PN-ADI-379

May 15, 2002

FINAL REPORT

Submitted to the Office Agriculture & Food Security
U.S. Agency for International Development

CHARACTERIZATION AND MANAGEMENT OF TOMATO AND PEPPER GEMINIVIRUSES

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Project Number: C16-020
Grant Number: TA-MOU-96-C16-020

Grant Project Officer: Larry Gumbiner

Project Duration: January 1st 1997 to December 31, 2001

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2. Executive Summary

Since 1991, farmers in south and central Jamaica have reported losses of up to 100% in their tomato and pepper crops. Increased losses were associated with viral diseases transmitted by the whitefly *Bemisia tabaci*. Owing to the potential threat to tomato and pepper production, the overall aim of this project was to provide an increased technological base for the development of disease management strategies for whitefly-transmitted geminiviral diseases affecting pepper and tomato in Jamaica. The specific objectives were:

1) To molecularly and biologically characterize the whitefly-transmitted geminiviruses infecting vegetable crops including tomatoes, peppers, and weeds in Jamaica.
2) To develop rapid diagnostic systems for the identification of geminiviruses associated with tomatoes and peppers, and use these techniques to provide geminivirus diagnosis for the Caribbean Basin countries.
3) To screen cultivated and wild species of tomato genotypes for resistance/tolerance to tomato-infecting geminiviruses.
4) To evaluate antiviral strategies for use in engineering tomatoes with resistance to infections of tomato yellow leaf curl geminivirus.
5) To produce and select begomovirus-resistant tomato varieties suitable for the Jamaica market.

Geminivirus DNA probes, polymerase chain reaction and nucleotide sequencing were used to identify and characterize geminiviruses from Jamaica and Barbados. The monopartite *Tomato yellow leaf curl virus* (TYLCV, Israeli strain), was identified in *Lycopersicon esculentum* (tomato) in Jamaica. Another, new, bipartite geminivirus, *Tomato dwarf leaf curl virus* (TDLCV) was found in tomato and Scotch Bonnet pepper (*Capsicum chinense*). Two distinct viruses infecting cabbage were identified. The common weeds *Sida* sp and *Wissadula amplissima* were found to contain geminiviruses *Sida golden mosaic virus* (SiGMV-JM) and *Wissadula golden mosaic virus* (WGMV), respectively. Three distinct geminiviruses were found in the weed *Macroptilium lathyroides*: SiGMV-JM and *Macroptilium golden mosaic virus* strains 1 and 2 (MacGMV-JM1, MacGMV-JM2). In Barbados, tomato, muskmelon and watermelon contained the same bipartite geminivirus, whereas, two distinct geminiviruses were associated with *M. lathyroides*. Phylogenetic relationships of the Jamaican viruses with other Western Hemisphere geminiviruses indicate that crop- and weed-infecting geminiviruses from Jamaica and Barbados are distinct, highly diverse, and have several geographical origins. It can be concluded that weeds are not host to crop-infecting geminiviruses in Jamaica.

A new strategy to genetically engineering tomato for resistance to TYLCV was developed. The *rep* mutant developed by this project in collaboration with the University of Wisconsin is now being engineered into tomato in the Jamaican laboratory. The virus coat protein gene V1 and the Rep protein gene *rep* have been used to transform tomato plants in Israel and Jamaica. A transgenic tomato line expressing the coat protein of TYLCV have been shown to be immune to whitefly-mediated inoculation at the University of Jerusalem. This line has been crossed with commercial varieties and hybrids are tested.

Tomato lines have been developed by the Hebrew University of Jerusalem using the wild tomato species *L. hirsutum* as a source for resistance to TYLCV. These lines showed high levels of resistance in field tests and in the laboratory. Hybrids have been made and a large-scale field trial has been conducted in Jamaica. Some of the lines showed excellent levels of resistance and marketable fruit qualities.

Cooperation between the project partners has been implemented by several long- and short-term research visits of the researchers and their collaborators in the participating laboratories.

3. Research Objectives

Since 1991, farmers in south and central Jamaica have reported losses of up to 100% in their tomato and pepper crops (McGlashan *et al.*, 1994). Increased losses were associated with viral diseases transmitted by the whitefly *Bemisia tabaci*. Preliminary analyses using the polymerase chain reaction (PCR), DNA hybridization and nucleotide sequencing have showed that whitefly-transmitted viruses from the geminivirus group, or begomoviruses, infected tomato and peppers. Two begomoviruses were detected which infect tomato; one was practically identical to the monopartite *Tomato yellow leaf curl virus* from Israel (TYLCV) and the other was a bipartite begomovirus unrelated to other characterized geminivirus. Peppers (*Capsicum annum* and *Capsicum frutescens),* which are also hosts to a number of begomoviruses causing leaf curling and other leaf distortion symptoms have never been associated with TYLCV.

