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Survey and Biotype Identification of Whitefly, *Bemisia tabaci* Transmitting Tomato Leaf Curl Virus in Andhra Pradesh, India

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The whitefly, *Bemisia tabaci* (Gennadius) is an important pest of tomato which transmits *Tomato leaf curl virus* (ToLCV), a Gemini virus, causing heavy losses round the year in Andhra Pradesh, India. Several biotypes of *B. tabaci* were reported from different parts of the world and most recently a new biotype–B was confirmed from kolar district of Karnataka, India.. But very little information is available on the biotype status of *B. tabaci* in Andhra Pradesh. There is a great concern about the spread of B-biotype whiteflies from border districts of Karnataka to Andhra Pradesh through material transfer and trade. Surveys undertaken during 2007 and 2008 in the major tomato growing areas of Andhra Pradesh revealed that *Tomato leaf curl virus* (ToLCV) incidence was wide spread in all the five districts under study *Viz.*, Rangareddy, Medak, Guntur, Prakasam and Chittoor and was in severe form during summer, moderate in *Rabi* and less in *Kharif* season. Molecular characterization studies of whiteflies collected from different districts of Andhra Pradesh were analyzed through RAPD-PCR technique. The present study revealed the presence of the 750 bp amplification specific to B-biotype of whitefly in Shamshabad and Moinabad of Rangareddy district, Patancheru of Medak district and Madanapalli of Chittoor district with similar banding pattern to whiteflies (B-biotype) collected from Kolar district of Karnataka. There is every possibility that this biotype may spread to other parts of the state and may cause substantial losses to vegetable production. This is the first report of existence of *B.tabaci* biotype-B from Telangana region of Andhra Pradesh.

Keywords: Whitefly, *Bemisia tabaci*, B-biotype, RAPD-PCR, Tomato leaf curl virus.

Introduction:

The whitefly *Bemisia tabaci* (Gennadius) is one of the most economically important pest throughout the world and causes extensive damage in more than 500 species of crops (Greathead, 1986). In tomato besides causing direct damage as a pest, it transmits *Tomato leaf curl virus* (ToLCV), a Gemini virus,

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which causes heavy losses round the year in tropical and sub tropical tomato growing regions of the world (Green and Kalloo, 1994). *Bemisia tabaci* consists of several biotypes (Brown *et al*., 1995) that have been distinguished largely on the basis of biochemical and molecular diagnostics. The most widespread biotypes of *B. tabaci* in Southern Europe and the middle-East are referred to as the B and Q types (Guirao *et al*., 1997). In India, the severe outbreak of *ToLCV* occurred during 1999 which caused complete failure of tomato crop in Kolar district of Karnataka was attributed to the result of a new biotype-B of *B. tabaci* which was confirmed by RAPD-PCR (Banks *et al*., 2001). Verylittle information is available on the biotype status of *B. tabaci* in Andhra Pradesh. There is a great concern about the spread of B-biotype whiteflies from border districts of Karnataka to Andhra Pradesh through material transfer and trade. The invasion of the B-biotype and displacement of indigenous biotype is of greater importance which could threaten insecticide resistance management strategies of *B. tabaci* and consequent spread of viral diseases in tomato. The molecular methods of RAPD have proved useful in distinguishing B biotype and indigenous populations of *B. tabaci*. The primer OPB 11 amplifies the 750 bp DNA fragment which was specific to B biotype and was absent in indigenous whitefly population. Due to specificity of amplification, these bands were used as molecular markers for the identification of B biotype.

Materials and Methods

A roving field survey was conducted during *Rabi* 2007, Summer 2007 and *Kharif* 2008 to record the incidence of tomato leaf curl virus disease (ToLCV) and prevalence of whitefly vector, *Bemisia tabaci* population in five major tomato growing districts of Andhra Pradesh *viz*., Chittor, Guntur, Rangareddy , Medak and Prakasam (Plate 1). In each field, five plots of ten m^2 were marked and whitefly population was counted in five randomly selected plants by observing six leaves (top two, middle two and bottom two leaves) from each plant. The adult whiteflies were collected with the help of aspirator and stored in 80% alcohol for the molecular characterization of whitefly biotypes. The incidence of ToLCV was recorded by counting the number of diseased and healthy plants in 10 m^2 area and converted to per cent incidence. The ToLCV infected plant samples were collected and further maintained in the glass house by graft transmission method for confirmation of ToLCV and molecular characterization studies.

