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# Field Screening of tomato breeding lines for improved resistance against Tomato Yellow Leaf Curl Virus (TYLCV)

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## Abstract

The tomato represents an extensively cultivated crop within tropical and subtropical regions of the world for their fresh market and processing attribute. However, its production frequently encounters substantial setbacks due to notable losses associated with diseases such as Tomato Yellow Leaf Curl Virus (TyLCV). To address this challenge, the current study aimed to assess the resistance or susceptibility of selected 25 tomato breeding lines for TyLCV under natural field screenings to mimic real-world scenarios in accordance with the disease reaction score of Banerjee and Kalloo (1987). The field screening results showed that, the check Arka Vishes line demonstrated high resistance (HR) to TLCV, with reduced PDS, PDI values and a low coefficient of infection (CI). Several lines, including CBE SL 101, CBE SL 105, CBE SL 108, CBE SL 110, and CBE SL 114, exhibited a resistant (R) reaction, while others, such as CBE SL 102, CBE SL 107, CBE SL 112, CBE SL 120, and Arka Rakshak, displayed moderate resistance (MR). Conversely, lines CBE SL 103, CBE SL 104, CBE SL 106, CBE SL 109, CBE SL 111, CBE SL 115, CBE SL 117, and CBE SL 123 showed moderate susceptibility (MS), and the remaining lines, namely CBE SL 113, CBE SL 116, CBE SL 118, CBE SL 119, CBE SL 121, and CBE SL 122, were deemed susceptible to TLCV. The varying disease responses observed across these lines provide valuable insights into the complex dynamics of host-pathogen interactions in tomato plants, informing strategies for disease management and breeding efforts aimed at enhancing resistance to TLCV.

**Key words:** Tomato leaf curl virus, disease incidence, disease severity, coefficient of infection, resistant reaction.

## Introduction

Tomato (*Solanum lycopersicum* L.) holds a prominent position as one of the extensively cultivated and consumed solanaceous vegetables, under tropical and sub-tropical regions worldwide (Howladar, 2016). The per capita consumption of fresh tomatoes and related products is recorded at 20.2 kg annually (FAOSTAT, 2022). The tomato fruit plays a crucial role in the human diet, serving as a significant source of essential minerals such as phosphorous and iron, vitamins including A, B, and C, proteins, fibre, sugars, organic acids, and phytochemicals (Alluqmani & Alabdallah., 2022). Universally recognized as protective foods, tomatoes are esteemed for their antioxidant content, notably lycopene. (Gerhardt *et al.,* 2009). For decades, there has been a consistent increase in both the area and production of tomatoes. However, the productivity per unit area remains low, primarily attributed to the prevalence of both biotic and abiotic factors. Tomatoes exhibit susceptibility to over 200 distinct pathogenic groups (Gry, 1994; Bai and Lindhout, 2007), among which a cumulative total of 136 viral species emerge as significant contributors to pronounced yield decrements in tomato cultivation (Islam *et al.,* 2022). Among these, the Tomato yellow leaf curl is a highly destructive viral disease affecting tomatoes, belong to the family Geminiviridae, of genus begomoviruses (Navot *et al.,*1991) and transmitted by the adult whitefly (*Bemisia tabaci*) in a persistent and non-propagative manner (Pan *et al.,* 2012). The virus exhibits twinned icosahedral (geminate) particles. Its genome structure can be either monopartite, consisting of a single circle of ss DNA approximately 2.7 kb in size, or bipartite, characterized by two circles of ss DNA, each around 2.7 kb. As of now, numerous isolates of viruses causing tomato yellow leaf curl or tomato leaf curl have been sequenced and characterized, totalling several hundred. The mutation rate observed for the entire TYLCV genome stands at $2.88 \times 10^{-4}$ substitutions per site per year, demonstrating comparability to that of RNA viruses (Duffy and Holmes, 2008). This extensive sequencing effort reveals a high level of genetic diversity and a widespread geographical distribution of these viruses across different parts of the world. (Kaushal *et al.,* 2020).

