






**Molecular Characterization and management of Tomato leaf curl virus and its vector, *B. tabaci*, using cultural methods like guard crops and mulches in Tomato.**



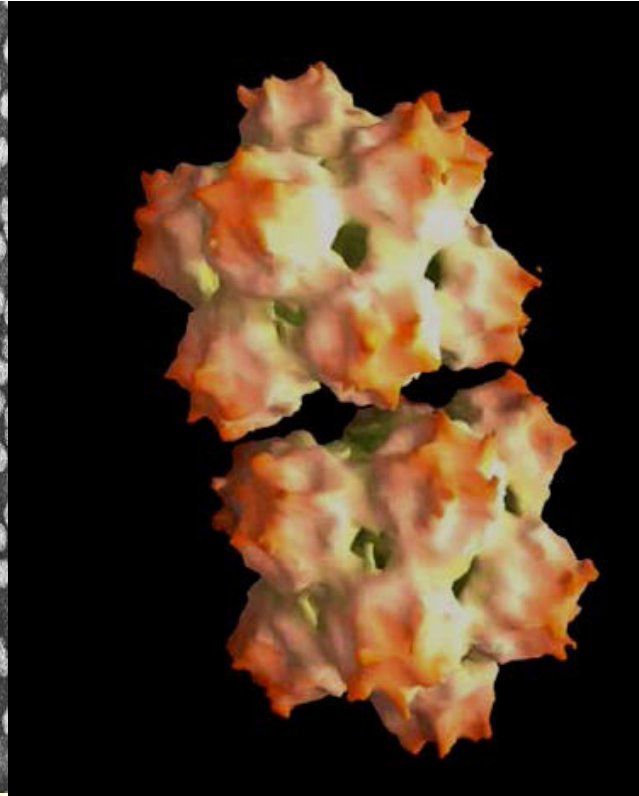
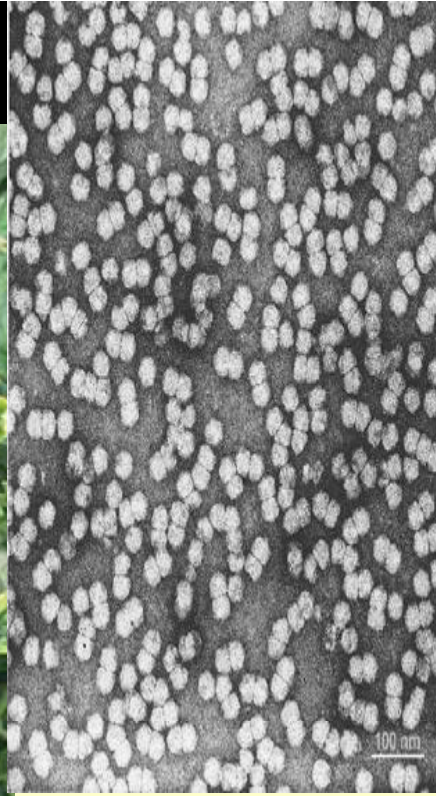
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P.J.T.S.Agril. University  
Hyderabad, India*

# Introduction

-  Tomato- most important vegetable crop & Rich source of Vit A ,C ,other minerals ,Lycopene ,carotinoids and anti-oxidants etc.,
-  *Tomato leaf curl virus (ToLCV)* , Geminiviridae is an important viral disease in tomato .
-  Symptoms - Upward curling ,reduction in size of new leaves, slight chlorosis, crinkling and puckering of leaflets, stunting of plants.
-  Whitefly, *B.tabaci* - Polyphagous, Biotypes, insecticide resistance, vector of viral diseases .
-  Information -ToLCV incidence, viral strains, whitefly biotypes and their role in Tomato leaf curl transmission .



# *Tomato Leaf curl virus*



- + Upward curling*
- + reduction in size of new leaves, slight chlorosis,*
- + crinkling and puckering of leaves*
- + stunting of plants.*

## *Monopartite*

*Tomato leaf curl Kerala virus (ToLCKeV) - Dr SK Mukherjee, ICGEB*

*Tomato leaf curl Ranchi virus (ToLCRnV) - Dr S Chakraborty, JNU*

*Tomato leaf curl Patna virus (ToLCPaV) - Dr S Chakraborty, JNU*

*Tomato leaf curl Rajasthan virus (ToLCRaV)*

*Tomato leaf curl Pune virus (ToLCPV) - Dr S Chakraborty, JNU*

*Tomato leaf curl Lucknow virus (ToLCLuV)*

*Tomato leaf curl Bangalore virus (ToLCBaV)- Dr VG Malathi, IARI*

*Tomato leaf curl Karnataka virus (ToLCKaV) - Dr VG Malathi, IARI*

*Tomato leaf curl Joydebpur virus (ToLCJoV) - Dr VG Malathi, IARI*

*Tomato leaf curl Gujarat virus (ToLCGuV) - Dr VG Malathi, IARI*

## *Bipartite:*

*Tomato leaf curl New Delhi virus (ToLCNDV) - Dr VG Malathi, IARI*

*Tomato leaf curl Palampur virus (ToLCPaIV) - Dr VG Malathi, IARI*

# *Whitefly* *Bemisia tabaci*



- 6 life stages: egg, 4 nymphal instars, adult
- Life cycle can be as short as 2 weeks



# *Nymphs*



- In Favorable conditions, 15 generations per year*
- Females Do not Require a male to reproduce*
- All stages prefer the underside of leaves*