DNA extraction from whiteflies was carried out using De Barro and Driver method (1997). A single whitefly adult was transferred into a 1.5 ml micro centrifuge tube and 25 µl of extraction buffer (50 mM KCI, 10 mM Tris - HCl pH 8.4, 0.45% Tween 20, 0.2% gelatin, 0.45% NP40, 500 µg/ml proteinase K) was added. The whitefly was ground thoroughly using a micro pestle. The homogenate was incubated at 65^0 C for 30 min in a hot water

bath.After incubation, a small hole was made at the top of the micro-centrifuge tube to release the pressure while boiling at 100^0 C for 10 minutes to inactivate proteinase K. 25 µl of 1 x TE buffer was added to yield a final sample volume of 50 µl and samples were stored at -20^0 C.Two µl of sample was used for the PCR analysis. The decamer oligonucleotide random Operon primer, OPB-11 was used for detailed RAPD-PCR analysis of whitefly biotype studies. RAPD-PCR analysis was carried out by using the DNA extracted from adult whiteflies.

PCR reactions were performed in 25 µl of reaction mix containing 2 µl of template DNA, 3 units of *Taq* DNA polymerase, 2.5 mM $MgCl_2$, 2.5 mM dNTPs, and 5 pmol of the primer (OPA-13) in 10 X reaction buffer (20 mM (NH_4) SO_4, 75 mM Tris-HCI, pH 9.0).Single distilled water (SDW) control was included in all reactions as no DNA (negative) control.Each reaction was run for 40 cycles in a PCR thermal cycler (Eppendorf) under the following amplification cycle: denaturation at 94^0 C for 2 min, annealing at 35^0 C for 1 min, extension at 72^0 C for 1 min 30 seconds and final extension at 72^0 C for 10 min.A 20 µl volume of PCR product was mixed with 4 µl of Orange G loading dye (15% (w/v) Ficoll type 400, 0.25 per cent (w/v) orange –G, 40 mM EDTA, pH 8.0 in 100 ml of SDW and electrophoresed at 5 V cm^{-1} through a 1.0 per cent (w/v) agarose gel with ethidium bromide solution (0.5 µg ml^{-1}) in 0.5x TBE buffer (4.5 mM Tris-borate, 0.1 mM EDTA). The DNA size marker 1 kb Ladder (Fermentas) was included to allow the size of the DNA bands to be determined. Amplified products were visualized using gel documentation unit (B & L Image system).The banding patterns of the different whitefly biotypes were compared.

Results and Discussion:

Surveys undertaken during 2007 and 2008 in the major tomato growing areas of Andhra Pradesh revealed that *Tomato leaf curl virus* (ToLCV) incidence was wide spread in all the five districts under study *Viz.*, Rangareddy, Medak, Guntur, Prakasam and Chittoor and was in severe form during summer, moderate in *Rabi* and less in *Kharif* season. A total of the 311 farmer's fields surveyed during 2007-08, out of which, 92 fields were surveyed during summer 2007, 121 during *Rabi* 2007 and 98 fields during *Kharif* 2008. The survey revealed the severe incidence of *ToLCV* during summer (51.80% to 93.60%), moderate incidence during *Rabi* (21.55 to 54.7%) and comparatively less incidence (16.72 to 33.5%) during *Kharif* 2008.

During summer 2007, highest incidence of *ToLCV* (93.6%) was recorded from Prakasam district followed by 85.27% incidence in chittoor district while less incidence of 51.8% was recorded from Medak district.

During *Rabi* 2007, highest *ToLCV* incidence of 54.7% was recorded from rangareddy district followed by 49.8% recorded from Guntur district while prakasam district recorded less incidence (21.58%). During *Kharif* 2008, the maximum *ToLCV* incidence of 33.5% was observed in Medak district while less incidence of 16.47% was recorded in Prakasam followed by Chittoor with 18.35% incidence. In Guntur district the ToLCV incidence was not recorded for summer and *Kharif* seasons, as the crop will be raised only during *rabi* period in this district.

