Phytology

Assessed in tomato hybrid germplasm has been South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), under greenhouse conditions, bacterial wilt (*Ralstonia solanacearum*) causes harm to tomato field

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Abstract

During the spring-summer seasons of 2019 and 2020 (April-September), fourteen different genotypes of hybrid tomato were evaluated under poly-house at the Vegetable Research Farm, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur to estimate genetic variability among horticultural traits and to evaluate these hybrids for protected cultivation. *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the biggest hazards to tomato agriculture areas after its pest outbreak. Sequencing and characterization of the internal transcribed spacer (ITS) region 12S primers were used to identify all pest up to species level. To explore the tomato germplasms *T. absoluta* infestation, two field trials were established on April 1st and September 21st, 2019-2020. 14 genotypes, including check verities, were assessed using similar approaches. Leaf damage, on the other hand, increased over time and peaked at 100% in 10–11 weeks after transplanting in all genotypes. Within 11 weeks following transplanting, the greatest leaflet damage recorded ranged from 64.18 percent to 100 percent, with a marketable fruit yield of 7.62–7.97 (m2/kg) in both experiments. In the current study, 14 tomato cultivars were evaluated for bacterial wilt disease resistance.

Keywords: *Tuta absoluta*, resistance, *Ralstonia solanacearum*, *Solanum lycopersicum* (L.)

1. Introduction

The fast spread of foreign invasive insect pests around the world was aided by international trade and the transportation of planting material from one country to another. Because they lack natural enemies, alien species are more dangerous in new regions, causing a variety of economic and ecological consequences such as habitat degradation, extinction of native flora and fauna, changes in ecosystem functioning, and facilitation of further invasions (Ragsdale *et al*., 2011; Asplen *et al*., 2015) [28, 3]. The *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera), also known as the tomato leafminer, tomato borer, South American tomato moth, and South American tomato pin worm, is a neotropical oligophagous pest that can cause up to 100% damage to tomato plants (Miranda *et al*., 1998; Desneux *et al*., 2011) [24, 10]. During her lifetime, a female *T. absoluta* can lay up to 260 eggs on the fragile leaves (Desneux *et al*., 2010; Kasi *et al*., 2021a) [11, 20]. Young larvae pierce leaves, stems, or fruits after hatching to feed and develop (Desneux *et al*., 2010; Urbaneja *et al*., 2013) [11, 41]. Tomatoes are the world's second most important vegetable crop, after potatoes. It is one of India's most important vegetables, with an area of 880 thousand hectares and a yield of 18.2 million tonnes (NHB 2014) [27].

T. absoluta was initially discovered on tomato in the mid-hills of Himachal Pradesh, India, which is located in the North-Western Himalayan region. Between 30 22' 40"–3312' 20"N latitudes and 75 45 55"–79 04' 20" longitudes, Himachal Pradesh is located at heights of 350–6975 m above mean sea level. It is bordered on the north by Jammu and Kashmir, the north-east by Tibet (China), the east/south-east by Uttarakhand, the south by Haryana, and the west/south-west by Punjab. Some tomato leaves were found with large, irregular, blotch-like mines during a survey visit to the tomato field planted at the experimental farm of The Department of Entomology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) India in November 2015.

The insect was identified on the bases of keys and characters described by (Roditakis *et al*., 2010; Sridhar *et al*., 2014) [30, 37] and the identity of the miner was confirmed by Dr. V. Shridhar, Principal Scientist, Indian Institute of Horticultural Research, Hessaraghatta, Karnataka, India and Dr. Biondi, University of Catania, Italy. The study indicated low to moderate level of infestation with up to 60% of plants damaged with the leafminer and each infested plant had 5–17 number of mines during November 2015. These mines were collected and examined using a binocular stereo zoom microscope (SZ2, Olympus make, Japan). After carefully separating these mines under a microscope, tiny greenish larvae feeding on the

mesophyll, which appeared to be *T. absoluta*, were discovered (Sharma and Gavkare, 2016) [34]. After that, a survey was carried out in other tomato fields in the nearby area in order to collect a larger number of mines and larvae. The larvae were then grown in the laboratory at a temperature of 25 ± 0.5°C and a relative humidity of 70 ± 5% to produce pupae and adults for identification. Wilt induced by *Ralstonia solanacearum*, a bacterial disease, is a severe limiting factor in tomato cultivation around the world (Hayward, 1991; Genin and Denny, 2012) [16, 13]. The pathogen has a wide host range and can persist in soil, crop debris, or weed hosts, making disease control difficult unless disease resistant cultivars are used (Hayward, 1991; Jyothi *et al*., 2012; Kasi *et al*., 2021b) [16, 18, 21]. To encourage the growth of wilt-susceptible but otherwise superior genotypes, disease control strategies must evolve. Crop rotation, the use of antagonistic microbes, and soil amelioration are all used to achieve this goal (Jyothi *et al*., 2012; Ramesh, and Phadke, 2012; Waiba *et al*., 2021) [18, 29, 44]. We discovered that direct delivery of inoculum to the shoot tissue interfered with the resistance characteristic of proven resistant genotypes while evaluating alternative seedling-stage inoculation methods for screening tomato (*Solanum lycopersicum*) genotypes against R. solanacearum (Thomas *et al*., 2014; Kasi *et al*., 2020) [39, 22]. Root injury and inoculation on two-week-old seedlings found to be the best method for distinguishing between known resistant and susceptible genotypes, with the majority of susceptible plants knocked down before field planting. While conducting screening experiments using the root-injury inoculation method, illness incidence appeared to be lower than expected in some of the studies. A review of the data from similar studies revealed that the age of the seedlings utilised in the screening programme may have influenced the disease incidence. The current research was carried out to examine if the age of tomato seedlings at the time of challenge in the nursery or at the point of pathogen exposure in the field had any bearing on disease incidence or susceptibility to R. solanacearum.

Due to several factors such as susceptibility to various insect/pests and illnesses, which results in poor yield, the current study on the production of local tomato varieties in a protected environment is an unprofitable endeavour. To meet these obstacles, hybrids are more suited for ensuring yield in such harsh agro-ecological conditions as those found in Himachal Pradesh. Evaluation of new combinations/hybrids under protected conditions in Himachal Pradesh's temperate highlands is the need of the hour for increasing tomato production in the state. As a result, the current research was conducted to assess the performance of new hybrid combinations, as well as public and private sector tomato hybrids, in a modified naturally ventilated poly-house during the spring and summer seasons, with the goals of estimating genetic variability among horticultural traits and evaluating tomato hybrids for marketable yield.

2. Materials and methods

2.1. Experimental conditions

The experiment was carried out in a controlled environment at the Department of Vegetable Science and Floriculture, CSKHPKV, Palampur, during the spring-summer of 2019 (April-September). The poly-house depicts the mid-hill zone of Himachal Pradesh, India, and is located at an elevation of 1290.80 m above mean sea level with 32 º6'N latitude and 76 º3' E longitude with East-West orientation. It has a humid and sub-tropical climate, with 80 percent of rainfall falling between June and September due to harsh winters, moderate summers, and high rainfall (2500 mm). This region's mean monthly (RH) throughout a year range from 46 to 48 percent. Due to high rainfall after July, it is difficult to cultivate tomatoes in open fields at Palampur.

2.2. Plant materials

Twelve different tomato genotypes, including Palam Tomato Hybrid-1 as a control, were examined in a 250 m2 naturally ventilated poly-house. The germplasms of tomato hybrids, as well as their sources, are listed in (Table 1).