Because of the worldwide occurrence of epidemics associated with begomoviruses and their potential threat to crop production, rapid and specific procedures for geminivirus detection are needed in both the whitefly vector *Bemisia tabaci* and plants to aid in epidemiology and disease management. Traditionally, serological methods have met limited success with begomoviruses because it has been difficult because different geminiviruses share similar epitopes making it difficult to differentiate closely related geminiviruses. The progress in recombinant DNA techniques offers relatively simple procedures to obtain viral cloned DNA which can be used for the production of highly specific and sensitive molecular probes. Techniques such as dot blot, squash blot, Southern blot hybridization and the polymerase chain reaction (PCR) provide sensitive and specific technique for the detection and identification of begomoviruses in plant tissue and whitefly vectors. These techniques can be used to answer precise questions about pathogen populations and diversity, and may serve as diagnostic tools.

Controlling diseases caused by begomoviruses has proven to be extremely difficult, expensive and environmentally unfriendly when conventional methods are used. Insecticides applied generally twice per week to reduce the whitefly populations have brought only limited success and in a very short time insecticide resistance in *B. Babaci.* Continuing efforts in breeding for resistance are providing new hope for controlling TYLCV. Several wild tomato species have been screened for their response to TYLCV and some accessions of *Lycopersicon pimpinellifolium, L. cheesmanii, L. hirsutum, L. peruvianum,* and *L. chilense* were found to have varying resistance (Zakay *et al.*, 1991). Breeding programs ongoing for the last 20 years have been aimed to introgress the resistant genes(s) from the wild tomato species into the domesticated tomato *L. esculentum.* Several hybrids are commercially available which show various levels of tolerance to the viral disease. Many experimental lines with good resistance have been developed during the last years. Viral DNA assays indicate that the tolerance mechanism operates through the reduction of the multiplication and/or spread of the virus in the plants or at the interface between the whitefly and the leaf (Lapidot *et al.*, 1997; Vidavsky and Czosnek, 1998). More recently, have efforts been devoted to breeding tomatoes for resistance to New World geminiviruses, based on the wild tomato genitor *L. chilense* (Griffiths and Scott, 2001). Biotechnology approaches utilizing antisense (Day *et al.*, 1991) and coat protein (Kunik *et al.* 1994) strategies have shown promise for begomovirus resistance in plants.

Owing to the potential threat to tomato and pepper production, the overall aim of this project was to provide an increased technological base for the development of disease management strategies for whitefly-transmitted geminiviral diseases affecting pepper and tomato in Jamaica. The specific objectives were:

1) To molecularly and biologically characterize the whitefly-transmitted geminiviruses infecting vegetable crops including tomatoes, peppers, and weeds in Jamaica.

2) To develop rapid diagnostic systems for the identification of geminiviruses associated with tomatoes and peppers, and use these techniques to provide geminivirus diagnosis for the Caribbean Basin countries.
3) To screen cultivated and wild species of tomato genotypes for resistance/tolerance to tomato-infecting geminiviruses.
4) To evaluate antiviral strategies for use in engineering tomatoes with resistance to infections of tomato yellow leaf curl geminivirus.
5) To produce and select begomovirus-resistant tomato varieties suitable for the Jamaica market.

4. Methods and Results

Objective 1. Molecular and biological characterisation of geminiviruses of tomato, pepper and weeds from Jamaica

Molecular characterization of *Tomato dwarf leaf curl virus*
The bipartite tomato/pepper geminivirus has been given the name *Tomato dwarf leaf curl geminivirus* (TDLCV) because of the severe dwarf and leaf curling symptoms associated with infected plants. This geminivirus was first isolated from tomato (Roye *et al.*, 1999b). Oligonucleotide overlapping primers designed to amplify the entire genome (2.6-kb) of TDLCV was used with polymerase chain reaction (PCR) to obtain the entire genome of the DNA-A using *vent* DNA polymerase. This full-length clone was tested for its ability to replicate in a tobacco cell suspension system and then sequenced. Nucleotide sequence identity of the common regions of DNA-A and DNA-B, and partial nucleotide sequences of the *ac1*, *av1*, *bc1*, and *bv1* genes confirmed the presence of a new bipartite geminivirus, *Tomato dwarf leaf curl virus* (TDLCV) in *L. esculentum* and *C. chilense*. Nucleotide sequence analysis of the common region, *ac1* and *av1* indicate that geminivirus DNA was associated with *C. papaya* (papaya virus, PaGV). Phylogenetic relationships of the Jamaican viruses with other Western Hemisphere geminiviruses indicate that crop- and weed-infecting geminiviruses from Jamaica and Barbados are distinct, highly diverse, and have several geographical origins (Roye *et al.*, 1999a). It can be concluded that weeds are not host to crop-infecting geminiviruses in Jamaica.