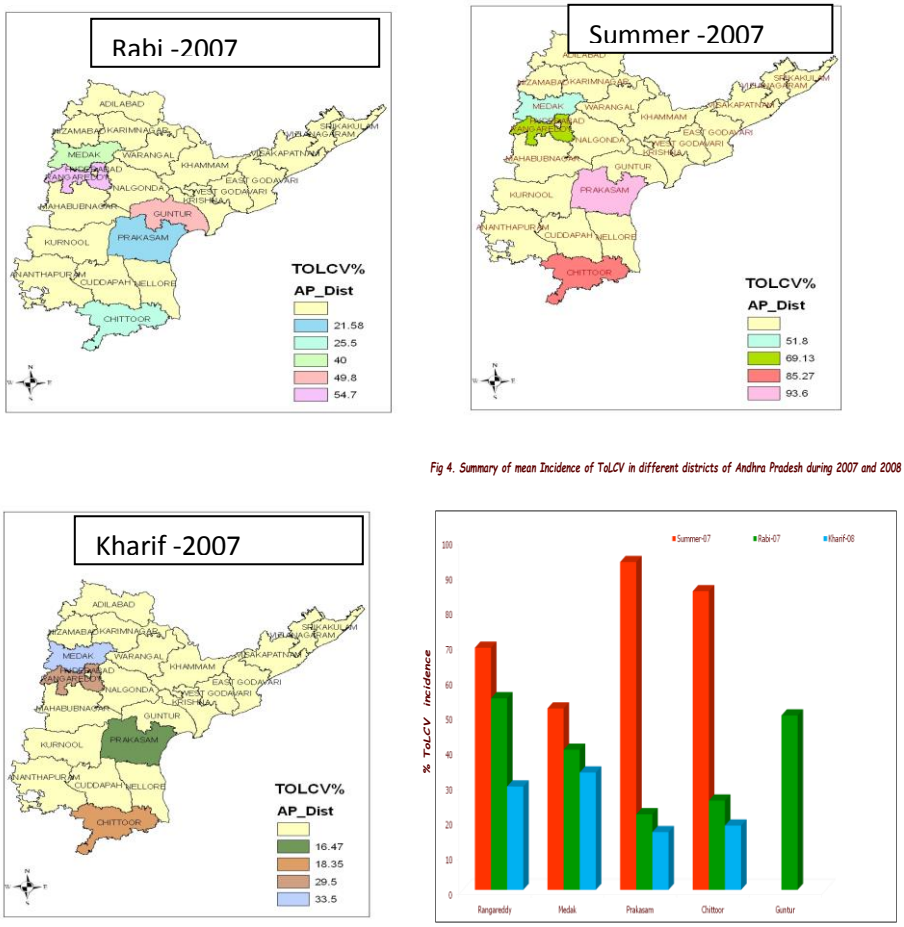
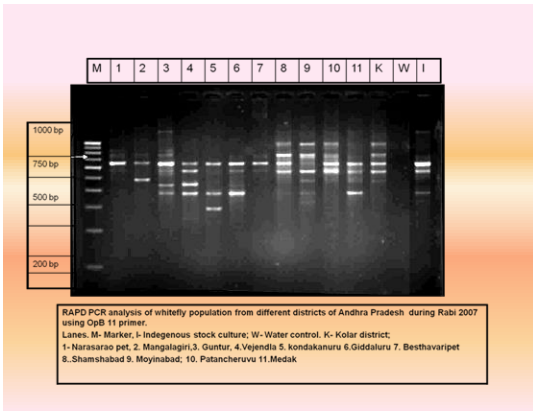


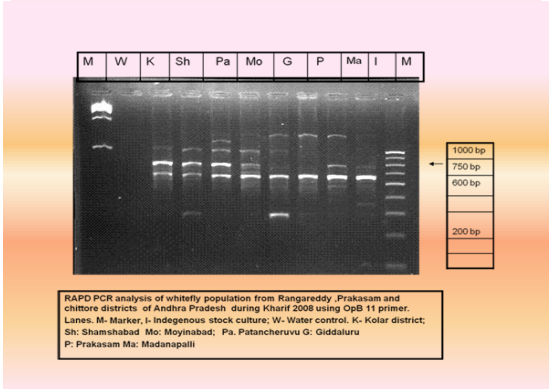
Fig 4. Summary of mean Incidence of ToLCV in different districts of Andhra Pradesh during 2007 and 2008