Table 1: List of tomato (*Solanum lycopersicum* L.) hybrids and their sources

Sr. No.	Hybrids	Source
1	15-2 × 12-1	CSKHPKV, Palampur
2	7-2 × Palam Pride	CSKHPKV, Palampur
3	CLN2126 × Palam Pride	CSKHPKV, Palampur
4	CLN1314 × Palam Pride	CSKHPKV, Palampur
5	7-2 × 16-B	CSKHPKV, Palampur
6	15-2 × CLN1314G	CSKHPKV, Palampur
7	16-B × Palam Pride	CSKHPKV, Palampur
8	12-1 × 16-B	CSKHPKV, Palampur
9	12-1 × CLN1314G	CSKHPKV, Palampur
10	12-1 × BWR-5	CSKHPKV, Palampur
11	CLN2126 × CLN1314G	CSKHPKV, Palampur
12	Hybrid R.K-312	R. K. Seeds Pvt. Ltd.
13	Palam Tomato Hybrid-1 (check)	CSKHPKV, Palampur
14	Roma (BW susceptible check)	IAR

2.3. Evaluation of tomato cultivars for diseases resistance

In the polyhouse, all 12 tomato cultivar nurseries were cultivated individually on sterilised potting mixture in germination trays. The polyhouse's daily temperature ranged from 25 to 27 degrees Celsius. When necessary, the trays were watered.

Table 2: Disease rating scale for bacterial wilt of tomato caused by *R. solanacearum* (Winstead and Kelman 1952)

Rating	Reaction observed
0	No wilting
1	Less than 10 % wilted plants
2	11–25 % wilted plants
3	26–50 % plants wilted
4	51–75 % plants wilted
5	>75 % plants wilted

Table 3: Scale based on disease index for the categorization of tomato germplasm

Disease index	Reaction
0.00–0.2	Highly resistant
0.21–0.3	Resistant
0.31–0.4	Moderately resistant
0.41–0.5	Moderately susceptible
0.51–0.6	Susceptible
0.61–0.9	Highly susceptible
0.91–1.0	Extremely susceptible

2.4. Field experiment

The experiment was carried out in three replications inside a modified naturally ventilated poly-house (25 m × 10 m) in (RBD). In each replication, ten plants of each hybrid were spaced 70 × 30 cm apart and trained on two stems. Chemical fertilizers were applied in addition to vermicompost at a rate of 5 tonnes per hectare, as per CSKHPKV's adhoc suggestion for protected cultivation.

2.5. Pest identification

2.2.1. Extraction of genomic DNA

The sample was picked up and placed in a mortar and homogenized with 1 ml of extraction buffer and the homogenate was transferred to a 2 ml-microfuge tube. An equal volume of Phenol: Chloroform: Isoamlyalcohol (25:24:1) was added to the tubes and mixed well by gently shaking the tubes. The tubes were centrifuged at room temperature for 15 min at 14,000 rpm. The upper aqueous phase was collected in a new tube and an equal volume of Chloroform: Isoamly alcohol (24:1) was added and mixed. The upper aqueous phase obtained after centrifuging at room temperature for 10 min at 14,000 rpm was transferred to a new tube. The DNA was precipitated from the solution by adding 0.1 volume of 3 M Sodium acetate pH 7.0 and 0.7 volume of Isopropanol. After 15 min of incubation at room temperature the tubes were centrifuged at 4ºC for 15 min at 14,000 rpm. The DNA pellet was washed twice with 70% ethanol and then very briefly with 100% ethanol and air dried. The DNA was dissolved in TE (Tris-Cl 10 mM pH 8.0, EDTA 1 mM). To remove RNA 5 µl of DNAse free RNAse A (10 mg/ml) was added to the DNA.

2.2.2. PCR amplification

Internal transcribed spacer (ITS) region from total genomic DNA of individual nematode strain was amplified with specific primers (Vrain *et al*. 1992) [43]. The amplified with ITS Universal primer HC02198 (50 TAAACTTCAGGGTGACCAAAAAATCA 30) and LCO1490 (50 GGTCAACAAATCATAAAGATATTGG 30). These primers were procured through the custom primer synthesis facility of Integrated DNA Technologies, Inc. Coralville, IA, USA and dissolved as described earlier in section 3.4.1. PCR reactions were performed in 50 l reaction, each containing 25ng template DNA solution (5 l), 1 mM dNTPs mix (12.5 l), 10 µM primer (F+R) (5 l), Taq polymerase 5 units/µl (1.5 l), 15mM MgCl2 in 10X Taq reaction buffer (5 l) and Sterile Milli-Q H2O (21 l). All PCR- amplifications were accomplished in a programmable DNA thermocycler (Mastercycler Gradient- Eppendorf TM) using a PCR amplification program consisting of 95 °C for 5 min (preheating), 95 °C for 1 min, 52 °C for 1 min, 72 °C for 2 min (30 cycles), 72 °C for 10 min (final extension) and stored at 4 °C until used.

2.2.3. Sequence analysis

Sequencing the double-stranded rDNA obtained from PCR through the dideoxy ribonucleoside chain termination procedure (Sanger *et al*., 1977) [32] and PCR products were purified using a kit method at BioKart India Pvt. Ltd., Bangalore, India.

2.2.4. BLAST and phylogenetic analysis

To identify species comparison of the partially edited nucleotide sequences, 12S rDNA was performed using the BLASTn program from NCBI. ClustalW aligned nucleotide sequences were given to construct a phylogenetic tree by using the maximum likelihood method with MEGA Version 11.0 program (Tamura *et al*., 2021) [38].

2.5. Pest and Diseases management

The pheromone lures were fixed in the lure holder and changed once a month by TLM Lure [Pest Control (India) Private Limited, Bangalore]. The traps were used to keep track on what was going on. To record the total moth catches, the traps were observed at weekly intervals from seedling stage to final harvest (11 weeks). The insecticide spraying schedule (sequential application) was created using *T. absoluta* susceptibility pattern to chemical pesticides (chlorantraniliprole).

The most common route of infection is through roots that have been damaged during transplantation or damage control during cultural operations. Tomato bacterial wilt is difficult to control, and no single technique has proven to be 100 percent effective in the past.

2.6. Data collection and analysis

2.6.1 Pest Assessment

The averages per plant were calculated using data obtained from five plants in the middle of each plot. Observations began two weeks after transplantation and continued weekly for the next 11 weeks. The proportion of leaves impacted (mined) by *T. absoluta* was measured for leaf damage, while leaflet damage was measured for the percentage of leaflets affected by *T. absoluta* on three leaves in the middle canopy of each plant (Cocco *et al*., 2014) [9] (m^2/kg) total yield.

2.6.2 Disease assessment

As previously stated, data were analysed for single factor ANOVA using the Microsoft Excel-2007 data analysis software (Thomas *et al*., 2014) [39]. The reaction of different age groups was examined using the following scale: 0% to 10%, high resistance (HR); 10% to 40%, moderate resistance (MR); >40% to 70%, moderate susceptibility (MS); >70%, high susceptibility (HS) (Thomas *et al*., 2014) [39].

2.6.3 Statistical procedures

The effect of treatments on the measured parameters was determined using analysis of variance (ANOVA). Prior to ANOVA, a normality check was performed, and the appropriate transformation (log or square root) was applied to achieve a normal distribution and match the ANOVA criteria. Tukey's honestly significant difference (HSD) test at $p \leq 0.05$ was used to distinguish the means of statistically different treatments.

3. Results

3.1. Molecular identification of pest, 12 S rDNA gene sequencing

The selected two nematodes' isolates were molecular characterized by 12S rDNA Gene Sequencing. The 12S rDNA gene was amplified with an ITS universal primer for the chosen

nematode isolates having larvicidal activity. The analysis of the amplified PCR product revealed a DNA size of 500 bp for all four strains (Fig. 1). Further, the isolates were identified at the species level by using BLASTn analysis. The result indicated that these bacteria belonged to the genus *T. absoluta*. Homology search against non-redundant nucleotide database identified the isolates were.