In India, the first report of tomato leaf curl disease dates back to 1948, documented by Vasudeva and Samraj (1948). Since its

initial identification, the disease has rapidly spread throughout nearly all tomato-growing regions in the country. This widespread prevalence has transformed the disease into a significant production constraint, with regular outbreaks impacting tomato cultivation in India. TYLCV constitutes a principal constraint on tomato cultivation during the summer in the southern region of India (Saikia and Muniyappa, 1989), and during autumn in the northern territories (Banerjee and Kalloo, 1987). Tomato yellow leaf curl disease (TYLCD) or tomato leaf curl disease (ToLCD) can be identified by distinct symptoms, including yellowing, curling, and cupping of leaves. Additionally, the disease causes severe stunting and abortion of flowers and fruits. The cumulative impact of these symptoms can lead to a significant reduction in yield losses of up to 100% on susceptible tomato plants infected with the virus (Abhary *et al.*, 2007). Given this background, the current study was designed to assess the degree of resistance or susceptibility among specifically chosen breeding lines of tomatoes. The evaluation was conducted in open field conditions, utilizing natural screening to simulate real-world scenarios.

## Materials and methods

**Experimental site:** The experiment was conducted at the university orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Were the average temperature ranged from Min 19°C to Max 34°C with the mean annual rainfall of 715 mm. Soil consist of clay loamy to sandy loamy with rich organic matter content.

**Plant materials:** A total of 25 breeding lines were collected from the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University. Among these lines, 23 (CBE SL) were identified as true breeding lines characterized by their high yield capacity. Additionally, two high yielding F1 hybrids, namely Arka Rakshak and Arka Vishesh are tripled disease resistant $F_1$ hybrids harbouring tomato yellow leaf curl disease (TyLCV) resistant genes, *Ty* -2, *Ty*-1 and *Ty*-3, obtained from ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru. The primary objective of this endeavour was to meticulously screen these 25 breeding lines, with a specific focus on evaluating their resistance to tomato yellow leaf curl disease (TyLCV) (as detailed in Table 1). The findings derived from this rigorous screening process were subsequently integrated into the ongoing tomato breeding program. This integration was conducted with a core scientific approach, ensuring that the acquired insights into resistance traits were effectively leveraged to enhance the overall efficacy and resilience of future tomato varieties.

**Nursery and layout:** Tomato seeds were sown in protrays under insect-proof nets to prevent early infection of TyLCV by whitefly and standard nursery practices were followed by without any pesticide application. After reaching 25 days of age, the tomato seedlings were transplanted into the open field by maintaining a spacing of 65 cm x 45cm between rows and plants under randomized complete block design with replicated thrice. The recommended package of practices outlined in the crop production guide from TNAU -2022 was strictly adhered to throughout the crop production period. Notably, no pesticides were applied during this time, allowing for the natural field infection of Tomato Yellow Leaf Curl Virus. To augment viral inoculation, susceptible host, PKM-1, were interspersed at regular intervals, specifically in every second row of the experimental plot. This approach was undertaken to enhance the presence and dissemination of Tomato Yellow Leaf Curl Virus for experimental efficacy.

**Screening for disease resistance:** The screening for Tomato Yellow Leaf Curl Virus (TYLCV) was conducted during the summer season, spanning from February to June in 2022, coinciding with the peak incidence of TYLCV and heightened populations of its vector (whitefly). The incidence of TYLCV disease was assessed at 15-day intervals, commencing from 30 days after transplanting by counting the TyLCV infected plants to its healthy plants formula given below and mean per cent disease incidence (PDI) was utilized to evaluate the TYLCV disease

Based on the incidence, per cent disease incidence (PDI) was calculated using the following formula:

$$\text{PDI (\%)} = \frac{\text{No. of TyLCV infected plants}}{\text{Total No. of plants observed}} \times 100$$

The Tomato Yellow Leaf Curl Virus (TYLCV) disease severity per cent was assessed by score the each and every individual plant at every 15-day intervals from 30 days after transplanting in accordance with the 0-4 disease resistant scale of Banerjee and Kalloo (1987) given in Table 1. Based on these scoring per cent disease severity (PDS) was calculated using the formula given below and mean per cent disease severity (PDS) was utilized to evaluate the TYLCV disease

Based on the disease score, per cent disease severity (PDS) was calculated using the following formula:

$$\text{PDS} = \frac{\text{Sum of numerical rating}}{\text{Total no. of plants observed x Maximum disease grade}} \times 100$$

The coefficient of the infection (CI) was calculated based on the per cent disease severity (PDS) and per cent disease incidence (PDI) The calculated CI value was compared with the standard disease reaction scale of Banerjee and Kalloo (1987) .

