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PROCEEDINGS OF THE GROUP MEETING ON WHITEFLIES AND EMERGING CHALLENGES

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ABSTRACT : A group meeting on whiteflies was organized jointly by the Out Reach Programme on Sucking pests of Horticultural Crops (ORP-SP) and the Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE) on the 17th May, 2010 at Indian Institute of Horticultural Research, Bangalore. About 60 participants comprising scientists from networking centers of the ORP-SP, different ICAR institutes and State Agricultural Universities, students and representatives from private sector participated in the meeting. The meeting deliberated on various aspects of emerging problems due to whiteflies both as pests and vectors. The meeting came out with several recommendations and the major ones include preparing a whitefly distribution map, strengthening research on insecticide resistance and biotypes of whitefly transmitted diseases and encouraging systematics, both traditional and molecular. The detailed proceedings are presented in this paper.

Keywords: Biotypes, Out Reach Programme, whiteflies

A group meeting on 'Whiteflies and emerging challenges' was organized jointly by the Out Reach Programme on Sucking pests of Horticultural crops and the Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE) on the 17th May, 2010 at Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. About 60 participants comprising researchers from networking centers of the (ORP-SP), different ICAR institutes and State Agricultural Universities, representatives from private sector and students participated in the deliberations. Dr. N. K. Krishna Kumar, Principal Investigator, welcomed the delegates and introduced the theme of the meeting. In his introductory remarks, Dr. Kumar emphasized the importance of whiteflies under protected cultivation conditions both as pests and a vector of several viral diseases. He mentioned that emerging biotypes, insecticide resistance, new viruses and break-down of resistance in plant varieties were the major challenges which need to be addressed and hence the group meeting was organized to take stock of the national scenario and chalk out strategies to overcome these challenges. Three objectives envisaged for this meeting were,

a) Understanding whitefly species complex, diversity, molecular systematics, biotypes, ecology, physiology and endosymbionts

b) Addressing the problem of whitefly

species both as pests and vectors and understanding the vector potential of *Bemisia tabaci* in the light of emerging biotypes.

c) Breeding for disease resistance to whitefly transmitted viruses using traditional and molecular approaches.

Prior to the proceedings, Dr. B. Vasantharaj David, the doyen of Entomology was honored with the *Life Time Achievement Award* of AAPMHE for his remarkable contribution to Entomology in general and whiteflies in particular. The award consisted of a silver plaque and a citation.

After felicitation, Dr. Vasantharaj David delivered key note address on **"Whiteflies (Hemiptera: Aleyrodidae) - their systematic history and problems of conventional taxonomy".** He highlighted the unusual lifecycle of whitefly which resembles holometaboly despite all Hemiptera being hemi-metabolous. He further added that in the real sense the term "puparium for the sessile larvae and puparial case for the vacated exoskeleton" used in biology is incorrect. He stated that the so called puparium and pupal case are required to identify whiteflies in most cases.

Dr. David stated that the original name of whitefly combination, *Phalaena* (Tinea) *proletella* reveals how Linnaeus was misled by the appearance of adult whiteflies, for the Tineidae that include the domestic clothes moth. It is not difficult to see why this mistake was made after all, the creature apparently has a proboscis the body and the four wings have a fine coating easily mistaken for lepidopterous scales and further briefed on the following points:

- Although the first genus of whiteflies Aleyrodes, was proposed by Latreille (1796), it was subsequently widely misspelled as Aleurodes by many, the introduction of this unjustified emendation being attributed to Burmeister. The incorrect spelling continued well into the 20th century. The family too, was often mistakenly called 'Aleurodidae'. This term of being misspelled sometimes used even today. The original description of one of the best-known and most notorious of whitefly species, *Bemisia tabaci*, was woefully poor and provides a graphic illustration of this historical descriptive malaise.
- A small hemipterous insect, called by the workers a fly, which for some time had been attacking tobacco. The insect is recognisable as *Aleurodes*, of the family Aleurodidae, which has recently been monographed by Signoret. On comparing it with the species described in that monograph, it resembles none of these, and consequently it is a new species not yet described. He named it as *Aleurodes tabaci* Gen. The workers on the tobacco fields call it a fly because, in its perfect [i.e. adult] state it has the form of a very small fly, although it is easily distinguished by the whiteness of the wings and the great numbers in which it occurs. The immature stage is found adhering, for most part, on the lower surface of the leaves, and it is the size of the common louse, and pale yellowish in colour. Armed with a sucker, it penetrates the epidermis and sucks out the juice. In dry years the leaves are covered by enormous numbers and such leaves dry up as they mature. A sweetish liquid is distilled from these leaves, like that on leaves bearing lice and scales. When such tobacco is smoked, it is unpleasant to the taste.'
- This is where the preservation of original voucher ('type') material is so important in systematic studies. Because Gennadius' original material is still available to scientists, being housed at the Smithsonian Institution's collection in United States, the true identity of *B. tabaci* can be clarified further in detail at any time, the material being re-examined as new questions arise. As techniques are refined, such archival dried material is likely to be of increasing use in

chemical or molecular studies. The extraordinary density of, and the level of damage caused by, the populations that were drawn to his attention in late 19th century Greece are actually the most pertinent aspects of Gennadius' description, being remarkably reminiscent of what we now refer to as the 'B' biotype of *B. tabaci*, a form that has become known as a notorious agricultural pest, implicated in the transmission of many viral diseases of plants. Dr. David made a comparative assessment of advantages and disadvantages of using adults and puparium.