Molecular characterization of *Tomato yellow leaf curl virus*
In April 1997 tissue from eight tomato plants with severe dwarfing and leaf curling symptoms were brought from Jamaica and successful grafted onto tomato plants in a growth chamber at the University of Wisconsin-Madison. All the grafted plant showed typical symptoms of infected plants from Jamaica. Polymerase chain reaction and DNA hybridization showed that 7 of the 8 plants had mixed infection of TYLCV and the bipartite geminivirus from Jamaica, while one sample had TYLCV only. These plants were used as a source of the full-length infectious clones. Overlapping primers for the full-length of TYLCV have been used with PCR to amplify a 2.8-kb fragment of TYLCV form Jamaica using *Vent* DNA Polymerase (New England Biolabs). This fragment was cloned in an *Nco*I site of the plasmid p-GEM5zf(-) (Promega Corp.), since *Nco*I sites were engineered in the 5' ends of the overlapping primers. The full-length clone of TYLCV from Jamaica was partially characterized (Wernecke *et al.*, 1997). TYLCV also infected hot pepper (*Capsicum chinense*) (Brown *et al.*, 1999).
Molecular characterization of weed infecting geminivirus
Weed samples *Sida*, *Macroptilium* and *Wissadula* were collected and analysed for geminiviruses using DNA hybridization and PCR. The geminivirus DNA was then sequenced

and the phylogenetic relationships determined. *Sida golden mosaic virus* (SiGMV-JM), and *Macroptilium golden mosaic virus* (MacGMV-JM1) were closely related to *Potato yellow mosaic geminivirus* (PYMV). The viruses associated with *W. amplissima*, *Wissadula golden mosaic virus* (WGMV), *C. papaya* (PaGV), *M. lathyroides* (MacGMV-JM2) were closely related to each other. Nucleotide identity of the common regions of DNA-A and DNA-B, and partial nucleotide sequences of the *ac1*, *av1*, *bc1*, and *bv1* genes confirmed the same bipartite geminiviruses (from Barbados) in tomato, muskmelon and watermelon and two distinct geminiviruses in *M. lathyroides*.

Molecular characterization of cabbage infecting geminivirus

PCR with degenerate primer for DNA-A and DNA-B (Rojas *et al.*, 1993) amplified the expected fragments of 1.3-kb for DNA-A, 1.4-kb for DNA-B and coat protein primers (Wyatt and Brown 1996) amplified a fragment of size of 0.9-kb (expected fragment size 0.50-kb). Partial DNA sequence of the 0.9-kb fragment showed that it was 91% identical to CLCV from Florida. This is the first report of CLCV outside of Florida.

A full length DNA-B clone of a cabbage-infecting geminivirus from Jamaica was obtained. Nucleotide sequence analysis showed that DNA-B of the Jamaican cabbage virus was >96% identical to *Cabbage leaf curl virus* from Florida (CLCV-Fl). Approximately 92% or 2400 of the 2600 nucleotides of a replicating full length DNA-A clone of the cabbage-infecting geminivirus from Jamaica was sequenced. Nucleotide sequence analysis of DNA-A of the Jamaican cabbage virus showed that 1600 nucleotides which include the entire *av1*, *ac2*, and *ac3* genes and the c-terminal part of the *ac1* gene were 90% identical to CLCV-Fl. The remaining 800 nucleotides was <70% similar to CLCV-Fl and showed greatest nucleotide similarity (86%) to *Sida golden mosaic geminivirus* (SiGMV-JM) from Jamaica. Only partial nucleotide sequence of the common region for the DNA-A of the Jamaican cabbage virus was obtained. However, nucleotide sequence of this partial common region sequence to homologues region of DNA-B showed <70% similarity. This suggests that the common regions of DNA-A and DNA-B of the Jamaican cabbage virus are distinct and that two distinct geminiviruses exists in our cabbage sample. The common regions of DNA-A and DNA-B of the same bipartite geminivirus must be >90% identical to be considered the same virus. However nucleotide identity of >90% for the entire *av1*, *ac2*, and *ac3* genes and the c-terminal part of the *ac1* gene suggest that DNA-A component of the cabbage virus from Jamaica maybe a recombinant virus between CLCV-Fl and SiGMV-JM. Recombination among geminiviruses is a new phenomenon and has been shown to be a major evolutionary tool among geminiviruses.

