WHITEFLY-*BEMISIA TABACI*: BIOTYPES, INDIAN CASSAVA MOSAIC VIRUS AND ITS BIOCONTROL AGENTS IN INDIA

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Bemisia tabaci (Gennadius) is considered as the most economically important and ubiquitous insect pest attacking over 600 plant species and transmitting more than 60 plant viruses. The research on whiteflies in India had been mainly concentrated on biology, ecology and chemical management. Basu (1995) and Palaniswami and Nair (1995) indicated the presence of host-associated strains in *B. tabaci.*

I. BIOTYPES

B. tabaci was collected from different parts of India, Kerala, Karnataka. Maharashtra and Orissa and colonized on cassava and sweetpotato plants. Biological assays, isoenzyme studies, cross breeding studies and RAPD-PCR studies were undertaken for determination of biotypes in India.

A. Biological Assay

The cassava whitefly (CWF) and sweet potato whitefly (SPWF) were separately maintained on the respective host plants. Two sets of experiments were designed- No-choice studies (Clip cage studies) and Choice tests.

- For no choice test CWF and SPWF were confined in leaf cages on cassava, sweet potato, tobacco, eggplant and cotton. Oviposition was recorded in all host plants
- The highest percentages of adult emergence of CWF occurred on cassava (94%), followed by eggplant (86%) and tobacco (81%)
- No CWF nymphs emerged beyond the first instars from eggs laid on sweet potato
- Adult emergence of SPWF was significantly highest on sweet potato followed by tobacco, cotton and eggplant.
- There was no pupal formation or adult development of SPWF on cassava
- For choice tests, plant pairs used for the study using cassava whitefly were cassava/cassava, cassava/sweet potato, cassava/eggplant, cassava/cotton, cassava/tomato and with sweet potato whitefly, the plant pairs were sweet potato/sweetpotato, sweet potato/cassava, sweet potato/eggplant, sweet potato/cotton, sweet potato/tomato
- The CWF females, when given a choice of cassava and sweet potato, laid 484 eggs on cassava and 30 eggs on sweet potato
- The highest percentage of adult emergence of CWF was 67% on cassava followed by 65% on eggplant, 29% on tomato and 4% on cotton
- No adult emergence was recorded from the eggs laid on sweet potato
- The SPWF females, when given a choice, laid 777 eggs on sweet potato compared with 293 on cassava. None of the eggs laid on cassava developed beyond first instar

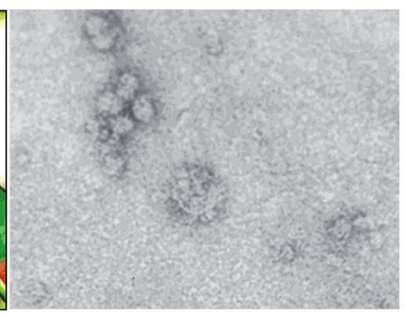
Thus the CWF reproduced on cassava, eggplant, tomato and tobacco, but not on cotton and sweet potato. Conversely, the SPWF reproduced on sweet potato, cotton, eggplant, tomato and tobacco, but not on cassava.

B. Electrophoresis

- Five isozymes, Esterase, Malate dehydrogenase, Alcohol Dehydrogenase, Phosphoglucomutase, Phosphoglucoisomerase were used in vertical slab native polyacrylamide gel electrophoretic studies.
- Of the 15 detected electrophoretic alleles, only 4 (26.67%) were shared by CWF and SPWF.
- There were five non-shared alleles to CWF and 6 to SPWF.
- Thus 73.33% of the alleles represented fixed differences between CWF and SPWF.