Advantages of using adults

- Almost certainly, they do not display intra-specific variation
- Male aedeagal characters definitive

Disadvantages

- Require slide-mounting
- Notoriously difficult to examine, either dry or on slides
- Extremely fragile
- Must be collected into spirit, or will shrivel when dried
- Tendency for segments to collapse osmotically in slide-making
- Frequently found as vagrants on non-hosts
- Often found only for very short periods each year
- Cuticle requires staining, but body contents often difficult to clear and also stains
- Coxae ventrally-directed making dorsoventral mounting almost impossible,
- Genitalia of both sexes rarely display significant characters
- Wing venation so reduced that is of little use in whiteflies
- If macerated, wings curl so wings must be treated separately if they are needed
- After a century of puparium-based taxonomy: adults of majority of described species are unknown and adult characters still poorly appraised.

Advantages of using puparia for taxonomic studies

- Specimen is sessile, hence avoids the possibility of erroneous host information
- Puparia rigid and mostly flattened, so easy to manipulate despite small size
- Many characters displayed
- Specimens can be easily collected and stored on dried leaves for later study
- Often apparently diapausing, thus puparia found for long periods each year

Disadvantages

- Known tendency to display character states that vary with the host-plant surface
- Require slide-mounting
- Host-induced variation makes generic definition particularly difficult
- A unique terminology used
- Sometimes waxy secretions can render preparation difficult – but a minor problem
- Black puparia require bleaching – again a minor problem

Dr. David explained in detail about the invasive species, *B. tabaci*, known under various common names like tobacco whitefly, cotton whitefly or sweet potato whitefly, which is the most investigated of all whitefly species. Until recently *B. tabaci* was regarded as a morphologically variable single species with an exceptionally wide range of host plants. However, recent investigations have shown many field-collected populations to have small host plant ranges, with some behaving as though monophagous. Indeed, Mound had observed the transfer of populations from one host to another

to be the greatest obstacle in his experimental work on host-induced morphological variation. Since the early 1980's *B. tabaci* has been causing escalating problems to world agriculture. A high degree of insecticide resistance has developed within many field populations of *B. tabaci* associated with agricultural crops. The problem of insecticide resistance is compounded by the ability of *B. tabaci* to transmit over 60 different plant geminiviruses.

Several population *biotypes* have been recognized for some years, but the development of new techniques for the study of cytology and DNA sequencing has led to a situation of great complexity and nomenclatural confusion. In USA, the existence of two distinct biotypes (termed A and B) was recognized in early 1986. Through the late 1980s and early 1990 the "B" strain caused huge economical losses, estimated at half a billion dollars for 1991.With such economic stimulus, the study of *B. tabaci* intensified greatly. Investigation of the DNA profiles of these biotypes was added to the study of more traditional aspects of biology. With the A and B types having demonstrably different fecundity, virus transmission ability, feeding damage profiles and DNA spectra, biotype B was eventually given its own species name, *B. argentifolii* (Bellows et al. 1994) and its own common name, Silver leaf Whitefly. There are indeed two distinct biological forms in the USA, the situation in other countries is much more complex, and several other biotypes have now been recognized. Further, no reliable morphological characters have been demonstrated which allow the Silver leaf Whitefly to be distinguished visually from other biotypes of *B. tabaci.*

Bemisia tabaci biotypes in India

Biotype 1: Brinjal/Cotton (Delhi, Ahmedabad, Parbhani and Chennai)

Biotype 2: Sunflower/Soyabean

Biotype 3: Tomato/Tobacco/Lantana

Biotype 4: Brinjal/Cassava/Cotton (Coimbatore and Kerala)

Host associated strains – Cassava strain and Sweet potato strain

Dr. David concluded that adult whiteflies will almost certainly come to assume a more important supporting role and, indeed, adult males are already necessary for species confirmation. However, up to now it has been a matter of, as our children say, 'adults do have some uses on occasions'. There is nothing inherently wrong with the use of immature stages for identification. The problem is that, with our current poor understanding of the true significance of many puparial characters, we are yet to maximize their value. The examples discussed here graphically illustrate, traditional whitefly taxonomy, based on poorly understood larval characters, has climbed high, perhaps surprisingly high, up a metaphorical ladder, but has now encountered a trapdoor, to some degree 'padlocked' by the conundrum of puparial character plasticity. There are high hopes, as we head into the 21st century, that developing molecular and biochemical techniques will help to release the puparial lock' and enable us to determine with added confidence, which taxa are true genera and species and which are not.

Dr. M. S. Palaniswamy gave a brief presentation on **"Whitefly, *Bemisia tabaci* and its parasitoids with reference to Cassava and Sweet potato".** He presented studies on *B. tabaci* collected from different parts of Kerala, Karnataka, Maharashtra and Orissa that colonized on cassava and sweet potato plants. Biological assays, Isoenzyme, cross breeding studies and RAPD-PCR were undertaken for determination of biotypes. He explained the cross breeding using ♀ of sweet potato whitefly (SPWF) and ♂ Cassava Whitefly (CWF) resulted in mean progeny of 2.8 ♀ and 17.7 ♂. The cross between ♀ CWF and ♂ SPWF gave 1.33 ♀ and 10.17 ♂. Breeding within each group yielding higher population of ♀ (13-16) and ♂ (14-18).

