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CHARACTERIZATION AND MANAGEMENT OF TOMATO AND PEPPER GEMINIVIRUSES

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1. Scientific summary

Overall aim

The overall aim of this project is to provide an increased technological base for the development of disease management strategies for whitefly-transmitted geminiviral diseases affecting pepper and tomato in Jamaica.

Specific Objectives

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.

Summary of achievements

Geminivirus DNA probes, polymerase chain reaction and nucleotide sequencing were used to identify and characterzse geminiviruses from Jamaica and Barbados. A new bipartite geminivirus, tomato dwarf leaf curl virus (TDLCV) was found in *L. esculentum* (tomato) and *C. chinense* (Scotch Bonnet pepper). 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* (macroptilium golden mosaic virus strains 1 and 2, MacGMV-JM1, MacGMV-JM2 and SiGMV-JM). 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. Additional mutants in the coat protein gene V1 and the rep protein gene have been developed and at the University of Jerusalem and are 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 will be tested shortly.

A first small-scale field trial has been conducted in Jamaica that involved tomato lines developed by the University of Jerusalem; some of the lines show appreciable levels of resistance. A full-scale trial will be performed at the beginning of 2001.

Eight new gene sequences from geminiviruses were deposited in the gene bank (http://www.ncbi.nlm.nih.gov/GenBank). Four publications were generated from this work along with several presentations locally and internationally.

Achievements

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

Molecular characterization of tomato dwarf leaf curl virus: Oligonucleotide overlapping primers designed to amplify the entire genome (2.6-kb) of a new tomato/pepper infecting geminivirus tomato dwarf leaf curl virus (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. chinense*. Nucleotide sequence analysis of the common region, *ac1* and *av1* indicate that geminivirus DNA was associated with *C. papaya* (papaya virus, PaGV).

Molecular characterization of tomato yellow leaf curl geminivirus: A full-length clone of tomato yellow leaf curl geminivirus from Jamaica TYLCV was cloned and partially characterized.

Molecular characterization of weed infecting geminivirus: Weed samples sida, macroptilium and wissadula were collected and analysed for geminiviruses using DNA hybridisation and PCR. The geminivirus DNA sequenced was then sequenced and the phylogenetic relationships determined. SiGMV-JM, and MacGMV-JM1 were closely related to potato yellow mosaic geminivirus (PYMV). The viruses associated with *W. amplissima* (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: A full length DNA-B clone of a cabbage-infecting geminivirus from Jamaica was obtained. Nucleotide sequence analysis using the DNAStar program 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 includes the entire *av1*, *ac2*, and *ac3* genes and the c-terminal part of the *ac1* gene was 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) was labelled 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 by Rojas et al. (1993) and Dellaporta et al., (1983). 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 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.

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

A limited test was performed in Jamaica to screen tomato germplasm developed at the Hebrew University of Jerusalem. 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. The following table summarizes the results.

Plant Number	1st Hybridization Result	2nd Hybridization Result	PCR (IR primers kb)*	Shows Symptoms
Favi 9-1	-	Died		
9-2	-	-		-
9-3	-	Died		
9-4	+	+++	0.3	+
9-5	+	+	0.3	+
9-6	-	+++		+
9-7	+	-		+
9-8	-	-		-
9-9	+	+		+
9-10	-	-		-
Favi 16-1	-	-		
16-2	+++	+		+
16-3	++++	+	0.3	+
16-4	++++	++	0.3	+
16-5	+	++		+
16-6	+++	+		+
16-7	++++	+++		+
16-8	++++	++++		+
16-9	+++	Died		
16-10	++++	+++		+
Favi 17-1	+	+	0.3	+
17-2	-	+/-		-
17-3	-	+/-		-
17-4	-	-		-
17-5	-	-		-
17-6	+/-	Died		
Favi 18-1	-	-		-
18-2	-	-		-
18-3	-	-		-
18-4	+/-	+	0.3	-
18-5	-	+	0.3	-
18-6	-	-		-
18-7	-	+		+
18-8	-	+	0.3	+

First Hybridization 3 weeks post inoculation; Second hydridization 3 months post inoculation
* only a few random samples were used for PCR
+ = 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.