3.2. Phylogenic analysis

The detection of homology towards closely related *Tuta absoluta* isolates from the evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) [31]. The optimal tree is shown. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) [26] and are in the units of the number of base differences per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 35 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There was a total of 605 positions in the final dataset. Evolutionary analyses were conducted in MEGA11. (Tamura *et al*., 2021) [38].

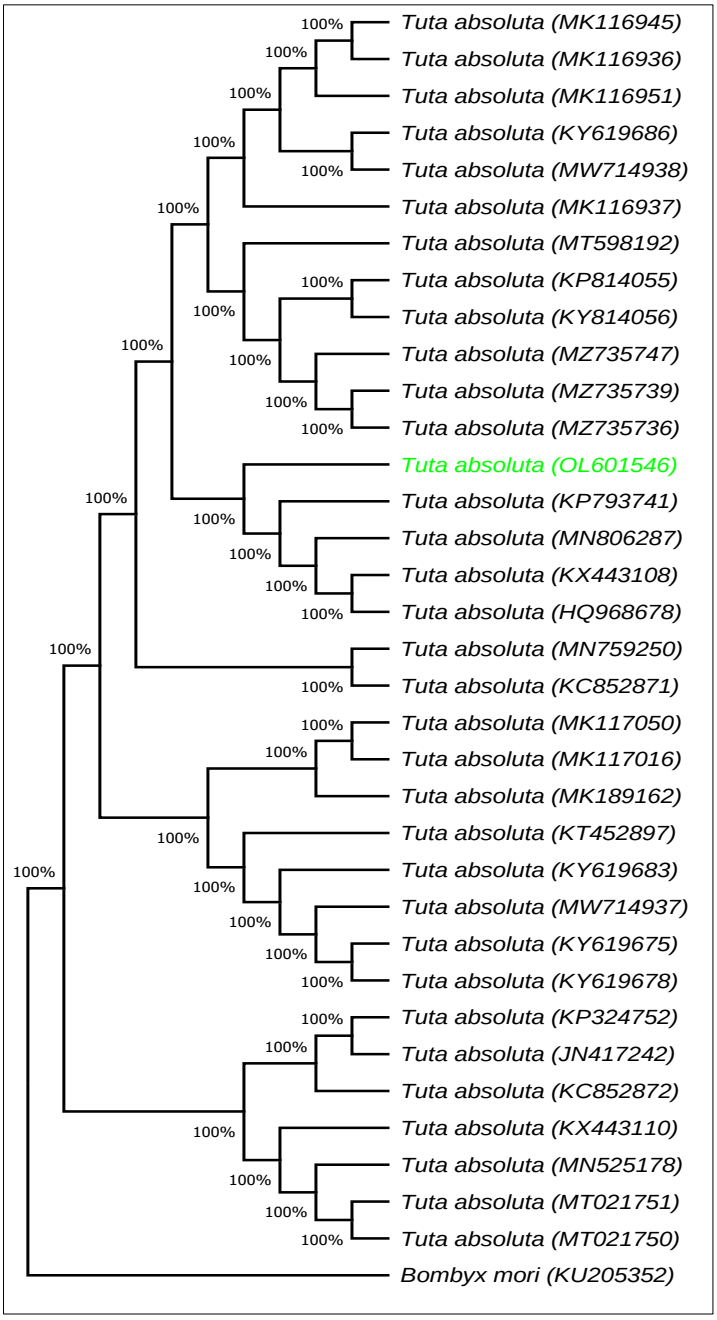

Fig 1: Phylogenetic relationships of the one *Tuta absoluta* isolate found during the survey based on analysis of ITS rDNA regions. Numbers indicated at the nodes represents bootstrap proportion values (50% or more, 500 replications). Numbers after each species and isolate indicate the GenBank Accession numbers. *T. absoluta* (OL601546).

3.3. Leaf damage

In both trials, the genotypes evaluated had a substantial (p<0.05) impact on *T. absoluta* leaf damage. For all genotypes in trials one and two, leaf damage increased throughout time and peaked at 100 percent in 9–11 weeks after transplantation (Table 4). In both trials, there was no significant difference in

leaf damage across genotypes on weeks two, nine, ten, and eleven after transplantation, however there was a significant difference on week eight in trial two. In trials one and two, documented lesser leaf damage and were not substantially different from one other throughout the other times of observation. When compared to the control verity, they all considerably (p<0.05) reduced leaf damage. Their efficacy was comparable to germplasm, although the latter caused much more leaf damage in weeks 6 and 7 of trial one, and week 4 of trial two.

3.4. Leaflet damage

Except for week two of both trials, there was a significant difference in leaflet damage percentage among treatments (p< 0.05). (Table 4). Leaflet damage that did not differ considerably from one another. During weeks seven, ten, and eleven of trial one, and all weeks of trial two, check verity leaflet damage was not substantially different from them or one of them. In hybrid germplasm, there was more leaflet damage, but the differences were not substantial for the first few weeks after transplanting.

3.5. Total and marketable yields

Higher total and marketable yields lose were obtained in all hybrid which were not significantly different from one another in trials one and two. Lower yields were recorded with check verity in both trials (Fig. 1).

3.6. Response of tomato germplasm to Ralstonia solanacearum

The reaction of tomato germplasm to *R. solanacearum* is summarized in Table 5. Assessment of cultivars on the basis of disease index (Table 5) showed that none of the cultivar was immune or highly resistant to *R. solanacearum*. Ten cultivars 15-2 × 12-1, 7-2 × Palam pride, CLN 2126 × Palam pride, 7-2 × 16-B, CLN 2126 × CLN 1314G, 15-2 × CLN 1314G, 16-B × Palam Pride, 12-1 × 16-B, 12-1 × CLN 1314 G, and Hybrid R.K-312 F1 were found resistant (R), and none of the cultivar was moderately resistant (MR) and moderately susceptible (MS) and two cultivar was CLN131 4 × Palam pride, 12-1 × BWR-5 were found susceptible (S) with DI ranging 0.51–0.6 respectively, none of the found cultivars was highly susceptible (HS) and Extremely susceptible (ES) to the bacterium (Tables 4).

Table 4: Leaf damage (%) (mean ± SD) caused by *Tuta absoluta* on hybrid tomato germplasm on experimental research trials

