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Full Length Research Paper

Genetic variation among active and inactive transmitters of *Maize streak virus* within a population of *Cicadulina storeyi* China (Homoptera: Cicadellidae)

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This study was undertaken to delineate active transmitters from inactive ones within a population of *Cicadulina storeyi* China, vector of *Maize streak virus* (MSV), genus *Mastrevirus* using Mendelian crosses, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and isozyme esterase analysis. This study indicated that the variation is genetic. Four out of six Mendelian crosses perfectly fitted the expected ratio. Molecular studies with random amplified polymorphic DNA (RAPD)-PCR and isozymic studies with esterase analysis showed significant polymorphism and were useful to separate active and inactive transmitters within *C. storeyi* population. MSV was detected within the vectors through PCR and IC-PCR. IC-PCR was used to show that there was no difference in the distribution of MSV within active and inactive *C. storeyi* as the virus was detected in the head, thorax and abdomen of active and inactive transmitters.

Key words: *Cicadulina stroreyi*, crosses, active/inactive transmitters, polymorphism.

INTRODUCTION

Maize streak virus (MSV), genus *Mastrevirus*, is an indigenous African virus and causes an important disease of maize (*Zea mays* L.). It is restricted to Africa and its neighbouring islands (Rose, 1978; Thottappilly et al., 1993). MSV is transmitted in a persistent manner by *Cicadulina* leafhoppers (Homoptera: Cicadellidae). The vector is able to retain the virus throughout its lifespan.

From the early works of H.H. Storey (Storey 1932, 1933, 1938, 1939a, b), *C. mbila* (Naude) populations of South Africa consisted of active and inactive races. While the active races were able to transmit MSV, the inactive races could not transmit the virus during normal feeding activities. He postulated that in inactive races, although virus was acquired while the vector fed on infected plants, the virus could not cross the gut wall and pass into the haemolymph and the salivary glands from where it should pass into another plant during feeding. He also postulated that the ability of MSV to permeate the gut wall of the vector depends on a major gene that was linked to the sex chromosome (Storey, 1932, 1933). Active and inactive races in *C. mbila*, *C. storeyi* China (Okoth et al., 1988), *C. ghaurii* Dabrowskii and *C. arachidis* China (Asanzi et al., 1995) have also been reported in Nigeria. Natural populations of *C. mbila* and *C. storeyi* had more active transmitters than populations of *C. ghaurii* and *C. arachidis* (Effron et al. 1989).

Since the early works of Storey in South Africa, and other studies on biology of the vectors as it affects epidemiology of maize streak virus disease in Nigeria (at International Institute of Tropical Agriculture, Ibadan), no effort has been made to study the genetics of MSV transmission by *Cicadulina* species. This study was undertaken to delineate active transmitters from inactive ones within a population of *C. storeyi* using Mendelian crosses, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR**)** and isozyme esterase analysis.

MATERIALS AND METHODS

Mendelian genetics of MSV transmission by *C. storeyi*

Third or fourth instars of *C. storeyi* from IITA mass-reared colonies were confined with polyvinylchloride (PVC) tubes, in batches of 100, for 2 days on maize seedlings (open-pollinated, susceptible variety *Pool 16*) that had severe MSV symptoms. The nymphs were then caged singly on 7-10 day-old healthy seedlings for 15-20

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days. During this period, the nymphs developed to adults and also transmitted MSV to the maize seedlings on which they were confined. The adult leafhoppers were then separated into active and inactive males and females: active because the vectors transmitted the virus to the test plant or inactive because the leafhoppers did not transmit MSV to the test plant. Appearance or otherwise of symptoms on test seedlings indicated transmission activity of the leafhoppers. A table lamp with a magnifying lens was used to separate males from females by observing the presence (female) or absence (male) of ovipositor in the last abdominal segment.

