (plant viruses consisting of two ‘twined’ or ‘geminate’ particles, encapsidating one or two single-stranded DNA genomes). The geminiviruses transmitted by B. tabaci belong to the genus Begomovirus (sigla for the type species, Bean golden mosaic virus), according to current taxonomic classification (Regenmortel et al., 2000). These viruses infect a large number of cultivated plant species, following their transmission by B. tabaci from wild or other cultivated hosts (Padidam et al., 1999). Currently, over 100 begomoviruses are transmitted by at least two biotypes of the whitefly B. tabaci, to more than 20 different cultivated species of socioeconomic importance. The main food crops affected by whitefly-transmitted geminiviruses are: common bean, mung bean, blackgram, lima bean, soybean, cowpea, tomato, potato, eggplant, pepper, chili peppers, melon, watermelon, squash, okra and cassava (Muniyapa, 1980; Brown, 1994).

The extreme pathogenicity and severe yield losses induced by begomoviruses, and absence of immune genotypes in most of the plant species attacked, has led to considerable pesticide abuse in order to control the whitefly vector. As a result, B. tabaci has developed resistance to most of the insecticides used in the past.

Breeding for begomovirus resistance

Despite the lack of immune cultivars recorded for the majority of the crops affected by B. tabaci-transmitted geminiviruses, breeding for disease resistance has proven to be the most successful and sustainable of the integrated whitefly/begomovirus control methods implemented to date. A comparative analysis of the three main cases of important food crops improved for begomovirus resistance, is presented here.

Breeding for resistance to African cassava mosaic virus

The case of cassava (Manihot esculenta Crantz) represents one of the earliest attempts at breeding for resistance to an important group of geminiviruses, collectively known as African cassava mosaic virus (ACMV). ACMV is transmitted by Bemisia tabaci, but the main method of dissemination is through the vegetative propagation of ACMV-infected cuttings (Swanson and Harrison, 1994). Currently, ACMV spreads in East and West Africa (Hong et al., 1993).

The search for resistance to African cassava mosaic viruses began in East Africa in the 1920s. Initially, several ACMV-tolerant cultivars were identified in large cassava germplasm collections evaluated in Madagascar and Tanzania (Jennings, 1994). Despite the initial successes in achieving notable levels of ACMV resistance following intraspecific crosses (Cours, 1951), cassava breeders resorted to interspecific crosses, using Manihot glaziovii, M. dichotoma, M. catingae, and a genotype called “tree cassava” (probably a natural M. esculenta X M. glaziovii hybrid), seeking higher levels of cassava mosaic resistance (Jennings, 1957). The first generation of M. glaziovii X M. esculenta produced in 1937, had non-tuberosous roots and became infected by ACMV under field conditions. Backcrossing was necessary to restore root quality. Resistance to
ACMV seemed to have improved slightly following three generations of backcrosses, probably due to the use of *M. esculenta* parents with intermediate levels of ACMV resistance.

All other interspecific crosses were discarded due to various agronomic problems. Unfortunately, the high expectations for the *M. glaziovii* X *M. esculenta* hybrids, were realized in some but not all regions of East Africa. A high proportion of the hybrid cassava lines selected, succumbed to ACMV in the coastal areas of Kenya. However, we now know that there are different ACMV strains and, more important, distinct species in what was originally considered as African cassava mosaic (Hong *et al.*, 1993), which may explain the differential reaction exhibited by the improved cassava genotypes in different locations of eastern Africa. On the other hand, the average proportion of *M. glaziovii* genes in the hybrid progenies had been reduced to 1/16 in the backcrossing process, and resistance to ACMV appeared to be multigenic and recessive. Thus, the expression of *M. glaziovii* genes was probably low, and the resistance achieved was the result of the accumulation of genes from moderately resistant *M. esculenta* parents used for backcrossing. Later on, some of the most resistant backcross hybrids were intercrossed to concentrate genes for resistance, which may have become dispersed among the various breeding lines, as well as to increase the levels of homozygosity of recessive resistant genes.

**Breeding for resistance to begomoviruses infecting common bean**

Four main begomoviruses transmitted by *B. tabaci* have been reported to attack common bean (*Phaseolus vulgaris* L.) in the Americas (Morales, 2000). *Bean golden mosaic virus* (BGMV) in Brazil, Argentina and Bolivia; *Bean golden yellow mosaic virus* (BGYMV), in southern Mexico, Central America and the Caribbean region; *Bean dwarf mosaic virus* (BDMV) in northwestern Argentina, and *Bean calico mosaic virus* (BCaMV) in northwestern Mexico. The first attempts at breeding for BGMV resistance were made in Brazil. Pompeu and Krantz (1977) initially selected symptomless individual plants within field populations of three BGMV-susceptible common bean cultivars. These selections were shown to be susceptible to the virus in subsequent evaluations (Costa, 1987). Another plant improvement strategy pursued in Brazil, was the use of radiation to create genotypic mutants possessing resistance to BGMV (Tulman-Neto, 1979). One of these common bean mutants, TMD-1, showed resistance to the virus but its yielding ability was poor, and its use in conventional breeding programs did not produce any outstanding progenies.

A parallel breeding project was initiated in Guatemala, in 1974, to solve the bean golden yellow mosaic problem in Central America, Mexico and the Caribbean. Approximately, 7,000 germplasm bank accessions of common bean were evaluated under natural disease pressure in southeastern Guatemala, but no immune genotypes were observed. Among these accessions, a group of black-seeded genotypes, namely Turrialba 1, Porrrillo 70, Porrillo Sintetico, ICA-Pijao and ICA-Tui, was selected for their better performance under natural BGYMV pressure. Due to their tolerance (acceptable yielding ability despite expressing noticeable foliar yellowing), Porrillo Sintetico, Turrialba 1 and ICA-Pijao were ultimately selected as potential parental materials (Yoshii *et al.*, 1979). The best lines derived from different crosses between the selected parental genotypes, were soon released in Guatemala as cultivars ICTA-Quetzal, ICTA-Jutiapán and ICTA-Tamazulapa (Yoshii *et al.*, 1980). In the absence of pesticide applications, ICTA-Jutiapán, ICA-Pijao and the local black-seeded cultivar ‘Rabia de Gato’, sustained yield losses of 38%, 53% and 86%, respectively.