Objective 2. Rapid diagnosis of geminiviruses

Geminivirus nucleic acid was detected in infected plant tissues by dot blot hybridization using non-radioactive molecular probes and PCR technology (Rojas *et al.*, 1993). General and specific probes from cloned geminivirus DNA representing the major phylogenetic clusters (Faria *et al.*, 1994) were labeled using the Genius II Labeling and Detection Kit (Boehringer Mannheim, IN). PCR with degenerate primers developed at the UW-Madison and non-radioactive DNA hybridization with digoxygenin-labeled probes was used for the detection of viral nucleic acids in plant tissues. Plant samples collected were dried at 50°C in a vacuum oven and stored over a desiccant at room temperature. Viral DNA was extracted essentially as described (Rojas *et al.*, 1993). To improve viral DNA recovery from the dried plant material, microfuge tubes containing the plant material and the extraction buffer was placed on ice for

30 min before grinding of the plant tissue. Dot blot hybridization was used in preference to direct squashing of the leaf on the membrane, as our experience has shown that false hybridization signals resulted from non-specific reaction with the plant tissue when digoxygenin-labeled DNA probe was used with the squash blot method.

DNA hybridization with geminiviral probes and polymerase chain reaction with degenerate primers for DNA-A and DNA-B (Rojas *et al.*, 1993) were used to detect geminiviruses in tomato, pepper, watermelon, muskmelon and several weeds including *M. lathyroides*, *L. nepthefolia* and two unidentified weeds from Barbados. DNA-A and DNA-B fragments from a tomato, a pepper and a *M .lathyroides* sample have been cloned and partial sequence. Initial DNA sequence analysis indicates that the geminivirus in the *M. lathyroides* and the tomato and pepper samples are distinct. Several tomato samples from the island of St. Lucia with leaf crumple symptoms did not hybridize with geminiviral probes and gave no PCR-amplified fragments with the degenerate PCR primers for DNA-A and DNA-B.

Objective 3. Screening of tomato genotypes for resistance to or tolerance to tomato-infecting geminiviruses.

Evaluation of commercially available tomato genotypes in field tests in Jamaica in 1997
A field test has been conducted where tomato cultivars have been evaluated for disease symptoms induced by whitefly-transmitted geminiviruses and for yields. The commercial varieties, "GemStar" and "Peto98" from Peto Seeds, "Castle Rock" from castle seeds, and "UC82" from Newman Seed Co, were evaluated. "GemStar" showed the best tolerance although the fruit was too soft. Under low whitefly populations this variety performed well. At three weeks after planting, the plants did not show symptoms. DNA hybridization tests using TYLCV probes showed that 24 % of the plants were infected with TYLCV. Under high whitefly population, 13 weeks after planting, 28% of the plants had symptoms. The other tomato varieties were all susceptible to TYLCV.

Objective 4: Virus derived strategies for conferring virus resistance

Construction of potential trans-dominant lethal mutants in the *rep* of TYLCV
Full-length infectious clones of TYLCV-Is isolate Egypt1 (Ismailia governorate) (TYLCV-EG1) clone pTYEG14 was used as a wild type template for mutant preparation. Mutants were designed to provide a *rep* product that might interfere in *trans* with the replication of wild-type TYLCV. These mutants were K^{104} to R, and D^{259} to R. The former is a conserved lysine within the putative nicking motif III identified by Koonin and Ilyina (1992). The latter mutant is conserved residue within the NTP binding site of *rep* (Gorbalenya and Koonin, 1989).

Following a Kunkle-based mutagenesis procedure using pTYEG14, colonies were generated containing mutant sequences from mutagenic primer *rep*v2000, and *rep*v2467 (Figure 1). Primer *rep*v2000 matches part of the putative NTP-binding motif and contains the codon change D^{259} to R. Primer *rep*v2467 matches part of the putative nicking motif and contains the codon change K^{104} to R. Colonies were produced for each site directed mutagenesis reaction. Colonies were named for the plasmid they contained with p and the single letter amino acid (AA) abbreviation followed by the AA position and the mutant AA with a designated colony number (i.e. $pTYD^{259}R$ # 5). Plasmid $pTYD^{259}R$ # 5 was determined to contain the codon change D^{259} to R. Plasmid $pTYK^{104}R$ # 1 was determined to contain the codon change K^{104} to R. These colonies were stored as glycerol stocks at -80°C.