# ToLCV- Virus –Vector interaction studies



Whitefly culture maintenance



Virus culture maintenance



Raising healthy plants





# Whitefly culture maintenance

- Glass house at NBPGR, Hyderabad



Insect proof cages for vector maintenance



Brinjal - preferred host for whitefly, *B. tabaci*



Maintenance of Tomato plants in *Glass house*





# Virus culture maintenance under glass house



# Molecular characterisation of *Tomato leaf curl virus*

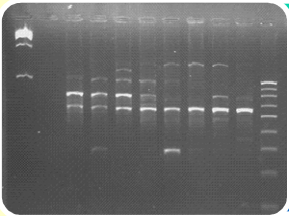


## Genomic DNA extraction from ToLCV infected leaf sample

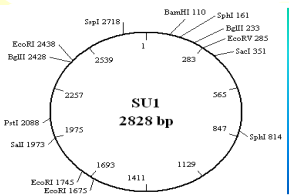
- Gem-CTAB method



Amplification of circular DNA  
Rolling circle amplification



Restriction with endo nucleases ( BamHI, HindIII, XbaI)



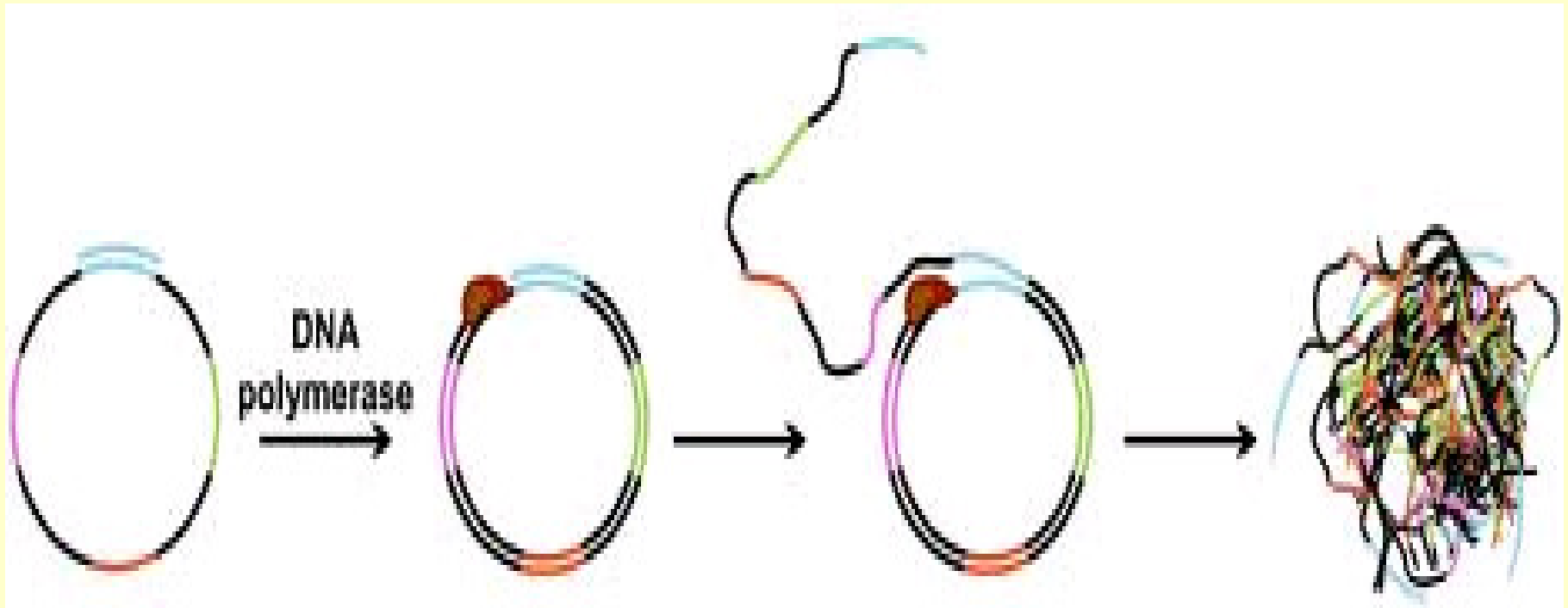
Cloning, Transformation, Screening and Sequencing

```
GCGTGAATCCGTGA  
TCATGGCAGTTGAT  
CGATTGGTAATACG  
ACAACCCACACGGT
```