However, yield losses for the DOR lines fluctuated proportionally with viruliferous *B. tabaci* populations, and no progress was made in breeding for BGYMV resistance in the red-seeded common bean cultivars preferred in Costa Rica, Nicaragua, Honduras and El Salvador (Morales, 2000). Fortunately, a serendipitous event took place in the mid 1980s. A common bean line improved for its upright architecture (A 429), showed a high level of BGYMV resistance under field conditions in Guatemala. A progeny test revealed the existence of a common bean
genotype belonging to the Mexican Durango race, which does not react with the characteristic yellowing when inoculated with BGYMV, despite being systemically affected by plant malformation and flower abortion by the virus. This Mexican common bean genotype, called ‘Garrapato’, combined with the Mesoamerican black-seeded source of BGYMV resistance, Porrillo Sintetico (also a parent of A 429), was associated with the high level of BGYMV resistance found in A 429 (Morales and Niessen, 1988). A 429 soon became one of the most widely used sources of begomovirus resistance in common bean breeding programs in Latin America (Singh et al., 2000). Subsequent research identified the gene bgm-1, responsible for conditioning mosaic resistance in Garrapato (Morales and Niessen, 1988; Blair and Beaver, 1993a).

Later on, a red kidney line, DOR 303, was also selected for its apparent high level of BGYMV resistance under field conditions. An evaluation of the parental materials selected to produce this line, revealed the presence of a red kidney genotype of Andean (race Nueva Granada) origin (Singh et al., 1991), besides the traditional black-seeded source of resistance, Porrillo Sintetico. In subsequent tests, this genotype was shown to behave as a tolerant genotype, producing flowers and pods despite its striking mosaic/yellowing foliar symptoms (Morales and Niessen, 1988). Porrillo Sintetico has considerable vigor, which often allows plants to escape infection, particularly in cases where the virus is inoculated after the first 2-3 weeks after emergence of the plants (Morales and Niessen, 1988). Thus, a second favorable interracial recombination of Mesoamerican and Andean genes had occurred, to produce a red-seeded common bean genotype possessing high levels of BGYMV resistance. The BGYMV-resistance gene in DOR 303 was later identified as bgm-2 (Velez et al., 1998).

A selection of diverse grain types was evaluated in different countries of Latin America, from Argentina to northern Mexico, in order to identify different mechanisms of virus resistance and sources of resistance to the different begomoviruses present in these regions. At least 10 new sources of begomovirus resistance were identified in the P. vulgaris accessions evaluated (grain colors different than black), as well as different mechanisms of resistance. Among the most interesting bean begomovirus-resistance mechanisms identified, were: disease escape, low mosaic expression, virus localization, low flower abortion, and low pod malformation (Morales and Niessen, 1988). The mean squares of the general combining ability of these traits was highly significant (P<0.01) and larger than values for specific combining abilities, which indicates that selection for the various traits evaluated was possible in true breeding lines, due to the existence of significant additive genetic variance (Morales and Singh, 1991). In subsequent studies, 83 recombinant inbred lines (RIL) selected from a population generated from the cross between a Mexican (Pinto UI 114) and a Mesoamerican (ICA-Pijao) common bean genotypes, were evaluated for their reaction to BGYMV. Of these lines, 11 did not show symptoms, 24 lines had mean disease incidence of 8%, 28 lines had a disease incidence of 26.6% and developed intermediate mosaic symptoms, and 20 lines were more susceptible than either of the parents. Thus, values for the 83 RILs transgressed the values of the two parents at both extremes (resistance and susceptibility) for both disease incidence and mosaic expression. These results show that the BGYMV-resistance genes present in the two parental materials (common bean races) are different and complementary to each other, and, consequently, that gene pyramiding could be attempted. Subsequent interracial crosses have generated highly resistant lines adopted as bean cultivars in different countries of Central America. Begomovirus multiplication in these improved genotypes is highly restricted according to molecular tests performed on these lines (Morales, 2000), and this type of resistance has also been associated to quantitative traits (QTLs), which reduce symptom expression (Miklas et al., 1996). On the contrary, common bean genotypes derived from intraracial populations, usually behave as moderately resistant under severe whitefly/virus pressure (Singh et al., 2000).
**Breeding for resistance to tomato begomoviruses**

A major boom of non-traditional export crops has taken place in most of the agricultural regions affected by whitefly-transmitted geminiviruses around the world. One of the predominant plant species found in these new cropping systems, is tomato (*Lycopersicon esculentum* Mill.). This is a highly profitable but costly crop, due to the amount of chemical inputs usually required to protect tomato from the various pests and diseases that attack this crop. The well documented pesticide abuse associated with tomato production has greatly contributed to the development of pesticide-resistant *B. tabaci* populations. This whitefly species is capable of vectoring over 20 different begomoviruses that attack tomato in tropical and subtropical regions of the world (Polston and Anderson, 1997; Zeidan *et al.*, 1999).

Although most of the begomoviruses that attack tomato are found in the New World, very little breeding work has been done to minimize the severe damage that these viruses cause to tomato plantings in this region. Moreover, despite its tropical American origin, most of the tomato breeding work has been conducted in temperate countries. Hence, tomato growers in tropical and subtropical America have relied almost exclusively on pesticides to control *B. tabaci* and the geminiviruses this vector transmits.

The situation in the Old World is similar, due to the severe damage caused by a group of geminiviruses transmitted by *B. tabaci* in tomato plantings throughout the Mediterranean region, the Middle East, north Africa, central Africa and southeast Asia (Czosnek and Laterrot, 1997). These related although distinct geminivirus species, are collectively referred to as *Tomato yellow leaf curl virus* (TYLCV). This geminivirus was accidentally introduced in the last decade into the Americas in the early 1990s (Nakhla *et al.*, 1994), where it has already caused millions of dollars worth of industrial and fresh tomato production losses.

Early efforts to identify sources of resistance to TYLCV within *L. esculentum*, only revealed the existence of some moderately resistant or tolerant genotypes (Cohen and Harpaz, 1964; Nitzany, 1975; Abu-Gharbieh *et al.*, 1978). However, Cohen and Nitzany (1966) observed that some wild relatives of tomato, namely *L. pimpinellifolium* and *L. peruvianum*, possessed a higher level of resistance to TYLCV, although they were not immune. Crosses between *L. esculentum* and *L. pimpinellifolium* (currant tomato/accession LA 121) and genetic analyses of F$_{1-3}$ and backcross generations, indicated the existence of incomplete dominance of resistance over susceptibility, suggesting a monogenic control of resistance (Pilowski and Cohen, 1974). A dominant gene (*Tylc*) was later proposed for the resistance gene in *L. pimpinellifolium* (Kasrawi, 1989). The progenies derived from this cross showed only moderate symptoms, but their yield was markedly reduced. Nevertheless, among the *Lycopersicon* species, *L. pimpinellifolium* is one of the most compatible for crossing with *L. esculentum* (Picó *et al.*, 1996).