Table 1. A scale for categorizing the disease reaction of Tomato Leaf Curl Virus (TLCV) accordance with the reaction by Banerjee and Kalloo (1987)

| Symptom | Score | Response value | CI | Reaction |
|---|---|---|---|---|
| Symptoms absent | 0 | 0 | 0-4 | Highly resistant (HR) |
| Very mild curling up to 25% | 1 | 0.25 | 5-9 | Resistant (R) |
| Curling, puckering of 26-50% | 2 | 0.5 | 10-19 | Moderately resistant (MR) |
| Curling, puckering of 51-75% | 3 | 0.75 | 20-39 | Moderately susceptible (MS) |
| Severe curling, puckering> 75% | 4 | 1.00 | 40-69 | Susceptible (S) |
| | | | 70-100 | Highly susceptible (HS) |

CI = Coefficient of infection

## Results and discussion

The study evaluated the response of different tomato lines for Tomato Leaf Curl Virus (TLCV) disease incidence. The key parameters considered include percentage disease incidence (PDI), percentage disease severity (PDS), and the observed resistant reactions. (Table 2 and 3) These lines, demonstrated a dynamic disease response across distinct growth stages. Certain

Table 1. Mean performance of the selected tomato breeding lines for tomato yellow leaf curl virus (TyLCV) disease reaction

| Lines | PDI value | Trans-formed PDI | PDS Value | Trans-formed PDS | CI | Resistant Reaction |
|---|---|---|---|---|---|---|
| CBE SL 101 | 17.50 | 24.73 | 30.00 | 33.21 | 5.25 | R |
| CBE SL 102 | 22.50 | 28.32 | 50.00 | 45.00 | 11.25 | MR |
| CBE SL 103 | 32.50 | 34.76 | 62.50 | 52.24 | 20.31 | MS |
| CBE SL 104 | 40.00 | 39.23 | 65.00 | 53.73 | 26.00 | MS |
| CBE SL 105 | 20.00 | 26.57 | 30.00 | 33.21 | 6.00 | R |
| CBE SL 106 | 42.50 | 40.69 | 72.50 | 58.37 | 30.81 | MS |
| CBE SL 107 | 27.50 | 31.63 | 45.00 | 42.13 | 12.38 | MR |
| CBE SL 108 | 17.50 | 24.73 | 30.00 | 33.21 | 5.25 | R |
| CBE SL 109 | 45.00 | 42.13 | 72.50 | 58.37 | 32.63 | MS |
| CBE SL 110 | 20.00 | 26.57 | 35.00 | 36.27 | 7.00 | R |
| CBE SL 111 | 45.00 | 42.13 | 60.00 | 50.77 | 27.00 | MS |
| CBE SL 112 | 30.00 | 33.21 | 60.00 | 50.77 | 18.00 | MR |
| CBE SL 113 | 57.50 | 49.31 | 70.00 | 56.79 | 40.25 | S |
| CBE SL 114 | 20.00 | 26.57 | 32.50 | 34.76 | 6.50 | R |
| CBE SL 115 | 42.50 | 40.69 | 75.00 | 60.00 | 31.88 | MS |
| CBE SL 116 | 55.00 | 47.87 | 80.00 | 63.44 | 44.00 | S |
| CBE SL 117 | 52.50 | 46.43 | 65.00 | 53.73 | 34.13 | MS |
| CBE SL 118 | 60.00 | 50.77 | 67.50 | 55.24 | 40.50 | S |
| CBE SL 119 | 62.50 | 52.24 | 67.50 | 55.24 | 42.19 | S |
| CBE SL 120 | 32.50 | 34.76 | 42.50 | 40.69 | 13.81 | MR |
| CBE SL 121 | 62.50 | 52.24 | 70.00 | 56.79 | 43.75 | S |
| CBE SL 122 | 72.50 | 58.37 | 77.50 | 61.68 | 56.19 | S |
| CBE SL 123 | 55.00 | 47.87 | 60.00 | 50.77 | 33.00 | MS |
| Arka Rakshak | 22.50 | 28.32 | 45.00 | 42.13 | 10.13 | MR |
| Arka vishesh | 20.00 | 26.57 | 20.00 | 26.57 | 4.00 | HR |
| Grand Mean | 39.00 | 38.27 | 55.40 | 48.20 | 24.09 | |
| S.D. | 16.75 | 10.15 | 17.46 | 10.41 | 15.15 | |
| C.V. | 42.94 | 26.51 | 31.52 | 21.60 | 62.90 | |
| S. Ed. | 3.35 | 2.03 | 3.49 | 2.08 | 3.03 | |
| C.D. at 5% | 6.90 | 4.18 | 7.19 | 4.29 | 6.24 | |