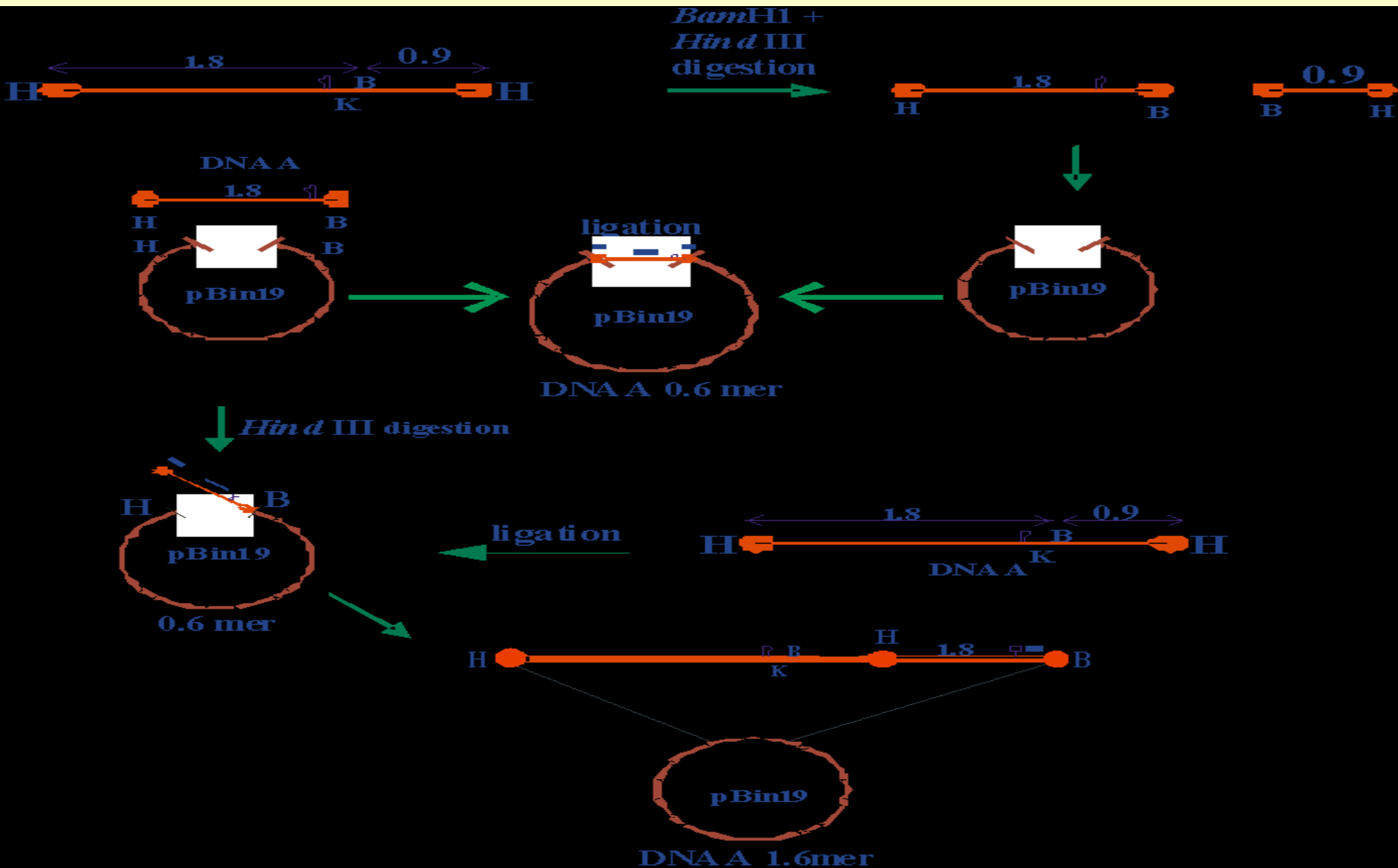
Phylogenetic analysis of genome sequence with selected known sequences in gene bank using blast analysis.



# Rolling circle amplification (RCA)



# Cloning



# Molecular characterization of *Tomato leaf Curl Virus*

## Genomic DNA extraction

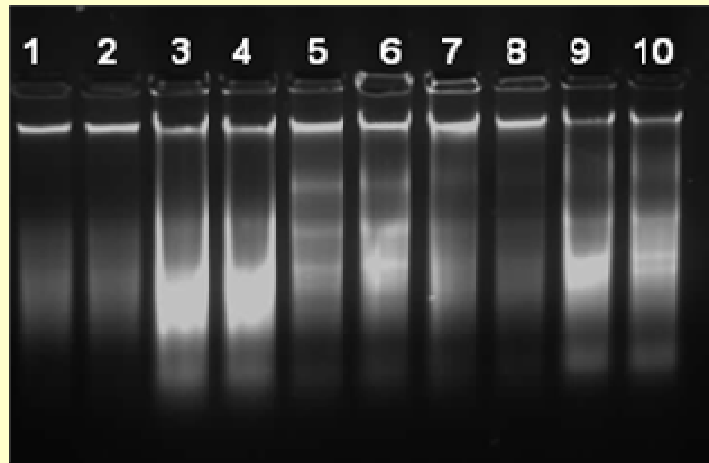


Fig. DNA extraction from tomato leaf curl samples collected from A.P. Lane 1&2 – Hyderabad, 3 &4- Shamshabad, 5&6 – Moyirabad, 7&8 – Patancheruvu, 9&10-Medak