Individual males and females were then caged in pairs on another set of 7-10 day-old healthy seedlings. This pairing allowed them to mate and produced offspring. Four types of crosses were made viz: active male X active female, active male X inactive female, inactive male X active female, inactive male X inactive female. After five weeks, the progenies from each parent-pair were collected and caged for two days on MSV infected maize leaves with severe symptoms. This step was to allow the progenies to acquire MSV. They were then caged individually on healthy 7-10 days -old seedlings for another 15-20 days to test whether they were able to transmit MSV or not. MSV susceptible maize variety *Pool 16* was used as test plant.

The data collected included: the total number of progenies produced per cross, the number that transmitted MSV (i.e. active population) and the number that did not transmit MSV (inactive population) . Goodness of fit to the expected F1 ratio for each cross was tested by χ^2 analysis.

Random amplified polymorphic DNA

DNA extractions from individual insects were done according to Livak (1984). Single insects were collected in 1.5 ml eppendorf tubes and homogenised in 200 µl 2X CTAB buffer, using a plastic micropestle. 200 µl 20% SDS was then added. The samples were then vortexed and incubated at 65°C for 10 min. After incubation, the samples were allowed to cool on ice and equal volume of phenol/chloroform mixture (1:1) was added. These were mixed thoroughly followed by centrifugation at 14,000 rpm (for 10 min at 4°C) to precipitate DNA. The supernatant was immediately poured off and the nucleic acid pellet was left to air -dry at room temperature. Finally, 150 µl sterile distilled water was used to dissolve the DNA at 65°C for 10 min. For subsequent PCR, 5 µl of this stock DNA were used.

Genetic variation studies using RAPD- PCR were undertaken following the protocol previously used by Puterka et al. (1993), Mendel et al. (1994) and Dowdy and McGaughey (1996). DNA preparation from individual insects was as described by Livak (1984). Amplification reactions were carried out in 25 µl reactions volumes each containing 50 ng of DNA, 100 µM each of deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP), 1.7 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl and 0.4 mM of one oligonucleotide decamer primer. Reactions were performed in a thermocycler model 9600 (Perin- Elmer Cetus, Norwalk, Com., USA) programmed for 1 cycle at 94°C for 3 min, followed by 45 cycles of 94°C for 1 min, 35°C for 1 min and 72°C for 2 min. After the 45 cycles, the samples were held at 72°C for 7 min (final extension step) and then stored at 4°C until they were electrophoresed. Amplification products were separated on 1.4% agarose gels using 1X TAE buffer (45 mM Tris-acetate, 0.1 mMEDTA, pH 8.0) stained with ethidium bromide (10 µg/ml) and photographed with UV light with polaroid film type 667.

The primers used to study genetic variation of active and inactive races of *C. storeyi* were purchased from Operon Technologies (Alameda CA, USA) and each was 10 nucleotides long.

The genetic variability patterns were scored on the basis of the presence or absence of amplified products. For each primer, the presence (1) or absence (0) of amplified DNA bands were scored in a table using only those major bands whose presence or absence could be easily distinguished. Since RAPD products are dominant, a locus was considered polymorphic if the presence of bands was observed in only some individuals. A monomorphic locus had bands present in all individuals. Pairwise distance matrices were compiled using the Jaccard's coefficient of similarity (Jaccard, 1908) present in NTSYS- PC 1.8 software packages (Hohlf, 1993). Dendograms were created by UPGMA cluster analysis (Sneath and Sokal, 1973).

Detection of MSV within vectors

Two types of PCR techniques were used to detect MSV within adult *C. storeyi*. These were the standard PCR involving the amplification of whole insect DNA extracts, and immunocapture PCR (IC-PCR) which amplifies the DNA of the virus previously trapped by antibodies. IC- PCR detection of streak viruses in insect samples was carried out following the protocol of Jansen et al. (1990) and Hoffmann et al. (1997). Individual insects or insect parts were ground in 50, 100 or 200 µl (depending on sample size) of PBS-T-PVP in 1.5 ml sterile Eppendorf tubes using sterile micropestles. The samples were then centrifuged (14000 rpm at 4°C) for 1 min. The supernatant was used for IC-PCR assays

0.5 ml Eppendorf tubes were coated with MSV antibodies by loading 50 µl of MSV polyclonal antiserum diluted 1:1000 in a coating buffer (15 mM $Na_2CO)_3$, 34.9 mM $NaHCO_3$ pH 9.6). The tubes were incubated for 2 - 3 h at 37°C and then washed with PBS- Tween (three times with three min soaking). 50 µl of streak virus saps were then added to the coated tubes and the tubes were incubated overnight at 4°C. The tubes were washed three times with PBS-Tween after which 25 µl of PCR cocktail was added to each tube.