* PDI- Per cent disease incidence, PDS- Per cent disease severity, CI- Coefficient of the infection, Arcsine- Arcsine data transformation value, HR- Highly resistant, R- Resistant, MR- Moderately resistant, MS- Moderately susceptible, S- Susceptible, S.D- Standard deviation, C.V- Coefficients of variation, S. Ed- Standard error difference, C.D- Critical difference at 5 per cent level of significance.

plants exhibited an initial phase of resistance, subsequently transitioning to susceptibility, particularly during the fruiting period. Conversely, some plants manifested susceptibility from the onset of their growth stages. This variability underscores the nuanced and stage-specific nature of the plant's interaction with the pathogen, highlighting the need for a comprehensive understanding of the temporal dynamics of its disease resistance.

The findings from the field screening for Tomato Leaf Curl Virus (TLCV) disease revealed that the check Arka Vishesh line demonstrated high resistance (HR), as evidenced by a reduced percentage of disease severity (PDS) at 20.00, along with a corresponding decrease in percentage disease incidence (PDI) at 20.00. Additionally, the coefficient of infection (CI) recorded at 4.00 further signifies a diminished susceptibility of the line to TLCV (Table 2 and 3). The outcomes align with the findings of Sadashiva *et al.* (2022), who developed and screened for TYLCV, revealing that the hybrid Arka Vishesh displayed a highly resistant response to TYLCV by governing the Ty-3 genes associated with TYLCV resistance. The lines CBE SL 101, CBE SL 105, CBE SL 108, CBE SL 110, and CBE SL 114 manifest a resistant (R) reaction to Tomato Leaf Curl Virus (TLCV) disease. CBE

Table 3. Classification of tomato breeding lines based on disease resistant reactionTable

| Lines | Reaction | Symptom |
|---|---|---|
| Arka vishesh | Highly resistant (HR) | No Symptoms |
| CBE SL 101, CBE SL 105, CBE SL 108, CBE SL 110, and CBE SL 114 | Resistant (R) | Very mild curling up to 25% |
| CBE SL 102, CBE SL 107, CBE SL 112, CBE SL 120, and Arka Rakshak | Moderately resistant (MR) | Curling, puckering of 26-50% |
| CBE SL 103, CBE SL 104, CBE SL 106, CBE SL 109, CBE SL 111, CBE SL 115, CBE SL 117, and CBE SL 123 | Moderately susceptible (MS) | Curling, puckering of 51-75% |
| CBE SL 113, CBE SL 116, CBE SL 118, CBE SL 119, CBE SL 121, and CBE SL 122, | Susceptible (S) | Severe curling, puckering> 75% |

SL 101 and CBE SL 108 exhibited a 17.50% disease incidence, while the remaining three lines, namely CBE SL 105, CBE SL 110, and CBE SL 114, displayed a 20.00% disease incidence. Correspondingly, the severity percentage for CBE SL 101, CBE SL 105, and CBE SL 108 was 30.00%, whereas CBE SL 110 and CBE SL 114 show 35.00% and 32.50% disease severity, respectively. In terms of the Coefficient of Infection (CI), CBE SL 101 and CBE SL 108 demonstrate values of 5.25, while CBE SL 105, CBE SL 110, and CBE SL 114 register values of 6.00, 7.00, and 6.50, respectively (Table 1&3).