## Rolling circle Amplification (RCA)

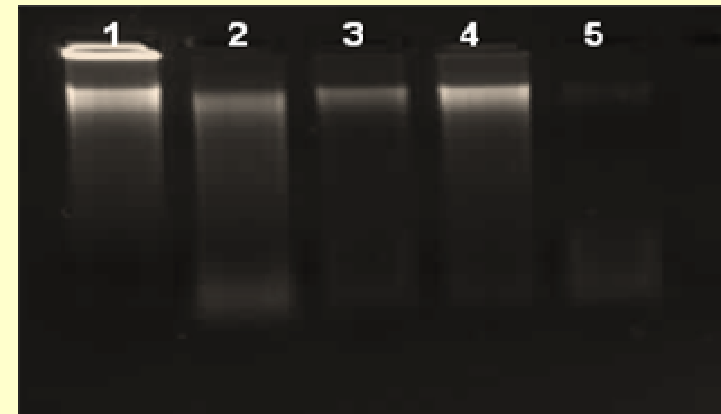


Fig. Agarose gel electrophoresis of RCA product

## Restriction with different endonucleases

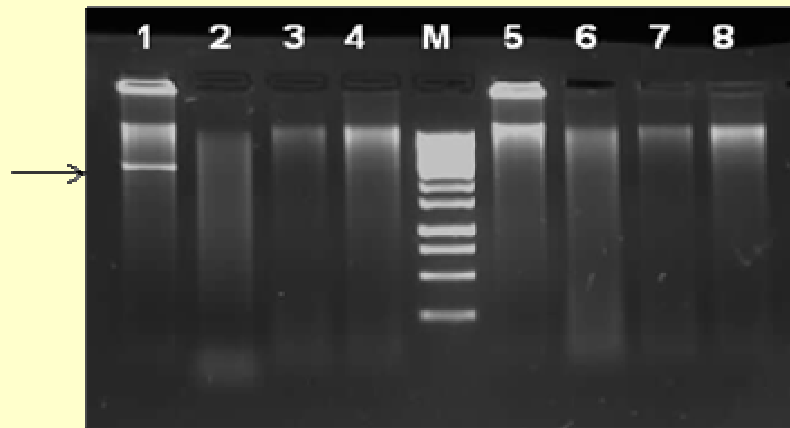


Fig. The RCA product was restricted with BamHI, HindIII, XbaI and KpnI restriction endonucleases. 2.7 kb band in lane 1 (Hyderabad sample)

## Cloning

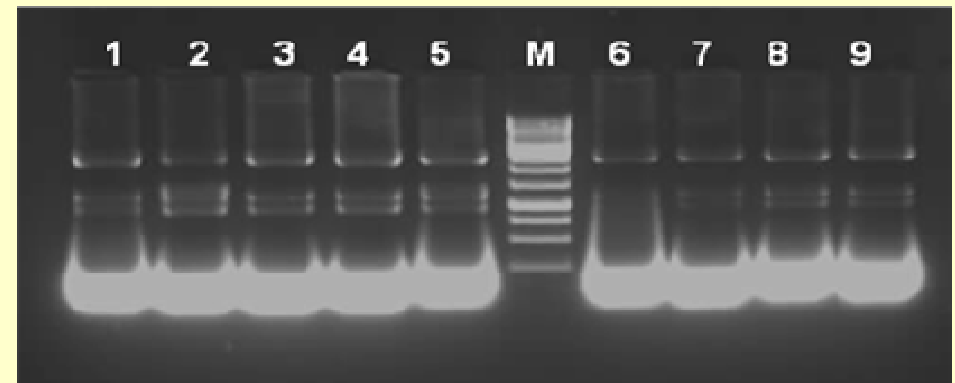


Fig. All clones were showing vector (pUC18) cut into 2 fragments with BamHI/BglI releasing 2.7kb insert.

# *Phylogenetic sequence analysis of ToLCV through BLAST Analysis*

Virus	Query coverage	% identity	Accession no.
ToLCNDV-AVT1	96%	93%	AY42876
ToLCNDV-Svr	99%	93%	U15015
ToLCNDV-Chilli	96%	93%	EU309045



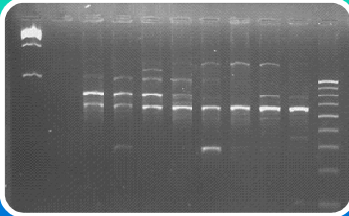
# Molecular characterisation of whitefly, *B.tabaci*



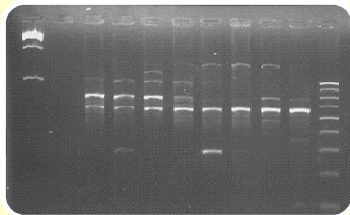
DNA extraction from single whitefly adults using Debarro method



Amplification using Primer OPB-11 in PCR thermocycler



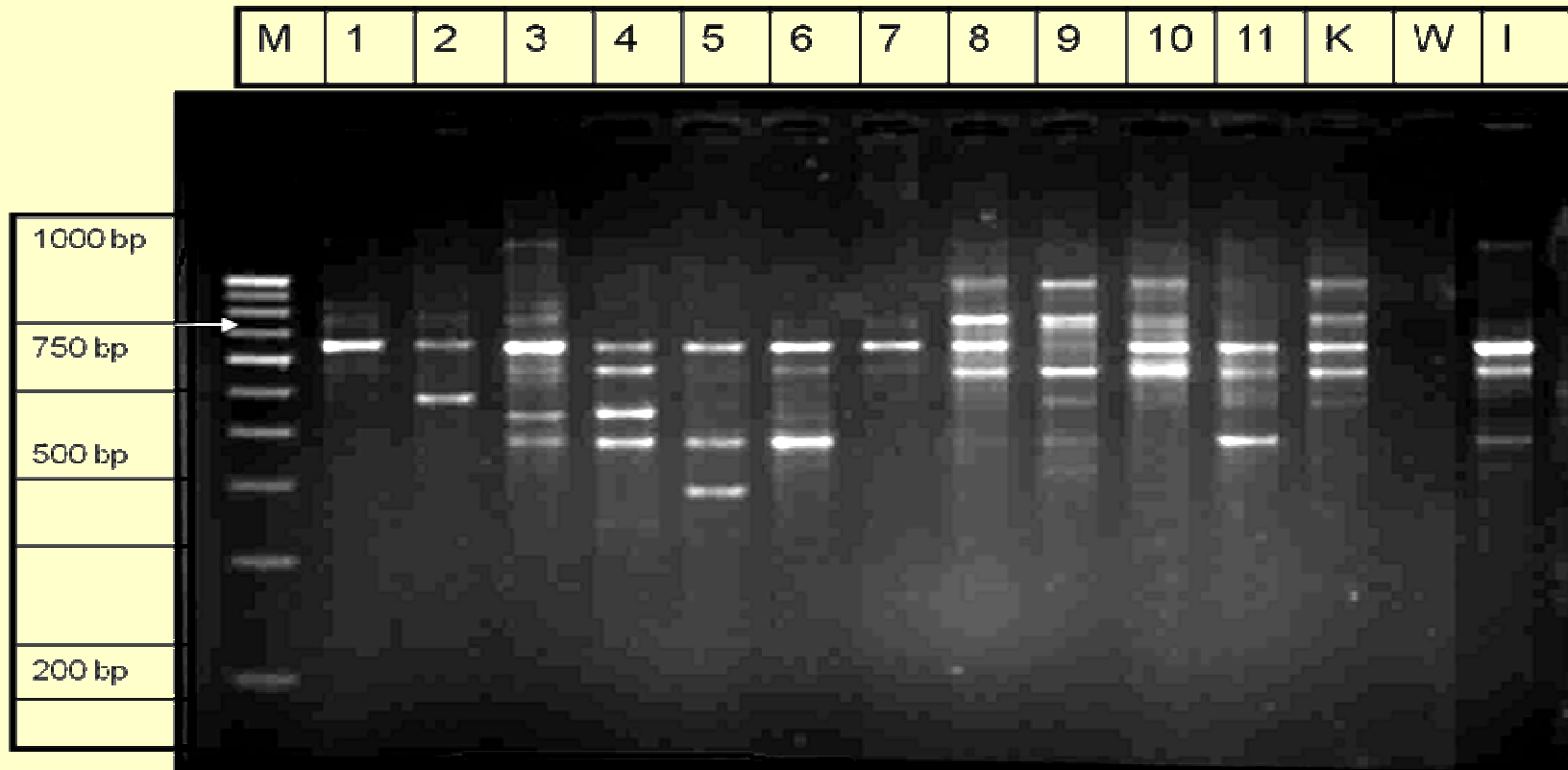
Gel electrophoresis using 1.5% Agarose gel