Dr. Palaniswamy highlighted the whitefly transmission work using clip cage confined transmission that showed, that there was more than 85 per cent mortality during 48h acquisition access feeding period (AAFP) using sweet potato whitefly (SPWF) for Indian Cassava Mosaic Virus (ICMV) transmission from cassava. Surviving adults of SPWF when released on young cassava seedling for inoculation access feeding period (IAFP) (48h) did not express ICMD symptoms even after 3months indicating non-transmission of ICMV by SPWF. There was 71.43 per cent transmission from cassava to cassava using cassava whitefly (CWF). Higher rate of transmission was observed with 42-48h AAFP and 48-120h IAFP. Increase in number of whitefly (1-8) did not influence percentage transmission but was directly related to AAFP and IAFP. He detected virus using ICMV serological assays Dot- blot Immunoassay/TAS-ELISA, Nested primer for ICMV coat protein-CTCRI-USIF a molecular tool for rapid diagnosis for ICMV identification, and also detected by PCR. Dr. Palaniswamy further, highlighted on biological control of whiteflies. Survey of *B. tabaci* and it's 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, Maharastra, Andhra Pradesh, Bihar, Chatisgarh and West Bengal. Where in ten Aphelinid parasitoids, 11predators and 2pathogens were recorded. Among these *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.

There was a discussion after Dr. Planiswamy's presentation and following are some of queries raised by delegates:

Dr. C. A. Virakthmath: How you define biotype with respect to reproduction?

Dr. M. S. Palaniswamy: Based only on reproduction we can't define biotypes

Dr. K. S. Mohan: Is there any weed host which supports Cassava whitefly?

Dr. M. S. Palaniswamy: Jatropha is one such weed.

Dr. V. G. Malathi spoke on the **"Advances in Geminiviruses' transmission by Whiteflies in South Asia".** She stated that Geminiviruses form the second largest family of plant viruses, the *Geminiviridae*, represented by four genera: *Mastrevirus, Curtovirus, Topocuvirus* and *Begomovirus.* During the last two decades these viruses have emerged as devastating pathogens, particularly in the tropics and subtropics, causing huge economic losses and threatening crop production. Epidemics caused by re-emerging and newly emerging geminiviruses are becoming frequent even in regions that were earlier free from these viruses. Compared to Mastreviruses and Curtoviruses, Begomoviruses have emerged as more serious problems on a variety of crops, for example, cassava, cotton, grain legumes and vegetables. Major contributory factors for the emergence and spread of new geminivirus diseases are the evolution of variants of the viruses, the appearance of the whitefly 'B' biotype and the increase in the vector population. Variability in geminiviruses has arisen through mutations, recombination and pseudo-recombination.

Gene recombination in geminiviruses, not only between the variants of the same virus but also between species and even between genera, has resulted in rapid diversification. From the disease point of view, most virulent variants have developed through recombination of viral genomes such as those associated with cassava mosaic, cotton leaf curl, and tomato leaf curl diseases. Heterologous recombinants containing parts of the host genome and/or sequences from

satellite-like molecules associated with monopartite begomoviruses provide unlimited evolutionary opportunities. Human activity has also played an important role in the emergence of serious geminivirus diseases across the globe, like the changes in cropping systems, introduction of new crops, the movement of infected planting materials and introduction of host susceptible genes through the exchange of germplasm.

Dr. Malathi explained that viruses of the genus *Begomovirus* (Family: *Geminiviridae)* have genomes consisting of either one or two genomic components. The component of bipartite begomoviruses known as DNA-A is homologous to the genomes of all geminiviruses and encodes proteins required for replication, control of gene expression, overcoming host defenses, encapsidation and insect transmission. The second component, referred to as DNA-B, encodes two proteins which functions in intra- and intercellular movement in host plants. The origin of the DNA-B component remains unclear. The study described here was initiated to investigate the relationship between the DNA-A and DNA-B components of bipartite begomoviruses with a view to unraveling their evolutionary histories and providing information on the possible origin of the DNA-B component as the two components share little sequence identity with the exception of a ~200 nucleotide sequence with typically greater than 85% identity known as the common region (CR). The CR encompasses an absolutely conserved (among geminiviruses) hairpin structure containing, within the loop, the nonanucleotide sequence (TAATATTAC) that marks the origin of virion-strand DNA replication, and repeated sequences (known as "iterons") that are the recognition sequences for binding of the DNA-A-encoded replication-associated protein (Rep: a rolling-circle replication initiator protein that is the only virus-encoded product required for viral DNA replication). The CR thus functions to maintain the integrity of the multiplying genome, ensuring that replication of both components can be initiated by the DNA-A-encoded Rep.

Dr. Malathi also made a comparative statement of phylogenetic and exhaustive pair wise sequence comparison of all DNA-A and DNA-B components of begomoviruses demonstrates that the two molecules have very distinct molecular evolutionary histories and likely are under very different evolutionary pressures. The analysis highlights that component exchange has played a far greater role in diversification of begomoviruses than previously suspected, although there are distinct differences in the apparent ability of different groups of viruses to utilize this "sexual" mechanism of genetic exchange. Additionally she explored the hypothesis that DNA-B originated as a satellite that was captured by the monopartite progenitor of all extant bipartite begomoviruses and subsequently evolved to become the integral (essential) genome component that is recognized today. The situation with present-day satellites associated with begomoviruses provides some clues to the processes and selection pressures that may have led to the "domestication" of a wild progenitor of the DNA-B component. She clearly explained virus pathway inside the body of vector translocation of virus, GroEL protein interaction with virus.

