CROP PROTECTION PROGRAMME

**Project Title:** Sustainable management and molecular characterisation of *Bemisia tabaci* and tomato leaf curl virus disease (ToLCVD) on tomato in India (phase II)

R No. 7460 (ZA0323)

**FINAL TECHNICAL REPORT**

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Executive Summary

This project developed sustainable technologies and management practices for one of the most serious pest and disease problems of tomato in India. Three tomato leaf curl virus disease (ToLCVD)-resistant tomato varieties, TLB111, TLB130, and TLB182 were bred and evaluated extensively in on-station and participatory on-farm trials in Karnataka. Yield of one of the varieties, TLB 182, was not reduced significantly when 14-day-old seedlings were inoculated rigorously with tomato leaf curl virus (ToLCV). This variety, therefore, does not require netting protection as seedlings, which reduces farmers’ costs.

Data were collected throughout the project on the performance and end-user acceptability of the project’s technologies and management recommendations. In particular, data on the horticultural acceptability of the tomato lines to farmers was used in the selection and breeding programme. This resulted in the production of tomato varieties that have characteristics desired by tomato farmers and consumers. Official release approval documentation has been completed and the varieties’ official release is imminent.

A simulation model showed that targeting more than one of the sensitive parameters of the system is likely to be necessary for a successful ToLCV-management strategy. In particular, the use of protective netting for ToLCV-susceptible seedlings, barriers for transplanted tomato crops combined with ToLCV-resistant varieties reduces *B. tabaci* immigration to the crop and virus inoculation within the crop.

Molecular data were collected on the variability of ToLCV and its whitefly vector. An important discovery was the presence of the non-indigenous, B biotype of *Bemisia tabaci* associated with a severe ToLCVD epidemic in Kolar district. This ‘aggressive’ biotype has now also been identified in Gujarat, more than 1500 km away. Two new ToLCV species were discovered that were <88% similar to previously described viruses.

Five peer-reviewed scientific publications were produced. Five presentations were given at four conferences and these generated significant interest and discussions. Three variety release booklets were prepared. These promotional activities resulted in numerous requests for ToLCV-resistant tomato seed.

The project’s outputs have the potential to improve greatly the livelihoods of the rural poor in India and are being adopted by sections of the farming community and seed companies. To facilitate greater impact, further funding is being sought for an impact maximization and promotion phase. Throughout most of India, where severe ToLCVD is present, widespread uptake of the project’s outputs would result in, (1) greater than 100% increase in yields compared to susceptible varieties and benefit to cost ratios as great as 6.6 to 1; (2) a 50-75% reduction of insecticides applied for control of whiteflies and geminiviruses; (3) improved farmer and consumer health through reduced pesticide residues; (4) increased tomato production during ToLCVD-epidemic periods, leading to reduced seasonality of tomato supply and lower prices for consumers; (5) lower production costs and higher productivity leading to higher farmer income and other stakeholders involved in the supply chain; (6) reduced risk of crop loss from ToLCV that may encourage more poor farmers to grow tomatoes; (7) increased understanding and improved awareness of whiteflies and ToLCVD amongst stakeholders and the general public.
Background

After China, India is the second largest vegetable producer in the world with 5.5 million ha of land under vegetable crops and an annual production of 74 million tonnes. At present, vegetable production in India is limited by losses caused by insect pests and diseases to the extent that the per capita consumption of vegetables is only 25 - 33% of the daily minimum requirement.

Whiteflies have recently reached worldwide prominence as important crop pests, causing direct and indirect losses through phloem feeding, the excretion of honeydew and the transmission of viruses. In 1987-88 in India, the estimated yield loss due to whitefly transmitted viruses was 1.2 million tons, representing an annual loss of approximately US$53 million. The global demand for research in this area was recognised by the task force that initiated the Inter Centres Initiative on Whiteflies. More specifically in Asia, the demand was recognised by AVRDC’s current five year plan which includes under Project 1, Solanaceous Vegetables; Activity 2, Integrated technologies to control tomato geminiviruses.

In several studies, a very strong correlation has been recorded between the incidence of ToLCV and the size of the B. tabaci population (Anzola & Lastra, 1985; Ioannou & Iordanou, 1985; Cohen et al., 1988; Saikia & Muniyappa, 1989; Verma et al., 1989a; Singh, 1990). When populations of B. tabaci are high, 90 - 100% of susceptible plants can become infected with a consequent yield loss of between 40 - 100% (Shaheen, 1983; Jeyarajan et al., 1988; Saikia & Muniyappa, 1989). Disease symptoms are particularly severe if the plants are infected at an early stage of development (Saikia & Muniyappa, 1986).

B. tabaci has a wide host range and Sastry (1984) identified 5 weed and 3 ornamentals as hosts and sources of inoculum for ToLCV. In the Kalyani area, India, B. tabaci and virus were found on 17 plant species including tomato, okra and bean, Phaseolus vulgaris (Verma et al., 1989b). In the off-season in Rajasthan, B. tabaci and TCLV survived on cucurbits and weeds (Bhardwaj & Kushwaha, 1984). Saikia & Muniyappa (1989) found that B. tabaci lives on 173 plant species around Bangalore and can transmit ToLCV to 23 of them. The incidence of ToLCV is at its highest in areas where tomato is grown continuously, year after year, and lowest where tomato has only recently been introduced (Ramappa et al., 1994).

As part of a response to this serious problem, the UASB decided to focus on the tomato leaf curl virus disease (ToLCVD) problem and screened 1306 Lycopersicon genotypes for resistance to ToLCV. This was undertaken with the long term objective of introducing ToLCV-resistance genes into edible tomatoes through conventional plant breeding techniques (Muniyappa et al., 1994). The UASB is committed to an Integrated Pest Management (IPM) approach to pest problems and has uptake channels for research through its Directorate of Extension and its laboratory-to-land programme which conducts field trials with farmer participation. It also has links with the National Centre on Integrated Pest Management (NCIPM) which has a project on the management of ToLCV in India.

From April 1996 to 31 March 1999, a CPP-funded project involving the NRI, the UASB, the Asian Vegetable Research and Development Centre (AVRDC), the International Institute of Biological control (IIBC) and the International Institute of Entomology (IIE), was initiated to work on the “Sustainable Management of Tomato Leaf Curl Virus and B. tabaci in India”. This first phase’s purpose was to develop sustainable and cost-effective management practices for two principal researchable
constraints, *Bemisia tabaci* and ToLCV, thereby improving both the quantity and quality of Indian tomato production.

In the first year of the first phase, a socio-economic survey was carried out to assess farmers’ perceptions of the problem and 100% of farmers reported ToLCV to be their most serious problem. Research activities involved field experiments into the effect of beneficial insect augmentation, mycopesticides, and ToLCV resistant varieties on the rate of spread of ToLCV into the tomato crop. Of these options, the ToLCV-resistant tomato lines showed the most promise in terms of delivering developmental impact and this was the output for which there was the greatest demand from tomato farmers. This was especially evident from the feedback obtained from farmers who attended the end of project farmer-field day/workshop. Epidemiological data were also collected and incorporated into a mathematical model that was used to assess and identify potential novel control techniques. Those that, respectively, increased and decreased the vector emigration and immigration rate had the greatest potential for reducing the spread of the disease.

The outputs of the project were:
- the production of more than ten inbred F5 ToLCV-resistant tomato lines with acceptable horticultural characteristics;
- *B. tabaci* parasitoids and predators (beneficial insects) in Karnataka identified and their potential for reducing *B. tabaci* populations and ToLCV incidence assessed;
- Indian fungal isolates assessed and compared with existing mycopesticides in field trials against *B. tabaci* and ToLCV;
- a simulation model built to determine the potential impact and conditions under which the proposed IPM practices are likely to be most successful;
- a report on farmers’ perceptions and management practices related to *B. tabaci* and ToLCV and on socio-economic factors affecting the adoption of new varieties and IPM management strategies;
- recommendations for pest management practices, developed through farmer participation, for improved tomato production through control of *B. tabaci* and ToLCV;
- IPM recommendations published as a leaflet in Kannada accessible to extension workers and farmers and peer-reviewed research articles published in international journals.

This research contributed directly to DFID’s developmental goals by developing and disseminating measures that ameliorate the effects of ToLCV and *B. tabaci* on tomato growers in South India. However, the development of the project’s ToLCV-resistant tomato genotypes with horticulturally acceptable qualities appeared particularly important as there was strong demand expressed for this technology by farmers.

Before the resistant genotypes could be released officially, however, they were required to be subjected to rigorous multilocation testing and trials in farmers’ fields, as well as being approved by the official variety release programme. These activities formed a part of the second phase of the project, which is detailed in this Final Technical Report.
References


Project Purpose

Indicative output 1.15. Improved methods for the management of insect pests of fruit and vegetables particularly whitefly and spider mites developed and promoted.

The outputs of this project will facilitate improvement in the quantity and quality of Indian tomato production through the sustainable and cost-effective management of two principal researchable constraints, the whitefly, *Bemisia tabaci* and ToLCVD.

Research Activities

1.1 Participatory, variety-selection, multi-location trials in farmers’ fields in Karnataka State were conducted using the ToLCV-resistant lines developed in the previous phase of the project. More than seven of the most promising ToLCV-resistant genotypes and susceptible checks were field tested at five geographically separate locations. The experimental layout will consist of a randomised, replicated (x 3 or x 2), block design, depending on land and water constraints. Data were collected on yield and horticultural characteristics. ToLCVD incidence and the *B. tabaci* populations were assessed and sampled, respectively, at regular intervals during the crop cycle (years 1 - 3).

1.2 In parallel with activity 1.1, the project’s ToLCV-resistant tomato genotypes were improved by the breeding programme at AVRDC in order to introduce other desirable horticultural and agronomic characteristics demanded by the farmers who attended the project’s farmer field day/workshop in Bangalore. These lines were screened at the UASB site (ongoing years 1 - 3).

2.1 At harvest, samples of symptomatic (if available) and non-symptomatic tomato genotypes were collected (two per replicate) and diagnosed for virus content using molecular PCR diagnostic techniques. Where present, up to five of the most common weed species both inside the experimental plots and in the surrounding areas were also collected and diagnosed for virus infection. Restriction enzyme digests of a subsample of PCR products of these isolates was carried out to examine the variability of the virus isolates in the different localities. This work was carried out jointly by both the UASB and NRI (years 1 - 3).

2.2 In order to identify the most important sources of immigrant viruliferous vectors, *B. tabaci* were collected from the different multilocation trial sites from symptomatic and non-symptomatic tomato genotypes and from weed-host species. These insects were then analysed and compared using RAPD-PCR. A sub-sample of 4 – 5 individuals were used for CO1 gene sequencing at NRI to determine the usefulness of this marker in identifying *B. tabaci* biotypes. Live *B. tabaci* colonies and virus isolates were maintained at NRI to facilitate this and activity 2.1 (years 1 & 2).

2.3 The viruliferous proportion of the *B. tabaci* population on ToLCV-susceptible tomato and four common weed-host species was estimated at the UASB Hebbal site, in the first three weeks after planting. The sampling protocol was as follows. All the adult *B. tabaci* were collected from 10 randomly selected plants of each host-plant type. Insects from the same host-plant type were pooled and twenty flies from each group were selected randomly and analysed molecularly as individuals by PCR for the presence of the virus. Randomisation was achieved by assigning each of the whiteflies a number and then using a random number generator to select the experimental insects. Detection of ToLCV in individual *B. tabaci* adults proved difficult and so this was achieved by single adult inoculation tests. The work was carried out jointly at both the UASB and at NRI (years 1 - 3).