IC-PCR conditions were similar to the standard PCR except that no DNA was added. The trapped antibody - antigen complex in the tubes served as DNA template. In addition, the volume of sterile distilled water was increased from 9.6 to 14.6 µl per tube. About 30 µl of sterile mineral oil was also layered on the surface of the solution in each tube to prevent evaporation during the PCR cycling. The reaction conditions were the same as the standard PCR.

The primers used for detection of MSV through PCR are listed in Table 1 with their different nucleotide sequences. Five primers were purchased from Integrated DNA Technologies Inc., USA. The first four were 17- mers (Rybicki and Hughes, 1990) while the fifth was 19-mer (Ed. Rybicki, personal communication). The sixth primer pair was produced by GIBCO, BRU, USA and supplied by Dr. P. G. Markham (John Innes Institute, U.K.) . The seventh primer pair was produced and supplied by Dr. S. Winter (Plant Virus Division, DSMZ, Germany).

Esterase analysis

Polyacrylamide gel electrophoresis (PAGE) was also used to analyze isozyme esterase activity in *C. storeyi* . The procedure described by Devonshire and Moores (1982) was followed.

Individual leafhoppers were ground in 1.5 ml Eppendorf tubes containing 30 µl of the extraction buffer (0.05M Tris-HCl pH7.1; 15% sucrose; 0.5% Triton X-100) using micropestles. The tubes containing the samples were centrifuged (Chermle, Z360) at 5000 rpm for 5 min at 4°C. The samples were then removed and kept on ice.

Polyacrylamide gels were prepared according to LKB 2050 Midget Electrophoresis Unit laboratory manual (Pharmacia LKB Biotechnology, Uppsala, Sweden). Electrophoresis (20 µl of samples) was performed on vertical unit (Bio-rad model 200 /2.0

Table 1. Oligonucleotide primers used in polymerase chain reactions to detect MSV.

Virus	Primer code	Nucleotide sequence 5' to 3'	Size
MSV	Forward 1F	TTC ATC CAA TCT TCA TC	17mer
MSV	Reverse 1R	GGA AAA TCT ACT TGG GC	17mer
WDV	Forward 2F	TTG AGC CAA TCT TCG TC	17mer
WDV	Reverse 2R	GGA AAG ACT TCC TGG GC	17mer
CSM	Forward 3F;	TGC AGC CAG TCT TCA TC	17mer
CSM	Reverse 3R	GGA AAG ACT TCT TGG GC	17mer
DSV	Forward 4F	TTC ATC CAA TCT TCA TC	17mer
DSV	Reverse 4R	GGA AAG TCT ACT TGG GC	17mer
MSV	Forward 5F	TTG GVC CGM VGA TGT ASA G	19mer
MSV	Reverse 5R	CCA AAK DTC AGC TCC TCC G	19mer
MSV	Forward 6F	GCT AGA ATT CAT GTC CAC GTC CCA AGA GG	29mer
MSV	Reverse 6R	GTC AGA GCT CTT ACT GGT TGC CAA CAC TCT TA	32mer
MSV	Forward 7F	TTG GVC CVM VGA TGT ASA G [§]	19mer
MSV	Reverse 7R	CCA AAD NKC ASC TCC TCC G [§]	19mer

§ where D=A/G/T; N=A/C/G/T; K=G/T; S=C/G; V=A/C/G; M=A/C

Table 2. χ^2 values and probabiliies of inheritance of MSV transmission ability of *C. storeyi* from different crosses between active and inactive populations.