The entire *rep* was cloned by PCR from the full-length mutant containing plasmids using the TA cloning kit from Invitrogen. The *rep* ORF's were amplified using primers *rep*v1707 and CRc4 and the PCR fragment cloned. Resulting colonies were streaked for single colony isolation and screened for the presence of the desired mutations by sequencing. Plasmids p*rep*D^{259}R # 2, p*rep*K^{104}R # 2 were determined to have the appropriate mutations. From this research eight new gene sequences from geminiviruses were deposited in the GenBank. The *rep* mutants have been used to transform tomato so as to develop genetically engineered tomatoes with resistance to TYLCV. Regenerated transgenic plants are currently tested for resistance to whitefly-mediated inoculation of TYLCV in the Jamaica laboratory.

Tomato plants expressing the TYLCV coat protein gene show immunity to whitefly-mediated inoculation

We have previously generated transgenic tomato lines expressing the TYLCV coat protein gene (Kunik et al., 1996). Upon whitefly-mediated inoculation, plants from some of these lines showed disease symptoms, which disappeared with time. This remission phenomenon was not observed in non-transgenic plants. In order to use these plants as source for resistance in the program for TYLCV resistance in Jamaica, we have re-evaluated the tomato lines. One plant was totally immune to infection: it did not show symptoms and the virus was undetectable by Southern blot hybridization and by PCR. The plant was multiplied vegetatively and selfed and crossed with superior tomato commercial varieties suitable for the Jamaican market. The progenies of selfing remained immune to whitefly-mediated inoculation of TYLCV. All the F1 offspring were susceptible to TYLCV whitefly-mediated inoculation. The F1 plants were backcrossed with the resistant parent. The BC1F1 plants are currently tested for susceptibility/resistance.

During a research visit of Ms Jodi Spence from Jamaica (June-July 1999), the TYLCV *Rep* gene was amplified using specific primers that included *Bam*HI (N-terminus) and *Sma*I (C-terminus). The *Sma*I/*Bam*HI digested PCR fragment was cloned into the pGEM intermediate vector followed by the binary vector pBI121 under the promoter of the 35S CaMV promoter. Regenerated plants were screened for the presence of the Rep sequence. Interestingly, all the transgenic plants showed disease-like symptoms of leaf curling and yellowing, although they were not inoculated with virus. Following whitefly-mediated TYLCV inoculation, all the transgenic plants showed exacerbated symptoms and contained large amounts of viral DNA.

Objective 5. Production and selection of begomovirus-resistant tomato varieties suitable for the Jamaica market

Establishment of a routine inoculation test for accurate selection of TYLCV-resistant/ tolerant cultivars

An inoculation routine was established to avoid escapes and to overcome the difficulties inherent to the age of the plant at the time of inoculation (the age of the plant at the time of inoculation is critical for the expression of the disease symptoms). Advanced tolerant and susceptible breeding lines as well as segregating populations originating from three wild tomato species described as tolerant to TYLCV, *L. peruvianum* EC104395, *L. pimpinellifolium* Hirsute and *L. chilense* LA1969, were used throughout. Clear differences were observed between susceptible symptomatic and tolerant symptomless tomato genotypes, indicating that the incertitude inherent to escapes, to different levels of inoculum, and to the time of inoculation, can be eliminated. The inoculation routine was further applied to a selection of resistant/tolerant individuals generated through a diallel F1 cross and F2 segregating populations (Vidavski *et al.*, 1998).

Breeding and evaluation of tomato lines tolerant and resistant to TYLCV
New tomato lines have been developed using two different accessions of the wild tomato species *L. hirsutum* as the source for resistance. Two TYLCV-immune plants from accessions LA 1777 and LA 386 of the wild tomato species *Lycopersicon hirsutum* have been crossed. The resulting immune F1 plants were crossed with the domesticated tomato *L. esculentum* and a series of selfing was performed. At each generation, individuals were selected for immunity (no symptoms and undetectable viral DNA) and tolerance (no symptoms but with detectable viral DNA), following controlled massive and repeated inoculations with viruliferous whiteflies. A stable BC1F4 line (denominated 902), which does not segregate for immunity was obtained. This line does not support virus replication even upon extensive whitefly-mediated of young seedlings and does not need protection with nets and/or insecticides. Another stable BC1F4 line (denominated 908) was tolerant to the virus. Both lines have good horticultural characteristics and bear 80-120 g red fruits. Analysis of segregation of susceptibility, tolerance and immunity during the BC1F1 to BC1F4 crosses indicated that tolerance is under the control of a dominant major gene and tolerance by 2 to 3 additive recessive genes. The immune and tolerant lines need not to be protected by insecticides and/or nets. These lines are completely immune to TYLCV from Israel (no virus, no symptoms, good yield).