3. Research in the previous phase of the project highlighted the advantages of using netting to protect ToLCV-susceptible tomato seedlings and recent evidence
from Israel has shown that up to 20% yield loss can still occur in non-symptomatic, but infected, ToLCV-resistant genotypes. PCR analysis has shown that some of our selected genotypes can become infected although they remain non-symptomatic and so an experiment was carried out to quantify any potential yield loss that occurred under these conditions. A cost-benefit analysis of the results was carried out to assist in deciding whether to recommend that our ToLCV-resistant genotypes should be grown in netting protected seedling beds before transplanting. Two ToLCV-resistant tomato genotypes and a susceptible variety underwent two treatments. Half the seedlings of both genotypes were inoculated rigorously with viruliferous B. tabaci and the other half were protected from infection. Both were planted in two replicates under netting in the field and yields assessed (year 2).

4. A new model was built to examine the epidemiological consequences of integrating partial varietal resistance with exclusion methods and other cultural control methods. Analysis of the model helped identify robust control methods, which will be effective under a range of environmental conditions (years 1-3). Additional modelling work was carried out to examine how host-mediated changes in vector fecundity enhanced the role of alternative hosts as sources of ToLCV (year 3).

5. Feedback from the farmers involved in the participatory, variety-selection, multi-location trials was collected by UASB project staff at least three times during the growth of the crop. Farmers were also invited to one of the multi-location trial sites at the UASB campus, during the peak ToLCV-epidemic season (March to June) to examine and comment on the performance of the selected genotypes with respect to ToLCV-resistance, yield and other horticultural parameters (years 1 & 2).

6. More than three research papers (including one using socio-economic data collected in the previous phase) were produced and submitted to peer-reviewed journals and results were disseminated at appropriate international conferences and workshops (years 1-3).

7.1 To facilitate the uptake of developed recommendations and technologies by resource poor farmers, the project’s results were prepared and presented to the Zonal Research and Extension Advisory Council Meeting organised by UASB, the agricultural and horticultural developmental departments and the State Horticultural Department. The UASB Directorate of Extension staff conducted trials in farmers’ fields to make their own assessment of our varieties and management recommendations (years 2 & 3).

7.2 The project’s Final Technical Report and Output to purpose Review document was prepared and compiled (year 3).

**Outputs**

Output 1. Three tomato varieties, TLB111, TLB130, and TLB182, were bred and evaluated extensively in on-station and on-farm trials in Karnataka; documents for their official release have been completed (Annex 1) and the final official release approval is expected soon. The varieties have:

- High levels of resistance to south Indian geminiviruses, which withstood the high inoculum pressure they experienced during the Kolar ToLCVD epidemic.
- Tolerance to bacterial wilt (caused by *Ralstonia solanacearum*) and resistance to tomato mosaic virus.
- Greater than 100% yield increases in comparison to susceptible varieties and benefit to cost ratios as great as 6.6 to 1.
- A 50-75% reduction of insecticides applied for control of whiteflies and geminiviruses.
- Fruit qualities acceptable to farmers and consumers.
The impact of these varieties is likely to be enormous, as open pollinated varieties account for 60% of the area of tomato production in India. These varieties may also generate significant impact if used by the private sector to produce ToLCV-resistant hybrids.

1.1 **Conduct participatory, variety-selection, multilocation trials in farmers’ fields in Karnataka State using the ToLCV-resistant lines developed in the previous phase of the project.**

The selection and development of the ToLCV-resistant tomato lines proceeded more rapidly than anticipated at the beginning of the project, due to funding becoming available for additional activities from the Competitive Research Facility (DFID project Number R7257(C)). This allowed more lines to be tested in the multi-location trials and in the State of Gujarat, as well as for participatory on-farm trials to be carried out in more than one season (2000 & 2001), which was a requirement of the State Variety Release Approval Procedure.

**Farmer participatory selection.** Forty-five to fifty farmers were invited from Bangalore and Kolar Districts to the UASB, Hebbal Farm, in the summer seasons of 1998 and 1999 to view the advanced ToLCV-resistant breeding genotypes and to provide feedback on their acceptability. In 1998, the number of promising advanced lines they assessed was 53 and in 1999, it was 21.

In all years during Krishi Mela, about five thousand farmers from different regions also had an opportunity to view the selected lines and to provide feedback on them. A leaflet containing the information on the fruit and the plant characters of the ToLCV-resistant tomato lines was prepared and distributed on these occasions. This process was a continuation of the participatory screening and selection process initiated in the previous phase of the project. After each selection process, further crosses were carried out at the AVRDC and the next generation sent to the UASB for screening and testing. At the end of this process, the three lines that the farmers selected as the best were TLB-111, TLB 130 and TLB 182. The opinions of farmers involved in these activities and in activity 5 (see below) are provided in Table 1.

**Multi-location trials.** These took place in the summer season of 1999 on research stations situated in the different agro-ecological zones of Karnataka, viz; Bangalore, Mandya, Arasikere, Shimoga and Nagenahalli. In the first season, nine lines were evaluated along with two susceptible checks and the hybrid, Avinash II. No ToLCV-symptomatic plants were recorded in any of the locations for the resistant genotypes TLB 111, TLB 119, TLB 130, TLB 147 and TLB 148. TLB lines 122, 129, 134 and 146, however, had symptomatic plants and therefore were not considered further (Tables 2 – 6). In the susceptible checks Arkavikas and Rashmi, up to 100% ToLCVD incidence was recorded at the high inoculum pressure sites of Bangalore, Arasikere and Nagenahalli. The lines that had no symptomatic plants were therefore genuinely resistant to the field viruses and not ‘escapes’. These resistant lines performed well with respect to growth and yield parameters (Tables 2 – 6).

A second multi-location trial took place in the summer season of 2000 using sites at Bangalore, Arasikere and Mandya (Tables 7 – 9). In the second year, twelve promising ToLCV-resistant lines, along with susceptible checks Arkavikas and Sun 176 were tested for ToLCVD resistance and yield. All of the ToLCV-resistant lines performed well except TLB 152 and TLB 221. The line TLB 182 was considered to be the best with respect to yield and firmness (Tables 1, 7 – 9).
Of all the lines tested in the these trials, TLB 111, TLB 130 and TLB 182 were selected for the on-farm trials in zone 4, 5, 6 and 7 of Karnataka State.

**Farmer participatory on-farm trials.** In the ToLCVD epidemic season of 2000 (summer), thirty-six farmers were given two lines TLB-111 and TLB-130 along with susceptible check Arkavikas in four agroclimatic zones viz., zone 4 (Chikamagalure), 5 (Bangalore, Kolar and Tumakur), 6 (Mysore and Mandya) and 7 (Chikamagalure and Hassan). No symptomatic plants of the ToLCV-resistant varieties were recorded by the farmers in any of their fields until the end of the crop, whereas up to 100 percent incidence was recorded in the susceptible check in the fields. A maximum yield of 64 tons/ha was obtained in TLB 111 with an average yield of 34.8 tons/ha. TLB 130 produced a maximum yield of 72 tons/ha with an average yield of 34.84 tons/ha in different agro-climatic regions of southern Karnataka (Tables 4 of variety release proposals in Annex 1 and summarised in Table 2 of the R7257(C) FTR). A late Kharif trial was also undertaken by the farmers of zone 5 and the project’s ToLCV-resistant lines performed well in this season also (Tables 10 - 12; Also tables 5 of variety release proposals in Annex 1).

In a second farm trial during the peak ToLCV-epidemic period of 2001, 25 farmers in four-agroclimatic zones viz., zone 4 (Chikamagalure, Chitradurga and Hassan), 5 (Bangalore, Kolar and Tumakur), 6 (Mysore and Mandya) and 7 (Shimoga, Chikamagalure and Hassan), were given three lines TLB-111, TLB-130 and TLB-182 and the susceptible check Arkavikas. ToLCV incidence was not noticed in any of the farmers’ fields until the end of the crop, whereas up to 100 percent incidence was recorded in the susceptible check. An average yield of 31.56, 31.36 and 37.27, tons/ha was obtained in TLB-111, TLB-130, and TLB-182, respectively (Tables 13 – 15; Tables 6 of variety release proposals in Annex 1).

1.2 *Improved ToLCV-resistant tomato genotypes.* AVRDC has developed more than 15 new whitefly-transmitted ToLCV-resistant tomato genotypes with larger and firmer fruit than the TLB varieties. These were characteristics identified by South Indian farmers that would be desirable under their conditions. Funding will be sought to introduce these lines to the private sector in India and to test them in field trials.

Output 2. Molecular data were collected on both ToLCV and *B. tabaci* variability. An important discovery was the presence of the non-indigenous, *B* biotype of *B. tabaci* associated with a severe ToLCVD epidemic in Kolar district. This ‘aggressive’ biotype has now also been identified in Gujarat, more than 1500 km away.

ToLCV variability in the different localities was examined and two new species were discovered that were <88% similar to the previously known ToLCVs.

The proportion of viruliferous *B. tabaci* arriving into a tomato crop was discovered to be remarkably high (up to 77%), which accounts for the rapid spread of ToLCVD in susceptible tomato crops.

2.1 *Variability of the ToLCV isolates in the different localities.* Virus isolates were collected from the different trial sites from symptomatic tomato genotypes and common weed species. The presence of ToLCV in these samples was confirmed by PCR. Restriction enzyme digests of a sub-sample of PCR products of these isolates was then carried out to examine the variability of the virus isolates in the different localities. Isolates that appeared different from each other were used for coat protein gene sequencing. 1300 bp of the Arsikere and Shimoga viruses were sequenced and these were found to be less than 88% similar to the known ToLVC
viruses. Viruses similar to Ban-1, Ban-2 and Ban-4 were also identified in the multilocation field-trial sites. At all sites and in the later trials in farmers’ fields, our selected tomato lines TLB 111, TLB 130 and TLB 182 did not develop ToLCVD symptoms and are resistant to the diversity of viruses present in these areas.

Virus isolates were also collected from within the severe epidemic of ToLCVD that occurred in Kolar district in May 1999. DNAs extracted from 35 symptomatic tomato-leaf samples collected within the epidemic region all gave the expected 500-600 bp amplicon with begomovirus-specific primers A/B (2). These primers amplify from the conserved nonanucleotide TAATATTAC in the common region of DNA-A to the conserved amino acid sequence CEGPCKYG within the coat protein gene. AluI and TaqI restriction patterns of all 35 PCR products were identical. One PCR product from an epidemic (GenBank no. AF321929) and a non-epidemic (AF321930) site (Bangalore) were cloned and sequenced. The two 531 bp inserts showed 96% nucleotide identity to each other and 94% nucleotide identity to the equivalent region of Tomato leaf curl Bangalore virus (ToLCBV-Ban-4) (AF165098), suggesting that the epidemic was caused by an indigenous ToLCV strain (Fig. 1).

2.2 CO1 gene sequencing at NRI to determine the usefulness of this marker in identifying B. tabaci biotypes. In order to identify the most important sources of immigrant viruliferous vectors, B. tabaci adults were collected from the different multilocation trial sites from symptomatic and non-symptomatic tomato genotypes and from weed-host species. These insects were compared molecularly using RAPD-PCR. A sub-sample was used for CO1 gene sequencing and the indigenous B. tabaci population on weeds grouped with that found on tomato (Fig. 2).

Discovery of the B-biotype of B. tabaci. In May 1999, in the Kolar district of Karnataka State, B. tabaci numbers on tomato increased by approximately 1000-fold that observed previously. Adult B. tabaci were collected from tomato plants at nine sites within the epidemic. DNA was extracted from 9-13 individuals per site and analysed by RAPD-PCR using primers OpB20 and OpB11. Eighty to 100% of individuals per site had identical patterns to those of B biotype individuals from Israel and Florida, which were different to the patterns produced by the indigenous Indian B. tabaci. Adult B. tabaci from the epidemic and non-epidemic (Bangalore) regions were cultured separately on zucchini plants (n = 20) vars. Fordhook and Ambassador. Distinct silverleaf symptoms appeared in all plants fed on by the epidemic B. tabaci, but not on those fed on by the non-epidemic whiteflies. Regular ripening of tomatoes was also a widespread problem in the epidemic area. Cytochrome oxidase I (COI) (720 bp) gene sequences were obtained for epidemic (AF321927) and non-epidemic (AF321928) B. tabaci, which had only 80% nucleotide identity to each other. A GenBank BLAST search showed that the former were most similar to B biotype whitefly from Israel (AF164667; 97%) and Texas, USA (AF164675; 99%). These data confirmed beyond doubt the presence of the B biotype in South India.