Cross	No of individual male and female crossed	Progenies			Expected ratio	χ^2 value	Probability
		Active	Inactive	Total			
Active male X active female	10	87	29	116	3:1		
Active male X active female	16	35	0	35	1:0		
Active male X Inactive female	2	16	11	27	1:1	0.926	0.30-0.50
Inactive male X active female	3	18	21	39	1:1	0.230	0.50-0.70
Inactive male X active female	4	46	9	55	1:0		
Inactive male X inactive female	3	0	25	25	0:1		

power supply). The gel was run for about 5 h at a constant 300 V with running buffer. After electrophoresis, the gels were stained for the esterase according to Wendel and Weeden (1989). The gel was then examined with a white light box (Vari Quest 100, white light, Fotodyne Incorporated). The isozyme patterns on the gel were documented with a Polaroid camera (Polaroid MP_4 Land Camera). Each band revealed by isozyme analysis was scored as a binary variable (0, 1) . If a band was present, it was scored '1", if absent'0'. Band sharing analysis was as described earlier.

RESULTS

Mendelian genetics of *C. storeyi* transmission ability

The results of experiments to study the Mendelian genetics of MSV transmission ability of *C. storeyi* are presented in Table 2. Out of the six possible crosses that were made, four perfectly fitted the expected ratio. The χ^2 values obtained when active males were crossed with inactive females (0.926) and when inactive males were crossed with active females (0.230) were not significant at 5% level of probability but showed 30-50% and 50-70% possibility of occurrence, respectively.

Separation of active and inactive transmitters of *C. storeyi* by RAPD-PCR

The results of RAPD- PCR experiments among active and inactive transmitters of *C. storeyi* are presented in Table 3 while Figure 1 illustrates the type of RAPD bands produced among active and inactive populations. The number of RAPD marker loci detected in the vector population ranged from 3 for primer OPM-12 to 13 loci for primer OPO- 13 and the size of the amplified fragments ranged from 200 to 2600 bp. A total of 79 amplified DNA bands were scored of which 28 (35.44%) were polymorphic. All the primers showed some polymorphism ranging from 12.50 to 83.33%. The highest level of polymorphic bands were from OPT-12, with 83.33% polymorphism.

Table 3. List and sequence of the 10-base oligonucleotide primers used to study variation among active and inactive *C storeyi* by RAPD-PCR showing the number of amplified fragments, the number of polymorphic bands and the percent polymorphic bands

Operon Code	Nucleotide Sequence 5' to 3'	No of fragment amplified	Noof polymorphic bands	Percent polymorphic bands
OPL-3	CCAGCAGCTT	7	1	14.29
OPL-8	AGCAGGTGGA	8	2	25.00
OPL-12	GGGCGGTACT	8	1	12.50
OPM-12	GGGACGTTGG	3	2	66.67
OPN-4	GACCGACCCA	7	3	42.86
OPN-8	ACCTCAGCTC	7	1	14.29
OPN-9	TGCCGGCTTG	11	5	45.45
OPN-10	ACAACTGGGG	9	3	33.33
OPO-13	GTCAGAGTCC	13	5	38.46
OPT-12	GGGTGTGTAG	6	5	83.33