In contrast, the inheritance of tolerance to TYLCV in *L. peruvianum* (PI 126935) is controlled by five recessive factors, according to Pilowski and Cohen (1990). This breeding program initiated in 1977, resulted in the release of the commercial hybrid TY-20, in 1988. This hybrid delays symptom expression and viral DNA accumulation in infected plants, resulting in acceptable yields (Pilowski and Cohen, 1990). Other tolerant/resistant TY-lines generated by this breeding program are: TY172, TY197, TY198, and TY536 (Lapidot *et al.*, 1997; Friedmann *et al.*, 1998).

In 1991, other wild tomato species: *L. chilense* and *L. hirsutum*, besides *L. peruvianum* and *L. pimpinellifolium*, were examined for the presence of viral DNA and symptom expression following their inoculation with whiteflies removed from TYLCV-infected tomato plants. Approximately 85 days after inoculation, all of the above species had infected plants with detectable levels of viral DNA, but *L. chilense* and *L. hirsutum* were the most resistant species, with the majority of the inoculated plants remaining symptomless, and only few containing viral DNA (Zakay *et al.*, 1991). The TYLCV resistance gene in *L. chilense* was identified as Ty-1 (Michelson *et al.*, 1994). The resistance to this virus in *L. hirsutum*, on the other hand, seems to be dominant and controlled by more than one gene (Mazyad *et al.*, 1982). *L. hirsutum* has been
crossed with *L. esculentum*, yielding tolerant and immune lines. One of the immune lines was crossed with *L. esculentum*, to produce the hybrid FAVI-9 or Line F1-901. The immune reaction was associated with 2-3 additive genes (Vidavski and Czosnek, 1998). Another promising species evaluated for TYLCV resistance, *L. cheesmani*, possesses recessive resistance to TYLCV. Breeding projects in the Mediterranean region have also used *L. cheesmani, L. peruvianum* and *L. pimpinellifolium* to control TYLCV in this region (Laterrot, 1990, 1992, Laterrot and Moretti, 1996). Some of the TYLCV-resistant lines obtained from this project are: Pimpertylc-J-13 and Chepertylc-92.

Interspecific hybrids obtained from crosses between *L. pimpinellifolium, L. peruvianum*, and *L. hirsutum*, show transgressive segregation for their reaction to TYLCV, suggesting the existence of different, complementary genes (Kasrawi and Mansur, 1994). In 1991, Muniyapa and coworkers reported that lines of *L. hirsutum* and *L. peruvianum* were resistant to another tomato geminivirus: *Tomato leaf curl virus* (ToLCV). The resistance mechanism in these wild species was subsequently associated with the presence of exudates from trichome glands on the leaf surface, in which whiteflies became entrapped (Channarayappa and Shivashankar, 1992). This is one of the few cases where genetic resistance to a viral disease has been achieved indirectly by incorporating genetic traits against *B. tabaci*. Nevertheless, there is sufficient evidence showing that different cultivars of plant species such as common bean and tomato, interact differentially with *B. tabaci*. For instance, in Sinaloa, northwestern Mexico, the common bean cultivar ‘Azufrado Peruano-87’, had 16% more nymphs/leaf than the geminivirus (BCaMV) resistant common bean cultivar ‘Azufrado Higuera’ (Lopez, 1996). Similar data has been obtained for tomato, although the preference shown by *B. tabaci* for some tomato cultivars, was not related to virus resistance/susceptibility traits in the tomato cultivars evaluated (Avilés, 1996).

**Discussion**

Many agricultural scientists have implied that there is no resistance to most whitefly-transmitted geminiviruses in cultivated species of cassava, common bean or tomato, probably confusing ‘resistance’ with ‘immunity’. It is important to understand that ‘resistance’ is a relative term, which can span a range of disease reactions from a low to a high level. ‘Tolerance’ is another term which is often misused in reference to the degree of symptom expression (e.g. mild mosaic or plant malformation symptoms). The term ‘tolerance’ should be used in relation to the ability of a plant genotype to yield an acceptable or expected quantity or quality of product (e.g. seed, fruits, flowers, etc.) despite being affected by a biotic or abiotic factor. Both genetic resistance and tolerance can be found in most cultivated species attacked by whitefly-transmitted geminiviruses.

The strategy followed to incorporate high levels of begomovirus resistance in common bean, strictly through the intraspecific recombination and pyramiding of different resistance traits found in diverse gene pools of *Phaseolus vulgaris*, confirms the feasibility of this approach. Moreover, the underlying mechanism of resistance (i.e. restricted virus multiplication) is similar in the three crops discussed here (Zakay et al., 1991; Lapidot et al., 1997; Fargette et al., 1996; Morales, 2000).

However, the important conclusion is that there is both direct and circumstantial evidence indicating the existence of adequate genetic variability in the primary and secondary gene pools of most cultivated species. This genetic variability can be exploited within and between cultivated species and their relatives. Interspecific hybridization in cassava, common bean and tomato, can be practiced not only in search of resistance to begomoviruses, but to other pathogens and pests as well (Nichols, 1947; Debouck, 1991). In the case of tomato, it is evident that the cultivars improved for TYLCV resistance, are also exhibiting acceptable levels of resistance to distinct New World begomoviruses attacking tomato in the Americas and Asia (Muniyapa et al., 1991; Piven et al., 1995). Another neglected but possible begomovirus control method is the
incorporation of genetic resistance to the whitefly vector, *Bemisia tabaci*, as it has been suggested by limited investigations in cassava (Fargette *et al.*, 1996), common bean (Blair and Beaver, 1993b), and tomato (Channarayappa *et al.*, 1992).

Undoubtedly, the utilization of all the genetic diversity present in the primary and secondary gene pools of these plant species, will require both conventional and advanced crop improvement techniques, such as molecular marker assisted selection (Chavarriaga *et al.*, 1999; Singh *et al.*, 2000; Zamir *et al.*, 1994).

References


Michelson, I., Zamir, D., Czosnek, H., 1994. Accumulation and translocation of tomato yellow leaf curl virus (TYLCV) in a Lycopersicon esculentum breeding line containing the L. chilense TYLCV tolerance gene Ty-1. Phytopathology 84, 928-933.