A brief pathway is as follows:

Enter through stylets – esophagus – gut-haemocoel - salivary gland

- This polarity is never changed
- Non-transmissible virus/mutants get stuck in midgut

The major barrier- Gut to haemocoel, *Trialeurodes vaporariorum* have same barrier. Receptor- that will facilitate translocation to haemocoel is yet to be characterized.

Translocation of the virus

- Difficult to detect in stylet
- First detected in head after 10 min AAP
- Detected in midgut after 40 min
- Detected in haemolymph after 90 min
- Detected in salivary glands 5.5 hr after detection in haemolymph and
- 6-7 hr AAP ready for inoculation

Role of Groel homologue protein from endosymbiont bacteria

- A 63 kDa protein isolated from Whitefly feeding explant
- TYLCV showed affinity to this protein
- Feeding on *Buchnera* GroEL antiserum block the transmission
- The protein protects the virus from destruction

Transgenic resistance to TYLCV using GroEL gene of endosymbiont

GroEL gene was incorporated into tomato plants. Expression of GroEL homologue mainly in phloem, virion was complexed by the protein, which could be trapped by GroEL antibody. Plants expressing GroEL exhibited mild or no disease symptoms.

Plants contained TYLCV-GroEL complex. Transgenic plants equally served as inoculum replication/transcription of the virus inside vector

- TOMOV and TYLCV were compared. Whiteflies fed on virus infected tomato plants
- Transferred to cotton (non-host)
- Real - time PCR used to detect transcripts
- ToMoV transcripts become undetectable TYLCV transcripts increased after transfer to cotton

Peculiar findings in TYLCV-I

- Transovarial transmission: Virus detected in eggs, first and second instar and adults that developed on cotton after feeding on infected tomato plants
- TYLCV DNA in progeny of insects that acquired the virus through egg
- TYLCV can be transmitted through the egg for at least two generations
- Viruliferous female were able to transmit the virus to non viruliferous male and vice versa
- Transfer does not happen between male B type and female of Q biotype

Bemisia tabaci - biotypes

She defined biotypes as biological types which manifest host preference, fecundity, vector competency, phytotoxin, endosymbionts, invasiveness and insecticide resistance. Based on mtCOI and ITS, SSR markers, about 40 biotypes are recognized

- Whether they constitute the same or different species?
- Sexual selection followed by mating incompatibility
- Sympatric speciation formation of two or more descendent species from a single ancestral species
- Mating between and among biotypes mixed results
- Several biotype subscript crossing have resulted in unsuccessful F1 hybrids (for eg. B and Q in Israel)
- Incompatibility could be due to endosymbiont load
- Mating/courtship behavior between males and females of different populations do occur

Dr. Malathi explained some progress made in understanding the Whitefly Genome **Three cDNA libraries**:

1. Non-viruliferous Whiteflies (eggs, immature instar and adults)

2. Adult fed on tomato plants. One infected by TYLCV and one by ToMoV

Sequence of 18,976 clones

Eggs	201
Instars	1816
Non adult	2093
TYLCV/Adult	2296

1000 bp of *Candidatus portiera*

Gene silencing- a probable approach

- Infection of dsRNA between mesothorax and metathorax dsRNA targetting exonic sequences
- Infection of dsRNA targeting *Drosophila chikadae* (homolog of profilin a small actin binding protein) caused severe disruption of normal *B. tabaci* oocyte development
- siRNA expressed in plants on which whiteflies are feeding
- B biotype EST in progress

Some of the points raised after the presentation were:

Dr. Asokan : Is beta DNA also an RNAi suppressor? Which is more effective protein for transmission; is it CP (Coat protein) or GroEL?

Dr. Malathi : Yes. Beta DNA action is suppressing RNAi and we are trying to find out the transcripts of beta DNA. It has one ORF, which has to be transcripted and translated; tracing transcripts in whitefly gives a clear idea about transmission.

Among CP and GroEL, CP is quick transmitter because by mutation atleast we may get infection effectively. So transmission mediated through CP is much faster.

Dr. Mohan : What are the true whitefly factors responsible for contributing in transmission? If we identify the factor the potential of RNAi will be effectively utilized.

Dr. Malathi : Morphology of insect! In most cases viruses are detected in anterior gut i.e. in foregut. In case of TLCV, it is easily detected in posterior gut of whitefly because of structural differences.

Dr. Palaniswamy : How gene silencing is used in IPM?

Dr. Asokan : Gene silencing is wonderful tool for IPM, targeting *Helicoverpa* as a model and different groups of gene. In insect embryogenesis transcription factor gene involved in digestion, physiology, vitalogenesis, pheromones production, pheromone suppression, reproduction are targeting different group of genes to make dsRNA in understanding physiological important of gene, some gene are expressed in neural tissue of brain.

Dr. N. K. Krishna Kumar : How do you define virulence? Is virulence related to plant or is it a vector perspective?

Dr. Malathi : Virulence is based on plant perspective, while cloning it is important to see viral gene factor and virus suppressor protein functions as virulence factor with one particular ORF, functions as elicitor. But still the totality of perfection is unclear.