Our most recent work shows that the B biotype is now established on weeds and that the indigenous B. tabaci populations on brinjal group separately from the tomato/weed populations. A brinjal B. tabaci colony has been established at NRI for further work.

2.3 The viruliferous proportion of the B. tabaci population on ToLCV-susceptible tomato and four common weed-host species. Indian B. tabaci cultures and tomato plants infected with the Bangalore-4 isolate of ToLCV were established at NRI and
maintained throughout the project. These whiteflies and ToLCV-infected plants were used to optimize and validate the PCR technique.

Adult *B. tabaci* were collected from the field in Maderahalli, Kurubur, Talagavara, Vadagur, Chatrakodihalli, and Keelukote. A second group of *B. tabaci* were allowed virus acquisition access periods (AAPs) of 1.5h, 24h, and 72h by feeding them on ToLCV-infected tomato plants maintained in the NRI insectary. A third group was given a 24h AAP on ToLCV-infected plants in India. This group included sub-groups of B-biotype *B. tabaci*, and indigenous *B. tabaci*. Virus-free whiteflies were used as the control.

In year 3, *B. tabaci* field samples were also collected from a range of locations and crops in districts of Coimbatore (Malemchanapatty -tomato, Mamballi -tomato, -cotton, -*Ageratum conyzoides*, -unidentified weed, Makkad -brinjal, Harishepalyam-*Parthenium hysterophorus*, Saravi -*Parthenium hysterophorus*, Pollachi -*Croton bonplandianum*, Nachipalyam -*Acanthospermum hyspidum*, Kunithakodavu-*Cucurbita moschata* and Kiran-Kolar (Talagawara -*Acanthospermum hyspidum*, Cheluvanahalli -*Parthenium hysterophorus*, Vadagur -tomato, -brinjal, -*Ageratum conyzoides*, -*Croton bonplandianum*, -*Euphorbia geniculata*). *B. tabaci* were also collected directly from tomato plants at two and four weeks after transplanting and from yellow traps.

In the first year of the project, two total DNA extraction methods from single whiteflies were compared. Method A involved heating (65°C, 15 minutes) the whitefly in 50 µl of proteinase K buffer (5 mM Tris-HCl, 0.5 mM EDTA, 0.5% Nonidet P-40, and 1 mg/ml proteinase K, pH 8.0), followed by heat inactivation at 95°C for 10 minutes, and centrifugation (13000 g, 1 minute). Method B involved heating (94°C, 11 minutes) the whitefly in PCR mix before addition of Taq polymerase and cycling. Method B was found to generate similar results to Method A and hence to be more suitable for the rapid processing of single whitefly samples.

Single whiteflies were tested in 25 µl PCR reaction mixes containing 1x reaction buffer (supplied by polymerase manufacturer), 1.5 mM MgCl₂, 150 µM dNTPs, 1 µM of each of primers Deng A (5'–TAATATTACCKGWKGVCCSC–3') and Deng B (5'–TGGACYTTRCAWGGBCCTTCACA–3') and 0.5U polymerase (for all polymerases other than Hot Prime Taq polymerase (Q-BIO gene, France), the enzyme was added individually to each reaction (using a sterile tip) after the 11-minute 94°C initial incubation to minimize heat inactivation of the enzyme.

Reactions were placed in a thermocycler programmed as follows: 94°C 1 minute, 55°C 1 minute, 72°C 1 minute for one cycle, followed by 94°C 1 minute, 55-57°C 1 minute, 72°C 1 minute for 35 cycles followed by 72°C for 10 minutes, after which the reaction was kept at 4°C, before 10 µl of it was run on a 1.2-1.5% (w/v) agarose in 0.5xTBE gel. Bands were visualised by staining in 1 µg/ml ethidium bromide followed by observation under UV (305 nm), and their size estimated by comparison with a 1 kb size marker (Gibco-BRL Ltd. UK) included on each gel.

Initial experiments to validate use of the PCR technique in identifying viruliferous *B. tabaci* appeared promising, and transmission experiments confirmed that the methodology appeared to be working, as those individuals in which virus was detected also transmitted ToLCV to test tomato plants. However, during further repetitions with this technique, it was noticed that on higher resolution agarose gels that the bands being scored in the whitefly samples were, in many PCR runs, slightly smaller than the expected size band of 530 base pairs (bp). Bands were also too
faint to be scored accurately. The annealing temperature in the first amplification cycle was lowered to 52°C to try to increase the amount of PCR product but this had the undesired effect of generating a large number of non-specific bands.

Two similar sized products were present in many of the whiteflies with AAPs of 1.5, 24 and 72h, but as these were not exactly the same size they could not be scored as positive. Cloning and sequencing of the slightly smaller product at a later stage confirmed that it was a non-specific 490 bp product, and not part of any ToLCV or other geminivirus genome.

Individual parameters in the PCR technique were examined and the source of polymerase was determined to have a great effect on the bands generated. All nine tested single whiteflies were from a control batch of whiteflies known to be >90% viruliferous. Although all three whiteflies gave rise to the 530 bp diagnostic band using Red Hot polymerase, only one out of the three whiteflies was scored positive using Super Taq polymerase (HT Biotechnologies, Ltd. UK) and all three were scored negative using the other Taq polymerase (Eurobio, France).

The methodology was altered to try to remove the non-specific bands generated in single whitefly PCR reactions but clear bands of diagnostic size were only generated in the two positive DNA controls.

Further experiments to optimize the technique also failed to produce a reproducible diagnostic PCR test for detecting whether single whiteflies were viruliferous. As the DengA/B primer pair is known to amplify up all known ToLCVs from total plant DNAs, it was considered that the most probable cause of the PCR failing was that the whitefly extracts were too crude for sensitive detection with this highly degenerate primer pair. Both Deng A and DengB are highly degenerate primers, with each “primer” actually being a mix of 48 and 24 primers respectively. It is therefore not surprising that with a mixture of 72 primers in a single PCR mix that problems arose with non-specific products. It is in fact more remarkable that these primers have been used very successfully for detecting geminivirus infections in total DNAs from infected plants.

Due to the difficulties experienced with use of the above PCR technique for determining the level of virus infectivity of field B. tabaci, transmission experiments were carried out in India and these generated the data required on whitefly infectivity (see section below). As single adult inoculation work is very labour-intensive, efforts are still being made to develop a rapid PCR technique. Another set of slightly less degenerate primers has been screened but this has also led to amplification of too many non-specific products for the primers to be of use. Moreover it is known that they do not detect the large number of different geminiviruses now known to be present in tomato in India. Cloning of coat protein sequences of the latter geminiviruses is now in progress. Once sequences are obtained, new primer sets will be developed to amplify up all viruses/strains known to be present in tomato samples, aiming for the least level of degeneracy in the primers. Should it prove impossible to design suitable degenerate primer sets, or such sets not work in practice, then it will be necessary to amplify aliquots of a DNA preparation from a single whitefly with different sets of primers specific for known ToLCV viruses of interest.

2.3(i). Assessment of the proportion of immigrant adult B. tabaci collected from a tomato field that was able to transmit ToLCV by single adult inoculations. The proportion of the B. tabaci population that was viruliferous was determined using
single adult $B.\ tabaci$ inoculations to healthy ToLCV-susceptible tomato plants ($Arka\ vikas$). From the first day after transplanting, immigrant $B.\ tabaci$ were collected and used individually to inoculate seedlings. On the first day, 25% of the females collected transmitted ToLCV to tomato. The proportion of viruliferous $B.\ tabaci$ adults then increased to c. 68% by the end of the first week and 77% in female $B.\ tabaci$ 14 days after transplanting.

The infectivity of adult $B.\ tabaci$ collected from weeds in the field. $B.\ tabaci$ adults were collected from weeds in the field. The percentage of $B.\ tabaci$ that successfully transmitted ToLCV was highest for all plant species from April to May and ranged from 40-80%. For all weed species except $P.\ hysterophorus$, the highest proportion of transmission occurred in April. From October to January, $B.\ tabaci$ adults were hard to find on the weed hosts and very few were able to transmit ToLCV. These results support the hypothesis that the $B.\ tabaci$ population in the weeds is highly viruliferous, particularly in the summer months of March to May, and that movement of adult $B.\ tabaci$ from the weeds to tomato results in the rapid infection of ToLCV-susceptible tomato varieties.

Output 3. Yields of TLB 182 were not reduced significantly when 14-day-old seedlings were inoculated rigorously with ToLCV. The seedlings of this variety, therefore, do not require netting protection, which will reduce farmers’ production costs. A significant yield reduction, however, was observed for TLB 111 and so vector-proof netting should be used to protect seedlings of this variety.

3. Potential for using netting to protect ToLCV-resistant tomato seedlings. The ToLCV-resistant varieties used for this experiment were TLB111 and TLB 182 and these were compared with the ToLCV-susceptible check Arka Vikas. Half the plants were inoculated by viruliferous $B.\ tabaci$ using the ToLCV isolate from Dr Muniyappa’s laboratory (Ban-4), and the other half were protected from whiteflies by growing them from seed under vector-proof netting. Twenty-four plants from each variety were transplanted with two replications in two net houses. The data were analysed in GenStat as a Two-way ANOVA (in randomised blocks) and the effect of inoculation, variety and the interaction effect were all significant at $P=0.009$, $P<0.001$ and $P=0.015$, respectively. Mean yields per plant (kg) for the different groups and treatments are provided in the following table.

<table>
<thead>
<tr>
<th></th>
<th>TLB 111 (kg)</th>
<th>TLB 182 (kg)</th>
<th>ArkaVikas (sus. ck) (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated</td>
<td>0.291</td>
<td>0.352</td>
<td>0.079</td>
</tr>
<tr>
<td>Not inoculated</td>
<td>0.372</td>
<td>0.324</td>
<td>0.288</td>
</tr>
</tbody>
</table>

Standard errors of differences of means are 0.0331, 0.0405 and 0.0573 for the inoculation, variety and interaction effects, respectively.

TLB 182 showed no reduction in yield from having been inoculated, whereas the control showed a dramatic reduction in yield. None of the inoculated plants of either TLB line produced symptoms and viral DNA could not be detected by PCR in them. This result shows an impressive level of resistance shown particularly by TLB 182.

Output 4. The model showed that targeting more than one of the sensitive parameters is likely to be necessary for a successful ToLCVD management strategy. In particular, the use of protective netting for ToLCV-susceptible seedlings, barriers for transplanted tomato crops, combined with ToLCV-resistant varieties has the potential to reduce $B.\ tabaci$ immigration to the crop and virus inoculation within the crop.
4(i). Simulation model to examine the epidemiological consequences and durability of integrating partial varietal resistance with exclusion methods and other cultural control methods. Holt et al. (1999) used biological and epidemiological data to develop a mathematical model of the dynamics of ToLCD in south India, where irrigated tomato production takes place year round and alternative host plants of both virus and vector are abundant. The model was developed to allow targeted research on effective and sustainable disease management strategies in a year-round vegetable production system.

The assumptions made in the model are described in full by Holt et al. (1999) and were considered sufficiently appropriate to justify the use of equations similar to those of Jeger et al. (1998). All reproduction was assumed to take place on alternative host species, which were also hosts for the virus. A constant rate of immigration, \( \mu \), was assumed to take place from these alternative hosts, with a proportion \( \theta \) being infective, so arrivals of infective and non-infective vectors were given by \( \theta \mu \) and \( (1-\theta)\mu \), respectively. Of the vectors present at any one time, a proportion was assumed to die or depart per day, given by a total vector loss rate, \( g \).