Production of hybrids showing resistance to TYLCV in Israel and in Jamaica
The TYLVC tomato resistant line 902 developed from accessions LA1777 and LA386 of the wild tomato species *L. hirsutum* was used to develop the new resistant lines referred to as "Favi". Line 902 was a stable line that is resistant to TYLCV from Israel, had *L. esculentum* plant morphologies, were self compatible, and produced red fruits weighing between 80 and 120 g (Vidavsky and Czosnek, 1998). Subsequent crosses between Line 902 and a very prolific and large size tomato line but susceptible to TYLCV resulted in the hybrid Favi-9. Favi -9 was resistant to TYLCV-Is. Six resistant tomato lines, Favi -21, Favi -22, Favi -23, Favi - 24, Favi -25 and Favi -27 were derived form the hybrid Favi -9. All the Favi lines and were found to be resistant to TYLCV in Israel. These lines were also heat resistant in Israel and gave fruits at high temperature (40 °C) while other commercial lines were fruitless.

Testing tomato lines resistant/tolerant to TYLCV from Israel in Jamaica
Germplasm of commercially available varieties and of experimental tolerant breeding lines developed by the Israeli group have been tested in the field in a limited trial during July 1999. Four lines were tested which included plants resistant and susceptible to TYLCV as tested in Israel. Because of the conditions that were not optimal, yields were not appraised. The presence of the virus was tested by hybridization with virus-specific probes and the appearance of symptoms was monitored. A few samples were also tested by PCR. Six weeks after planting, the immune tomato breeding lines and their F1, the plants in the TYLCV-infected field are symptomless. The results are summarized in Table 1. Line Favi-9 segregated in the field; some plants were resistant and others susceptible. The tomato line Favi-16 was susceptible but the line Favi-18 showed good levels of resistance.

A large-scale field test took place, beginning of 2001, at Bodles Agricultural Research Station, (Min. Agric), St. Catherine, Jamaica, under the supervision of Mr. Don McGlashan. The plants were evaluated during the months of May and June 2001. The test included resistant Favi lines, the susceptible check line Favi -26 along with a susceptible commercial variety UC82 (Cal Seed Inc. USA). Seedling were germinated in seedling trays in potting mix and maintained in a screen house for 6 weeks. The seedlings were then transferred to the open

field where they were naturally exposed to viruliferous whiteflies. Tomato lines were grown on single beds in clay-loam soil type. Plants were fertilized at flowering and at fruiting. Pest and disease were controlled with applications of Karate (for cutworm), Tambo (for pinworms), Sevin (for cucumber beetle) and Ridomil to control fungus. Whiteflies were not treated. Plants were watered using drip irrigation and supported using a trellis system. Each tomato plant was evaluated for TYLCV disease by symptom expression. Tomato fruits from 10 plants per plot were harvested and weighed. The shelf life of the fruit was determined by harvesting at different stages of ripeness. The results are summarized in Table 2. The commercial variety UC82 and the susceptible Favi-26 were the only two lines that were infected by TYLCV and had 90% symptom expression, with yields of 10 and 11.4 kg per 10 plants respectively. All the Favi lines had *L. esculentum* characteristics and produced red fruits weighing between 150 and 200 g. The yield ranged between 10 and 41 kg per 10 plants. Fruits harvested from stages 1 (mature, no red) to 4 (red fruits) and held at room temperature (25°C) remained firm for periods ranging from 2 weeks at stage 4 to 6 weeks from stage 1. The fruits produced were of high quality and are suitable for the hotel and supermarket businesses. The fruits were firm, which is the preference of farmers since they would be transported without damage to the markets.

5. Impact relevance and Technology Transfer

This projects has helped develop the ties between the three partners which have established a strong collaboration implemented by:

1. meeting in Jamaica to evaluate problems and provide solutions
2. hosting trainees from Jamaica in the relevant laboratories in Israel and the USA
3. transferring technologies such as production of virus-derived resistant transgenic tomato, cloning and diagnosis of tomato and pepper whitefly-transmitted geminiviruses
4. testing tomato germplasm and breeding tomato for resistance to whitefly-transmitted geminiviruses in Jamaica

Field tests have been conducted where germplasm has been evaluated and selected for resistance against TYLCV under climatic conditions and for the Jamaican market. The following abstract summaries perfectly the gains of this project for Jamaica.