Dr. Mohan : Can we have whitefly complete genome sequence in next year?

Dr. Malathi : Whole genome sequencing is not a solution to find the prevalence of biotypes and interestingly it gives the structural genome not functional genome. We don't have basic information of prevalence of B-biotype, its distribution.

Dr. A. T. Sadashiva made presentation on **"Breeding for whitefly transmitted disease resistance in vegetables".** He stated that whitefly-transmitted geminiviruses cause significant yield losses in many vegetable crops (tomato, chilli, capsicum, okra, French bean & cucurbits) throughout the world. The use of resistant genotypes (varieties/hybrids) offers many advantages; and when used with integrated pest management methods, these can ensure successful crop. Several begomoviruses are prevalent in different parts of the country warranting search for stable sources of resistance to most prevalent virus strains. Systematic breeding work at IIHR has resulted in the

identification of eight stable sources of resistance to *ToLCBV* in tomato. Of these IIHR-2101 (*Solanum habrochaites* LA-1777) was also observed to be resistant to *ToLCNDV* under field conditions. Resistance from IIHR-2101 has been successfully transferred to cultivated tomato through backcross. Molecular marker (*Ty-2*) linked to TOLCV resistance was validated and was used in MAS to develop high yielding advanced breeding lines and hybrids of commercial acceptance. Of the 148 chilli accessions screened against Chilli Leaf curl Virus (ChLCV) in Tamil Nadu, sixteen lines were highly resistant. Of the 370 lines screened against ChLCV at Varanasi, three lines were observed to be resistant. In case of okra though several YVMV resistant varieties have been released for commercial cultivation, resistance is not stable due to prevalence of different begomoviruses and there is an urgent need to breed for a stable variety. Of the 126 French bean genotypes screened against MYMV, eight lines were resistant. RAPD marker OPP 07730 linked to MYMV resistance has also been identified in French bean germplasm IC-525260. Advanced breeding lines with high level resistance to MYMV with high yield potential have also been evolved at IIHR, Bangalore. Breeding for stable resistance to begomoviruses involve identification of stable sources of resistance to predominant virus/strain in major vegetable crops, adoption of efficient screening techniques (artificial inoculation/PCR testing), identification of molecular markers and pyramiding of resistance genes through MAS.

Following clarifications were sought after presentation.

Dr. V. Muniyappa: In case of combined resistance genotype to ToLCV and Bacterial wilt, was your material tested in West Bengal and Jharkhand? What is the performance?

Dr. A. T. Sadashiva: Yes, We have tested our materials in these location and other areas. We are going to get result by next January or February.

Dr. V. Muniyappa: Is there any experience in pathogenic/virulence earlier?

Dr. A. T. Sadashiva: Earlier we had sent hybrid TLBR-H6 which has Ty-2 based resistance to ToLCV and bacterial wilt. We observed excellent result in West Bengal as it escapes hot season to give high yield and good seed quality.

Dr. V. Muniyappa: Do we have only one race across India with bacterial wilt? Were the parents used are resistance to both the diseases?

Dr. A. T. Sadashiva: Yes. Ty-2 and bacterial wilt resistance; here both parents are resistant to Bacterial wilt and ToLCV. But these hybrids do not survive in Kerala, but have given high level of tolerance in West Bengal, Himachal Pradesh and Jhawalpur. Among our varieties L390 is a susceptible genotype to bacterial wilt and Hawaii-7996 is highly resistant to bacterial wilt.

Dr. V. Muniyappa: We have come across the problem of *Tospo*, is it not considered under the project?

Dr. A. T. Sadashiva: We received about 13 accessions from AVRDC; two have shown resistance in hot spots like Hyderabad and Coimbatore. We are increasing the intensity of screening work on *Kharif* hybrids. Screening all *Tospo* reported source of resistance from Pearl Harbour, none of them were resistant to GBNV under Indian condition, and also none of the commercial variety is showing resistance.

Dr. N. K. Krishna Kumar: Is there any relation between tospo resistance and leaf curl resistance? Is it independent or mutually exclusive or one influencing each other?

Dr. A. T. Sadashiva: it is mutually independent.

Dr. N. K. Krishna Kumar: Suppose there is breakdown in resistance, determining the reason for breakdown of resistance then approaches to over come it will be more meaningful.

Dr. A. T. Sadashiva: Recombination in begomovirus is faster, resistance doesn't

breakdown easily. Since new viruses are emerging many of our gene don't work for those new viruses but we are able to identify the new sources.

Dr. N. K. Krishna Kumar: Breeding and resistance should look from the perspective of whitefly biotypes, resistance in biotypes, in combination with accidental introduction taking place to identify what exactly is happening.