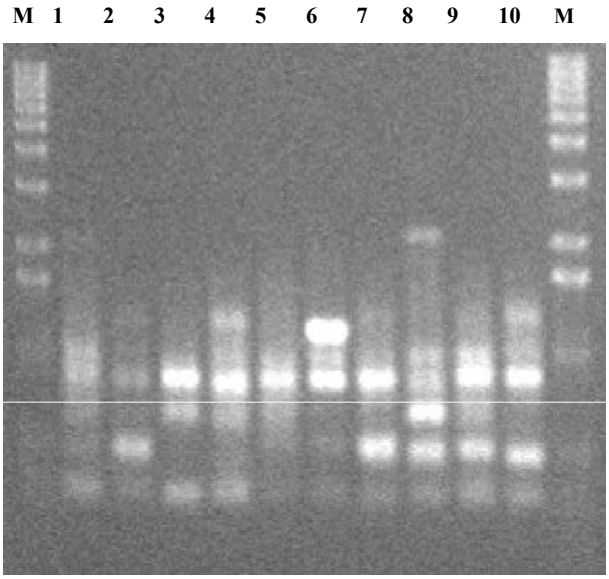

Figure 1. Example of RAPD bands while screening for polymorphism between active and inactive transmitters of *C. storeyi* females generated by 10-base nucleotide primer OPL-8. Lanes 1-5, active transmitters; Lanes 6-10, inactive transmitters. Column M is 1 kb ladder DNA molecular size markers.

Relationship dendograms were drawn from the data produced from each primer. Figure 2 illustrates the dendogram produced from OPN-10 primer. The dendogram showed that the five insects that transmitted MSV (denoted by T) were closely related while the other five insects that could not transmit MSV (denoted by NT) were also closely related. At 65% coefficient, the dendogram splits into four clusters. The first cluster consisted all five inactive insects. These inactive transmitters showed 65-90% similarity. The other three clusters were

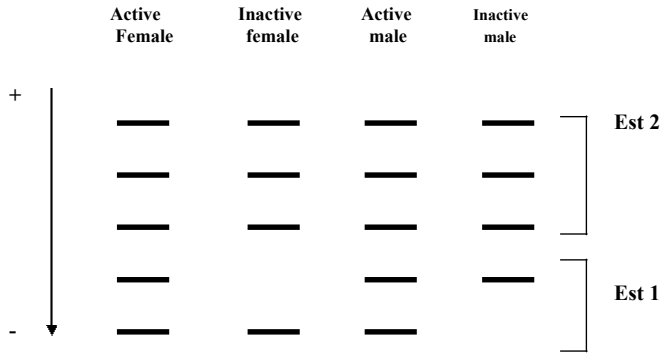

Figure 3. Isozyme banding patterns detected for esteras in active and inactive transmitters of male and female *C. storeyi*.

made up of the five active transmitters with 52.5-77.5% similarity among the samples.

Separation of active and inactive transmitters of *C. storeyi* by esterase analysis

Polymorphism was observed in the esterase analysis of active and inactive male and female *C. storeyi*. The observed zymogram pattern is illustrated in Figure 3. The gels showed two zones of activity. The fastest migrating zone (Est 1) was polymorphic with absence of bands (for inactive males), single-banded (for active males) and double-banded patterns for females. However, the position of the second band for active and inactive females was different. The lower migrating zone (Est 2) was polymorphic with four-banded and five-banded patterns. Active males and females had five-banded patterns while inactive males and females had four banded patterns.

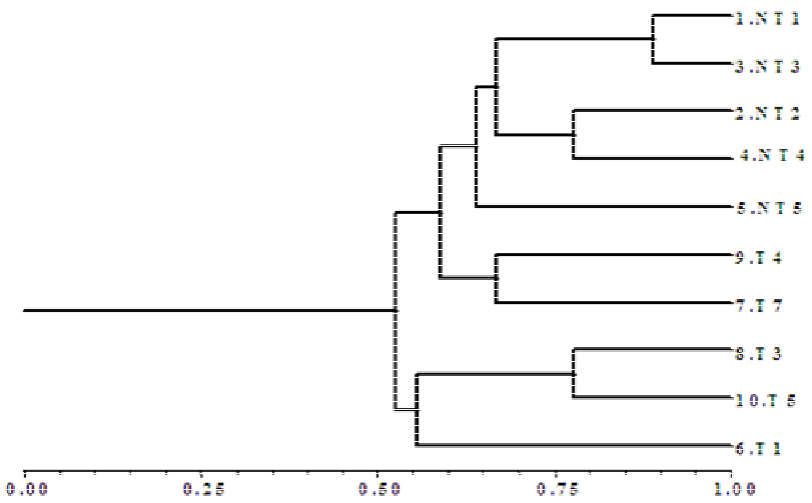

Figure 2. Dendogram from RAPD-PCR showing genetic similarity between active (denoted as T) and inactive (denoted as NT) transmitters of *C. storeyi* females using OPT-12 primer.