Documentation of gel pictures using Gel Doc system



# *RAPD PCR Analysis of Whitefly*



**Plate 4:**

RAPD PCR analysis of whitefly population from different districts of Andhra Pradesh during Rabi 2007 using OpB 11 primer.

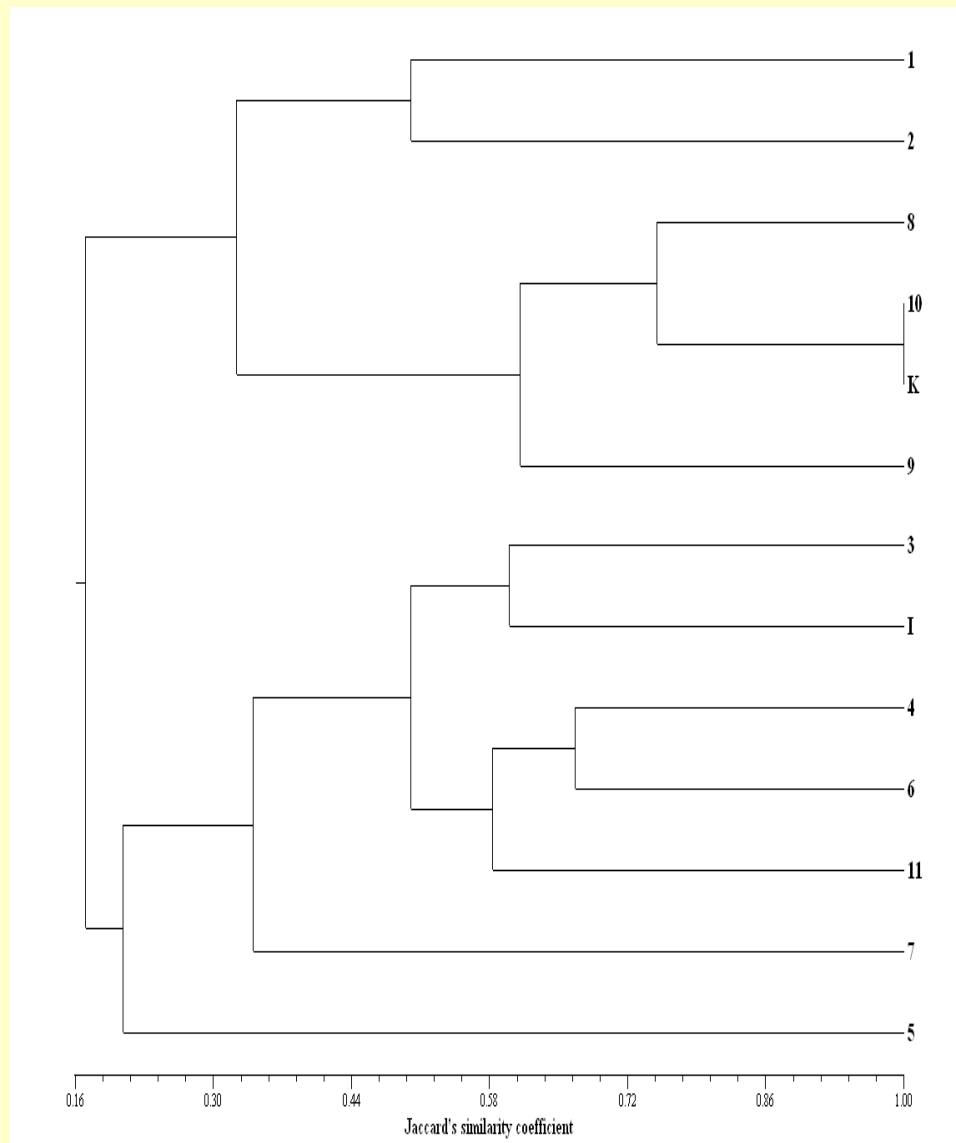
Lanes. M- Marker, I- Indigenous stock culture; W- Water control. K- Kolar district;

1- Narasarao pet. 2. Mangalagiri, 3. Guntur, 4. Vejjendla 5. kondakanuru 6. Giddaluru 7. Besthavaripet  
8. Shamshabad 9. Moyinabad; 10. Patancheruvu 11. Medak





# Dendrogram showing the grouping of whitefly populations of A.P in comparison with biotype –B of Kolar district in Karnataka.