	2 Wat	3 Wat	4 Wat	5 Wat	6 Wat	7 Wat	8 Wat	9 Wat	10 Wat	11 Wat
Trial one										
G1	9.3±0.6 ^{ab}	13.3±1.4 ^{a}	19.3±0.6 ^{bc}	32.0±1.0 ^{a}	50.0±3.4 ^{c}	64.6±2.4 ^{a}	77.6±0.3 ^{c}	84.0±3.0 ^{ab}	89.0±3.0 ^{ad}	94.6±2.6 ^{a}
G2	10.3±1.2 ^{ab}	13.3±0.8 ^{d}	20.6±0.8 ^{d}	32.3±0.3 ^{ac}	47.3±2.6 ^{b}	70.3±2.3 ^{a}	82.6±1.7 ^{a}	85.0±1.5 ^{cd}	89.3±1.3 ^{ab}	97.0±0.5 ^{a}
G3	10.6±1.2 ^{ab}	13.3±0.8 ^{d}	21.6±1.8 ^{ac}	32.6±1.3 ^{a}	49.6±2.3 ^{b}	67.0±2.5 ^{a}	80.0±3.6 ^{ab}	84.0±2.0 ^{d}	85.0±2.8 ^{cd}	94.3±1.2 ^{a}
G4	11.0±2.0 ^{d}	13.6±0.3 ^{ac}	22.0±1.1 ^{ab}	34.6±0.8 ^{d}	52.6±3.3 ^{ab}	70.3±2.4 ^{a}	82.3±1.6 ^{d}	82.6±1.2 ^{ab}	89.0±2.0 ^{d}	95.3±1.4 ^{a}
G5	9.0±0.5 ^{ab}	14.0±1.0 ^{a}	23.0±1.5 ^{ab}	35.3±1.6 ^{b}	49.0±3.6 ^{b}	67.6±2.6 ^{b}	82.0±2.3 ^{ab}	84.3±0.8 ^{d}	88.0±0.5 ^{c}	96.0±0.5 ^{a}
G6	8.6±1.2 ^{ab}	15.0±0.5 ^{ac}	23.0±2.1 ^{a}	33.6±1.2 ^{ab}	49.3±1.7 ^{a}	72.0±1.0 ^{d}	83.3±2.0 ^{d}	86.6±0.8 ^{c}	89.6±0.3 ^{cd}	96.3±1.4 ^{a}
G7	10.3±2.1 ^{ab}	15.0±1.5 ^{a}	21.6±2.0 ^{d}	36.3±0.8 ^{d}	55.3±1.4 ^{c}	71.3±1.8 ^{ab}	81.0±0.5 ^{c}	85.6±1.6 ^{ab}	90.3±1.8 ^{c}	98.0±1.1 ^{a}
G8	8.3±1.6 ^{ab}	14.0±1.0 ^{a}	22.0±2.1 ^{a}	36.0±3.0 ^{cd}	52.6±0.6 ^{c}	73.0±1.0 ^{d}	81.6±0.8 ^{c}	85.3±0.3 ^{c}	89.3±0.3 ^{cd}	95.6±1.8 ^{a}
G9	10.0±1.5 ^{ab}	16.6±1.7 ^{a}	22.6±1.2 ^{a}	35.0±1.7 ^{a}	53.0±0.5 ^{d}	72.6±1.3 ^{d}	82.6±1.4 ^{ab}	87.3±1.3 ^{ab}	93.0±1.0 ^{a}	95.6±0.3 ^{a}
G10	9.6±0.8 ^{ab}	15.3±0.3 ^{ac}	22.6±0.3 ^{ac}	34.0±2.0 ^{d}	58.6±3.2 ^{ab}	71.6±2.3 ^{d}	83.0±1.5 ^{ab}	86.6±1.4 ^{cd}	91.3±0.8 ^{a}	97.6±0.3 ^{a}
G11	11.0±0.5 ^{ab}	19.3±1.2 ^{ab}	20.6±0.3 ^{ac}	34.0±1.1 ^{ab}	51.6±4.3 ^{bc}	69.6±2.8 ^{ab}	81.6±0.8 ^{c}	88.3±0.8 ^{c}	93.0±0.5 ^{a}	97.6±0.8 ^{a}
G12	12.0±0.5 ^{ab}	17.0±0.5 ^{ac}	23.0±1.1 ^{ab}	34.6±2.3 ^{ab}	51.6±0.3 ^{c}	74.6±0.8 ^{d}	84.3±1.2 ^{cd}	87.6±1.3 ^{ab}	92.3±1.2 ^{a}	99.0±1.0 ^{a}
G13	12.3±1.2 ^{ab}	17.3±0.8 ^{ac}	23.3±0.8 ^{d}	37.6±1.3 ^{ab}	55.0±1.1 ^{c}	75.0±1.7 ^{a}	83.6±0.8 ^{ac}	89.0±0.5 ^{c}	95.0±0.1 ^{a}	99.3±0.3 ^{a}
G14	10.6±0.6 ^{ab}	21.0±1.0 ^{ab}	24.3±0.8 ^{ac}	34.6±0.6 ^{c}	55.6±0.3 ^{c}	72.3±1.2 ^{a}	85.6±0.8 ^{d}	90.3±0.3 ^{ad}	95.3±0.6 ^{a}	99.6±0.3 ^{a}
CV	19.69	11.51	8.54	7.5	7.49	4.62	3.21	2.62	2.61	1.94
P	0.4664	<0.0001	0.2242	0.3963	0.079	0.0307	0.1504	0.0128	<0.0008	0.0224
Trial Two										
G1	11.0±0.5 ^{ab}	14.0±1.0 ^{a}	20.6±0.8 ^{c}	33.6±0.8 ^{ac}	48.6±2.0 ^{d}	57.0±4.0 ^{b}	72.3±1.4 ^{ab}	87.0±1.5 ^{cd}	94.3±1.4 ^{a}	100.0±0.0 ^{a}
G2	10.3±0.8 ^{a}	15.0±1.0 ^{a}	21.6±1.8 ^{ab}	35.3±0.8 ^{ac}	47.3±1.8 ^{ab}	71.6±3.7 ^{ad}	80.3±1.3 ^{ab}	86.6±2.0 ^{d}	94.0±1.0 ^{a}	99.3±0.6 ^{a}
G3	11.6±1.2 ^{ab}	15.6±0.3 ^{a}	24.6±1.3 ^{ab}	35.6±0.8 ^{ac}	47.6±2.9 ^{ab}	64.3±2.3 ^{a}	74.6±2.4 ^{d}	86.6±1.4 ^{cd}	96.0±0.5 ^{a}	99.3±0.6 ^{a}
G4	11.6±2.0 ^{d}	16.3±0.6 ^{a}	24.0±1.5 ^{ab}	33.3±1.3 ^{ab}	51.0±2.0 ^{d}	66.3±3.4 ^{a}	76.3±2.7 ^{ad}	86.3±0.8 ^{d}	95.3±1.2 ^{a}	100.0±0.0 ^{a}
G5	9.6±0.3 ^{ab}	16.6±0.3 ^{c}	25.6±1.7 ^{a}	33.3±1.7 ^{a}	48.0±2.0 ^{d}	64.0±3.6 ^{a}	78.0±2.0 ^{d}	88.3±0.6 ^{c}	96.0±0.5 ^{a}	99.3±0.6 ^{a}
G6	10.6±0.8 ^{ab}	16.0±0.5 ^{c}	26.3±1.7 ^{a}	35.0±2.6 ^{ad}	50.0±1.5 ^{ab}	69.3±0.8 ^{d}	78.6±0.3 ^{c}	88.6±0.8 ^{d}	96.0±1.1 ^{a}	99.6±0.3 ^{a}
G7	10.0±1.1 ^{a}	16.3±0.8 ^{c}	23.6±2.6 ^{bc}	36.3±1.2 ^{ab}	50.6±0.8 ^{ac}	64.3±2.9 ^{a}	77.0±0.5 ^{c}	87.0±2.6 ^{cd}	96.0±1.1 ^{a}	100.0±0.0 ^{a}
G8	8.3±0.6 ^{ab}	15.3±1.3 ^{ab}	24.3±2.1 ^{a}	34.6±1.4 ^{ab}	48.3±1.2 ^{ab}	70.0±1.0 ^{d}	77.3±0.8 ^{c}	88.3±0.6 ^{c}	96.3±0.6 ^{a}	99.3±0.6 ^{a}
G9	10.6±0.8 ^{ab}	17.0±0.5 ^{c}	25.3±1.8 ^{a}	35.6±1.2 ^{ab}	49.3±0.8 ^{ac}	70.3±1.2 ^{d}	76.6±2.9 ^{ab}	88.0±2.6 ^{cd}	96.0±0.0 ^{a}	99.6±0.3 ^{a}
G10	12.0±1.0 ^{ab}	17.6±0.3 ^{c}	26.6±0.8 ^{ac}	35.0±2.0 ^{d}	49.6±0.6 ^{ac}	68.6±2.0 ^{ab}	78.6±1.4 ^{d}	89.3±2.3 ^{bc}	98.0±0.0 ^{a}	99.3±0.3 ^{a}
G11	13.0±0.5 ^{a}	18.3±0.6 ^{ac}	25.0±1.5 ^{ac}	34.6±1.8 ^{ab}	50.0±1.7 ^{a}	64.6±2.9 ^{a}	75.3±2.3 ^{ab}	88.0±1.0 ^{c}	96.6±0.3 ^{a}	100.0±0.0 ^{a}
G12	14.3±0.3 ^{a}	19.0±0.5 ^{c}	26.6±1.2 ^{ab}	35.6±2.0 ^{d}	50.3±0.8 ^{ac}	71.0±1.5 ^{a}	80.0±1.0 ^{d}	88.3±0.8 ^{c}	97.0±1.0 ^{a}	99.3±0.6 ^{a}
G13	13.3±0.8 ^{ab}	19.3±0.8 ^{c}	27.3±0.8 ^{c}	39.0±1.1 ^{ab}	52.0±0.5 ^{c}	71.3±1.8 ^{a}	81.3±1.2 ^{ab}	91.3±0.6 ^{a}	98.6±0.3 ^{a}	100.0±0.0 ^{a}
G14	12.6±0.8 ^{ab}	20.6±1.8 ^{ab}	28.3±0.8 ^{c}	38.0±0.5 ^{ac}	53.0±0.5 ^{c}	70.3±1.3 ^{ab}	79.0±0.5 ^{c}	92.0±1.5 ^{a}	97.6±0.8 ^{a}	100.0±0.0 ^{a}
CV	14.85	9.01	9.58	7.05	4.01	6.74	3.66	3.16	1.56	0.76
P	0.0143	<0.0006	0.0334	0.3014	0.0605	0.0228	0.045	0.4088	0.0431	0.8821