GEMINIVIRUS RESISTANCE DERIVED FROM \textit{Lycopersicon chilense} ACCESSIONS LA 1932, LA 1938, AND LA 2779

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A bipartite geminivirus appeared in Florida in the late 1980's and it was later termed tomato mottle virus (ToMoV). Accessions from several species were screened for resistance and the best resistance came from accessions of \textit{Lycopersicon chilense} Dunal (Scott and Schuster, 1991). Twelve accessions were selected for introgression based on their lack of virus symptoms and larger leaf size; LA 1932, LA 1938, LA 1959, LA 1960, LA 1961, LA 1963, LA 1968, LA 1969, LA 2747, LA 2762, LA 2774, and LA 2779 (Scott et al., 1996). Interspecific crosses with embryo rescue and from \textit{L. esculentum} pollen mixtures were equally effective but both resulted in only 1 F\textsubscript{1} plant per 40 crosses. No F\textsubscript{1}'s were obtained from LA 1969 (only few crosses made), LA 2747, LA 2762, or LA 2774. LA 1960 crosses were fertile and red fruited. During introgression, resistance was often associated with indeterminate plants with small leaves and orange fruit. Early in the introgression work, resistant determinate plants obtained from LA 1932 and LA 1961 were small with sparse growth. Determinate resistant plants from LA 1932 also had potato-like leaves even though no potato leaf (c) parents were used.

Introgressed lines with ToMoV resistance also had resistance to tomato yellow leaf curl virus (TYLCV) in the Dominican Republic (Scott et al., 1996). Further testing has shown that ToMoV bred lines often have resistance to various geminiviruses, but no one line has had resistance to all viruses tested. In contrast, most lines bred for resistance to TYLCV have been susceptible to ToMoV with a few exceptions. ToMoV resistance has been obtained from Tyking, selections from CHILTYLC 94-2 and -3, Ty 197, and a selection from Ty 34. The best resistance to ToMoV has been obtained from LA 1932 or LA 2779 alone and LA 1938 combined with Tyking. P. D. Griffiths (1998) used RAPD markers to locate ToMoV resistance genes. Three regions were located on chromosome 6 by using the \textit{sp} and \textit{c} loci as anchors. LA 2779 and LA 1938 had RAPD polymorphisms in a region analogous to the \textit{Ty-1} region (Zamir et al., 1994). A second region near the \textit{sp} locus was present in lines derived from LA 1938 and LA 1932, and a third region on the telomeric side of the \textit{sp} and \textit{c} loci was found in lines derived from LA 1932. Inheritance studies using LA 1932 estimated 2 effective factors with primarily additive gene action (Griffiths and Scott, 2001). Later we found that lines derived from LA 2779 selected for ToMoV resistance are susceptible to gray leaf spot despite numerous crosses to \textit{Sm} resistant recurrent parents. Thus, i appears a fourth gene has been located on chromosome 11 linked to \textit{Sm}\textsuperscript{+}.

An unusual yellow mosaic ToMoV symptom was found to be associated with tomato lines that carried tomato mosaic virus resistance at the \textit{Tm-2} locus. More detail on this relationship is in the abstract written for the 1999 Tomato Breeder’s Roundtable Meeting that is in this publication.

In summary, four additive geminivirus resistance genes derived from \textit{L. chilense} sources have been found. For ToMoV resistance, a line requires two homozygous genes. ToMoV resistant lines are usually resistant to TYLCV and often other geminiviruses. These genes provide a high level of resistance, but plants often have slight disease symptoms. Further work is underway to determine if any of the RAPD markers are linked closely enough to be used in marker assisted selection. Markers will also be used to synthesize lines with all combinations of resistance genes so these can be tested with cooperators around the world to determine specific effects of the genes on various geminiviruses. Undesirable repulsion linkages such as the one with the \textit{Sm} locus or the
one in the *Ty-1* region with *Mi* will limit the usefulness of some geminivirus resistance genes unless these linkages can be broken.

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Development of a universal and reliable scale for evaluation of TYLCV-resistance level in tomato plants

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Tomato yellow leaf curl virus (TYLCV) is one of the most devastating viral pathogens of cultivated tomatoes in tropical and subtropical regions. The virus is a monopartite geminivirus, transmitted by whiteflies. Control measures in infected regions are based on limitation of the vector population, which usually requires heavy pesticide use and/or physical barriers such as 50 mesh nets. However, control of the viral vector by mechanical barriers is difficult, expensive, and hardly applicable to open-field cultivation, while heavy pesticide use has a deleterious effect on the environment. Thus, genetic resistance in the host plant is an ideal defense against whitefly-transmitted viruses, since it requires no chemical input and may be stable and long lasting. As all tomato (L. esculentum) cultivars are extremely susceptible to TYLCV, breeding programs have been based on the transfer of resistance genes from accessions of wild origin into the domesticated tomato. Since growth conditions have a strong affect on severity of disease symptoms induced by TYLCV, one of the major obstacles in the development of TYLCV-resistance is the assessment of resistance level displayed by the plant. We have developed a scale of differential hosts, which enables the determination and comparison of level of resistance to TYLCV expressed by resistant tomato plants. The scale is composed of seven different homozygous tomato genotypes that exhibit different levels of TYLCV resistance, ranging from fully susceptible to highly resistant. The resistant differential hosts composing the scale were inoculated with TYLCV under different environmental conditions. Four weeks after inoculation the plants were evaluated for disease symptom severity, and virus level in the inoculated plants was determined. While the score of each individual resistant genotype indeed changed under different environmental conditions, its position on the scale did not. Thus, in order to evaluate disease resistance of a given tomato genotype, the genotype in question is being inoculated alongside the differential hosts composing the scale, and within four weeks one can determine the relative level of resistance of the tested genotype related to its position on the resistance scale.
Distribution and genetic diversity of tomato-infecting geminiviruses in Brazil. F.M. Zerbini* and S.G. Ribeiro**, *Dep. de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil, 36571-000 (zerbini@mail.ufv.br); **EMBRAPA Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Brasília, DF, Brazil, 70770-900.