Whereas, the lines CBE SL 102, CBE SL 107, CBE SL 112, CBE SL 120, and Arka Rakshak exhibited a moderately resistant (MR) response to Tomato Leaf Curl Virus (TLCV), as evidenced by their respective percentage disease incidences (PDI) of 22.50, 27.50, 30.00, 32.50, and 22.50. Concurrently, they displayed TyLCV severity percentages of 50.00, 45.00, 60.00, 42.50, and 45.00. Additionally, the coefficient of infection (CI) for these lines were calculated to be 11.25, 12.38, 18.00, 13.81, and 10.13 (Table 1&3). The lines, including CBE SL 103, CBE SL 104, CBE SL 106, CBE SL 109, CBE SL 111, CBE SL 115, CBE SL 117, and CBE SL 123, were observed to manifest a state of moderate susceptibility (MS) to Tomato Leaf Curl Virus (TLCV) disease, as indicated by their respective percentage disease incidences (PDI) of 32.50, 40.00, 42.50, 45.00, 45.00, 42.50, 52.50, and 55.00. Furthermore, these lines demonstrated TyLCV disease severity percentages of 62.50, 65.00, 72.50, 72.50, 60.00, 75.00, 65.00, and 60.00. The coefficient of infection (CI) for these lines was calculated as 20.31, 26.00, 30.81, 32.63, 27.00, 31.88, 34.13, and 33.00, respectively (Table 1&3.). The remaining six lines, namely CBE SL 113, CBE SL 116, CBE SL 118, CBE SL 119, CBE SL 121, and CBE SL 122, exhibited a susceptible reaction to Tomato Leaf Curl Virus (TLCV) disease, as evidenced by their respective percentage disease incidences (PDI) of 57.50, 55.00, 60.00, 62.50, 62.50, and 72.50. Additionally, the percent disease severity (PDS) for these lines was determined to be 70.00, 80.00, 67.50, 67.50, 70.00, and 77.50. The coefficient of infection (CI) for these lines were found to be 40.25, 44.00, 40.50, 42.19, 43.75, and 56.19, respectively (Table 2 and 3).

The current field screening outcomes align closely with the findings of Singh (2014) who observed that the wild accession,

H-88-78-1, displayed immunity to Tomato Leaf Curl Virus (ToLCV), while three other genotypes, namely Hissar Lalima, TLBRH-6, and NS-515, exhibited resistance. Additionally, eight genotypes, including Hissar Anmol, Kashi Vishesh, Kashi Amrit, Kashi Sharad, KS-17, KS-118, Avinash-2, and US-1008, demonstrated moderate resistance. Dheemanth *et al.* (2020). Screened 13 commercial hybrids of tomato for TyLCV resistant and the results showed that seven hybrids exhibited resistant reaction, four exhibited moderately resistant, two exhibited moderately susceptible and none of the hybrids showed susceptible and highly susceptible reaction against TyLCV. Babu *et al.* (2018), revelled that AVRDC line containing crosses showed greater resistance to TyLCV along with high yield. Mandali *et al.* (2020) findings showed that out 34 germplasms screened for *ToLCV*, a wild tomato germplasm EC-251672 exhibited highly resistant reaction to ToLC. Out of 12 varieties screened for TLCV during kharif and rabi season, the lowest incidence and severity of tomato leaf curl disease were observed in the Advanta-1211, Lima, and T-1359 varieties. During the rabi season, the disease intensity was notably lower compared to the kharif season (Solangi *et al.,* 2022)

The field screening of 25 tomato breeding lines for Tomato Leaf Curl Virus (TyLCV) resistance revealed varying levels of susceptibility. While some lines demonstrated high resistance, others showed moderate resistance or susceptibility. The highly resistant line Arka Vishesh and other resistant lines such as CBE SL 101, CBE SL 105, CBE SL 108, CBE SL 110, and CBE SL 114 underwent further screening under controlled artificial conditions and molecular validation. From these, the resistant lines may be considered for utilization as pre-breeding lines or potentially released as varieties/hybrids. Arka Vishesh is exclusively employed as a parental breeding line for resistance breeding against TYLCV diseases and cannot be released as standalone varieties/hybrids due to its status as an F1 hybrid. These findings emphasize the importance of genetic diversity in breeding for TyLCV resistance. Future breeding efforts can leverage this information to develop resilient tomato hybrids/ varieties, crucial for sustainable tomato production in the face of TyLCV challenges.

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