G1: 15-2 × 12-1, G2: 7-2 × Palam Pride, G3: CLN2126 × Palam Pride, G4: CLN1314 × Palam Pride, G5: 7-2 × 16-B, G6: 15-2 × CLN1314G, G7: 16-B × Palam Pride, G8: 12-1 × 16-B, G9: 12-1 × CLN1314G, G10: 12-1 × BWR-5, G11: CLN2126 × CLN1314G, G12: Hybrid R.K-312, G13: Palam Tomato Hybrid-1 (check), G14: Roma (BW susceptible check). Means followed by the same letter (s) are not significantly different according to Tukey's test ($p \leq 0.05$).

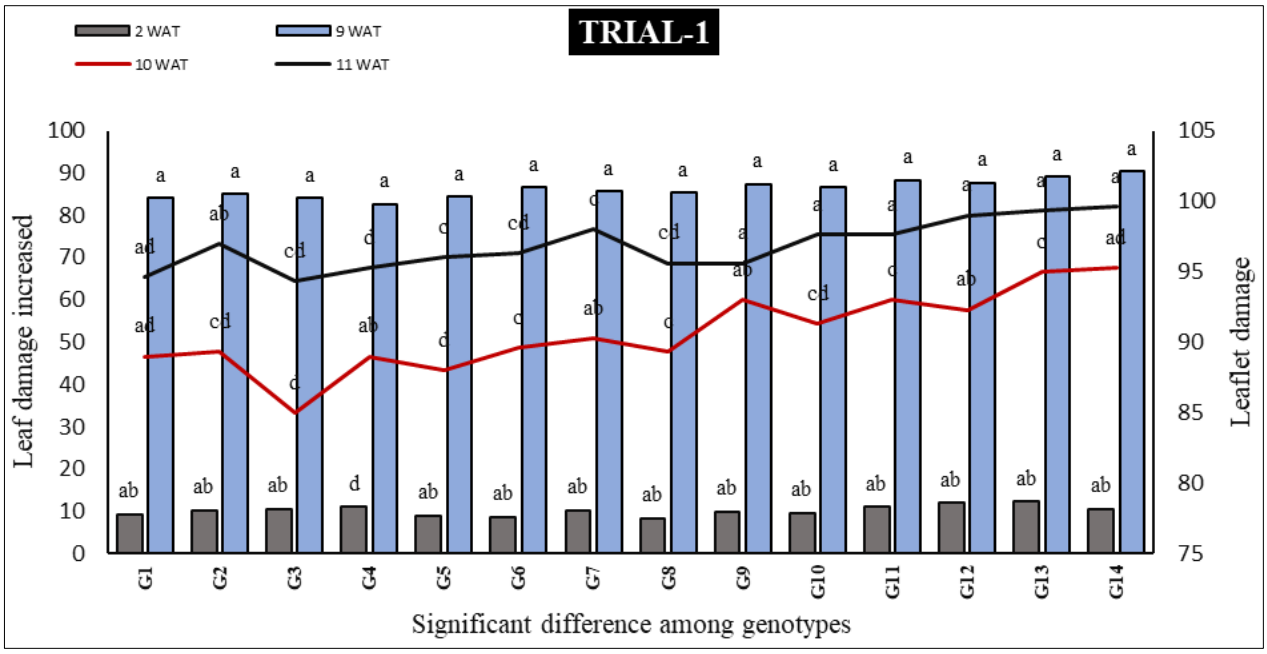

Fig 2

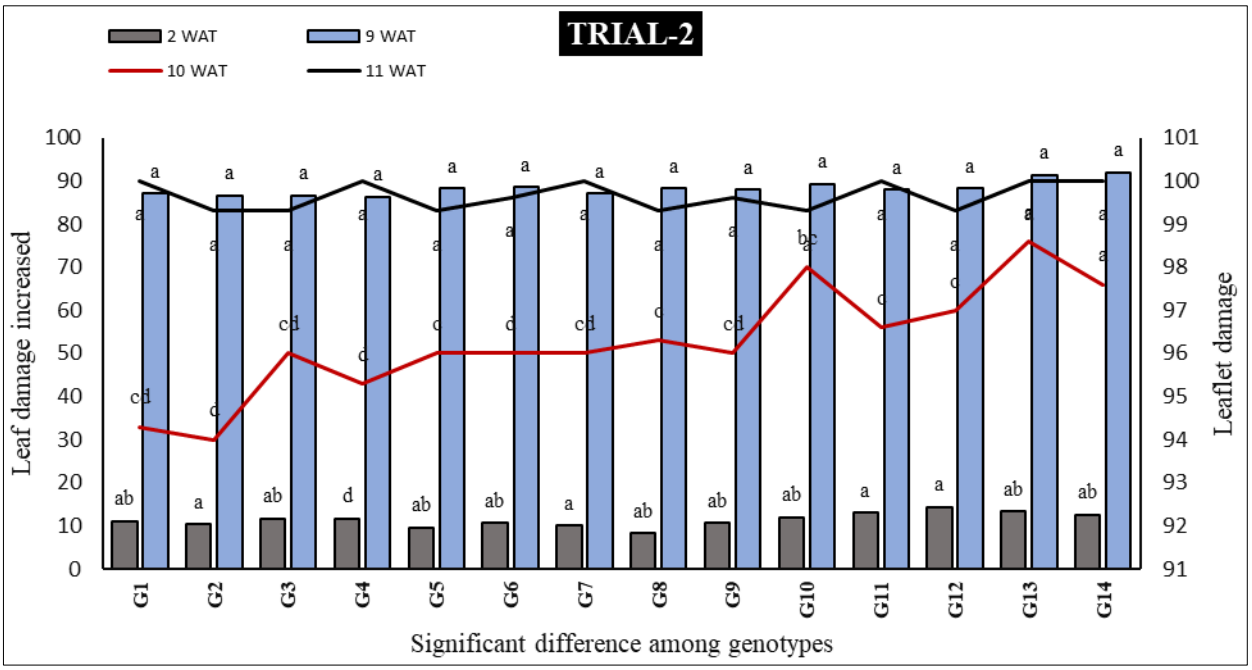

Fig 3

Table 5: Categorization of genotypes for bacterial wilt incidence (according to anonymous 2000)