Tomato-infecting geminiviruses have been reported throughout Brazil since the introduction of a new species of insect vector, the whitefly Bemisia argentifolii, in 1992. However, a detailed analysis of the biological and molecular properties of these viruses has not yet been carried out. As a first step towards the characterization of these viruses and the development of management strategies based on host resistance, we carried out a country-wide survey on the distribution and genetic diversity of tomato-infecting geminiviruses. Tomato samples with typical geminivirus symptoms were collected in seven different states, comprising the major tomato growing areas of the country, from 1994 to 1999. Geminiviruses were detected by PCR using universal primers for the genus Begomovirus. PCR-amplified fragments were cloned and sequenced. Based on sequence comparisons and phylogenetic analyses, at least five previously undescribed species of begomoviruses, plus two tentative new species, were found. Two of the new viruses were found exclusively in the Northeastern states, two exclusively in the Southeastern states, and one was found in both regions. Three of the new species were found in Minas Gerais, and two in Pernambuco. Sequence comparisons reveal strong evidence of recombination among the Brazilian geminiviruses. Together, the results indicate the existence of a high degree of pre-existing genetic diversity among tomato-infecting geminiviruses in Brazil and suggest that these viruses have emerged after being transferred from natural hosts to tomatoes, due to the introduction into Brazil of a novel polyfagous vector species.
Tomato Market
Central America and Caribbean
Javier Martinez, Seminis Vegetable Seeds

The total area planted with tomatoes in Central America and the Caribbean is 28,205 ha (69,690 acres) divided approximately 50-50% in each region.

The region plants four different types of tomatoes; Fresh Market tomatoes, Saladettes (pear shape for fresh market), processing shape for fresh market and processing for process (the last one just in the Dominican Republic and Panama).

Basically all the countries within the region have the same pests, diseases and the most important thing is that the entire region has enormous problems with viruses. When we talk about diseases, we have Pseudomonas (Bacterial wilt), Phytophthora (late blight), Alternaria (early blight), in the cases of pests, white fly, leaf miner, Spodoptera.

The region also has big heat pressure in some areas and during some months (April-July) and there is a lot of pressure with viruses.

The area planted in the region is divided by country as follows:

- **Guatemala:**
  - Saladette 4,544 ha (11,230 acres)
  - Processing for FM 980 ha (2,430 acres)
  - Fresh Market 70 ha (173 acres)

- **El Salvador**
  - Processing form FM 1,400 ha (3,460 acres)
  - Fresh Market 100 ha (247 acres)

- **Honduras**
  - Processing for FM 2,000 ha (4,940 acres)
  - Fresh Market 500 ha (1,235 acres)

- **Nicaragua**
  - Processing for FM 1,750 ha (4,325 acres)

- **Costa Rica**
  - Fresh Market 1,500 ha (3,700 acres)

- **Panama**
  - Fresh Market 250 ha (620 acres)
  - Processing 800 ha (1,980 acres)

- **Dominican Republic**
  - Processing 10,000 ha (24,710 acres)
  - Fresh Market 1,200 ha (2,960 acres)

- **English and French Speaking Islands**
  - Fresh Market 2,460 ha (6,080 acres)
  - Saladette* 654 ha (1,610 acres)

*These are indeterminate Saladettes.

We do not have information about Cuba, Haiti or Puerto Rico.
ATTEMPTS TO ELUCIDATE THE COMPONENTS OF TOMATO FLAVOR FOR IMPROVED BREEDING EFFICIENCY

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Cooperators on the tomato flavor project are Dr. Elizabeth Baldwin, USDA Winter Haven, FL, Dr. Rob Shewfelt of Food Science Dept., University of Georgia, and Dr. Harry Klee of Horticultural Science Dept., University of FL.

The chemistry of tomato flavor is not well understood despite considerable literature on the topic (see Baldwin et al., 2000). The reducing sugars fructose and glucose are important as are the acids; primarily citric and malic. Some literature suggests that breeding tomatoes for high levels of sugars and acids will result in varieties with improved flavor (Jones and Scott, 1984; Stevens et al., 1979). A more recent paper indicated that flavor improved with increasing sugar concentration, but that flavor improved with increasing acid concentration only to a point after which it decreased (Malundo et al., 1995). Much molecular marker work for flavor improvement is done with high sugars because they are important components of tomato flavor and because they can be easily measured. One approach to improve flavor is to incorporate a gene that increases the ratio of fructose to glucose since the former is the sweeter of the two (Levin et al., 2000). Another approach is to develop extended shelf life (ESL) tomatoes since this would facilitate breaker harvests and overcome problems associated with immature fruit in the green harvest system. The ESL approach includes use of \textit{rin}, \textit{nor}, or \textit{alc} genes in firm-fruited backgrounds or in developing very firm (ultrafirm) fruit without these genes. There have been flavor problems associated with \textit{rin} and \textit{nor} hybrids Kopeliovitch et al., (1982) and ultrafirm lines are often bland tasting(Scott, unpublished data). There have been unpublished reports that shelf life of \textit{alc} hybrids have not been improved under high temperature growing conditions. On the biotechnological side, PG antisense was used in the FlavrSavr® tomato developed by Calgene, but the shelf life increase was only modest. An alcohol dehydrogenase (ADH) gene has also been modified in ‘Flora-Dade’ which resulted in increased hexanol and cis-3-hexanol and improved ripe flavor (Speirs et al., 1998).

The other genetic component of flavor are the aromatic volatiles. There are hundreds of these but only 17 were found to be important in tomato based on odor threshold studies by Buttery, (1993). We have found that sugars and acids often do not explain differences in flavor perceived by sensory panels (Baldwin et al., 2000). Volatile levels probably account for these differences. However, it has been difficult to determine consistent volatile patterns associated with superior flavor and at present there is no real selection target.

Our present research on flavor involves attempts to understand the chemistry of some common flavor notes in tomato that we think are analogous to primary colors in that combinations of these notes may be responsible for all the flavors encountered in tomatoes. Six of these flavor notes are clearly undesirable; musty, bitter, astringent (associated with an orange hue), ethanolic, sour (spoiled), and metallic. Another note called vegetative is undesirable if present without enough sweetness to balance it out. A common undesirable flavor is bland and this is thought to result from a lack of sugars, acids, and volatiles. Three flavors; sweet, acid, and balanced probably relate strongly to the sugar and acid content of the tomatoes. Sweet can vary from mild to strong. If mild, this can become insipid or bland under some environmental conditions. Sweet (strong) and acid can be desirable depending on one’s taste. Balanced flavor has a good degree of sweetness and acidity and is considered desirable. We have found only one
other desirable note and that is fruity-floral. Our hypothesis is that a tomato variety with good levels of acids and sugars (balanced) and the fruity-floral note would be highly desirable. For three years efforts have been underway to fix lines for strong expression of the above flavor notes so they can be used for training taste panels and in defining their flavor chemistry with the ultimate goal of finding flavor selection targets. However, environmental factors play a profound role in tomato flavor and it has been difficult to fix lines for the flavor notes. This work is ongoing. One method we are using to overcome environmental effects is to taste individual fruit and when a flavor note is strongly expressed, use the other half of the fruit for chemical analysis. It is hoped that enough replications of each note will result in elucidation of the chemistry causing the flavor.