From the results shown in the table, tomato line Favi-9 segregates in the field; some plants are resistant while others are susceptible; the tomato line Favi-16 is susceptible but the line Favi-18 shows good levels of resistance. A large-scale field test is scheduled to take place in Jamaica the beginning of 2001 where the number of lines and of plants will be considerably extended.

Objective 4: Virus derived strategies for conferring virus resistance

1. Construction of potential trans-dominant lethal mutants in the *rep* of TYLCV

Full-length infectious clones of TYLCV-Is isolate Egypt1 (Ismailia governorate) (TYLCV-Is 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 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 $prepD^{259}R$ # 2, $prepK^{104}R$ # 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 are now being used to transform tomato so as to develop transgenic (genetically engineered) tomatoes with resistance to TYLCV.

2. 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 form 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 PCR. The plant was multiplied vegetatively and was crossed with superior tomato commercial varieties suitable for the Jamaican market. The offspring are currently evaluated.

2. Scientific issues

The results obtained during the third year of the research program do not justify any change to the proposal.

3. Managerial issues

The duration of the project has been extended with no additional costs to allow a full-scale field test in Jamaica of tomato plants that have been generated through breeding, and to evaluate the transgenic tomato plants in the laboratory.

4. Special concerns

None

5. Collaborative activities

Training

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

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

Research visits

Dr Marcia Roye visited the University of Wisconsin, May – July 2000.

6. 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**.

Roye, M. **E.**, M. E. Wernecke, W. A. McLaughlin, M. K. Nakhla and D. P. Maxwell. (1999). Tomato dwarf leaf curl virus, a new bipartite geminivirus associated with tomatoes and peppers in Jamaica and mixed infections with tomato yellow leaf curl virus. Plant Pathology **48**: 370-378

Roye, M.E. Burrell, P. D. McLaughlin, W.A., Nakhla, M.K. and Maxwell, D. P. 2000. A new tomato-infecting geminivirus related to the squash family of geminiviruses in Barbados. Plant Disease (Submitted).

Brown, S., Green, C._Roye, M.E., M., McLaughlin, W.A. Nakhla, M.K., Maxwell, D. P. 1999. Tomato yellow leaf curl geminivirus infecting hot pepper (*Capsicum chinense*) in Jamaica. Proceedings of the Fourth Conference of the Faculty of Natural Sciences, University of the West Indies Mona. pg. 38

Burrell, P. Roye, M.E., McLaughlin, W.A. 1999. Genetic diversity of geminiviruses from Barbados, Antigua and St Kitts. Proceedings of the UWI/OAS/NCST Regional Conference University of the West Indies, Mona, Kingston, Jamaica.

Roye, M.E., M., McLaughlin, W. Nakhla, M.K., Maxwell, D. P. 1999. Genetic diversity among geminiviruses infecting crops and weeds in Jamaica. VIIth International Plant Virus Epidemiology Symposium, Almeria Spain. pg. 78

Vidavski, S., Czosnek, H. 1999. Tomato lines immune to tomato yellow leaf curl virus infection. VIIth International Plant Virus Epidemiology Symposium, Almeria Spain.

Roye, M.E., M., McLaughlin, W. Nakhla, M.K., Maxwell, D. P. 1999. Genetic diversity and relationships among geminiviruses from Jamaica and Barbados. In Jamaica. Proceedings of the Fourth Conference of the Faculty of Natural Sciences, University of the West Indies Mona. pg. 37

M. E. Roye 1999 Geminiviruses infecting crops and weeds in Jamaica and their control. CALGENE (A Division of Monsanto) Davis, California USA.