DI	Genotypes	Reaction
0–0.2	-	HR
0.21–0.3	15-2 × 12-1, 7-2 × Palam pride, CLN 2126 × Palam pride, 7-2 × 16-B, CLN 2126 × CLN 1314G, 15-2 × CLN 1314G, 16-B × Palam Pride, 12-1 × 16-B, 12-1 × CLN 1314 G, Hybrid R.K-312 F1	R
0.31–0.4	-	MR
0.41–0.5	-	MS
0.51–0.6	CLN131 4 × Palam pride, 12-1 × BWR-5	S
0.61–0.9	Hybrid R.K-312, Roma (BW susceptible check)	HS
0.91–1.0	-	ES

It was also discovered that symptoms appeared 4 days after inoculation in HS cultivars. In HS cultivars, the symptoms started on the leaves and then spread to other parts of the plant, resulting in full wilting. Within sensitive cultivars, total wilting took 14 days. Brown staining was also seen in the vascular system of transversely sliced portions of HS plants. The disease was proven by reisolating the bacterium from susceptible plants. Symptoms emerged on leaves followed by chlorosis in R cultivars, but no wilting was seen even after 14 days of inoculation.

3.7. Crop management practices

T. absoluta can be controlled with the chemical pesticide chlorantraniliprole (Coragen™). Coragen™) reduced the amount of eggs laid by *T. absoluta* females, resulting in a considerable reduction in the number of tunnels in the treated leaves, as well as a reduction of up to 85 percent in tomato fruit damage due to cyantraniliprole foliar or soil spray against *T. absoluta*. Chemical pesticides, on the other hand, should only be used when absolutely necessary, and caution should be exercised to avoid resistance, which has already been observed in *T. absoluta* with chlorantraniliprole and other diamides in various parts of the world.

In both seasons, pheromone trapping in the IPM plots revealed a single peak in *T. absoluta* population build-up, which steadily fell during the later crop stages. The use of a sex pheromone trap as a monitoring tool in *T. absoluta* management has been documented. However, research on the deployment of the mating disruption strategy against *T. absoluta* found that mass-trapping alone was ineffective in minimising leaf and fruit damage. As a result, in the current investigation, pheromone traps were used as a monitoring method. As a result, the IPM package, which includes pheromone traps, microbial pesticides, neem, and the chemical pesticide, is as effective as farmers' calendar-based chemical pesticide application, but with additional economic and environmental benefits.

Phyto sanitation and cultural practises are the best strategies for controlling bacterial wilt in the field. These treatments have shown to be beneficial in some situations in areas where the disease is endemic. Chemical control using spying copper bactericides, soil fumigation (chloropicrin), and phosphorous acid application are similarly costly to implement; soil fumigation has been observed to have poor success when used in conjunction with other control measures. Chemical control should be used in conjunction with other approaches to lessen pathogen resistance selection pressure.

4. Discussion

Germplasm evaluation of tomato hybrids A rapid spread of foreign invasive pests is necessary for improved crop performance and subsequent production since they can cause significant damage and even total devastation. Desneux and colleagues (2010) [11]. *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is a major threat to tomato (Solanum lycopersicum L.) production in various parts of the world, according to Biondi *et al* (2018) [6]. Despite the fact that there is widespread agreement that vegetable and seedling trade is a major driver of *T. absoluta* spread (Karadjova *et al*., 2013; Campos *et al*., 2017) [19, 8], previous modelling efforts have only focused on establishment potential Tonnang *et al*., (2015) [40] and spatial dispersion Guimapi *et al*., (2016) [15]. This is the first study to investigate human-mediated mechanisms in *T. absoluta* Venkatramanan *et al*., (2017) [42].

The crop management package tested in this study consisted of a series of chemical pesticide applications (chlorantraniliprole). The results showed that using the IPM package reduced the damage caused by *T. absoluta* larvae. According to Devi *et al*., Andhra Pradesh, together with Uttar Pradesh, Maharashtra, Punjab, and Haryana, accounted for 70% of overall pesticide usage in India, with a positive growth trend (above 5%) (2017). This could raise the likelihood of *T. absoluta* developing resistance to all of the pesticides that are often used in the field. Diamides, for example, were one of the most efficient pesticide classes used to control *T. absoluta* Biondi *et al* (2018) [6]. In recent years, however, considerable resistance to these pesticides has been recorded in Brazil and Europe (Guedes, 2017; Silva *et al*., 2019) [8, 36].

In all seasons, pheromone trapping in crop management revealed a single peak in *T. absoluta* population growth, which steadily fell over the later crop stages. The use of a sex pheromone trap as a monitoring tool in *T. absoluta* management has been documented (Braham, 2014; Bhanu *et al*., 2017) [7, 4]. Pheromone traps are extremely valuable as a monitoring tool in crop management packages since early detection is achievable even before obvious *T. absoluta* damage appears (Bhanu *et al*., 2017) [4]. However, mass-trapping alone proved ineffective in minimising leaf and fruit damage, while investigations on the mating disruption technique's application against *T. absoluta* yielded mixed findings Megido *et al* (2013) [23].

As a result, in the current investigation, pheromone traps were used as a monitoring method. As a result, the crop management package, which includes pheromone traps, microbial pesticides, neem, and the chemical pesticide, is as successful as farmers' calendar-based chemical pesticide application, but with additional economic and environmental benefits.

Among the various management techniques for bacterial wilt in aubergines, using resistant cultivars is a low-cost, environmentally beneficial, and long-lasting option. Researchers in various nations have attempted to identify resistant aubergine sources against *R. solanacearum* (Gopalakrishnan *et al*., 2014; Bhavana and Singh, 2016) [14, 5]. Some of the aubergine cultivars studied in this study showed somewhat resistant reactions to R. solanacearum. Certain genes confer resistance to R. solanacearum.

The bacterial wilt resistance in tomatoes is also regulated by soil temperatures, with resistance in cultivars breaking down as the temperature rises, because this temperature promotes pathogen growth, but soil temperatures below 20°C are unsuitable for the disease. Wang and Lin are a couple (2005) [45]. At 30–35 °C, the bacterial wilt disease of tomato induced by R. solanacearum was severely harmed. Hernandez *et al*., (2012).