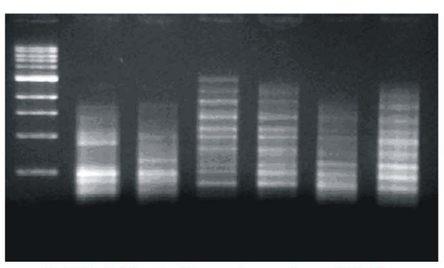
C. Cross Breeding Studies

- Cross breeding experiments using female SPWF and male CWF resulted in mean progeny of 2.8 females and 17.7 males
- The cross between female CWF and male SPWF gave 1.33 female and 10.17 males
- Breeding within each group yielded higher population of females (13-16) and males (14-18)

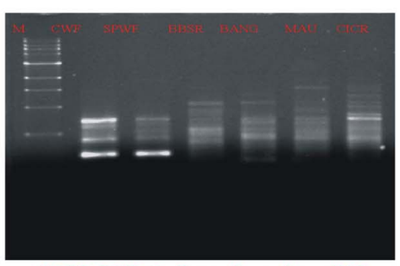


D. RAPD-PCR Studies

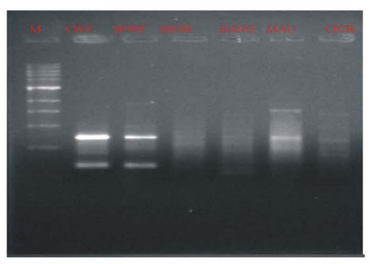
- Further molecular detection primer used was Operon H16 (5'TCTCAGCTGG3'), H9 (5' TGTAGCTGGG 3'), $(GTC)_5$.
- The banding pattern of the SPWF did not agree with the banding pattern shown by the B biotype (variation at the 300-600bp region).
- As a further step squash silver leaf assay was carried out using SPWF and no silver leaf symptom developed.



RAPD-PCR studies using primer $(GTC)_5$,



RAPD-PCR studies using primer H16,



RAPD-PCR studies using primer H9,

Lane 1 -1Kb marker, Lane 2-Cassava whitefly, Lane 3-Sweet potato whitefly, Lane 4- Bubaneshwar, Lane 5-Bangalore, Lane 6-7- Maharashtra

II. INDIAN CASSAVA MOSAIC VIRUS

A. Transmission studies

- Clip cage confined transmission studies showed that there was more than 85% mortality during 48h AAFP using SPWF for ICMV transmission from cassava to cassava. Surviving adults of SPWF when released on young cassava seedlings for IAFP (48h) did not express ICMD symptoms even after 3 months indicating non-transmission of ICMV by SPWF
- There was 71.43% transmission from cassava to cassava using CWF. Higher rate of transmission was observed with 42-48h AAFP and 48-120h IAFP
- Increase in number of whitefly (1-8 nos.) did not influence percentage of transmission but was directly related to AAFP and IAFP

B. ICMV serological assays Dot-blot immuno assay/TAS-ELISA

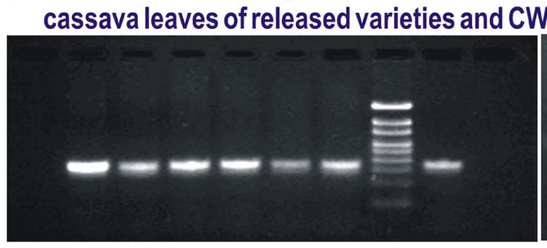
- Immuno-blot/TAS-ELISA showed the positive to ICMV in CWF biotype in different periods of AAFP. Whereas the SPWF did not show positive reaction
- CWF biotype with 48h AAFP showed maximum absorbance value

C. Detection of ICMV by PCR

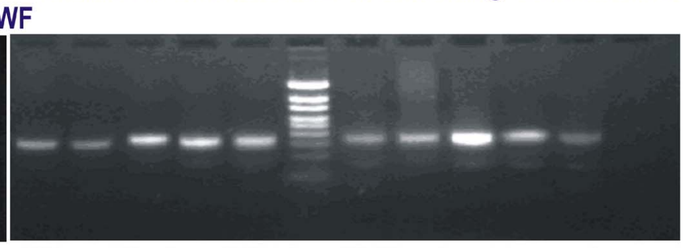
- Nucleic acid extract from ICMV acquired CWF adults and ICMV infected cassava leaves when used with two degenerate primers in PCR gave a specific amplification of 530 bp size fragment. The product amplification was not observed with SPWF.