These assumptions led to the following model, using the same notation as Holt et al. (1999):

\[
\begin{align*}
\frac{dH}{dt} &= \beta (L + S) - aHZ \\
\frac{dL}{dt} &= aHZ - bL - \beta L \\
\frac{dS}{dt} &= bL - \beta S \\
\frac{dX}{dt} &= -\lambda SX - gX +(1-\theta)\mu \\
\frac{dZ}{dt} &= \lambda SX - gZ + \theta \mu
\end{align*}
\]

where \( \beta = 1/\text{crop period; } a = \text{host plant infection rate per vector; } b = 1/\text{latent period; } \lambda = \text{vector acquisition rate per plant, } g = \text{vector loss rate from emigration or death; } \theta = \text{infectivity of arriving vectors (proportion); } \mu = \text{vector arrival rate per plant.} \)

The main conclusions drawn from analysis of the model were as follows:

I. Varietal resistance to ToLCV infection could be an important component of disease management, but whether or not infected tomato plants acted as a source of inoculum had little impact on disease incidence in the tomato crop. Very effective virus resistance is required otherwise supporting control measures such as protective netting will also be required to prevent substantial infection.

II. A low rate of vector immigration into a susceptible tomato crop was sufficient to cause almost total infection. In south India, vectors may migrate into tomato crops in numbers in excess of those required for disease 'saturation',
which explains the motivation for intensive use of conventional insecticides on many tomato crops in this region.

III. Disease incidence was sensitive to vector mortality only when vector numbers were low. Immigration of viruliferous vectors tended to make disease incidence insensitive to vector mortality within the tomato crop. This militates against the efficacy of insecticides for virus control.

IV. A disease management strategy, which targets more than one of the parameters to which the model proved sensitive is likely to be necessary. In particular, use of protective netting for ToLCV-susceptible varieties, combined with resistant varieties has the potential to reduce *B. tabaci* immigration to the crop and virus inoculation within the crop.

References


4(ii). The PPR (Jan. 02) reported that unexpectedly high infectivity rates were detected in whiteflies and this has important implications for disease control in tomato. It was proposed to redirect the remaining modelling work to estimate the importance of this effect, which is probably linked to observations of higher *B. tabaci* population growth on ToLCV-infected hosts or the presence of the newly introduced B biotype of *B. tabaci*.

Host-mediated changes in vector fecundity enhance the role of alternative hosts as sources of TYLCV

Field observations showed that infectivity in a random sample of the vector population (proportion infective) was higher than disease incidence (proportion infected) in the weed hosts. This seemed a surprising result because, due to their relative lifespan, the period of infectivity of the vector is much less than period of infection of the host. Further, the inoculation efficiency of the virus by vectors was also found to be very high. Knowing that vector fecundity on infected plants was higher than that on healthy plants, we consider whether the higher birth rate of vectors on infective plants combined with some restriction in vector dispersal between plants could lead to the type of infectivity and infection patterns observed. We then examined the implications of infection-enhanced vector fecundity for weed hosts as virus sources.

Model

In the last decade, a class of models has been developed for plant pathosystems to analyse both vector population dynamics and host infection. Most epidemiological models of this kind implicitly assume random mixing of the vector population. Here, we are concerned with different vector fecundities on healthy and infected hosts. Where vector parameters differ according to the host colonised, the simplifying assumption of random mixing is less defensible. To gain an understanding of the effects of differential fecundity, we partition the vectors between healthy and infected hosts. In doing so, we also recognise that vector movement between hosts is likely to be less fluid than implied by models which invoke random mixing.
The model is formulated with six variables:

\[ H \] healthy plants
\[ S \] infected plants
\[ X_H \] non-infective vectors on healthy plants
\[ X_S \] non-infective vectors on infected plants
\[ Y_H \] infective vectors on healthy plants
\[ Y_S \] infective vectors on infective plants

The terms describing virus transmission differ from those found in models where the vector population is not partitioned. Virus can only be acquired by those non-infective vectors feeding on infected hosts, \[ X_S \]. The remainder of the non-infective vector population \[ X_H \], does not have the opportunity to acquire virus because it is not in contact with an infected host. The acquisition term does not need to include the number of infected hosts as all vectors of category \[ X_S \] are in contact with infected hosts. Similarly, with virus inoculation, the subset of infective vectors \[ Y_H \] is, by definition, all in contact with healthy hosts. The virus acquisition and inoculation terms are thus \( k_1 X_S \) and \( k_2 Y_H \), respectively. The novel transmission terms give rise to the units, time\(^{-1}\) and host vector\(^{-1}\) time\(^{-1}\), for \( k_1 \) and \( k_2 \), respectively.

Vector movement between categories of hosts takes place. This occurs in two ways: by actual movement of the vectors and by the fact that when a host changes category from \( H \) to \( S \), the vectors associated with that host change category also. Physical movement is assumed to take place such that the vector population departs its current host at a rate \( m \) (time\(^{-1}\)).

The total number of non-infective and infective vectors available to colonise hosts per unit time are \( P_X = mX_H + mX_S \) and \( P_Y = mY_S + mY_H \), respectively. Assuming that these vectors land on hosts according to relative host abundance, then the additions to each vector category per unit time due to movement are: \( P_X H / (H + S) \), \( P_X S / (H + S) \), \( P_Y H / (H + S) \) and \( P_Y S / (H + S) \) for \( X_H, X_S, Y_H \), and \( Y_S \), respectively.

When a healthy plant becomes infected, the associated vectors change category from \( X_H \) to \( X_S \) and from \( Y_H \) to \( Y_S \). The proportion of hosts becoming infected per unit time is \( k_2 Y_H / H \). It is assumed that this proportion of the associated vector population also changes category, and the transfer terms are thus \( X_H k_2 Y_H / H \) and \( Y_H k_2 Y_H / H \).

Whitefly fecundity is known to be greater on diseased than on healthy host plants (Govindappa, unpublished data). No transovariole transmission occurs so that all newly hatched nymphs are non-viruliferous. Vector categories \( X_H \) and \( X_S \) therefore increase due to fecundity according to the terms \( r(X_H + Y_H) \) and \( rb(X_S + Y_S) \) where \( r \) (time\(^{-1}\)) is the fecundity on healthy hosts and \( b \) (dimensionless) the fold increase in fecundity on diseased plants. Whitefly mortality is assumed to occur at a constant rate \( c \) (time\(^{-1}\)).
Based on the above considerations, a model has the form:

\[
\begin{align*}
\frac{dH}{dt} &= -k_2 Y_H \\
\frac{dS}{dt} &= k_2 Y_H \\
\frac{dX_H}{dt} &= -X_H \frac{k_2 Y_H}{H} - mX_H + P_X \frac{H}{H + S} + r \delta H + Y_H - cX_H \\
\frac{dX_S}{dt} &= -k_1 X_S + X_H \frac{k_2 Y_H}{H} - mY_H + P_X \frac{S}{H + S} + rb \delta S + Y_S - cX_S \\
\frac{dY_H}{dt} &= -Y_H \frac{k_2 Y_H}{H} - mY_H + P_Y \frac{H}{H + S} - cY_H \\
\frac{dY_S}{dt} &= k_1 X_S + Y_H \frac{k_2 Y_H}{H} - mY_S + P_Y \frac{S}{H + S} - cY_S
\end{align*}
\]

(1)

We examined infection dynamics over a restricted period of the year, December to March, in which infection in the host population typically increases from less than 10% to nearly 100% (Govindappa, unpublished data). It is not necessary, therefore to make assumptions concerning host reproduction. Neither do we make assumptions concerning density dependent constraints to vector population increase. Both these assumptions are reasonable for the period concerned.

The model (Equations 1) could be simplified because, with no host reproduction or death, the population is constant, and the host dynamics could be described by a single equation. For clarity, however, both \( H \) and \( S \) are retained in the model.

Parameters

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
<th>Units</th>
<th>Estimated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( b )</td>
<td>fold of increase in fecundity on diseased plants</td>
<td>dimensionless</td>
<td>1.3</td>
</tr>
<tr>
<td>( c )</td>
<td>whitefly mortality</td>
<td>day(^{-1})</td>
<td>0.03</td>
</tr>
<tr>
<td>( k_1 )</td>
<td>Virus acquisition rate</td>
<td>Day(^{-1})</td>
<td>0.15</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>Virus inoculation rate</td>
<td>Host vector(^{-1}) day(^{-1})</td>
<td>0.15</td>
</tr>
<tr>
<td>( m )</td>
<td>Whitefly movement rate to other plants</td>
<td>Day(^{-1})</td>
<td>0.03</td>
</tr>
<tr>
<td>( r )</td>
<td>Whitefly fecundity</td>
<td>Day(^{-1})</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Starting with a known number of whiteflies placed on plants in the laboratory, the change in whitefly numbers were recorded on both healthy hosts and host infected with ToLVC. The instantaneous rate of increase \( r_m \) (day\(^{-1}\)) was calculated for each of the weed species studied, and from this the fold difference in \( r_m \) between vectors feeding on infected and healthy hosts.

<table>
<thead>
<tr>
<th>Host</th>
<th>Whitefly ( r_m ) on healthy host</th>
<th>Whitefly ( r_m ) on TYLCV-infected host</th>
<th>Fold of increase due to TYLCV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. hysterophorus</td>
<td>0.035</td>
<td>0.047</td>
<td>1.35</td>
</tr>
<tr>
<td>E. geniculata</td>
<td>0.038</td>
<td>0.058</td>
<td>1.51</td>
</tr>
<tr>
<td>A hispidum</td>
<td>0.041</td>
<td>0.051</td>
<td>1.22</td>
</tr>
<tr>
<td>A. conyzoides</td>
<td>0.041</td>
<td>0.052</td>
<td>1.28</td>
</tr>
<tr>
<td>Arka vikas (tomato)</td>
<td>0.028</td>
<td>0.033</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Estimates of \( r_m \) on weed species are close to 0.04 day\(^{-1}\). There is no information concerning fecundity on these weeds per se, or indeed of mortality under natural conditions. Given that the estimates of \( r_m \) are a result of both fecundity and mortality in the laboratory, fecundity was estimated, arbitrarily to be 50% higher than laboratory measurement of \( r_m \). Field mortality was also estimated, again arbitrarily, to be 50% that of fecundity. The fold of increase in \( r_m \) due to TYLCV infection was approximately 1.3 and this was used as an estimate of the difference in whitefly fecundity.

Virus transmission experiments were performed by placing whiteflies on infected hosts for a 24 hr period of acquisition, then transferring them to healthy hosts for a similar inoculation period. The number of healthy hosts that subsequently develop infection provides an indication of the combined acquisition and transmission rate. Groups of ten whiteflies gave infection rates between 80 and 100 %. Tests with individual whiteflies gave between 25 and 80 % infection. A transmission probability of 0.8 with 10 vectors is approximately equivalent to 0.15 with one vector. This was used as an estimate for both \( k_1 \) and \( k_2 \).

No information is available concerning the rate of movement of whiteflies between hosts. Very little movement of immature stages takes place but some movement of adults can be observed especially when disturbed. The rate of movement is arbitrarily estimated to be 0.03 day\(^{-1}\).

Field data for the period December to March 1999 indicate that TLYCV incidence in the weeds was about 10% at the start of this period. Whitefly numbers were too low to be measured. Reflecting these observations, a host infection rate of 10%, and a whitefly density of 1 per 50 plants was used to initiate the simulations.

**Results**

Comparisons with field-collected data

With the parameter values estimated above, the simulated patterns of host infection, vector abundance and vector infectivity were broadly similar to those observed. Host infection reached near-saturation by the end of the simulation. Whitefly numbers reached 2 to 3 per plant by the same time. Both these results are consistent with field observations. Also consistent with field observations, vector infectivity was considerably higher that host infection for the majority of the period concerned. The proportion of hosts infected was greater than the proportion of vectors infective only
at the beginning and end of the period. Thus, there was a strong qualitative agreement between the model and the observed infection and infectivity data (Fig 3).