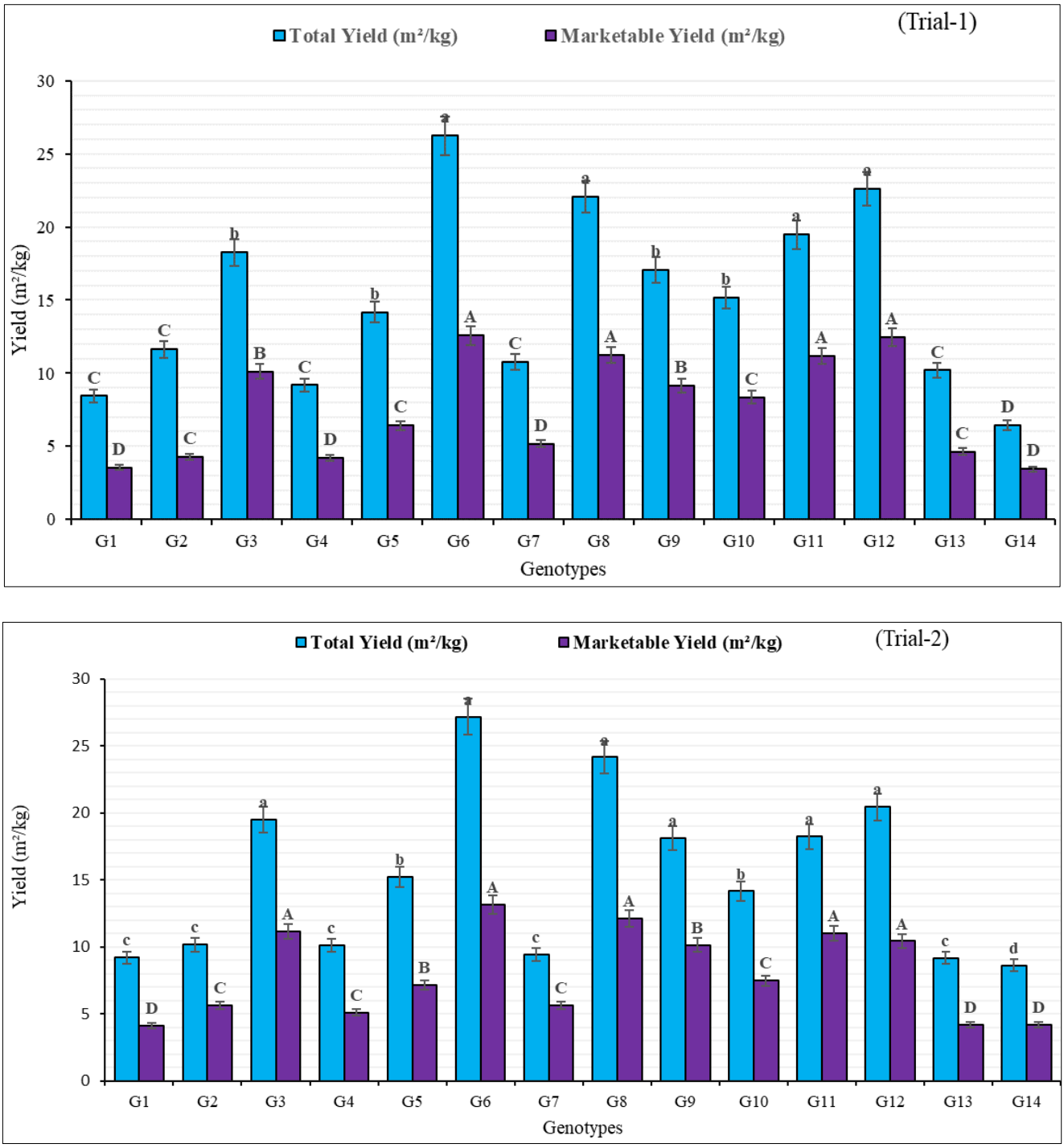

Fig 4: Total and marketable yield of tomato genotypes against *Tuta absoluta* in trials one (trial-1) and two (trial-2). (lowercase for total yield, uppercase for marketable yield) above the bars indicate nonsignificant difference according to Tukey's test ($p \leq 0.05$)

Resistance and susceptibility have also been linked to the onset of symptoms. Symptoms appeared after 4 days in susceptible genotypes and 14 days in resistant genotypes, which is consistent with the findings of (Sharma *et al*., 1997; Anith *et al*., 2004) [35, 1] who tested new tomato bred lines for resistance to *R. solanacearum* and recommended three of them that were moderately resistant and high yielding. The tomato cultivars Swarna Lalima, Swarna Naveen, and B-17 were determined to be resistant by Sharma *et al*. (2016) [34]. Scott *et al*. (2009) [33] also found a high level of *R. solanacearum* resistance in large-fruited breeding lines from eight F5 generation crosses.

In line with this, (Biondi *et al*., 2018; Silva *et al*., 2019) [6, 36] found lower tomato crop yields as a result of T. absoluta damage, emphasising the importance of carefully selecting and implementing efficient pest management techniques to avoid production losses of up to 100%.

5. Conclusion

Tuta absoluta infestation became one of the most serious dangers to tomato producing areas almost quickly. In the experiments, 14 hybrid tomato genotypes were assessed, as well as check verities that were also infested. In all genotypes, leaf damage increased with time and peaked at 100% in 10–11 weeks after transplantation. The highest leaflet damage was reported in both trials 11 weeks after transplantation. In the current study, 14 tomato cultivars were evaluated for bacterial wilt disease resistance. As a result, the (R) cultivars are suggested for use in integrated production systems and in the

development of novel resistant tomato cultivars.

Accession numbers

The genome sequence and its annotations are available at NCBI under the accession numbers OL601546.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit authorship contribution statement

Kanchhi Maya Waiba

Conceptualization, Methodology, Investigation, Resources, Data curation, Software, Formal analysis, Writing - original draft, Funding acquisition, Writing - review & editing. Kasi Indra Kumar: Conceptualization, Methodology, Resources, Supervision, Validation, Writing - review & editing. Praveen Sharma: Conceptualization, Methodology, Supervision, Validation, Writing - review & editing.

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References

1. Anith KN, Momol MT, Kloepper JW, Marois JJ, Olson SM, Jones JB. Efficacy of plant growth-promoting rhizobacteria, acibenzolar-S-methyl, and soil amendment for integrated management of bacterial wilt on tomato. Plant Disease, 2004; 88:669-673.
2. Anonymous. Screening of pest and diseases in tomato. In: Progress Report of AVRDC, Tiawan, 2000, p110-115.
3. Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, *et al*. Invision biology of spotted wing *Drosophila* (Drosophila suzukii): a global prospective and future priorities. J Pest Sci, 2015; 88:469-494. https://doi.org/10.1007/s10340-015-0681-z
4. Bhanu KRM, Walekar RVA, Mamatha B. Monitoring of *Tuta absoluta* (tomato leaf miner) using pheromone traps: a recent invasive pest in India. Environ. Ecol, 2017; 35:297-2976. http://refhub.elsevier.com/S0261-2194(20)30281-7/sref4
5. Bhavana P, Singh AK. Biodiversity in brinjal germplasm against resistance to bacterial wilt. Bangl. J. Bot, 2016; 45:737-739.
6. Biondi A, Guedes RNC, Wan FH, Desneux N. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, Tuta absoluta: past, present, and future. Annu. Rev. Entomol, 2018; 63:239-258. https://doi.org/10.1146/annurev-ento-031616-034933
7. Braham M. Sex pheromone traps for monitoring the tomato leaf miner, *Tuta absoluta*: effect of colored traps and field weathering of lure on male captures. Res. J. Agric. Environ. Manag, 2014; 3:290-298. http://refhub.elsevier.com/S0261-2194(20)30281-7/sref9
8. Campos MR, Biondi A, Adiga A, Guedes RNC, Desneux N. From the Western Palaearctic region to beyond: *Tuta absoluta* 10 years after invading Europe. Journal of Pest Science, 2017; 90(3):787-796. https://doi.org/10.1007/s10340-017-0867-7
9. Cocco A, Serra G, Lentini A, Deliperi S, Delrio G. Spatial distribution and sequential sampling plans for *Tuta absoluta* (Lepidoptera: Gelechiidae) in greenhouse tomato crops. Pest Manag. Sci, 2014; 71:1311-1323. https://doi.org/10.1002/ps.3931.
10. Desneux N, Luna MG, Guillemaud T, Urbaneja A. The invasive South American tomato pin worm, *Tuta absoluta* continuous to spreading Afro-Eurasia and beyond: the new threat to tomato world production. J Pest Sci, 2011; 84:403-408. https://doi.org/10.1007/s10340-011-0398-6
11. Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narvaez-Vasquez CA. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J Pest Sci, 2010; 83:197-215. https://doi.org/10.1007/s10340-010-0321-6
12. Devi PI, Thomas J, Raju RK. Pesticide consumption in India: a spatiotemporal analysis. Agric. Econ. Res. Rev, 2017; 30:163-172. https://doi.org/10.5958/0974-0279.2017.00015.5.
13. Genin S, Denny TP. Pathogenomics of the *Ralstonia solanacearum* Species Complex. Annual Review of Phytopathology, 2012; 50:67-89. http://dx.doi.org/10.1146/annurev-phyto-081211-173000
14. Gopalakrishnan C, Singh TH, Artal RB. Evaluation of eggplant accessions for resistance to bacterial wilt caused by *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al*. J. Hort. Sci, 2014; 9:202-205.
15. Guimapi RYA, Mohamed SA, Okeyo GO, Ndjomatchoua FT, Ekesi S, Tonnang HEZ. Modeling the risk of invasion and spread of Tuta absoluta in Africa. Ecological Complexity, 2016; 28:77-93. https://doi.org/10.1016/j.ecocom.2016.08.001
16. Hayward AC. Biology and Epidemiology of Bacterial Wilt Caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology, 1991; 29:65-87. http://dx.doi.org/10.1146/annurev.py.29.090191.000433
17. Hernández RJ, Ramírez RS, Ydrac MCJ. First report of *Ralstonia solanacearum* causing tomato bacterial wilt in Mexico. New Dis Rep, 2012; 26:22.
18. Jyothi HK, Santhosha HM, Basamma. Recent Advances in Breeding for Bacterial Wilt (*Ralstonia solanacearum*) Resistance in Tomato—Review. Current Biotica, 2012; 6:370-398.
19. Karadjova O, Ilieva Z, Krumov V, Petrova E, Ventsislavov V. *Tuta absoluta* (Meyrick) (Lepidoptera: gelechiidae): potential for entry, establishment and spread in Bulgaria. Bulg. J. Agric. Sci, 2013; 19(3):563-571.
20. Kasi IK, Singh M, Waiba KM, Monika S, Waseem MA, Archie D, *et al*. Bio-efficacy of entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the Cabbage butterfly (*Pieris brassicae* [L.]) under laboratory conditions. Egypt J Biol