**Narasaraopet/Guntur**

**Mangalagiri/Guntur**

**Shamshabad/Rangareddy**

**Patancheru/ Medak**

**Kolar district/Karnataka**

**Moinabad/Rangareddy**

**Guntur/Guntur**

**Indigenous culture**

**Vejendla / Guntur**

**Giddaluru/Prakasam**

**Gummadidala/Medak**

**Bestavaripeta/Prakasam**

**Kondakanuru/Prakasam**



# WHAT IS IPM?

Integrated Pest Management (IPM) is the coordinated use of pest and environmental information with available pest control methods to prevent unacceptable levels of pest damage by the most economical means and with the least possible hazard to people, property, and the environment (EPA definition). IPM is a sustainable approach to pest management that uses knowledge of pest, crop and environmental conditions to select the best combination of the following

## *IPM TACTICS:*

### **Cultural control**

modify farming practices to decrease pest problems

### **Biological control**

use beneficial organisms to regulate pests

### **Chemical control**

use chemical pesticides appropriately

### **Physical control**

kill pests directly or by disrupting their environments



## Guard crops

- 1. Tomato + Maize
- 2. Tomato + Jowar
- 3. Tomato + Bajra
- 4. Tomato + Brinjal
- 5. Tomato + Sunflower
- 6. Tomato + Redgram
- 7. Tomato sole

## Mulches

- 1. White polythene sheet
- 2. Black polythene sheet
- 3. UV reflective mulch
- 4. Paddy straw mulch
- 5. Saw dust mulch
- 6. Paddy husk mulch
- 7. Control