The pattern shown in Fig. 3 was very robust to changes in parameter values. The boost to fecundity was not responsible for this pattern and in absence of such a boost \( b = 1 \), the epidemic was slower to develop, final vector numbers were fewer, but the infectivity of the vectors remained well above the infection rate of the hosts. Higher rates of movement of the vectors (e.g. \( m = 0.1 \)) increased the rate of the epidemic and increased the final vector population. Vector infectivity plateaued at a lower proportion but prior to this, ‘led’ host infection as before. A higher fecundity (e.g. \( r = 0.1 \)) again increased the speed of the epidemic and, as with a higher rate of movement, vector infectivity plateaued at a lower proportion. With an increase in both fecundity and mortality (\( r = 0.1 \) and \( c = 0.07 \)) the epidemic developed at the similar rate to the original estimates but the proportion of infective vectors plateaued at a lower value and the final vector abundance was greater. An increase in mortality alone slowed the epidemic and decreased the final vector abundance. In summary, changes to the above parameters altered the numerical results. However, they did not change the qualitative finding, that the proportion of infective vectors is higher than the proportion of infected plants for the mid epidemic period, and that the reverse is true at the start and finish.

Changes to the transmission parameters give a different result. When the acquisition and inoculation rates are sufficiently low (e.g. \( k_1 = k_2 = 0.02 \)), the proportion of infective vectors remains relatively low (about 10%), but host infection eventually reaches saturation. The epidemic is of course slowed. A qualitatively different situation exists in which host infection ‘leads’ vector infectivity throughout. This is accompanied by considerably higher vector numbers, as disease saturation is approached. The same pattern emerges when only \( k_1 \) is reduced. This would represent a situation in which virus infection is easily inoculated but not easily acquired. When the reverse is true and only \( k_2 \) is reduced, the pattern reverts to that seen with the original estimates. This would represent the situation where the virus is easily acquired but not easily transmitted. In summary, the acquisition parameter appears to determine the relative infection and infectivity of the host and vector. Where this parameter is sufficiently large, infectivity is greater than infection. At disease saturation, vector numbers are also low because numbers do not need to build up to a very high level for saturation to occur. In contrast, where the acquisition rate is low, the proportion of vectors that are infective remains low. This situation is accompanied by much higher vector numbers as the host approach disease saturation. This is because higher vector numbers are needed to achieve disease saturation within a given period.

Of some surprise is that the virus infection-induced fecundity boost and the rate of movement of the vectors do not alter the observed pattern. Intuition would suggest that the fecundity boost should increase the relative numbers of vectors on infected plants and therefore the relative numbers that are infective. A reduction in movement would be expected to keep those high numbers of vectors on the infective plants, so further increasing the proportion exposed to infection as reproduction occurs. Neither of these processes appears to have much bearing on the development of infectivity in the vector population compared to infection in the host. More work is need to understand why this is so and the implications for weeds as virus sources.

Output 5. Data were collected throughout the project on the performance and end user acceptability of the project’s technologies and management recommendations.
In particular, data on the horticultural acceptability of the tomato lines to farmers was used in the selection and breeding programme. This resulted in the production of tomato varieties that have characteristics desired by tomato farmers and consumers.

5. Feedback from the farmers involved in the participatory, variety-selection field trials. UASB project staff collected feedback from the farmers during the different stages of activity 1. There were two types of farmers – those that supplied the local fresh produce markets and those that exported to the distant cities. The local producers were satisfied with the TLB lines, as they normally grew OPs and did not require tomatoes that were extremely firm. The main problem that the ‘exporters’ mentioned was that they would have liked a slightly firmer tomato to withstand the rigors of transportation to the cities. TLB 182 in particular was acceptable to them in this respect (Table 1).

Output 6. Five peer-reviewed scientific publications have been produced. Five presentations on the project’s research were given at four conferences and these generated significant interest and discussions. Three variety release booklets were prepared (Annex 1). These promotional activities have resulted in numerous requests for seed of the ToLCV-resistant tomato varieties and represent potentially new uptake pathways for the projects outputs.

6. At least three publications produced in peer-reviewed scientific journals and project data presented at one or more international conferences. The project delivered significantly more than the originally agreed output.

Published:


The following booklets were prepared as release proposals for the three ToLCV-resistant varieties, in which they were given the popular names, Sankranthi (TLB-111), Nandi (TLB 130) and Vybhav (TLB 182) (See Annex 1).

Release proposal of tomato variety Sankranthi (TLB-111) resistant to tomato leaf curl virus. 25 pp. UAS, Bangalore 560 065, Karnataka, in collaboration with NRI, UK and AVRDC, Taiwan.
Release proposal of tomato variety Nandi (TLB 130) resistant to tomato leaf curl virus. 25 pp. UAS, Bangalore 560 065, Karnataka, in collaboration with NRI, UK and AVRDC, Taiwan.

Release proposal of tomato variety Vybhav (TLB 182) resistant to tomato leaf curl virus. 25 pp. UAS, Bangalore 560 065, Karnataka, in collaboration with NRI, UK and AVRDC, Taiwan.

In press:


In preparation:

Two additional papers are in preparation for submission to peer-reviewed journals. These contain the molecular work on B. tabaci and virus variability.

Planned:

The project has generated sufficient data for a further epidemiological/modelling paper.

Conference Abstracts.


Output 7. Project data were presented successfully at eight Zonal Research and Extension Advisory Council Meetings as well as at the UASB Technical Programme Meeting. This has resulted in the imminent release of three ToLCV-resistant tomato varieties.
7.1 Facilitation of the uptake of developed recommendations and technologies by resource poor farmers, and presentation of the project’s results to the Zonal Research and Extension Advisory Council Meeting organised by UASB, the agricultural and horticultural developmental departments and the State Horticultural Department.

The farm trial results on TLB111, TLB 130 and TLB 182 were presented in Joint Meeting of the Zonal Research Extension Advisory Council (ZREAC) and Zonal Research Extension Formulation Committee (ZREFC) of zone 4 (central dry zone: Chitradurga, Tumkur, Hassan); zone 5 (eastern dry zone: Kolar, Bangalore, Tumkur); zone 6 (southern dry zone: Mandya, Mysore) and zone 7 (southern transition zone: Shimoga, Chikmagalur) of the Karnataka State held at the following dates.

6-7 February, 2001 at ZRAS, Tiptur
13-14 February, 2001, at RRS, Shimoga
8-9 March, 2001 at RRS, VC farm Mandya
10 October, 2001 at RRS, Shimoga
8-19 October, 2001 at RRS VC farm, Mandya
6-7 February, 2002 at RRS VC farm, Mandya
12-14 February, 2002 at RRS, Shimoga
19-21 March 2002, UAS, Hebbal, Bangalore

The farm trial results on tomato leaf curl virus resistant varieties TLB 111, TLB 130 and TLB 182 were presented in the Technical Programme Meeting of the discipline of Plant Pathology of the University of Agricultural Sciences, Bangalore at Regional Research Station, Mandya, on 24-25th January 2002.

A training programme on “tomato leaf curl virus disease, resistant varieties and other management options” was organised at University of Agricultural Sciences, Bangalore for farmers and extension workers from University, State Departments of Agriculture and Horticulture. Farmers and Extension specialists from different parts of Karnataka participated. The training included a field visit to Kolar district to see the performance of the resistant varieties and the spread of tomato leaf curl virus into susceptible varieties / hybrids.

Dissemination through media and extension programmes:
Prestigious dissemination outputs

CONVOCATION INVITATION. The ToLCV-resistant variety TLB-111 was featured on the 35th Convocation (30th March 2001) invitation of the UASB, which was sent to all the registrars of the State Agricultural Universities, the Director of ICRISAT, Karnataka State Government Ministers, Directors of the Development Departments, Education and Extension Departments, research stations and NGOs.

UNIVERSITY CALENDAR. The ToLCV-resistant variety TLB-130 was featured in the UASB calendar for the month of October, 2002. This will be widely distributed throughout Karnataka State and beyond.
NEWSPAPER ARTICLES.

“PA” London Press Service article. British Government’s overseas publicity effort sent to over 100 countries around the world. Title of article: “Virus resistant tomatoes improve health and profits”.

Write-up in DFID Natural Resources News No. 14, September 1999. Crop Protection Section – Whitefly and tomato leaf curl virus on tomato (R6627).


The Sanjevani daily newspaper 2nd July 2000, published an article entitled, “Whiteflies are responsible for leaf curl disease on tomato”.

The Janavarthe daily newspaper 23rd June 2001, published an article with photos on ToLCV-resistant varieties developed by the project entitled, “Field demonstration of ToLCV-resistant TLB lines”.

The widely circulated Prajavani daily newspaper 27th June 2001, published an article with photos on ToLCV-resistant varieties developed by the project entitled, “Tomato field demonstration of resistant lines”.

The Prajavani daily newspaper, 22nd June 2002. Three new tomato varieties from GKV soon to reach market.

The Deccan Herald, 24th June 2002, page 5. UAS develops three virus-resistant varieties of tomatoes.


Workshop on Tomato Leaf Curl Disease and Whitefly. 19th June 2001, UASB Hebbal Campus.

FIELD DEMONSTRATIONS OF TOLCV-MANAGEMENT TECHNOLOGIES AND PRACTICES.

Krishi mela. On 3-5 November 1999 and on 3-5 November 2000, the ToLCV-resistant varieties were demonstrated to more than 3000 farmers who attended the USAB organized event where farmers from all over the state come to see the new technologies developed by the university.

Farmer Participatory Programme on Tomato Leaf Curl Resistant Tomato Genotypes. 16th February 2000, Directorate of Extension, UASB Hebbal Campus.

A field day was organized on 20th June 2001 to demonstrate the farm trials on ToLCV-resistant varieties to farmers from different parts of the state, officers of the State Horticulture Department and scientists from Agriculture University.

Farmers participatory meeting and field day was organized on 25th June 2001. Farmers from different parts of Shimoga district, scientists of Agriculture College, Shimoga and officers of State Departments of Agriculture and Horticulture participated to see the performance of ToLCV resistant varieties TLB 111, TLB 130 and TLB 182.

The results and posters of the ToLCV resistant varieties were displayed in field day organized jointly by the Sericulture College, Chintamani, State Agriculture, Horticulture and Sericulture Departments, held on 13th November, 2001, where scientists, extension specialists, farmers and NGOs from different seed and Pesticide companies participated.
Amendments to project

Amendment 1. Additional funding for consumables for plant-virus transmission experiments. This funding was used to purchase additional consumables for the whitefly and ToLCV detection work (activity 2).

Amendment 2. To ensure that all socio-economic inputs and activities are covered as per the original project memorandum. This was achieved and the socio-economic paper has been published (see activity 6, Nagaraju et al., 2002).

Amendment 3. Application for GBP 2,000 under the 2002/03 financial year budget for the multiplication of ToLCV-resistant varieties (10 May 2002). As part of the variety release procedure, seed of the ToLCV-resistant varieties has been multiplied and is available from the UASB.

Amendment 4. Four month no-cost extension (19/09/02) to visit the UASB to facilitate the start of the next phase. Approval of the third phase was achieved in late December 2002 and a visit to the UASB is planned for the 15-23/1/03 to facilitate the approval of their sub-contract.

Contribution of Outputs to developmental impact

How the outputs will contribute towards DFID’s developmental goals.

We bred and tested rigorously three geminivirus-resistant tomato lines, which yield at least twice that of susceptible varieties in Karnataka on-farm trials. The lines have now progressed through the official variety release procedures and will be approved in the near future. The level of ToLCV-resistance in the varieties is considerable and intensive inoculation of TLB 182 seedlings, in particular, with viruliferous B. tabaci caused no significant yield loss.

We now have a reasonable picture of the extent of virus and B. tabaci variability in Karnataka and, consequently, were able to discover a non-indigenous and aggressive B. tabaci population and explain the mechanism driving the particularly severe ToLCV epidemic that occurred in Kolar district in 1999. This whitefly has recently been identified in Gujarat, which means that the threat to Indian agriculture has increased considerably.