Pest Control, 2021a; 31:125. https://doi.org/10.1186/s41938-021-00469-4
21. Kasi IK, Singh M, Waiba KM, Monika. Occurrence and distribution of entomopathogenic nematodes in soils of Solan and Sirmaur district of Himachal Pradesh, India. International Journal of Agriculture Environment and Biotechnology, 2021b; 14:393-397. https://doi.org/10.30954/0974-1712.03.2021.16.
22. Kasi IK, Waiba KM, Singh M. First report of natural infestation of *Ovomermis sinensis* (Nematoda: Mermithidae) parasitizing fall armyworm *Spodoptera* sp. (Lepidoptera: Noctuidae) in Himachal Pradesh, India. Indian Journal of Nematology, 2020; 50:148-149.
23. Megido RC, Haubruge E, Verheggen FJ. Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). A. Ndereyimana *et al*. Crop Protection 134 (2020) 105183 7 A review. Biotechnol. Agron. Soc. Environ, 2013; 17:475-482. http://hdl.handle.net/2268/154676
24. Miranda MMM, Picanco M, Zanuncio JC, Guedes RNC. Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Bio Sci Technol, 1998; 8:597-606. https://doi.org/10.1080/09583159830117
25. Ndereyimana A, Nyalala S, Murerwa P, Gaidashova S. Field efficacy of entomopathogens and plant extracts on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) infesting tomato in Rwanda. Crop Protection, 2020, 105183. https://doi.org/10.1016/j.cropro.2020.105183
26. Nei M, Kumar S. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 2000.
27. NHB. National horticulture database, 2014, p289.
28. Ragsdale DW, Landis DA, Brodeur J, Heimpel GE, Desneux N. Ecology and management of the soybean aphid in North America. Annu Rev Entomol, 2011; 56:375-399. https://doi.org/10.1146/annurev-ento-120709-144755
29. Ramesh R, Phadke GS. Rhizosphere and Endophytic Bacteria for the Suppression of Eggplant Wilt Caused by *Ralstonia solanacearum*. Crop Protection, 2012; 37:35-41. http://dx.doi.org/10.1016/j.cropro.2012.02.008
30. Roditakis E, Papachristos D, Roditakis NE. Current status of the tomato leafminer *Tuta absoluta* in Greece. EPPO Bull, 2010; 40:163-166. https://doi.org/10.1111/j.1365-2338.2009.02367.x
31. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 1987; 4:406-425.
32. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci, 1977; 74:5463-5467.
33. Scott JW, Vallad GE, Jones JB. High level of resistance to bacterial wilt (*Ralstonia solanacearum*) obtained in large-fruited tomato breeding lines derived from Hawaii 7997. Acta Horticulturae, 2009; 808:269-274.
34. Sharma PL, Gavkare O. New Distributional Record of Invasive Pest *Tuta absoluta* (Meyrick) in North-Western Himalayan Region of India. National Academy Science Letters, 2016; 40:217-220. https://doi:10.1007/s40009-016-0526-1
35. Sharma TVRS, Rathore RKS, Kumar S. Screening tomato for bacterial wilt resistance in Andamans. Journal of the Andaman Science Association, 1997; 13:32-36.
36. Silva EM, Crespo ALB, Farias ES, Bacci L, Queiroz RB, Picanço MC. Conventional Sampling Plan for Scouting *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) Eggs on Tomato Fruits. Journal of Economic Entomology, 2019, 1-8. https://doi.org/doi: 10.1093/jee/toz158
37. Sridhar V, Chakravarthy AK, Asokan R, Vinesh LS, Rebijith KB, Vennila S. New record of the invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India. Pest Manag Hortic Ecosyst, 2014; 20:148-154.
38. Tamura K, Stecher G, Kumar S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 2021. https://doi.org/10.1093/molbev/msab120.
39. Thomas P, Sadashiva AT, Upreti R, Mujawar MM. Direct Delivery of Inoculum to Shoot Tissue Interferes with Genotypic Resistance to *Ralstonia solanacearumin* Tomato Seedlings. Journal of Phytopathology, 2014; 163:320-323. https://doi:10.1111/jph.12281
40. Tonnang HEZ, Mohamed SA, Khamis F, Ekesi S. Correction: Identification and Risk Assessment for Worldwide Invasion and Spread of *Tuta absoluta* with a Focus on Sub-Saharan Africa: Implications for Phytosanitary Measures and Management. Plos One, 2015; 10(9):e0138319. https://doi.org/10.1371/journal.pone.0135283
41. Urbaneja A, Desneux N, Gabarra R, Arno J, Gonzalez-Cabrera J, Mafra-Neto A, *et al*. Biology, ecology and management of the tomato borer, *Tuta absoluta*. In: Pena JE (ed) Potential invasive pests of agricultural crops, CABI series, 2013, p98-125.
42. Venkatramanan S, Wu S, Shi B, Marathe A, Marathe M, Eubank S. Towards robust models of food flows and their role in invasive species spread. 2017 IEEE International Conference on Big Data (Big Data), 2017. https://doi.org/10.1109/bigdata.2017.8257955
43. Vrain TC, Wakarchuk DA, Levesque AC, Hamilton RI. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundam Appl Nematol, 1992; 15:563-74.
44. Waiba KM, Sharma P, Kasi IK, Chauhan S. Studies of Genetic Variability of Tomato (*Solanum lycopersicum* L.) Hybrids under Protected Environment. International Journal of Bio-resource and Stress Management, 2021; 12:264-270. https://doi.org/10.23910/1.2021.2211.
45. Wang JF, Lin CH. Integrated management of bacterial wilt of tomatoes. Shanhua (Taiwan): Asian Vegetable Research and Development Center. Publication, 2005, 05-615.