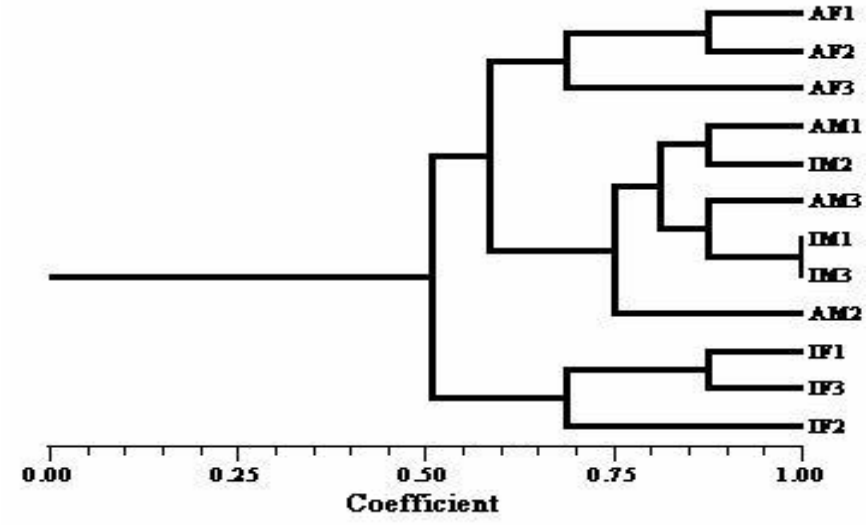

Figure 4. Dendogram showing genetic similarity, using esterase enzyme, among active(A) and inactive (I) female (F) and male (M) *Cicadulina storeyi.*

A dendogram created from a single gel (having three insects each of active and inactive males and females) is presented in Figure 4. There were 50-90% variations among the 12 *C. storeyi* samples. The dendogram separated the samples into three clusters at 0.70 coefficient of similarity. The first cluster consisted the three active females. The second cluster consisted all the six male samples. The third cluster consisted the three inactive female samples.

Distribution of MSV within bodies of active and inactive *C. storeyi*

MSV was also detected in the body parts (head, thorax and abdomen) of active and inactive *C. storeyi* by IC-PCR assays. There was no difference between the two populations. Figure 5 illustrates the amplified bands that were obtained. MSV was detected through PCR and IC-PCR in all parts of *C. storeyi* studied after they were given different acquisition access feeding periods.

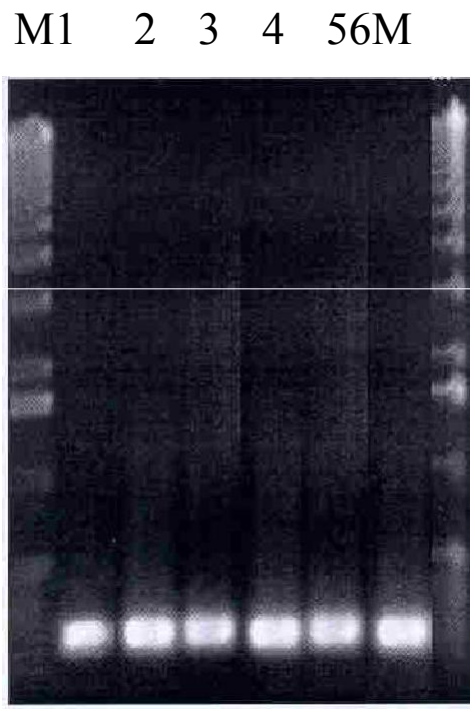

Figure 5. Example of amplified bands produced from IC- PCR in the detection of MSV within different parts of active and inactive *Cicadulina storeyi*. Lane 1= Head, active transmitter, lane 2= thorax, active transmitter, lane 3= abdomen, active transmitter, lane 4= head, inactive transmitter, lane 5= thorax, inactive transmitter, and lane 6= abdomen, inactive transmitter.