Dr. M. Krishna Reddy made a presentation on **"Emerging diseases transmitted by whitefly – A threat for future".** He stated that plant virus diseases are emerging as a serious constraint in improving productivity of horticultural crops in India. Viral diseases are an important limiting factor in many crop production systems. Because antiviral products are not available, control strategies rely on genetic resistance or hygienic measures to prevent viral diseases, or on eradication of diseased crops to control such diseases. Increasing international travel and trade of plant materials enhances the risk of introducing new viruses and their vectors into production systems. In addition, changing climatic conditions can contribute to a successful spread of newly introduced viruses or their vectors and establishment of these organisms in areas that were previously unfavorable. *Begomovirus* species and other whitefly-transmitted viruses are invading into new areas, and several recently described new viruses such as *Tomato torrado virus*. Among the most notable emergent arthropod vector-plant virus complexes is the whitefly *Bemisia tabaci* (Genn.) and genus *Begomovirus* (Family, Geminiviridae). The increased importance of begomoviruses as new and emerging plant viral pathogens is directly related to the adaptive capacity of *B. tabaci* and its ability to exploit agricultural systems, which increasingly impose upon their subtropical habitats. The greenhouse whitefly (GHWF) (*Trialeurodes vaporariorum* Westwood) has been a problem in greenhouses for many years, both as an insect pest capable of reducing plant productivity and longevity, as well as a virus vector for *Criniviruses*. In addition to the begomoviruses, *B. tabaci* transmits a limited number of viruses in the genera *Carlavirus*, *Ipomovirus* (Potyviridae), and *Crinivirus* (Closteroviridae). The only whitefly genus other than *Bemisia* identified as a virus vector is *Trialeurodes*. In contrast to the large number of viruses transmitted by *Bemisia*, only a handful of viruses have been found to be transmitted by *Trialeurodes*, all within the genus *Crinivirus*. The genus *Crinivirus* contains viruses transmitted by both *Bemisia* and *Trialeurodes*. The emerging viruses can be countered through the development of genetic resistance against the virus, or through the use of a range of farming practices based upon the propagation of virus-free plant material and the exclusion of the virus vectors from the growing crop.

A few issues were discussed after his presentation as under;

Dr. N. K. Krishna Kumar: What should be our approaches be in managing the emergence of new biotypes, recombinations that are taking place?

Dr. M. Krishna Reddy: Our theme should have a source of resistance and a virologist dealing with virus species complex, an entomologist dealing with vector and biotypes, a breeder should have a source of resistance in combination to ecological approach. Only a co-ordinated work in have lasting answer.

Dr. J. Amudha presented on **"Molecular methods for breeding resistance to cotton leaf curl virus".** She remarked that whitefly-transmitted (WFT) geminiviruses are a major threat to the productivity and quality of cotton grown in the subtropical and tropical regions of the world. The most important diseases known to be transmitted by the whitefly are the cotton leaf curl viral (CLCuV) disease and the cotton leaf crumple viral (CLCrV) disease. Cotton leafcurl viral disease was first reported in *G. barbadense* in Nigeria in the year 1912. This is a serious problem in the northern region and leads to yield loss up

to 58-69 per cent. There is some evidence that the destructive 'B' biotype of *B. tabaci* is present in the Middle East and possibly India. Most evidence suggests that indigenous populations are more virulent because of population pressures resulting from insecticide failures. In India the Cotton Leafcurl Disease (CLCuD) was first noticed in a few plants of *G. barbadense* in the experimental fields of IARI in 1989 and later on *G. hirsutum* near Sri Ganganagar in Rajasthan in 1993. It has spread to all the major cotton growing areas of Punjab, Haryana and Rajasthan and is a threat to cotton cultivation in the region.There are two approaches to tackle the problem i.e., Host plant mediated resistance approach by molecular breeding method, antisense and RNAi transgenic approach.

The genotypes can be identified with the molecular markers tagged for resistant traits and genetically distinct lines can be exploited for crop improvement program. The molecular marker will help the breeding program in screening the F^2 mapping population. HPR study on CLCuD conducted showed that a single dominant gene in *G. hirsutum* affords resistance. In their study, the mapping populations were developed between cotton leaf curl virus resistant (RS 875) and susceptible lines (F 846) and phenotyping and genotyping was done with the SSR markers. Phenotyping of F2 plants (160) reveal that 123 plants were resistant to the disease and 37 plants were susceptible which fit in to 3:1 Mendelian segregation ratio (Single dominant gene). Parental survey with 82 SSR primers (MGHES-EST) could reveal 12 informative primers. F2 genotyping with SSR primers namely MGHES 1A, 4, 7, 41 could produce 350 bp, 500bp, 600bp, 650 bp alleles respectively in the resistant parent and segregated in F2 population in Mendelian fashion. Parental survey with 127 JESPR SSR primers could reveal 15 informative primers. F2 genotyping with JESPR 242, 231, 234 primers could produce 290bp, 190bp and 250 bp, 350 bp alleles respectively as the resistant parent and segregated in F2 population in a Mendelian fashion. F2 were subjected to RAPD analysis with the decamer primers (OPA, OPB, and OPC). Sixty primers were used for RAPD analysis of the DNA samples. Out of 792 fragments produced and 26% of fragments were polymorphic. This fragment was developed into a SCAR (Sequence characterized amplified region) marker which segregated in F2 population in Mendelian fashion.