We determined that weeds are very important in geminivirus epidemics, both as sources of virus inoculum and hosts for whiteflies and showed that a high proportion of the B. tabaci adults are viruliferous. The modelling of the ToLCV pathosystem helped highlight the combinations of management practices that would be effective and emphasized the point that very effective ToLCV-resistance is required in tomatoes, otherwise supporting control measures such as protective netting are necessary to prevent substantial infection. The ToLCV-resistance of our varieties appears durable and probably the biggest threat to its sustainability would be the inadvertent introduction to south India of a non-indigenous virus.

The projects outputs have the potential to greatly improve the livelihoods of the rural poor in India and are already being adopted by sections of the farming community and seed companies. However, to facilitate a greater impact, further funding is being sought for proactive impact maximization and promotion that will ensure increased uptake of these technologies and management practices.
Throughout most of India, where severe ToLCVD is present, widespread uptake of the project’s outputs would result in:

- Greater than 100% increase in yields compared to susceptible varieties and benefit to cost ratios as great as 6.6 to 1.
- A 50-75% reduction of insecticides applied for control of whiteflies and geminiviruses.
- Improved farmer and consumer health through reduced pesticide residues.
- Increased tomato production during ToLCVD-epidemic periods, leading to reduced seasonality of tomato supply and lower prices for consumers.
- Lower production costs and higher productivity leading to higher farmer income and other stakeholders involved in the supply chain.
- Reduced risk of crop loss from ToLCV that may encourage more poor farmers to grow tomatoes.
- Improved understanding of disease epidemiology, which lead to the design of rational and environmentally friendly management techniques.
- Increased understanding and improved awareness of whiteflies and ToLCV amongst stakeholders and the general public.

*The identified promotion pathways to target institutions and beneficiaries.*

The project will benefit the poor directly by providing technology to manage the most important biological constraint to tomato production in India. Seed production of public varieties in Karnataka is the responsibility of the Karnataka State Seed Corporation. However, production of vegetable seed by public institutions in many developing countries has been problematic for financial, technical and marketing reasons. The Karnataka State Seed Agency may not be capable of producing and distributing sufficient seed of these varieties to meet demand, thus limiting their potential impact. We are proposing an additional phase to this project in order to ensure that the maximum impact is generated from the project’s outputs.

The promotion pathways for project outputs include:

- Karnataka seed Agency and the Karnataka State Seed Corporation for seed production and distribution of the varieties in Karnataka
- The AVRDC South Asian Vegetable Research Network and the CGIAR Tropical Whitefly IPM Project, and the AVRDC website for distribution of the technologies and information to other researchers worldwide
- Private seed companies, NGOs, and public institutions

What follow up action/research is necessary to promote the findings of the work to achieve their development benefit?

The outputs of this phase of the project have the potential for generating enormous impact throughout India as well as in other geographical regions such as Africa, the Caribbean and Bangladesh, where the project has established links. By involving private seed companies, distribution can be improved enormously and impact data recorded.

There is a strong private vegetable seed sector in India possessing the technical expertise and distribution channels to make the project varieties available throughout India. However, most private vegetable seed companies in India prefer to sell hybrids rather than inbred lines. Sale of hybrid seed generates greater profits and the company can maintain control of the hybrid parental lines and prevent other
companies from producing the hybrid. We know that several seed companies inside and outside India have or will use the project varieties as parents in development of new hybrids. It is important to collect data on this to record the economic impact of our work.

Lack of Plant Variety Protection (PVP) laws in India is a major reason reducing incentive to develop inbred vegetable varieties. Without PVP, inbred lines developed by one company or institution can be produced and sold by other private companies or farmers. However, several private companies do market inbred lines. We have initiated discussions between UASB and the Mahyco seed company about the possibility of Mahyco producing and marketing seed of the project lines in India. We have also initiated discussions with Syngenta, India, about the possibility of marketing the varieties with a seed dressing as part of an IPM package. Both these companies are very positive about collaborating in a development and promotional phase to the project.

Within Asia, the most immediate area where they would be of use to the rural poor is Bangladesh, where their uptake pathway would be through NGOs. A small amount of funding has been obtained from the European Union to do this.

The work on surveying and sampling the geminiviruses in India has shown that our previous model of the distribution of WTV requires updating. Their distributions are much more overlapping than thought previously, which has implications for breeding for ToLCV-resistance. In the future, more effort should be aimed at developing plants with resistances to ToLCVs from several different resistance sources. This will be particularly necessary in areas where the B biotype of *B. tabaci* becomes common, as this biotype in highly polyphagous and will pick up viruses from the weeds and introduce them to tomato. In other parts of the world, this has led to epidemics of so called novel viruses in tomato. The B biotype is also a pest in its own right, causing irregular ripening and other damage to vegetables. There is therefore a need now to incorporate vector resistance into the tomato lines.

Farmers were involved in the selection of resistant varieties. The farm trial results on ToLCV resistant varieties TLB111, TLB 130 and TLB 182 were presented in Joint Meeting of the Zonal Research Extension Advisory Council (ZREAC) and Zonal Research Extension Formulation Committee (ZREFC) of zones of the Karnataka State where technical Officers from the State Departments of Agriculture, and Horticulture and farmers representatives were present. During annual Krishi melas we demonstrated ToLCV resistant and susceptible varieties to farmers. Each year about 4000 farmers were able to see the varieties. Results of on-station trials, farmer participatory selection, multilocation trials and on farm trials were presented to state variety release committee and they will shortly be recommended for release.

The vice-chancellor of the UASB, Dr A.M. Krishnappa, has written a supporting letter requesting further support for a developmental, promotional and dissemination phase of the project.

For projects aimed at developing a device, material or process specify:

a. What further market studies need to be done?

In the future and in order to assess the impact of the project, baseline data needs to be collected from private companies on the distribution and sales of existing ToLCV-
susceptible open-pollinated tomato varieties. In order to assess the longer term developmental impact of the project, the farmers identified in this phase who are using the ToLCV-resistant varieties could be re-assessed two to three years after the end of the this phase in order to assess how the project’s technologies had improved their livelihoods.

b. How the outputs will be made available to intended users?

The project will benefit the poor directly by providing technology to manage the most important biological constraint to tomato production in India. Seed will be available to farmers through the Karnataka State Seed Corporation and the Karnataka State Seed Agency. We are also proposing an additional phase to this project in order to ensure that the maximum impact is generated from the project’s outputs.

The other ways the outputs will be available to the intended users are through the:

- UASB extension services.
- The media.
- Private seed companies such as Mahyco, Nagarjuna Group, Sungrow, Leadbetter. The former two companies have expressed a strong interest in obtaining the commercial rights on a non-exclusive basis for marketing these varieties. These companies specialise in the production of OP varieties and have distribution networks that cover India, with a particular focus on areas where farmers grow OPs.
- The AVRDC South Asian Vegetable Research Network and the CGIAR Tropical Whitefly IPM Project, and the AVRDC website for distribution of the technologies and information to other researchers worldwide.
- NGOs such as AME, who are involved in linkage development and livelihood systems, will be approached as potential additional uptake pathways.

c. What further stages will be needed to develop, test and establish manufacture of a product?

Sixty percent of the area under tomato production in India involves OP varieties and so, in order to maximise impact, it is desirable that one or more commercial seed companies are involved in the multiplication, distribution and marketing of the project’s tomatoes. Many private seed companies market inbred lines and we have initiated discussions between UASB and the companies listed above about the possibility of producing and marketing seed of the project lines in India. They have expressed a keen interest in using the varieties.

d. How and by whom, will the further stages be carried out and paid for?

A final phase of the project is necessary because it has been so successful in identifying several potentially extremely useful IPM measures and technologies that have the potential to impact greatly on the B. tabaci/ToLCV problem in India. A proposal has been prepared and approved by the DFID Crop Protection Programme to fund this work. The private seed companies and the NGOs that we work with will make a contribution in kind.
ANNEX 1
Table 1: Farmers participatory selection of ToLCV-resistant lines at UAS, Bangalore.

<table>
<thead>
<tr>
<th>Name and address</th>
<th>Farmers’ opinions and perceptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dinesh Kumar Peresandra, Chikkaballapura taluk</td>
<td>TLB-130 is good with respect to yield when compared to Rashmi</td>
</tr>
<tr>
<td>2. Sreenivasa Reddy Rayapalli Chintamani taluk</td>
<td>Disease resistant lines TLB-111 and TLB-130 are very good. Fruit is medium sized, taste slightly sour but accepted only for local market.</td>
</tr>
<tr>
<td>3. Narayanappa Seegenahalli, Mulabagal taluk</td>
<td>TLB-130 and TLB-111 are good and suitable for market. Colour, firmness, shape and taste is acceptable.</td>
</tr>
<tr>
<td>4. Abdul Latif Saab Rayapalli Chintamani taluk</td>
<td>Disease resistant lines TLB-111, TLB-130 are having good fruit size</td>
</tr>
<tr>
<td>5. M. Narayanappa Seegenahalli, Mulabagal taluk</td>
<td>TLB-134 is medium sized fruit suitable for market. TLB-111 and 130, are good in yield characters</td>
</tr>
<tr>
<td>6. Munivenkatareddy Petechamanahalli, Kolar taluk</td>
<td>TLB-130 is acceptable but skin is smooth.</td>
</tr>
<tr>
<td>7. Ramakrishna Harati, Kolar taluk</td>
<td>TLB-130 fruit and yield is good.</td>
</tr>
<tr>
<td>8. Narayanappa, S/o Hanumarayappa Byrapura Tippuru post, Doddaballapura taluk</td>
<td>TLB-111 and TLB-130 fruit shape and colour is good. Suitable for fresh market. TLB-182 fruit is firm and suitable for long distance market</td>
</tr>
<tr>
<td>9. M.R. Muniyappa S/o Ramappa Muduvatti Kolar taluk</td>
<td>TLB-130 and TLB-111 height and plant type is good. Fruit weight is good. A little more firmness is desirable</td>
</tr>
<tr>
<td>10. M. Narayanaswamy Bullahalli Vijayapura (Hobli) Devanahalli taluk Bangalore rural district</td>
<td>TLB-111 fruit size and shape are very good. Very much suitable for fresh market</td>
</tr>
<tr>
<td>11. V. Ramesh Vadaguru Kolar taluk</td>
<td>TLB-182 seems to be good yielding genotype with good fruit size.</td>
</tr>
<tr>
<td>12. M.R. Muniyappa S/o Ramappa Muduvatti Kolar taluk</td>
<td>TLB-182 has good fruit firmness and suitable for long distance transport</td>
</tr>
<tr>
<td>13. V. Ramesh Vadaguru Kolar taluk</td>
<td>TLB-182 has good fruit colour and firmness. Suitable for long distance transport</td>
</tr>
<tr>
<td>14. M. Narayanaswamy Bullahalli Vijayapura (Hobli) Devanahalli taluk Bangalore rural district</td>
<td>TLB-130 has good colour and fruit weight</td>
</tr>
</tbody>
</table>
12. G. Venkatesh
S/o Ganeshappa Bullahalli,
Harohalli Post
Devanahalli Taluk
Bangalore district

TLB-130 is good in shape, colour, taste, firmness and yield.
TLB-111 is very good genotype.
TLB-182 is oblong fruit with good taste, colour and firmness.

13. K. V. Jayaram
S/o S. Venkatappa Keelukote
Kolar Post
Kolar Taluk

TLB-130, TLB-111 and TLB-182 are good genotypes.
TLB-182 has good fruit firmness and suitable for long
distance transport. TLB-111 and TLB-130 are acceptable
for local market. All have good taste.

14. K. N. Subramani
Santhekallahalli
Chintamani taluk
Kolar district

TLB-130 and TLB-111 has good shape. TLB-182 has good
colour and firmness. All the genotypes can give good yield.
All the three genotypes have good resistance to ToLCV

15. Shaik Mehaboob
S/o Mastan Saab Shahenshahnagar
Kolar Post & Taluk

TLB-130 and TLB-182 has good colour. Fruit size is medium
in TLB-130.
TLB-182 can yield better than TLB-111 and TLB-130.
Taste is very good in TLB-111 and TLB-182.