D. Nested Primer for ICMV coat protein - CTCRI-USIF

- A new nested primer was designed from ICMV- Maha (NCBI/GenBank no. AJ314739) and ICMV- Tri (NCBI/GenBank no. Af423180)
- Amplification products of 390 bp and 310 bp size fragment were obtained
- CTCRI-USIF nested primer *S1* and *A1* and *S2* and *A2* were effective in screening ICMV-infected cassava leaves of released varieties and CWF



PCR amplification of DNA from ICMV-coat protein gene fragment from different released cassava varieties using S1 & A1 primer.



PCR amplification of DNA from ICMV-coat protein gene fragment from viruliferous whitefly vector Bemisia tabaci in different AAFP on ICMD infected cassava plants, using S2 & A2 primer.

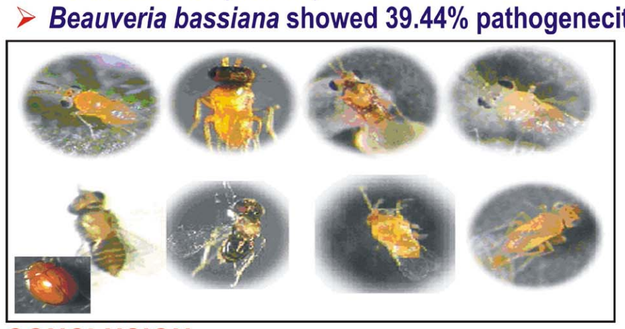
E. Coat Protein Gene Sequences And Phylogenetic Analysis

- Sequencing primers, *GEM-CO S1, GEM-CO S2, GEM-CO A1* and *GEM-CO A2* were designed from ICMV- Maharashtra
- By using the primer pair *CTCRI-USIF nested S1* and *A1*, and *GEM CO S1, A1, S2* and *A2*; ~771 bp CP gene (AV1) were sequenced and named as ICMV-Tri II.
- The comparison of nucleotide sequence showed the high level of homology (90-99%) between analyzed DNA and published sequences of coat protein gene of ICMV isolates originating from India and Sri Lanka.

III. BIOLOGICAL CONTROL AGENTS

Survey of *Bemisia tabaci* and their natural enemies was conducted in India during 1998-2003 on cotton, pigeon pea, sunflower, brinjal, cowpea, cucurbits, bitter gourd, tobacco, banana, cassia sp., cassava, sweet potato and several ornamental crops/crotons. The areas included all the 12 districts of Kerala, Tamil Nadu, Karnataka, Maharashtra, Andhra Pradesh, Bihar, Chatisgargh and West Bengal.

- Ten Aphelinid parasitoids, 11 predators and two pathogens
- *Encarsia transvena* and *E. bimaculata* are effective parasitoids (up to 19%) of *B. tabaci*
- *Eretmocerus mundus* failed to develop on CWF whereas it multiplied fast on SPWF
- *Serangium paracesetosum* showed predation efficiency of 91.45%, followed by *Cheilomenes sexmaculatus* (77.19%)
- *Beauveria bassiana* showed 39.44% pathogenecity on whitefly nymph



Molecular identification of parasitoids

- Nine different species of aphelinid parasitoids were identified by the amplification of D2 region of 28S rRNA using primers; FORWARD 5' GCG AAC AAG TAC CGT GAG GC 3', REVERSE 5' TAG TTC ACC ATC TTT CGG GTC 3'. Amplified

CONCLUSION

- The presence of biotypes in India, which was different from the B biotype. CWF transmit ICMV and not SPWF
- CTCRI-USIF nested primer was developed which can be used as a molecular tool for rapid diagnosis of ICMV in the plant and insect
- *E. transvena*, *E. bimaculata*, *Er. mundus* and *S. paracesetosum* were potential agents
- Use of molecular markers helped in the taxonomic identification of nine aphelinid parasitoids of which three are new species

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