*Tomato + Maize*

G  
U  
A  
R  
D



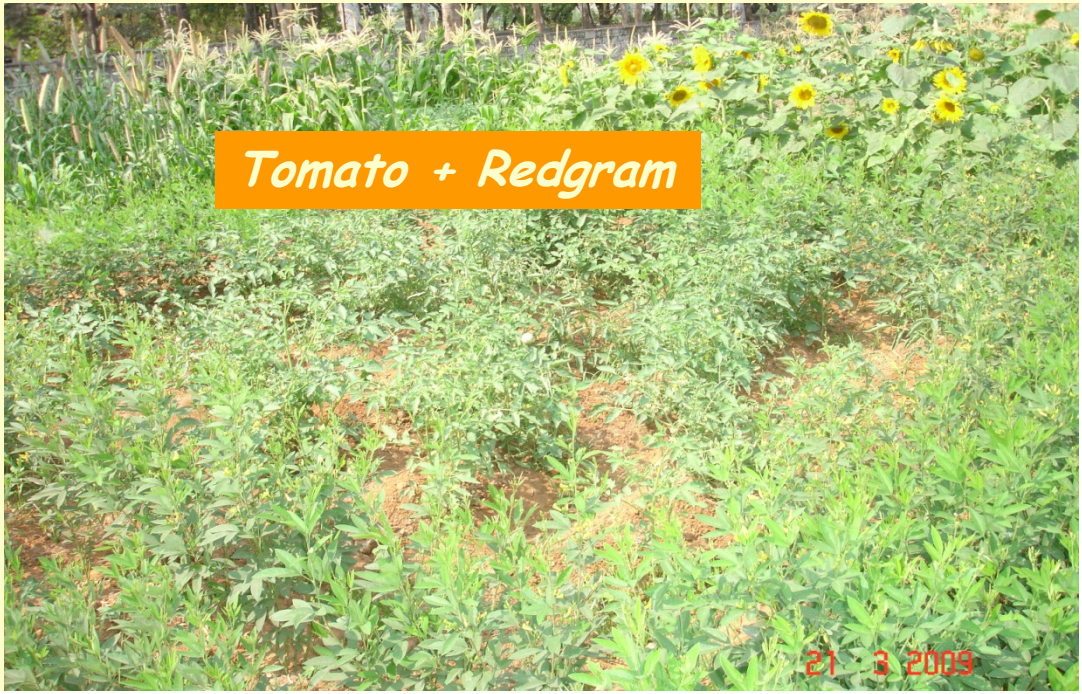
*Tomato + Sunflower*

C  
R  
O  
P  
S



*Tomato + Jowar*

C  
R  
O  
P  
S



*Tomato + Redgram*

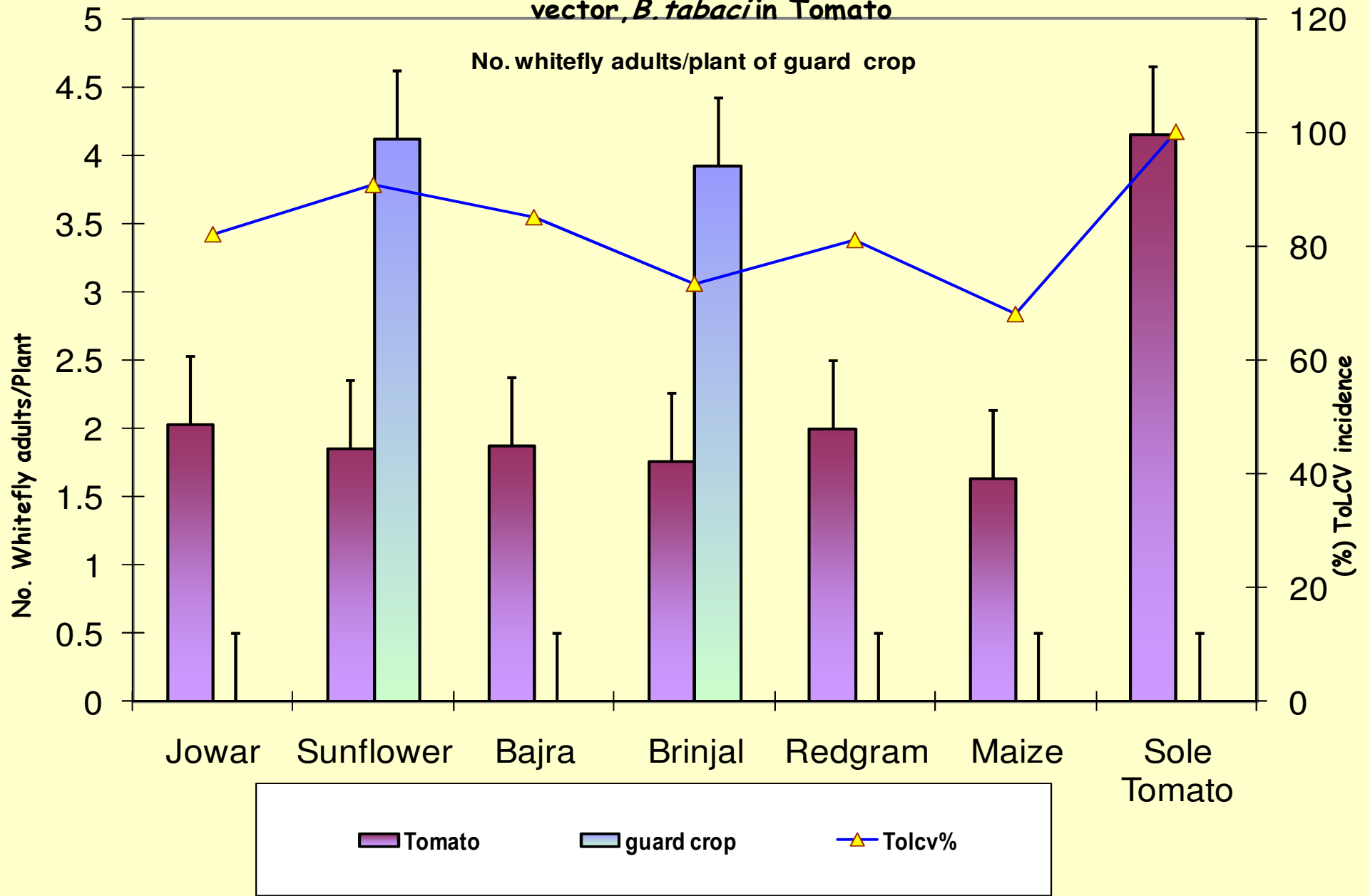
21 3 2009

## *Effect of guard crops on *B. tabaci* and incidence of ToLCV in tomato*

Guard Crop	* Mean number of whiteflies /plant		** (%) ToLCV incidence				Yield q/ha
	Mean		40 DAT	50 DAT	60 DAT	70 DAT	
	T	G					
Jowar	1.92 (1.56)	0 (0.71)	24.24 (26.12)	42.93 (40.92)	59.52 (50.54)	79.10 (63.16)	79.80
Sunflower	2.44 (1.71)	3.37 (1.96)	24.20 (29.63)	40.28 (39.41)	62.24 (52.17)	81.86 (64.77)	78.44
Bajra	3.06 (1.89)	0 (0.71)	22.57 (28.37)	49.37 (44.66)	71.10 (57.51)	95.23 (77.34)	69.88
Brinjal	1.45 (1.39)	5.03 (2.34)	13.16 (21.06)	25.71 (30.39)	44.47 (41.84)	63.13 (52.60)	101.88
Red gram	1.57 (1.44)	0 (0.71)	22.61 (28.29)	48.10 (43.90)	55.60 (48.22)	62.05 (51.95)	90.00
Maize	1.02 (1.23)	0 (0.71)	11.66 (19.70)	24.20 (29.39)	40.23 (39.27)	60.67 (51.25)	122.52
Sole Tomato	4.13 (2.14)	-	35.55 (36.53)	56.52 (48.74)	84.97 (67.41)	95.83 (79.91)	65.48
CD at 5%	0.16	0.17	4.03	5.55	8.63	11.47	22.66

*DAT: Days after transplantation; T: Tomato, G: Guard crop*

Effect of guard crops on the pooled mean incidence of ToLCV and its vector, *B. tabaci* in Tomato