Evaluation of new tomato lines with resistance to TYLCV in Jamaica.

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ABSTRACT

Two field trials were done at Bodles Agricultural Research Station, St. Catherine, Jamaica to evaluate new tomato lines for resistance to Tomato Yellow Leaf Curl Virus (TYLCV). The first trial was established in March 2001 and included 16 Favi lines, the susceptible check line Favi-26, along with a locally susceptible commercial cultivar UC 82B. In September 2001, another trial was implemented with five lines – Adonis, Nemo-Netta, breeding line 1651827, Jericho-9 (formerly favi-9) and Duke, which was a large fruited susceptible check. Seeds were germinated in polythene trays and maintained in screenhouse for five weeks. Seedlings were

then transplanted to field where they were naturally exposed to the TYLCV vectors, the whiteflies. Each tomato plant was evaluated for TYLCV based on symptomatology and southern blot assays. Results of the first trial showed that 25 days after transplanting, 6 Favi lines were negative for TYLCV. One of the six, Favi-9 was large fruited, highest yielding, possessing a deep red flesh colour and with good shelf life. In the second trial, Adonis and Jericho 9 were shown to be resistant to TYLCV, while the other lines were susceptible. There were significant differences ($p = < 0.05$) in yield among varieties, with Adonis and Jericho 9 being the highest yielders.

In the light of these achievements, we have discussed the possibility to establish a seed production facility in Jamaica to supply seeds to Jamaica and other Caribbean countries.

6. Project Activities, Outputs

Research visits

1. The three partners of the project met at the University of the West Indies, Kingston Jamaica, from April 3 till April 8, 1997. They reviewed the objectives of the proposal, discussed modalities of cooperation and defined the immediate tasks of each partner. The partners went to field trips to appraise the magnitude of the TYLCV disease on tomato. They visited a private farm and a research institution in the St. Catherine Parish, near Kingston.
2. Dr Marcia Roye from the University of the West Indies, Jamaica, visited the University of Wisconsin, May - July 2000. Dr. Roye has cloned TYLCV and another whitefly-transmitted geminivirus affecting tomato in Jamaica.
3. Dr. Favi Vidavski from the Hebrew University of Jerusalem, Israel, participated in the evaluation of tomato germplasm for resistance to TYLCV during a large field test, May-June 2001.

Training

Two graduate students for the University of the West Indies, Jamaica, received training.

- Ms. Jodi Spence (MPhil student) visited The Hebrew University, Jerusalem, July - September 1999, to learn techniques in plant transformation, whitefly biology, transmission procedures and tomato germplasm evaluation.
- Ms. Pamella Burrell (PhD student) visited The University of Wisconsin, USA, June - August 1999, to learn techniques in molecular biology.

Publications

Roye, M. E. W. A. McLaughlin, J. D. Spence and D. P. Maxwell (1999). The common weed *Macroptilium lathyroides* is not a source of crop infecting geminiviruses from Jamaica. Tropical Agriculture **76**.

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7. Project productivity

The project has achieved its goals. A fruitful collaboration has been achieved between all partners, based on research visits and collaborative experiments. Viruses affecting tomato and pepper have been characterized; diagnostics tools have been developed. Transgenic tomato lines have been developed using the virus-based approach, mostly as a research tool because of public acceptance of genetically modified organisms. Virus-resistance tomato lines have been developed which perform very well in Jamaica and which answer the needs of the local market.

8. Future Work

We are planning to set up a demonstration field at Bodles Agricultural Research Station during the summer of using the line Favi-9 of about 2,500 plants. Susceptible controls and commercial varieties will be included. The farming community, Ministry of Agriculture personnel, retailer and other interested parties will be invited to a field day to obverse the performance of new

TYLCV resistant tomato variety. Seeds of Favi9 (renamed Jericho 9) have been distributed to farmers for evaluation.
We have discussed the possibility to establish a seed production facility in Jamaica to supply seeds to Jamaica and other Caribbean countries.