Another approach is by development of transgenics using antisense and RNAi approach. Antisense RNA molecules (complementary RNA sequence) bind to the naturally occurring (sense) mRNAs and trigger mRNA degradation. Double stranded RNA (dsRNA) induces sequence specific Post Transcriptional Gene Silencing (PTGS) or Homology Dependent Gene Silencing (HDGS) by a process known as RNA interference (RNAi) and protects cells against invasive virus. *Agrobacterium tumefaciens* (EHA 105) gene constructs with Antisense coat protein (ACP), Sense coat protein (SCP) and antisense replicase protein (ARep) along with neomycin phosphor transferase (*npt II*) gene driven by 35 S CaMV promoter and NOS terminator were deployed for generation of transgenics in three elite cultivars viz., H 777, HS 6, F 846 grown in northern part of India. The transformed shoots were grown on Murashige and Skoog selection medium containing kanamycin 50µg/ml. The putative transformants were sub cultured on shoot elongation medium containing BAP and kinetin 1mg/l. The shoots were then transferred to rooting medium containing IBA 1mg/l and the established plants were hardened on pots containing peat, soil and sand in 1:1:1 ratio. Molecular characterizations of the transgenic plants were carried using the gene specific primers and by Southern blotting which revealed the presence of single copy gene integration. The confirmed events of HS 6, H777, F 846 with Antisense Coat protein, Sense Coat Protein and Antisense replicase gene by Southern and PCR were raised in the poly house for event selection study under RCGM (Research Committee on Genetically Modified Organism) contained green house trial

after getting the approval from GEAC (Genetic Engineering Advisory Committee). Screening the CLCuV transgenics with viruliferous whiteflies were carried in the polyhouse for event based selection. The transgenic plants were inoculated with the viruliferous whiteflies (24 hr after acquisition period) for four weeks. Three events namely HS 6 (ARep), HS 6 (ACP), H 777 (SCP) was found to be resistant after screening with viruliferous whiteflies and the plants were maintained in the polyhouse.

Dr. K. S. Shankarappa presented a talk on **"Development of diagnostic techniques for the quick and reliable detection of biotype B of the whitefly".** He explained the development of silver leaf and optimizes the silver leaf bioassay, esterase analysis and PCR-based techniques for quick and reliable distinguishing of biotype B of the whitefly, *Bemisia tabaci* (Gennadius), from Indian indigenous biotypes. Zucchini and squash readily develop silverleaf symptoms upon feeding by the B biotype, but these plants were not readily available in Indian markets. A local pumpkin variety 'Big' was used in silver leaf assay, which developed symptoms similar to those on zucchini and squash and can be used reliably to detect B biotype. Analysis of non-specific esterases of B and the indigenous biotypes indicated both quantitative and qualitative differences in esterase patterns. Two high molecular weight bands were unique to B biotype and they occurred in abundance. These esterases were used to develop quick and field-based novel detection methods for differentiating B from the indigenous biotypes.

He also explained the development of simple and cost-effective protocols which has wider application as they can be potentially used to identify other agricultural pests. Mitochondrial cytochrome oxidase - I gene sequences and randomly amplified polymorphic DNA (RAPD) polymorphisms, generated using the primer OPB11, were also observed useful for detecting *B. tabaci* biotypes. A B biotype-specific RAPD band of 800 bp was sequenced, which was used to develop sequence characterized amplified region (SCAR) marker. The SCAR marker involved the development of B biotype-specific primers that amplified 550 bp PCR products only from B biotype genomic DNA. Silver leaf assay, esterase's, RAPDs or a SCAR marker were used in combination to analyze whitefly samples collected from selected locations in India, and it was observed that any of these techniques can be used singly or in combination to detect B biotype reliably. The B biotype was observed in southern parts of India but not in north from 2004–06.

A few points discussed after the presentations were;

Dr. Mohan: What is the science involved in silver leaf symptoms by B-Biotype? Did you study mechanism in depth?

Dr. K. S. Shankarappa: May be physiological changes. Till now we have not focused on mechanism.

Dr. Muniyappa presented a talk on **"The status of Begomovirus in south India".** A begomovirus was recently shown to be causing Jatropha Mosaic Disease (JMD) on Jatropha for the first time in India. A typical begomovirus-like symptoms characterized by chlorotic specks on leaves, curling and malformation of leaves, severe reduction in leaf size, partial or complete sterility were seen on infected plants. In Karnataka state, South India, JMD caused significant yield losses by affecting the growth and by disease incidences of up to 47%. The putative Jatropha Mosaic India Virus (JMIV) was successfully transmitted through grafting, the dodder *Cuscuta subinclusa* and the whitefly, *Bemisia tabaci*. The JMIV was detected in infected plants and individual *B. tabaci* by polymerase chain reaction tests using two sets of begomovirus-specific degenerate primers. The core coat protein (CP) sequences of ~575 bases were obtained from two isolates collected at Bangalore and Dharwad, South India. Phylogenetic analysis of the core CP sequences with those of selected begomoviruses grouped JMIV in a separate cluster close to

Indian cassava mosaic virus and *Sri Lankan cassava mosaic virus* and shared highest nucleotide identities (90-95%) with them. The two JMIV isolates were 94% similar to each other. The begomoviruses causing JMD in the Americas grouped separately from JMIV and shared only 72.8-75.2% core CP nucleotide identities thus they are distinct. These results further confirm that JMD in India was caused by begomoviruses and they were most closely related to cassava mosaic viruses from the Indian sub-continent.