16. K. Sreenivas
S/o Narayanappa Santhekallahalli
Chintamani taluk
Kolar district

TLB-182 and TLB-111 plant height is very good.
TLB-111 fruit taste is very good but fruit is not as firm as in
TLB-182.

17. C. Doddayalasappa
Mallenahalli
Jangamakote Hobli
Shidlagatta Taluk
Kolar District

TLB-130 has good shape, colour and taste. Fruit is firm
TLB-111 is a good yielding genotype
TLB-182 colour is very good

18. Ramachandra N
S/o Narayanappa Annihalli
Kolar Taluk
Kolar District

TLB-182 has good foliage. Fruit has very good colour and
taste. Plant height is also good.
TLB-111 has good height and taste
TLB-130 has good plant height and taste

19. H. P. Venkatesh
Hodalavadi
Arahalli
Kolar Taluk

Fruit is round, firm and deep red in colour in TLB-130
TLB-111 has good colour and shape
TLB-182 has good fruit firmness and colour

20. H. V. Naganna
Kote Gavi Road
Hosakote
Bangalore District

TLB-130 has plant height, fruit shape, colour. Can give good
yield
TLB-111 and TLB-182 has good fruit firmness but TLB-111
has less fruit colour than TLB-182
Taste is good in all the genotypes
21. N. Venkatesh  
S/o Narayanappa. M Mallinahalli  
Jangamakote Hobli  
Shidlagatta Taluk  
Kolar District  

All the three genotypes, TLB- 111, 130 and TLB- 182 are good in respect of plant height, fruit size, taste and yielding ability. TLB-182 is good for long distance transport.

22. V. Govindappa  
S/o Venkateshappa S.B.Halli Post  
Annehalli  
Kolar Taluk  

TLB-130 fruit size and shape is medium but colour is very good. TLB-111 fruit size and shape is good. More number of fruits per cluster. Can yield better than TLB-130 and TLB- 111 TLB-182 colour and firmness is very good.

23. P. Byregowda  
S/o Puttanna Nagadenahalli  
Doddaballapura Taluk  

TLB-182 and TLB-130 have good colour and firmness. TLB-111 has good shape and size.

24. M. Ramesh  
Doddanallurahalli Kembadiganahalli Post  
Hosakote Taluk  
B'lore Dist.  

TLB-111 has very good shape, size and taste. TLB-182 has good plant height and good foliage cover.

25. N. Radhakrishna  
S/o Narayanappa Mallenahalli village  
Jangamakote Hobli  
Shidlagatta Taluk  
Kolar district 562102  

TLB-111, TLB-182, TLB-130 have good plant height and fruit taste. TLB-182 is good for long distance transport.

26. Timmegowda  
S/o Muttarayappa Byrapura  
Tippur Post,  
Doddaballapur Taluk  

TLB-130 and TLB-111 have good shape and colour.

27. A.B. Vhisweswaraiah  
Arakeri, Malur Post  
Kolar Taluk  

TLB-111 is very good in fruit shape, size and taste.

28. Narayanaswamy  
Jettypalya  
Tavarikare Hobli  
Bangalore South  
Bangalore District  

TLB-182 has very good colour, taste and shape. It has good yield potential. Fruits are very firm.

29. M. Ramachandra  
S/o Munirayappa Ittesandra,  
Nandigudi (Post)  
Hosakote Taluk  

TLB-111 has good bearing. Fruits per cluster are more. Size and shape of fruit is also very good. TLB-182 has very firm fruit and good colour but round shape is preferred.
Table 2: Performance of tomato genotypes at Hebbal, Bangalore.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% incidence at weeks after transplanting</th>
<th>Plant height (cm) at 70DAT</th>
<th>Days to 1\textsuperscript{a} maturity\textsuperscript{c} (From sowing)</th>
<th>Days to final maturity</th>
<th>No of branches /plant \textsuperscript{a,b}</th>
<th>No. of clusters /plant \textsuperscript{a,c}</th>
<th>No of fruits /cluster</th>
<th>No of fruits /plant</th>
<th>Fruit yield /plant \textsuperscript{a} (Kg)</th>
<th>Yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLB 111</td>
<td>0 0 0 0</td>
<td>68.7</td>
<td>69</td>
<td>120</td>
<td>6.5</td>
<td>13.9</td>
<td>4.5</td>
<td>34.1</td>
<td>1.2</td>
<td>28.3</td>
</tr>
<tr>
<td>TLB 119</td>
<td>0 0 0 0</td>
<td>60.4</td>
<td>73</td>
<td>110</td>
<td>6.3</td>
<td>16.15</td>
<td>2.3</td>
<td>30.8</td>
<td>1.1</td>
<td>24.0</td>
</tr>
<tr>
<td>TLB 122</td>
<td>0 13.33 42.5 90</td>
<td>54.9</td>
<td>74</td>
<td>120</td>
<td>5.9</td>
<td>11.4</td>
<td>4.5</td>
<td>27.7</td>
<td>0.5</td>
<td>22.7</td>
</tr>
<tr>
<td>TLB-129</td>
<td>0 2.94 5.88 6.06</td>
<td>83.4</td>
<td>72</td>
<td>130</td>
<td>7.4</td>
<td>22.6</td>
<td>5</td>
<td>39.1</td>
<td>1.5</td>
<td>35.2</td>
</tr>
<tr>
<td>TLB-130</td>
<td>0 0 0 0</td>
<td>75.5</td>
<td>72</td>
<td>120</td>
<td>6.5</td>
<td>18.7</td>
<td>4.5</td>
<td>27.9</td>
<td>1.3</td>
<td>28.8</td>
</tr>
<tr>
<td>TLB-134</td>
<td>2.67 7.83 23.47 51.8</td>
<td>77.8</td>
<td>69</td>
<td>120</td>
<td>7.5</td>
<td>19.3</td>
<td>5</td>
<td>31.1</td>
<td>1.2</td>
<td>27.2</td>
</tr>
<tr>
<td>TLB-148</td>
<td>0 0 0 0</td>
<td>60.4</td>
<td>76</td>
<td>100</td>
<td>5.6</td>
<td>10.9</td>
<td>5</td>
<td>25.25</td>
<td>0.8</td>
<td>19.2</td>
</tr>
<tr>
<td>TLB-147</td>
<td>0 0 0 0</td>
<td>53.9</td>
<td>84</td>
<td>115</td>
<td>6.4</td>
<td>13.3</td>
<td>3</td>
<td>16.95</td>
<td>0.7</td>
<td>17.6</td>
</tr>
<tr>
<td>TLB-146</td>
<td>10.5 25.78 68.85 100</td>
<td>53.0</td>
<td>72</td>
<td>120</td>
<td>5.1</td>
<td>5.1</td>
<td>3.5</td>
<td>20.8</td>
<td>0.6</td>
<td>13.4</td>
</tr>
<tr>
<td>Arka Vikas</td>
<td>2.6 21.61 66.15 100</td>
<td>61.7</td>
<td>69</td>
<td>113</td>
<td>5.1</td>
<td>9.7</td>
<td>3</td>
<td>15.2</td>
<td>0.6</td>
<td>13.5</td>
</tr>
<tr>
<td>Rashmi</td>
<td>3.69 55.20 95.5 100</td>
<td>43.7</td>
<td>82</td>
<td>120</td>
<td>6.1</td>
<td>6.1</td>
<td>4</td>
<td>17.2</td>
<td>0.8</td>
<td>18.1</td>
</tr>
<tr>
<td>Avinash-2</td>
<td>0 4.10 29.41 66.6</td>
<td>61.5</td>
<td>76</td>
<td>120</td>
<td>5.3</td>
<td>14.3</td>
<td>4</td>
<td>12.9</td>
<td>1.0</td>
<td>22.7</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Record observation in 10 plants; \textsuperscript{b} Record at maturity stage
\textsuperscript{c} Record before 1st harvest; \textsuperscript{d} Final harvest taken into consideration
Date of sowing: 6.4.1999; Date of transplanting: 3.5.1999; \textsuperscript{e} Days after sowing
Two replications per entry with 40 plants per plot.
Table 3: Performance of tomato genotypes resistant to ToLCV at RRS Mandya.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% incidence at weeks after transplanting</th>
<th>Plant height (cm) at 70DAT</th>
<th>Days to 1st maturity</th>
<th>Days to final maturity</th>
<th>No of branches/plant</th>
<th>No of clusters/cluster</th>
<th>No of fruits/plant</th>
<th>Fruit yield/plant (Kg)</th>
<th>Yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLB 111</td>
<td>0</td>
<td>55.0</td>
<td>51</td>
<td>86</td>
<td>4.6</td>
<td>10.2</td>
<td>3.3</td>
<td>33.6</td>
<td>1.78</td>
</tr>
<tr>
<td>TLB 119</td>
<td>0</td>
<td>56.2</td>
<td>54</td>
<td>86.5</td>
<td>3.6</td>
<td>8.3</td>
<td>2.8</td>
<td>23.2</td>
<td>1.17</td>
</tr>
<tr>
<td>TLB 122</td>
<td>3.57</td>
<td>7.14</td>
<td>7.14</td>
<td>7.14</td>
<td>54.0</td>
<td>51.5</td>
<td>88.0</td>
<td>3.9</td>
<td>33.5</td>
</tr>
<tr>
<td>TLB 129</td>
<td>0</td>
<td>58.5</td>
<td>50.0</td>
<td>85.5</td>
<td>3.5</td>
<td>8.6</td>
<td>2.1</td>
<td>18.0</td>
<td>1.23</td>
</tr>
<tr>
<td>TLB 130</td>
<td>0</td>
<td>45.9</td>
<td>54.5</td>
<td>85.5</td>
<td>4.3</td>
<td>8.1</td>
<td>2.42</td>
<td>20.6</td>
<td>1.31</td>
</tr>
<tr>
<td>TLB 134</td>
<td>3.33</td>
<td>6.66</td>
<td>6.66</td>
<td>6.66</td>
<td>58.8</td>
<td>51.0</td>
<td>86.0</td>
<td>4.6</td>
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</tr>
<tr>
<td>TLB 148</td>
<td>0</td>
<td>53.1</td>
<td>50.5</td>
<td>87.5</td>
<td>3.5</td>
<td>9.4</td>
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<tr>
<td>TLB 147</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>TLB 146</td>
<td>5</td>
<td>10</td>
<td>17.5</td>
<td>20</td>
<td>43.1</td>
<td>52.0</td>
<td>84.0</td>
<td>3.9</td>
<td>34.36</td>
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<td>12.5</td>
<td>53.5</td>
<td>50.5</td>
<td>85.5</td>
<td>5.6</td>
<td>9.4</td>
<td>25.47</td>
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<td>2.75</td>
<td>18.16</td>
<td>1.74</td>
</tr>
</tbody>
</table>

a Record observation in 10 plants; b Record at maturity stage

This table discusses the performance of tomato genotypes resistant to ToLCV at RRS Mandya. It includes data on % incidence, plant height, days to 1st and final maturity, and other plant characteristics. The data is presented for multiple genotypes, each with specific measurements and values. The table includes notes for record observation and harvest stages, as well as dates of sowing and transplanting. Two replications per entry with 40 plants per plot.
### Table 4: Performance of tomato genotypes at ARS Arasikere

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% incidence at weeks after transplanting</th>
<th>Plant height (^{a}) (cm) at 70DAT</th>
<th>Days to 1(^{st}) maturity</th>
<th>No of branches /plant (^{a,b})</th>
<th>No. of clusters /plant (^{a,c})</th>
<th>No fruits /cluster (^{a,c})</th>
<th>No of fruits /plant (^{a})</th>
<th>Fruit yield /plant (^{a}) (Kg)</th>
<th>Yield ((t/ha))</th>
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</thead>
<tbody>
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<td>4.15</td>
<td>47.0</td>
<td>1.46</td>
<td>32.5</td>
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<td>13.8</td>
<td>4.10</td>
<td>48.8</td>
<td>1.63</td>
<td>36.2</td>
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<td>9.0</td>
<td>4.42</td>
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<td>1.29</td>
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<td>14.2</td>
<td>4.39</td>
<td>53.3</td>
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<td>41.1</td>
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<td>12.1</td>
<td>3.59</td>
<td>33.9</td>
<td>1.08</td>
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<td>68.62</td>
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<td>10.9</td>
<td>3.42</td>
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<tr>
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<td>3.56</td>
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</tbody>
</table>

\(^a\) Record observation in 10 plants; \(^b\) Record at maturity stage
\(^c\) Record before 1st harvest; \(^d\) Final harvest taken into consideration

Date of sowing: 8.4.1999; Date of transplanting (DAT) 8.5.1999.