In summary, tomato flavor is a trait that has complex genetic control and strong environmental effects that make selection difficult. There are no acceptable selectable markers, thus the breeder must do a lot of tasting over several seasons and locations in order to define the flavors of a given line. Taste fatigue and time are some of the breeding problems. Of course, flavor must be integrated with other important breeding objectives such as yield, disease resistance, and horticultural quality. There are no easy solutions on the horizon.

**LITERATURE CITED**


Breeding for Color and Lycopene Content: The Role of Interacting Loci.

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We are interested in improving selection efficiency for color and lycopene content in tomatoes. The correlation between color and lycopene content suggests that selection for improved color may provide an inexpensive alternative to chemical measurement. Objective measurements of color, chromaticity values, have been used to estimate the concentration of lycopene as a tomato matures (D’Souza et al., 1992; Arias et al., 2000). Chemical measurement of lycopene can be accomplished by spectrophotometric methods of extracts and by High Pressure Liquid Chromatography (HPLC) using a polymeric C30 column (Emenhiser et al., 1995). The advantage of HPLC methodology is that individual pigments can be quantified and isomers can be separated. Thus the concentration of all-trans-lycopene, the most significant red pigmented isomer, can be accurately measured. Because the published regression equations are based on maturity stages, it is unclear how well they predict the lycopene content of ripe fruit from different genotypes. The results reported at this meeting were obtained from studies conducted to determine the relationship between color and lycopene content in a breeding population consisting of adapted germplasm, to compare the efficiency of direct selection for lycopene content with selection for color, and to gain insights into the genetic basis of variation for color and lycopene content within adapted germplasm.

Estimating Lycopene Content from Color. We developed regression equations for lycopene content using chromicity values and HPLC measurements of lycopene content obtained for 39 genotypes. Objective color measurements of tomato fruit were based on hue (tint of color), L (darkness or lightness of color), and chroma (vividness of color). These measurements were obtained using a Minolta CR300 colorimeter and the CIELAB color space (CIE, 1978). The a/b relationship common in tomato evaluation shows a strong negative correlation with Hue, but the relationship is truly linear through only a narrow range of Hue values. The content of lycopene for the 39 genotypes ranged from 8 to 14 mg/100 gm dry wt. when data were averaged across trial replicates. Three equations had significant linear correlations between lycopene concentration and objective measures of color:

\[
\begin{align*}
[l\text{ycopene}] &= -0.425 \times L + 29.36 \quad p = 0.0005 \quad r^2 = 0.66 \\
[l\text{ycopene}] &= -0.196 \times \text{Hue} + 21.32 \quad p = 0.0064 \quad r^2 = 0.551 \\
[l\text{ycopene}] &= 7.926 \times (a/b) + 4.93 \quad p = 0.0036 \quad r^2 = 0.582
\end{align*}
\]

In order to test the performance of these equations, we applied them to an independent population consisting of 19 selections and 3 high lycopene varieties. The observed range of lycopene was 8 to 25 mg/100 gm dry wt. Previously published equations failed to predict the lycopene content of ripe fruit within 30% of the measured levels (Table 1). In contrast, the three equations we developed had predictive value within 11% to 22% (Table 1). The greater ability to predict lycopene content was noteworthy because the correlation coefficients for our equations are lower than those published by D’Souza (1992) and Arias (2000). It is likely that these equations are biased due to the inclusion of unripe tomatoes, and therefore unsuitable to estimating the lycopene content of populations intended for once over harvest. The effect of including green tomatoes with very little lycopene results in a regression line with influential points that are outside of the portion of the curve relevant for predicting lycopene content in red fruit. At the same time these influential points have the effect of lowering the correlation coefficients by dramatically extending the line over a much larger range of values. The tendency
of both sets of published equations is to over-predict the lycopene content of ripe tomatoes is consistent with low lycopene green tomatoes leveraging the slope of the regression line.

Table 1. Prediction of lycopene content from linear regression equations

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>Average Difference $^2$</th>
<th>Population</th>
<th>D’Souza $^2$</th>
<th>Arias</th>
<th>Arias $^2$</th>
<th>This MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\frac{a}{b}$</td>
<td></td>
<td>$\frac{a}{b}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\frac{L}{Hue}$</td>
<td></td>
<td>5.73</td>
<td>4.66</td>
<td>4.72</td>
<td>1.39</td>
<td>1.69</td>
</tr>
<tr>
<td>$\frac{L}{Hue}$</td>
<td></td>
<td>44.4%</td>
<td>36.1%</td>
<td>36.6%</td>
<td>10.8%</td>
<td>13.1%</td>
</tr>
</tbody>
</table>

$^1$Regression equations are from D’Souza et al. (1992) Lycopene = 7.12 x $(a/b)^2$ + 5.60; Arias et al. (2000) lycopene = 11.848 x $(a/b) + 1.55$ and = 8.707 x $(a/b)^2$ + 1.52; and from this manuscript lycopene = -0.425 x $L + 29.36$, = -0.196 x Hue + 21.32, and = 7.926 x $(a/b) + 4.93$.

$^2$The absolute value of the average of all differences between the lycopene content estimated using the regression equation and the direct measurement of lycopene content.

$^3$The average of differences as a percentage between the estimated lycopene content based on regression and the direct measurement of lycopene content.

Relative efficiency of selection. Having established that we could estimate the content of lycopene from our color measurements, we wanted to estimate the relative efficiency of selection. Variance components were estimated for the genotypic component of each trait, and Heritability estimates were obtained according to the relationship $H = \sigma^2_G / [\sigma^2_G + (\sigma^2_E)/rep]$. In these analyses the traits were either objective measurements of color from replicated field trials or direct measurements of lycopene content using HPLC or UV/visual spectrophotometric ratios. Genetic correlations were calculated from the variance components of each pair of traits ($x, y$) and for the combined trait ($x+y$). The genetic correlation for traits was calculated as described by Falconer and MacKay (1996) as $\text{Corr} = \text{cov}(x,y) / \sqrt{\sigma^2(x) \times \sigma^2(y)}$ where $\text{cov}(x,y) = [\sigma^2(x+y) - \sigma^2(x) - \sigma^2(y)] / 2$ (Equation 19.2). The relative efficiency of selection was then estimated from the ratio of the heritabilities multiplied by the genetic correlation [Relative efficiency of selection = $r_{gen} \times (H_{selected\ trait} / H_{target\ trait})$]. Calculations are based on heritability estimates for two-year single-location data. Estimates are 18% to 21% lower when variance components from multi-year multi-location trials are used to estimate heritability. The relative efficiency of selection is high when $L$ values are used (Table 1).