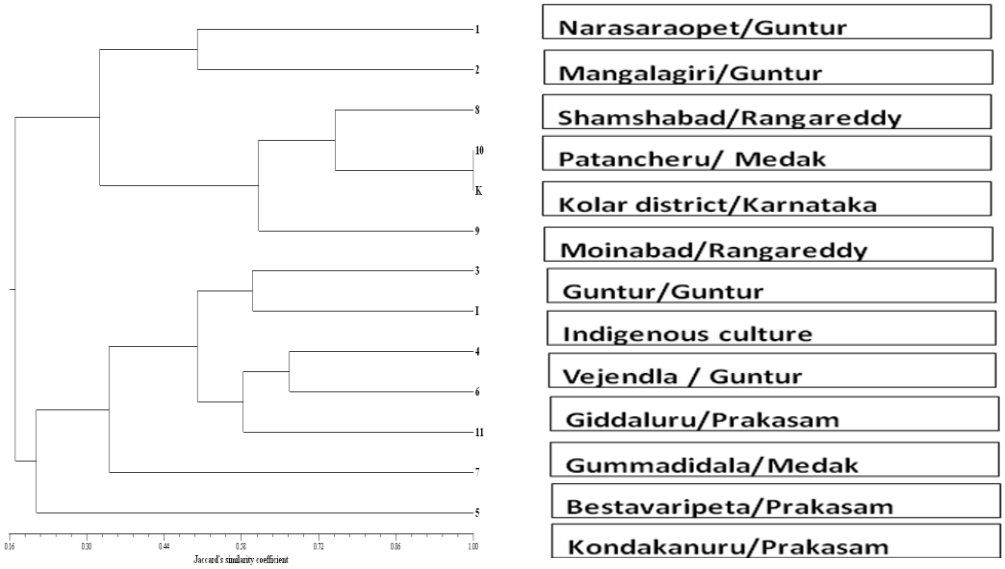
Fig 1. Survey for the incidence of ToLCV and whitefly, *B.tabaci* in different districts of A.P during 2007 and 2008.

The whitefly, *B. tabaci* isolates were collected from different locations of Andhra Pradesh Viz., Rangareddy, Medak, Guntur, Prakasam and Chittoor districts of and compared with the B biotype whiteflies collected from Kolar