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10. Appendices

Figure 1: Production of transgenic tomato plants using the *rep* dominant mutant approach: Changes in amino acid codons for the NTP-binding motif and DNA-nicking motif of *rep* ORF of TYLCV-Is EGI1

NTP-binding site mutation:

Wild type TYLCV-Is	AA	D V D **D** I V N Y
Wild type TYLCV-Is	nt[a]	GG GTC TAC GTC **ATC** AAT GAC GTT GTA CC
Wild type TYLCV-Ja	AA	D V D **D** I V N Y
Wild type TYLCV-Ja	nt[a]	GG GTC TAC GTC **ATC** AAT GAC GTT GTA CC
Wild type pTYEG14	AA	D V D **D** I V N Y
Wild type pTYEG14	nt[a]	GG GTC TAC GTC **ATC** AAT GAC GTT ATA CC
D^{259} to R	AA	D V D **R** I V N Y
Primer *rep*v2000	nt[a]	GG GTC TAC GTC **TCT** AAT GAC GTT GTA CC

Nicking motiff III mutation:

Wild type TYLCV-Is	AA	N G D **K** E V Y
Wild type TYLCV-Is	nt[a]	GTT TCC GTC **TTT** CTC CAC GTA GG
Wild type TYLCV-Ja	AA	S P D **K** E V Y
Wild type TYLCV-Ja	nt[a]	TCT CCC GTC **TTT** CTC CAC GTA GG
Wild type pTYEG14	AA	N G D **K** E V Y
Wild type pTYEG14	nt[a]	GTT TCC GTC **TTT** CTC CAC GTA GG
K^{104} to R	AA	N G D **R** E V Y
Primer *rep*v2467	nt[a]	GTT TCC GTC **TCG** CTC CAC GTA GG

[a] viral sense sequence; amino acids derived from complementary sense strand.

Table 1. Response of selected tomato lines to whitefly-mediated inoculation of TYLCV in Jamaica, Summer 1999.

Tomato line	Plant No	Hybridizations		PCR *	Symptoms
		First	Second		
Favi 9	1	-	Died		
	2	-	-		-
	3	-	Died		
	4	+	+++	+	+
	5	+	+	+	+
	6	-	+++		+
	7	+	-		+
	8	-	-		-
	9	+	+		+
	10	-	-		-
Favi 16	1	-	-		
	2	+++	+		+
	3	++++	+	+	+
	4	++++	++	+	+
	5	+	++		+
	6	+++	+		+
	7	++++	+++		+
	8	++++	++++		+
	9	+++	Died		
	10	++++	+++		+
Favi 17	1	+	+	+	+
	2	-	+/-		-
	3	-	+/-		-
	4	-	-		-
	5	-	-		-
	6	+/-	Died		
Favi 18	1	-	-		-
	2	-	-		-
	3	-	-		-
	4	+/-	+	+	-
	5	-	+	+	-
	6	-	-		-
	7	-	+		+
	8	-	+	+	+

First Hybridization 3 weeks post inoculation; Second hybridization 3 months post inoculation
* only a few random samples were used for PCR; a 0.3 kbp fragment from the IR is amplified
+ = Showed positive hybridization signals with TYLCV intergenic region probe.
+/- = weak hybridization signals with TYLCV intergenic region probe.
- = no hybridization signals with TYLCV intergenic region probe.

Table 2. Tomato fruit yields (kg/plot) from 10 plants per plot and plants infected by TYLCV. Field test conducted in Jamaica during winter spring 2001

Line/genotype	Average fruit weight (grams)	Yield (kg per 10 plants)	Plants with symptoms (%)
UC 82 (susceptible)	90 **	11.4	90
Favi-9	160-210	21.5	0
Favi -21	160-210	20.5	0
Favi -22	160-210	not determined	5
Favi -23	160-210	25.5	0
Favi -24	160-210	41	0
Favi -25	160-210	32	0
Favi -26 (susceptible)	160-210 **	10	90
Favi -27	150	20	0

Infection probably started at week 3 after planting
** Late infection

Field test, Bodles, Jamaica, Spring 2001

Dr Favi Vidavski from the Hebrew University of Jerusalem, examining tomato plants. Field test, Bodles, Jamaica, Spring 2001

Dr Douglas P Maxwell (DPM) from the University of Wisconsin, discusses test with Dr Don McGlashan (DMG) director of the Bodles Agricultural Research Station and Dr Marcia Roye (MR) from the University of the West Indies at Mona. Field test, Bodles, Jamaica, Spring 2001

TYLCV resistant tomato plants from line Favi 9 (Jericho 9). Field test, Bodles, Jamaica, Spring 2001

Tomato fruits from TYLCV-resistant tomato lines Favi 9 (left) and favi 25 (right). Field test, Bodles, Jamaica, Spring 2001

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