Table 1. Relative efficiency of selection$^1$ for lycopene content using chromicity values.

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>Hue</th>
<th>UV/vis</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L$</td>
<td>0.56</td>
<td>0.86</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Hue</td>
<td></td>
<td>0.45</td>
<td>0.40</td>
<td>0.82</td>
</tr>
<tr>
<td>UV/vis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evidence for Interacting Loci within L. esculentum Germplasm. Previous work in our program demonstrated that there is significant genetic variation for color within L. esculentum germplasm (Sacks and Francis, 2001). In order to further investigate the genetic basis of this variation, an $F_2$ population derived from crossing IBL 2349 with Ohio 8245 was evaluated for color in replicated
field trials. Objective color measurements of tomato fruit using the CIELAB color space (CIE, 1978) and the sampling scheme described by Sacks and Francis (2001) provided the phenotypic data for analysis. Realized heritability estimates for hue, L and chroma were moderate to high. By screening the population with molecular markers, two independent L. esculentum QTL were found to be associated with the improvement of tomato color. These QTL were linked to RAPD markers UBC-192 and OPBB-09 and linkage was confirmed in F₁ and F₂ families. The QTL marked by UBC-192 maps to chromosome 4 while the QTL marked by OPBB-09 maps to chromosome 11. Total phenotypic variation explained by UBC-192 ranged from 14-80% and phenotypic variation explained by OPBB-09 ranged from 24-44% within and across environments for F₁ and F₂ families.

Epistatic interactions were identified between UBC-192 and OPBB-09 (Table 3). Unexpectedly, the two L. esculentum QTL also appeared to interact positively with an introgression from LA407 on chromosome 7 of IBL 2359. This locus was not identified as a QTL in isolation. The QTL identified in this study and their epistatic interactions may provide alternative genes for the improvement of red-fruited tomatoes in breeding programs.

Table 3. Epistatic interactions between pairs of polymorphic RAPD and RFLP markers evaluated in an IBL 2349 x Ohio 8245 derived F₂ population.

<table>
<thead>
<tr>
<th>Marker¹</th>
<th>Chrm</th>
<th>Marker¹</th>
<th>Chrm</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hue</td>
</tr>
<tr>
<td>UBC-192</td>
<td>4</td>
<td>OPBB-09</td>
<td>11</td>
<td>0.0001</td>
</tr>
<tr>
<td>CT114</td>
<td>7</td>
<td></td>
<td></td>
<td>0.3769</td>
</tr>
<tr>
<td>TG199</td>
<td>7</td>
<td></td>
<td></td>
<td>0.0150</td>
</tr>
<tr>
<td>OPBB-09</td>
<td>11</td>
<td>UBC-192</td>
<td>4</td>
<td>0.0001</td>
</tr>
<tr>
<td>CT114</td>
<td>7</td>
<td></td>
<td></td>
<td>0.0004</td>
</tr>
<tr>
<td>TG199</td>
<td>7</td>
<td></td>
<td></td>
<td>0.0007</td>
</tr>
</tbody>
</table>

¹ UBC-192 allele of Ohio 8245; OPBB-09 allele of IBL 2349 (Hunt 100); CT114, TG199, TG216 alleles of L. hirsutum LA407.

References:
In Search of Breeder-friendly Markers Linked to Fr1

Ryan Walker and Mikal Stevens, Brigham Young University, Provo, Utah

Fusarium crown and root rot (FCCR) can cause severe economic losses in cultivated tomato. A disease resistance gene originating in L. peruvianum, designated Fr1, provides resistance to FCCR. Previous studies with this gene have placed it on the long arm of chromosome 9, near the centromere. We have previously determined through RFLP studies that this gene lies between RFLP markers CT208 and CD3, CD8, TG3A. We have also discovered 146 closely linked AFLP markers by using bulked segregant analysis (BSA).

By creating sequence tagged site (STS) PCR primers from the four RFLP markers and from six of the AFLP markers, we were able to amplify probes to screen through a binary bacterial artificial chromosome (BIBAC) library purchased from the Texas A&M BAC Center. The BIBAC library consists of over 42,000 clones from the FCCR resistant cultivar ‘Mogeor’. The screening yielded 82 clones that appeared to be in the region of interest. To create a physical map of the area, these clones were digested with a restriction enzyme, radioactively labeled, and run on a sequencing gel. Three possible contigs resulted from this test.

To further analyze these clones, a series of tests were performed. Of these tests, end sequencing has been the most powerful. Recent end sequencing has allowed us to discard 36 of the 82 clones (they were false positives). Interestingly, all of those discarded were from one the three contigs. We are currently working to analyze the remaining clones. We plan to sequence in from both ends, looking for PCR based polymorphic markers tightly linked to Fr1.
Breeding for resistance to bacterial spot: prospects for marker assisted selection

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Xanthomonas campestris pv. vesicatoria (Xcv) causes bacterial spot on both pepper (Capsicum spp.) and tomato (Lycopersicon spp.). The target population in the Mid-west U.S.A. has been identified as 67% tomato race 1 (T1), 29% T2, and 4% T3. Resistance to copper and streptomycin in X. campestris pv. vesicatoria strains in Ohio were significantly increased during sampling between 1994 to 1996 (Sahin and Miller, 1995; Sahin, 1997). The target population for our breeding efforts is therefore diverse.

The objectives of this project were to characterize resistance to race T1 of bacterial spot in a \(L. esculentum\) x \(L. esculentum\) cross, to identify polymorphic molecular markers linked to genes conferring that resistance, and to use markers in the context of an inbred backcross breeding program to develop breeding lines with improved resistance. Resistance to race T1 involves more than one locus, and the hypersensitive response does not predict field performance. Our strategy was to treat resistance as a quantitative trait in early generation field evaluation. However, our working hypothesis was that resistance is controlled by Mendelian loci that are genetically linked to existing resistance genes in tomato. We set out to test this hypothesis by mapping partial-resistance to Race T1 strains in \(L. esculentum\) x \(L. esculentum\) crosses by using markers developed from conserved domains of previously cloned resistance genes.