Two replications per entry with 40 plants per plot.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>% incidence at weeks after transplanting</th>
<th>Plant height(^a) (cm) at 70DAT</th>
<th>No of branches /plant(^a,b)</th>
<th>No of clusters /plant(^a,c)</th>
<th>No fruits /cluster(^a,c)</th>
<th>Fruit yield /plant(^a) (Kg)</th>
<th>Yield (t/ha)</th>
</tr>
</thead>
<tbody>
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<td>TLB 111</td>
<td>0 0 0 0 0</td>
<td>51.3</td>
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<td>23.3</td>
<td>4.9</td>
<td>1.07</td>
<td>23.8</td>
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<tr>
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<td>9.6</td>
<td>24.8</td>
<td>3.9</td>
<td>1.09</td>
<td>24.3</td>
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<td>48.6</td>
<td>9.0</td>
<td>16.2</td>
<td>4.6</td>
<td>1.04</td>
<td>23.2</td>
</tr>
<tr>
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<td>19.0</td>
<td>4.2</td>
<td>1.09</td>
<td>24.3</td>
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<td>4.8</td>
<td>1.11</td>
<td>24.7</td>
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<td>1.03</td>
<td>22.9</td>
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<td>9.8</td>
<td>22.5</td>
<td>4.1</td>
<td>0.98</td>
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<td>10.3</td>
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<td>1.04</td>
<td>23.22</td>
</tr>
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<td>14.6</td>
<td>4.6</td>
<td>1.04</td>
<td>23.9</td>
</tr>
</tbody>
</table>

\(^a\) Record observation in 10 plants; \(^b\) Record at maturity stage
\(^c\) Record before 1st harvest; \(^d\) Final harvest taken into consideration

Date of sowing: 9.4.1999; Date of transplanting(DAT) 12.5.1999.
Two replications per entry with 40 plants per plot.
### Table 6: Performance of tomato genotypes at ARS, Nagenahalli, Mysore.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% incidence at weeks after transplanting</th>
<th>Plant height(^a) (cm) at 70DAT</th>
<th>Days to (^1)st maturity</th>
<th>No of branches /plant(^b)</th>
<th>No.of clusters /plant(^c)</th>
<th>No fruits /cluster(^a,c)</th>
<th>No of fruits /plant(^a)</th>
<th>Fruit yield /plant (^a) (Kg)</th>
<th>Yield (^\text{t/ha})</th>
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<td>4.5</td>
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</table>

\(^a\) Record observation in 10 plants; \(^b\) Record at maturity stage
\(^c\) Record before \(^1\)st harvest; \(^d\) Final harvest taken into consideration

Date of sowing: 8.4.1999; Date of transplanting (DAT) 10.5.1999.

Two replications per entry with 40 plants per plot.
Table 7: Response of tomato genotypes to ToLCV at UAS, Bangalore, under field conditions during summer 2000

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% ToLCV incidence at weeks after transplanting</th>
<th>Plant height (cm)(^a/b)</th>
<th>Branch/plant (^a/c)</th>
<th>Cluster/plant (^d)</th>
<th>Fruits/Cluster</th>
<th>Fruits/plant</th>
<th>Yield/Plant (^e) (kg)</th>
<th>Yield/plot (kg)</th>
<th>Yield/ha (ton)</th>
</tr>
</thead>
<tbody>
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<td>1.2</td>
<td>30.00</td>
<td>26.66</td>
</tr>
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<td>27.26</td>
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<td>88.26</td>
<td>1.12</td>
<td>27.43</td>
<td>25.03</td>
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<td>28.80</td>
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<td>85.26</td>
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<td>55.00</td>
<td>48.14</td>
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<td>54.50</td>
<td>0.82</td>
<td>16.61</td>
<td>18.29</td>
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<td>79.40</td>
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<td>40.00</td>
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</tr>
</tbody>
</table>

\(^a\) Recorded observation in 10 plants; \(^b\) Recorded maturity stage; \(^c\) Recorded before 1\(^st\) harvest; Date of sowing: 7-3-2000; Date of transplanting: 3-4-2000.
No. of replications = 3, 30 plants per plot; Spacing: 2.5 x 2ft
## Table 8: Response of tomato genotypes to ToLCV at ARS, Arsikere

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Incidence at weeks after transplanting</th>
<th>Plant height (cm)</th>
<th>Branch/Plant a,b</th>
<th>Cluster/Plant a,c</th>
<th>Fruits/Cluster</th>
<th>Fruits/Plant</th>
<th>Yield/Plant a</th>
<th>Yield/Plot (kg)</th>
<th>Yield/ha (ton)</th>
</tr>
</thead>
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<td>4.36</td>
<td>22.10</td>
<td>3.86</td>
<td>30.7</td>
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<td>25.40</td>
<td>5.60</td>
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Date sowing: 9-3-2000  Date of planting: 4-4-2000
No. of replications = 3, 30 plants per plot;
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<th>Genotype</th>
<th>% ToLCV incidence at weeks after transplanting</th>
<th>Plant height (cm)</th>
<th>Branch/plant</th>
<th>Cluster/plant</th>
<th>Fruit/cluster</th>
<th>Fruit/Plant</th>
<th>Yield/Plant</th>
<th>Yield/Plot</th>
<th>Yield/ha</th>
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<td>TLB-152</td>
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<td>51.50 4.40 16.30 3.950 94.50 0.495 34.94 10.99</td>
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</tr>
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</tr>
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<td>SUN-176</td>
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<td>49.8 3.90 14.00 4.050 20.50 0.350 19.50 7.77</td>
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<tr>
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<td>47.5 4.85 10.95 4.850 53.50 0.504 22.96 11.2</td>
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Date sowing: March 2000 Date of planting: April 2000
No. of replications = 3, 30 plants per plot.
Table 10: On-farm trial of TLB-111 in Zone 5 during the Karif season of 2000.

<table>
<thead>
<tr>
<th>District</th>
<th>Taluk</th>
<th>Village</th>
<th>ToLCV incidence* (%)</th>
<th>Yield (ton/ha)</th>
<th>% increase in yield over Arkavikas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>TLB-111</td>
<td>Arka Vikas</td>
<td>TLB-111</td>
</tr>
<tr>
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<td>Bangalore Devanahalli</td>
<td>Varthur</td>
<td>0</td>
<td>40.0</td>
<td>63.5</td>
</tr>
<tr>
<td>Kolar</td>
<td>Kolar</td>
<td>Mudavathi</td>
<td>0</td>
<td>100</td>
<td>12.7</td>
</tr>
<tr>
<td>Tumkur</td>
<td>Madhugiri</td>
<td>Hariharapura</td>
<td>0</td>
<td>100</td>
<td>23.0</td>
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</table>

* Incidence of disease 12 weeks after transplanting

No. of trials: 6
Table 11: Farm trial on TLB-130 in the zone 5 during kharif 2000

<table>
<thead>
<tr>
<th>District</th>
<th>Taluk</th>
<th>Village</th>
<th>% ToLCV incidence*</th>
<th>Yield (ton/ha)</th>
<th>% increase in yield over Arkavikas TLB-130</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>TLB-130 Arka Vikas</td>
<td>TLB-130 Arka Vikas</td>
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<td>Bangalore</td>
<td>Varthur</td>
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<td>63.4</td>
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<td>Bangalore</td>
<td>Varthur</td>
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<td>63.4</td>
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<td>Madhugiri</td>
<td>Hariharapura</td>
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<tr>
<td></td>
<td>Mean</td>
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* Incidence of disease 12 weeks after transplanting
Table 12: Farm trials on TLB-182 in the zone 5, during kharif 2000

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<th>District</th>
<th>Taluk</th>
<th>Village</th>
<th>% ToLCV incidence*</th>
<th>Yield (ton/ha)</th>
<th>% increase in yield over Arkavikas</th>
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<td>Mean</td>
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*Incidence of disease 12 weeks after transplanting.
Table 13. On-farm trials on TLB-111 in zones 4, 5, 6 and 7 during Summer 2001

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<th>Zone</th>
<th>District</th>
<th>Taluk</th>
<th>Village</th>
<th>Name of the farmer</th>
<th>ToLCV incidence*(%)</th>
<th>Yield in tons per hectare</th>
<th>% increase in yield</th>
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<td>TLB-111 AV</td>
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<td>Anantha Kumar</td>
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<td>27.0</td>
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<tr>
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<td>Nagarahatta</td>
<td>Govindappa</td>
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<td>Timmapura</td>
<td>Jayanna</td>
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<td>Volgeripura</td>
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<td>Patalappa</td>
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<td>Thimmarai Gowda</td>
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</table>

*ToLCV incidence 12 weeks after transplanting
Table 14. On-farm trials on TLB-130 in zones 4, 5, 6 and 7 during Summer 2001

<table>
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<tr>
<th>Zone</th>
<th>District</th>
<th>Taluk</th>
<th>Village</th>
<th>Name of the farmer</th>
<th>% ToLCV incidence*</th>
<th>Yield in tons per hectare</th>
<th>Increase in yield over check (%)</th>
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<td>Arsikere</td>
<td>Bagalaghatta</td>
<td>Anantha Kumar</td>
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<td>32.0</td>
<td>30.0</td>
</tr>
<tr>
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<td>Chirradurga</td>
<td>Holalkere</td>
<td>Nagaraghatta</td>
<td>Govindappa</td>
<td>0</td>
<td>40.0</td>
<td>31.0</td>
</tr>
<tr>
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<td>Chikmagalur</td>
<td>Kadur</td>
<td>Timmapura</td>
<td>Jayanna</td>
<td>0</td>
<td>31.0</td>
<td>32.0</td>
</tr>
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<td>Volgeripura</td>
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*ToLCV incidence 12 weeks after transplanting
Table 15. Farm trials on TLB-182 in zones 4, 5,6 and 7 during Summer 2001

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<th>Zone</th>
<th>District</th>
<th>Taluk</th>
<th>Village</th>
<th>Name of the farmer</th>
<th>% ToLCV incidence*</th>
<th>Yield in tons per hectare TLB-182</th>
<th>Arka Vikas</th>
<th>Increase in yield over check (%)</th>
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<td>Total= 25</td>
<td>Mean 0</td>
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Figure 2. Phylogenetic comparison based on cytochrome oxidase I gene sequences (720 bp) of the Kolar B-biotype B. tabaci in relation to other whiteflies.

Cassava B. tabaci, Bangalore
Cassava B. tabaci, Trivandrum
Watermelon B. tabaci, India w2
Watermelon B. tabaci, India w3
Watermelon B. tabaci, India w1
Tomato indigenous B. tabaci, Kolar
E. geniculata B. tabaci, Bangalore
Cotton B. tabaci, UAS Bangalore
B-biotype B. tabaci, Texas
Tomato B-biotype B. tabaci, Kolar
B-biotype B. tabaci, Israel
Cassava B. tabaci, Uganda
Cassava B. afer, Uganda
T. vaporariorum, Bangalore
Figure 1. Phylogenetic analysis of the Kolar epidemic ToLCV with other begomoviruses using a 531 bp sequence amplified by Deng primers.
Figure 3. The lag between vector infectivity and host infection