Attempts to detect MSV in parts of the body through PCR failed probably because of the small size of the parts that were used to extract DNA which might have made DNA extraction difficult. However, MSV was detected through IC-PCR in the head, thorax and abdomen of *C. storeyi* indicating that there was no difference between active and inactive transmitters in the distribution of MSV within the vectors.

DISCUSSION

The results of the experiments to study the Mendelian genetics of MSV transmission ability of *C. storeyi* show that MSV transmission ability is a genetic trait as four out of six crosses perfectly fitted the expected ratio. The χ^2 values for the other two crosses were not significant probably because of the small number of crosses.

These results support the hypothesis of Storey (1932) that "in the species *C. mbila*, the male is heterozygous for sex, that the factor for activity is dominant to that for inactivity".

Genetic variability was identified between active and inactive *C. storeyi* in molecular traits. This study shows that RAPD markers are highly polymorphic and that they are useful to analyse genetic diversity at the level of active and inactive transmitters within *C. storeyi* populations. RAPD-PCR is a genetic technique that has been used successfully to identify genetic markers that characterize species in several insect orders, including Diptera, Homoptera and Hymenoptera (Black et al., 1992; Kambhampati et al., 1992; Haymer and McInnis 1994). Additionally, it has been useful in identifying the geographic origin of some introduced insect species (Mendel et al., 1994; Wiiliams et al., 1994). Puterka et al. (1993) used RAPD -PCR to characterize phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diauraphis noxia* (Mordvilko). Dowdy and McGaughey (1996) also used RAPD -PCR to differentiate six populations of Indianmeal moth *Plodia interpuntella* (Hubner) and examined the genetic similarity within and among populations.

Esterase analysis also showed polymorphism and revealed genetic differences among the samples of active and inactive *C. storeyi* that were studied. Esterase analysis has been used to study different populations of other insects including *Sitophilus* spp. (Coleoptera: Curculionidae) (Pintureau et al., 1991) and whiteflies (Homoptera: Aleyrodidae) (Wool and Greenberg, 1990; Wool et al., 1989). Wool et al. (1989) reported that esterase gave reliable, species discriminating patterns in adults of Israeli and Colombian whiteflies.

The results are thus consistent with the hypothesis that transmission ability is a genetic trait for *C. mbila* (Storeyi, 1932). This study shows that esterase analysis and RAPD-PCR techniques are useful in studying insects that transmit plant viruses and in understanding why there should be active or inactive vectors within the same species. Molecular markers provide a quick and reliable method for estimating genetic relationships among genotypes of any organism (Thormann et al., 1994).

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REFERENCES

Asanzi MC, NA Bosque-Perez, C.R. Nault, Gordon DT, Thottapplly G (1995). Biology of *Cicadulina* species (Homoptera: Cicadellidae) and transmission of maize streak virus. Afr. Entomol. 3 (2): 173-179.

Black WC, Duteau NM, Puterka GJ, Nichols JR, Pettorini JM (1992). Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (*Homoptera: Aphididae*). Bull. Entomol. Res. 82: 151-159.

Devonshire AL, Moores GD (1982). A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach –potato aphids (*Myzys persicae*). Pest Biochem. Physiol. 18: 235-246.

Dowdy AK, McGaughey WH (1996). Using random amplified polymorphic DNA to differentiate strains of the Indianmeal moth (*Lepidoptera: Pyralidae*). Environ. Entomol. 25(2): 396-400.

Efron T, Kim SK, Fajemisin JM, Mareck JH, Tang CY, Dabrowski v, Rossel HW, Thottappilly G (1989). Breeding for resistance to Maize streak virusi: a multidisciplinary team approach. Plant Breeding 103: 1-36.

Haymer DS, McInnis DD (1994). Resolutions of populations of the Mediterranean fruitfly at the DNA level using random primers for the polymerase chain reaction Genome 37: 244-248.