Previous studies suggested that three loci may be involved in a hypersensitive response to Race T1 of bacterial spot in tomato (Wang et al., 1994b; Yu et al., 1995). These studies were based on a wide cross between the resistant variety H7998 and \(L. pennellii\) LA716, and the genetic markers from that cross are not appropriate for transferring resistance from H7998 into \(L. esculentum\) breeding lines. The identification of polymorphic markers within \(L. esculentum\) is considered difficult due to restricted genetic variation (Miller and Tanksley, 1990; Williams and St. Clair, 1993). Our approach was to use conserved sequences from previously cloned resistance genes to identify polymorphisms. This strategy was based on the observation that resistance genes in plants tend to be clustered in a number of plant species, and the observation that high levels of polymorphism are associated with resistance genes. Using this approach, we have identified genetic markers that are polymorphic between H7998 and elite processing tomato lines adapted to the midwest. Two of these markers are linked to loci identified in the wide cross.

Data was collected from a greenhouse screen as bacterial populations present in leaf tissue 6 days post inoculation. This assay results in a population difference of over \(10^2\) between resistant and susceptible parents. The marker linked to the Rx1 locus explains 12% of the total variation in the backcross population and only 1% of the variation in the F\(_2\) population. This PCR based marker is dominant and genetic information is therefore lost in the F\(_2\) population, a fact that may explain some of the difference between the two populations. However, these results cannot be interpreted as evidence that Rx1 plays a role in resistance. This result contrasts with previous studies (Wang et al., 1994b; Yu et al., 1995) and may be due to differences between the bacterial strains used, differences in the procedure used to score resistance (hypersensitive reaction vs. bacterial populations), or differences in growth environment. In contrast, the chromosome 5 marker (linked to Rx3) explains as much as 45% of the variation in the greenhouse test and does appear to be linked to a locus that controls resistance (P < 0.008).

Greenhouse results were confirmed in the field using an advanced backcross (AB) population (O 88119 X H 7998 BC3 to 88119). 91 families were planted in plots consisting of 8 plants from each AB family. Families were completely randomized and replicated in two blocks.
and plants were inoculated with the race T1 isolate, 110C. Data were collected on a Horsfall-Barrett scale on individual plants within a plot. Ratings of disease severity were done twice. Analysis of variance was performed using genotypic means of plot high score, plot low score, and plot average. Genotype differences were highly significant (P<0.002) and block differences were not significant. As in the greenhouse studies, a locus on chromosome 5 is significantly associated with resistance to race T1 (P<0.05) in both rating periods. The locus on chromosome 1 was not associated with resistance to Race T1.

The locus on chromosome five, Rx3, is the most important locus for field resistance against the T1 strain, 110C. It explains 45% of the variation in both greenhouse and field studies, and is thus a robust locus. Markers from chromosome 1 (Rx1) and chromosome 5 (Rx3) are being used in a backcross breeding program aimed at introducing resistance to bacterial spot into cultivars used in the Midwest. We have yet to develop markers linked to Rx2. Populations designed to facilitate the selection of recombination events that will incorporate resistance to both bacterial speck and bacterial spot on chromosome 5 have been developed. The DNA based markers developed for genetic mapping will facilitate selection of recombinant plants. Selections from 2 families, AB129 and AB147, have been advanced for breeding purposes based on horticultural characteristics and disease resistance.

A second source of resistance, PI 114490, confers resistance to Race T1, T2, and T3 of bacterial spot (Scott et al., 1997). Heritability for T2 resistance is moderate to high based on F2 to F3 parent-offspring regression. We have developed a large (approx. 160 lines) Inbred Backcross population based on PI114490 as a donor of resistance and the adapted high lycopene variety Ohio 9242 as a recurrent parent. This population has been evaluated for two years in our T2 resistance nursery. Estimates of gene number are in the range of 2-3. A better understanding of the genetic basis of resistance from PI114490 will allow us to combine resistance to T1 based on Rx3 and resistance to T2.

References:
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Sahin, F. 1997 PhD Thesis Department of Plant Pathology, The Ohio State University, Columbus, OH.

e-mail for corresponding author: francis.77@osu.edu.
Ethics and Attitudes Seminar
Bob Heisey, Seminis Vegetable Seeds

A discussion of ethics and attitudes bring out differences in how we as breeders relate to other members of our industry. As a prelude to the discussion, we asked breeders to fill out a questionnaire. Twenty-nine people responded.

Although we as breeders have much in common, we disagree on many issues, based on the responses.

I will be the first to admit that some of the questions were unclear or ambiguous, so the results are not always a true indication of attitudes. Nevertheless, the results are interesting:

- The majority (59%) think that public breeding programs should not be involved in patenting or PVPing germplasm, and that breeders should be allowed to use germplasm from public programs with no strings attached.

- A significant majority (78%) agrees that patenting, PVPing, and other Intellectual Property Protection will slow down progress in plant breeding.

- Regarding germplasm acquisition or scouting, 66% of respondents would take germplasm samples from crop production fields, 52% from farm advisor/extension trials, 52% from seed dealer’s trials, 54% from grower’s trial; 89% would not take samples from breeder’s nurseries, 76% would not take samples from public breeder’s trial, 93% would not take samples from seed production fields. We were almost evenly split (54 to 46%) on selecting selfs in a production seed lot.

- 60% feel that there is not a lack of genetic variability in adapted tomato germplasm, 60% feel that we do have the tools to measure variability; we are almost evenly split on the reliability of data from small plot trials.

- Regarding the future of tomato breeding and breeders, a significant majority (76%) feels that universities are not training enough breeders; 72% feel that consolidation will mean fewer jobs. The overwhelming majority feels that research in conventional breeding is being under funded because of diversion of funding to genomics and biotech.

- An overwhelming majority feels that GMOs or GEOs are not unsafe, and that biotech will provide some useful tools to breeders. Most (61%) feel that biotech has been drastically oversold, and will not replace conventional breeding. We are almost evenly split (55-45%) on whether biotech will be important in solving the food deficit in the Third World.

The hour ended with a discussion of the possibility of developing a “code of ethics” for tomato breeders. It was clear that it would be difficult to come up with a meaningful code that everyone could support. Such a code would have to resolve the conflict between what is “legal” and what is “moral” on ethical, and it was clear that that argument could continue until the next TBRT, without resolution.
All programs of the Ohio Agricultural Research and Development Center are available to all potential clientele without regard to race, color, creed, religion, sexual orientation, national origin, gender, age, disability, or Vietnam-era veteran status.