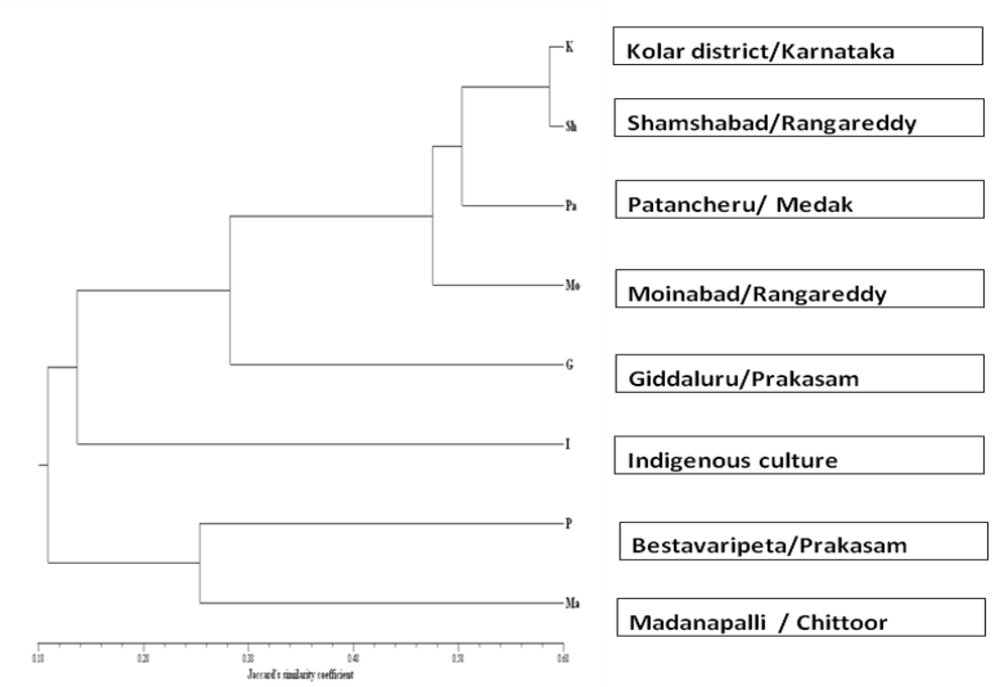
district of Karnataka through RAPD – PCR analysis using OPB 11 primer. Out of 11 and 8 whitefly samples collected during survey from different districts of Andhra Pradesh analyzed during *Rabi* 2007 and *Kharif* 2008, respectively.The 750 bp specific to biotype-B whitefly was identified in Shamshabad, Moinabad mandals of Ranga Reddy and Patancheru mandal of Medak district during *Rabi* 2007 and from Shamshabad and Moinabad mandals of Ranga Reddy, Patancheru of Medak and Madanapalli of Chittoor district during *Kharif* 2008. It clearly reveals that the biotype has spread to Andhra Pradesh from the neighboring Kolar district of Karnataka. Therefore the RAPD-PCR technique using OPB 11 can be used for differentiating the biotypes. It is evident from the results that B biotype is now present in some parts of Andhra Pradesh and is spreading rapidly from its original point of introduction i.e Kolar district of Karnataka. The existence of biotype B in Shamshabad, Moinabad, Patancheru and nearby mandals of Hyderabad which are far away from Kolar district of Karnataka indicates the fast spread of B-biotype into Andhra Pradesh. There is every possibility that this biotype may spread to other parts of the state and may cause substantial losses to vegetable production.







Dendrogram showing the grouping of whitefly populations of A.P in comparison with biotype –B of Kolar district in Karnataka during Rabi, 2007



Dendrogram showing the grouping of whitefly populations of A.P in comparison with biotype –B of Kolar district in Karnataka during Kharif, *2008*

Many researchers have used RAPD-PCR technique for distinguishing the indigenous population of *B. tabaci* from those of introduced B biotype (Gawel and Bartlett, 1993; Perring *et al.,* 1993; De Barro and Driver, 1997; Guirao *et al.,* 1997; Moya *et al.,* 2001; Shankarappa *et al.*,2007). Although RAPD - PCR analysis is not suitable for defining the taxonomic status of insects, it is quite useful in distinguishing the closely related populations (Gawel and Barlett, 1993; Guirao *et al.,* 1997).

Rapid spread and complete replacement of local indigenous strain of *B. tabaci* by B biotype in few years of its introduction in many countries have been already documented by many workers (Costa et al., 1993; Frohilich et al; 1999; Brown 2001). The first report of Biotype –B from Karnataka was recognized because it caused a havoc of ToLCV epidemic in Kolar district(Banks et al.,2001).Present investigation on whitefly populations on tomato revealed the presence of biotype –B of B.tabaci in Shamshabad and Moinabad of Rangareddy district, Patancheru of Medak district and Madanapalli of Chittoor district with similar banding pattern to whiteflies (B-biotype) collected from Kolar district of Karnataka. This is the first report of existence of biotype-B from Telangana region of Andhra Pradesh. This indicates the real and increasing threat posed by the spread of B- biotype and its potentiality to act as a vector of the deadly Gemini viruses causing severe losses to vegetable production in Andhra pradesh.

Genetic variability assessment in whitefly *B. tabaci* populations originating from different districts of Andhra pradesh, India indicates that the population is diversified based on the geographical distribution of the pest. Clustering pattern observed in the dendrogram showed that at least two distinct biotypes exist among the populations collected within the narrow region of a state. These differences may be influencing the virus vectoring capabilities of the whitefly population and also their susceptibility to insecticides. In order to clarify the complexity of these whiteflies, further experimental evidence is required especially on biology, mating compatibility, alternate hosts, transmission ability, insecticide resistance and other biological characteristics of different populations.

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