Dr. V. J. Shivankar made a presentation on **Citrus blackfly.** The major whiteflies of citrus cultivars in India are *Aleurocanthus woglumi* Ashby and *Dilaeurodes citri* (Ashmead) and among these, *A.woglumi* attained a major pest status of citrus which caused economic loss to the tune 20 crores rupees in Nagpur and Amravati districts of Maharashtra. It invaded sweet orange orchards in Punjab in epidemic proportion during nineties. The nymphs and adults desap the leaves, fruits and tender shoots and also excrete copious honeydew which invites sooty mould infestation leading to the formation of a black layer over the infested parts termed as 'Kolshi' in colloquial language in Maharashtra. It can be effectively managed by the timely application of systemic insecticides at 50 per cent egg hatching stage, during which the nymphs will be less chitinised and hence more vulnerable to insecticidal treatments. It coincides with first fortnight of April during Ambe, second fortnight of July during Mrig and first fortnight of December during Hasta season. Several bioagents are observed effective in bringing down the population levels of citrus balckfly. The major predators of citrus blackfly are *Mallada boninensis* Okamota, and *Seragium parcestosum* Sicard. Field evaluation of *M.boninensis* revealed that release of 30 larvae per tree twice in each season reduced blackfly population by 30-40%. Though aphelinid parasitoids like *Encarsia opulenta* (Silvestri), *E.bennetti* Hayat *and Amitus hesperidium* Silvestri are known to parasitise the nymphs of blackfly, mass multiplication was not quite successful since they are extremely stage specific. Since last decade, blackfly population shows a declining trend and are below economic threshold level whereas population levels and the damage caused by other pests like *citrus psylla, citrus thrips and mites* are progressing at an alarming rate.

Dr. A. Krishnamoorthy outlined on the **"Prospects of biological control of whiteflies".** Biological control of whiteflies across the globe was attempted and obtained partial to complete success. Complete control of whiteflies has been obtained in some countries through parasitoids rather than other agents, wherever the pests did not act as a vector. In India too biocontrol of spiraling whitefly, *Aleurodicus disperses* through parasitoids is a success. He further enlisted attempts made on biocontrol of whiteflies and suggested future possibilities.

Plenary session:

The summary of presentations of all the technical sessions was presented in the plenary session followed by a discussion. Important suggestions that emerged out of this session are briefly mentioned below.

Representative from Advanta: It is suggested to have natural and artificial whitefly consortium screening, so that we all can participate on payment basis.

Dr. Malathi : DBT is seriously thinking about this, IIHR can be one nodal centre. An artificial inoculation with infectious clones can be done. But breeders will oppose since the load of inoculum is very high.

Dr. Muniyappa : Begomovirus is spreading criss-cross; specific sampling of ToLCV is required. We have whitefly culture; Dr. Nagaraj is involved in screening Bangalore ToLCV. Hence I request Dr. Sadashiva to give susceptible tomato variety.

Mr. Sourabh : Good knowledge about Whitefly. Suggestion made by Dr. Krishna Reddy about collaboration between breeders, entomologists and pathologists in developing a molecule which can spot the movement of virus

in whitefly gives good idea.

Dr. N. K. Krishna Kumar : Resistance to whiteflies can be a challenging task in future. It is desirable to have a centre of excellence where all people can look for expertise. Industries should come forward to fund students to carry out their research work on these lines.

Recommendations:

1. The house felt that a comprehensive approach involving plant breeders, virologists, and entomologists is the need of the hour to evolve varieties/hybrids resistant to whitefly transmitted viruses. The house felt that the advanced breeding lines showing resistance to whitefly transmitted viruses should be evaluated against different biotypes of whiteflies so that the resistance is more durable in time and space. For this there is a need to have different centers that may be requested to maintain specific whitefly biotypes.
2. A distribution map indicating species, biotypes**,** whitefly transmitted diseases and whitefly parasitoids needs to be prepared.
3. The greenhouse whitefly, *Trialeurodes* is emerging as a major problem both in polyhouse and outside. There is a need to determine its status as a pest in different regions of the country.
4. The research work on biotypes should be addressed in two fronts.
   a. Insecticide resistance and biotypes where whitefly damage is observed more as a pest.
   b. Biotype in relation to virus transmission, emergence of new viral disease and break down in host plant resistance.
5. There is a need to investigate and document the factors responsible for the spectacular decline of the citrus black fly which was a major pest during 1975-2000.
6. A critical evaluation on the status of whiteflies in Andaman and Nicobar has to be carried out.
7. Species specific and biotype specific symbionts and their variability at molecular level need to be documented.
8. The marker developed for whitefly and whitefly transmitted virus resistance should be broadly shared.
9. There is a need to support both traditional and molecular systematics with regard to whiteflies.
10. As a consolidated information on whiteflies is lacking, it is proposed to publish the presentations made in this meeting in the form of a comprehensive publication.

List of Participants:

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21. Dr. P. N. Krishnamurthy, IIHR, Bangalore
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9. Mr. H. M. Mahadevaswamy
10. Mr. R. Ellango
11. Mr. B. N. Chaitanya
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Participants from Industry

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8. Mr. Susheel Kumar, Advanta, Bangalore
9. Mr. M. Amaresha, Bioseeds, Bangalore
10. Mr. Sunil kumar, Bayer Crops Science, Ltd, Bangalore
11. Mr. S. M. Mohan, Nunhems, Bangalore
12. Mr. Devaraju, Nunhems, Bangalore
13. Mr. K. C. Raju, Nunhems, Bangalore
14. Dr. V. Muniyappa, Bangalore

Plate 1 : Dr. B. Vasantharaj David being honoured with the Life Time Achievement Award of the AAPMHE

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