Hoffmann K, Sackey ST, Maiss E, Adamako D, Vetten HJ (1997). Immunocapture polymerase chain reaction for the detection and characterization of cacao swollen shoot virus 1A isolates. J. Phytopathol. 145: 205-212.

Hohlf FJ (1993). NTSYS-pc. Numerical taxonomy and multivariate analysis System. Exeter, New York.

Jaccard P (1908). Nouvelles recherches sur la distribution floale. Bull. Soc. Vandoise Sci. Natl. 44: 223-270.

Jansen RW, Siegl G, Lemon SM (1990). Molecular epidemiology of human hepatitis A virus defined by an antigen-captured polymerase chain reaction method.Proc. Natl. Acad. Sci. USA 87: 2867-2871.

Kambhampati S, Black WC, Rai KS (1992). Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): Techniques, statiscal analysis and applications. J. Med. Entomol. 29: 939-945.

Livak KJ (1984). Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. Genetics 107: 611-634.

Mendel Z, Nestel D, Gafny R (1994). Examination of the origin of the Israeli population of *Matsucoccus josephi* (*Homoptera: Matsucoccidae*) using random amplified polymorphic DNA-polymerase chain reaction method. Ann. Entomol. Soc. Am. 87(2): 165-169.

Okoth VAO, Dabrowski ZT, Thottappilly G, Van Emden HF (1988). Comparative analysis of some parameters affecting maize streak virus (MSV) transmission by various *Cicadulina* spp. populations. Insect Sci. Appl. 8 (3): 295-300.

Pintureau A, Grenier AM, Nardon P (1991). Polymorphism of esterases in three species of *Sitophilus* (Coleoptera: Curculionidae). J. Stored Prod. Res. 27(3): 141-151.

Puterka GJ, Black IV, Steiner WC, Burton RL (1993). Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordivilko), inferred from allozyme and RAPD-PCR markers. Heredity 70: 604-618.

Rose DJW (1978). Epidemology of maize streak disease. Ann. Rev. Ent. *23*: 259-282.

Rybicki EP, Hughes FL (1990). Detection and typing of Maize streak virus and other distantly related geminiviruses of grasses by polymerase chain reaction amplification of a conserved viral sequence. J. Gen. Virol. 71: 2519-2526.

Sneath PH, Sokal RR (1973). Numerical taxonomy: The principles and practice of numerical classification. W. H. Freeman and Co., San Francisco, USA. p. 573.

Storey HH (1932). The inheritance by an insect vector of the ability to transmit a plant virus. Proceedings of the Royal society of London (series B) 112: 46-60.

Storey HH (1933). Investigations of the mechanism of the transmission of plant viruses, by insect vectors: 1. Proceedings of the Royal Society of London (Series B). 113: 463-485.

Storey HH (1938). Investigation of the mechanisms of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission. Proceedings of the Royal Society of London (Series B): 125: 455-477.

Storey HH (1939a). Investigations of the mechanism of the transmission of plant viruses by insect vectors III, The insects saliva. Proceedings of the Royal Society of London (series B) 127: 526-543.

Storey HH (1939b). Transmission of plant viruses by insects. Bot. Rev. 5: 240-272.

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994). Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor. Appl. Genet. 88: 973-980.

Thottappilly G, Bosque-Perez NA , Rossel HW (1993). Viruses and virus diseases of maize in tropical Africa. Plant Pathol. 42: 494-509

Wendel JF, Weeden NF (1989). Visualization and interpretation of plant isozymes, Pages.5-45 In: D.E. Soltis, PS Soltis (eds.) Isozymes in plant biology. Diocorides, Portland, Oregon, USA.

Wool D, Greenberg DW (1990). Esterase activity in whiteflies (*Bemisia tabaci*) in Israel in relation to insecticide resistance. Entomol. Exp. Appl. 57: 251-258.

Wool D, Gerling D, Nolt BL, Constantino LM, Bellotti AC, Morales FJ (1989). The use of electrophoresis for identification of adult whiteflies (Homoptera: Aleyrodidae) in Israel and Colombia. J. Appl. Ent. 107: 344-350.