# Effect of different kinds of mulches on the incidence of *B. tabaci* transmitting ToLCV in Tomato.



*White polythene sheet mulch*



*UV reflective mulch*



*Black polythene sheet mulch*



*Paddy Straw mulch*

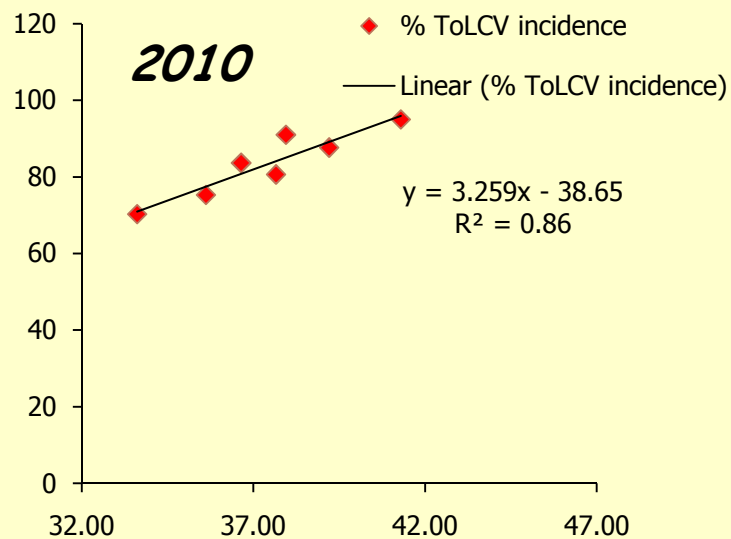
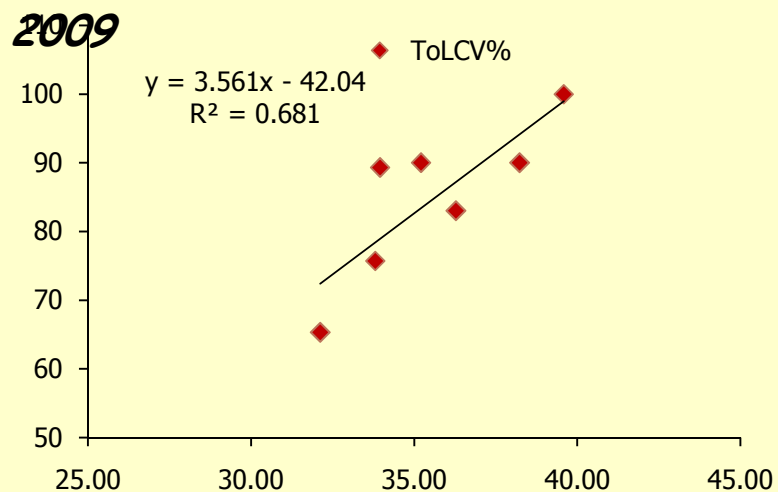
## Effect of different mulches on *B.tabaci* populations and incidence of *ToLCV* in Tomato

Treatment	* Mean No. of whiteflies / plant						** (%) <i>ToLCV</i> incidence				Yield q/ha
	30 DAT	40 DAT	50 DAT	60 DAT	70 DAT	Mean	40 DAT	50 DAT	60 DAT	70 DAT	
White polythene mulch	0.0 (0.71)	1.87 (1.52)	2.67 (1.77)	2.2 (1.64)	2.0 (1.58)	1.75 (1.49)	27.9 (31.82)	42.19 (40.45)	51.9 (46.09)	80.6 (63.94)	79.0
Black polythene mulch	0.33 (0.91)	2.4 (1.69)	2.93 (1.85)	2.4 (1.71)	2.2 (1.62)	2.07 (1.59)	35.93 (36.79)	41.8 (40.27)	60.0 (50.79)	91.0 (72.56)	84.78
UV reflective mulch	0.0 (0.71)	0.87 (1.17)	1.07 (1.24)	1.33 (1.35)	0.93 (1.19)	0.84 (1.16)	20.9 (27.9)	25.21 (30.06)	47.3 (43.45)	70.3 (57.05)	108.60
Paddy straw mulch	0.13 (0.79)	2.2 (1.62)	3.13 (1.85)	2.8 (1.80)	2.2 (1.64)	2.09 (1.61)	44.0 (41.55)	68.72 (55.95)	73.7 (59.17)	87.6 (69.47)	74.60
Saw dust mulch	0.53 (1.01)	2.53 (1.72)	3.33 (1.95)	2.4 (1.69)	2.5 (1.72)	2.26 (1.66)	29.2 (32.63)	32.53 (34.74)	60.0 (50.79)	83.6 (66.17)	79.0
Paddy husk mulch	0.27 (0.87)	1.33 (1.35)	2.07 (1.58)	1.83 (1.53)	1.53 (1.42)	1.41 (1.38)	23.4 (28.91)	28.25 (28.74)	47.8 (43.73)	75.3 (60.28)	98.48
Control	1.60 (1.42)	3.47 (1.98)	4.81 (2.29)	5.6 (2.45)	3.59 (2.01)	3.81 (2.06)	44.47 (41.74)	65.2 (53.99)	75.5 (58.07)	95.0 (79.55)	60.92





## Correlation between mean mulch Temperature and ToLCV incidence on tomato



*Temp oc*

Mulches	Temp oc *		RH% *		(% )ToLCV incidence	
	2009	2010	2009	2010	2009	2010
White polythene	37.66	33.95	30.90	36.45	80.6	89.3
Black polythene	37.95	35.21	33.96	35.49	91.0	90.0
UV-Reflective mulch	33.62	32.12	38.69	42.59	70.3	65.3
Paddy straw mulch	39.2	36.29	33.13	38.95	87.6	83.0
Saw dust mulch	36.65	38.24	41.10	44.00	83.6	90.0
Paddy husk mulch	35.62	33.81	39.416	41.14	75.3	75.7
Bare soil	41.29	39.58	36.828	40.79	95.0	100.0
Correlation coefficient (r value)	<b>**0.93</b>	<b>**0.83</b>	<b>-0.31</b>	<b>-0.30</b>		

*\* Significant at 1% level*



# Future Thrusts

- ✦ Regular Monitoring of the spread of B biotype Bemisia tabaci in other parts of Andhra Pradesh and Telangana*
- ✦ Studies on biology, host range, alternate hosts, bio-control agents of whitefly B-biotype.*
- ✦ IRM studies with different insecticides against Whitefly biotypes.*
- ✦ Studies on genetic diversity in Indian whitefly population by molecular approaches.*
- ✦ Identification of different virus strains involved in ToLCV on Tomato*
- ✦ Development of IPM package against TOLCV through vector management by integrating all suitable methods.*



Thanks

