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Phenotypic evaluation and utilization of tomato germplasm resistance for ToLCV and its vector *Bemisia tabaci* under natural condition

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Abstract
During May- June 2019, about 50 genotypes of tomato along with susceptible check, Arka Vikas were screened for resistance against the sweet potato whitefly, *Bemisia tabaci* and the begomovirus Tomato leaf curl virus (ToLCV) in a Randomized Block Design with two replications having a plot size of 5×4 m at Linga nayakan pati, Usilampatti Taluk, Madurai district. The population of whitefly was recorded on tomato at weekly interval from appearance of the whiteflies. The infestation found to be more at earlier weeks thereafter the population declined abruptly. The average minimum (0.24/plant) and maximum (3.41/plant) whitefly population during crop season was observed on EC-520078 and EC-165700. According to the mean of whitefly population the highest resistance was found in EC-520078 followed by EC-631364, EC-315477 and EC-620389. Among all the accessions observed for ToLCV resistance, the accession EC-520078 recorded only 7.88% disease incidence and found statistically significant as compared to other accessions while the maximum disease incidence was observed in EC-620372 (94.25%).

Keywords: whitefly, ToLCV, tomato germplasm, field screening

Introduction
Tomato (*Solanum lycopersicum* L., 2n=24, Family: Solanaceae), a fruit vegetable originated in Peru Ecuador region of Latin America and was introduced in India by Portuguese merchants, has coveted a respectable share as a fresh vegetable. Tomato cultivation is seen in almost all parts of the country occupying an area of 7.78 lakh hectares with a total production of 193 lakh tonnes, and productivity is around 24 tonnes per ha [1]. However, like all other vegetables, tomato also face production hurdles due to its perennial growth at a larger scale likewise unfavorable temperature, moisture-stress, cracking pollination, pests and diseases etc., Among these, problems imposed by pests and diseases are very critical. Sucking pest such as thrips, whiteflies and aphids cause severe damage to crop by transmitting virus diseases rather than direct feeding [2]. Besides fungal, bacterial and phytoplasmal infections, viral diseases are of large in number to affect tomato and are of great importance due to its severity in infection causing huge economic losses up to 20-90 % in tomato and other vegetable crops. Worldwide, about 146 viruses belonging to 33 genera are reported to infect tomato [3].
Among the viral diseases affecting tomato cultivation, Tomato leaf curl virus (ToLCV), a whitefly (*Bemisia tabaci* Gen.) (Hemiptera: Aleyrodidae) transmitted geminivirus comes under limelight due to its severity causing even 100% loss, sometimes, worldwide. *B. tabaci* is recognized as one of the world's top most 100 invasive pests and is the only known vector of ToLCV. This tiny insect causes excessive damage to commercially important crops either through direct feeding or through transmission of more than 120 plant viruses predominantly belonging to the genus Begomovirus (family Geminiviridae).
ToLCV has risen to alarming proportions in the plains of India. Symptoms caused by physiological reasons may misguide the farmer for proper identification of ToLCV too. Since chemical control measures are ineffective against ToLCV and *B. tabaci* biotype B, and are hazardous to environment and human health a search for alternate strategy is always attempted. In nature a lot of diversity is found with tomato accessions for different agronomic

characteristics including resistance to ToLCV and *B. tabaci*. Therefore, the first and foremost work is the identification of the best germplasm with resistance against the vector *B. tabaci* and to the ToLCV, so as to offer sustainable tomato production is possible in the fields with reduced menace of the leaf curl disease. Intended to develop resistant varieties or lines against ToLCV and the vector is a profound way of dealing the issue.

Developing resistant lines or varieties to ToLCV and its vector, *B. tabaci* by exploring resistant sources through challenging with whiteflies, their selection and introgression into cultivable susceptible lines is considered to be the most sustainable practice in checking ToLCV on tomato [4]. Keeping this option of utilizing resistance technology in mind tomato germplasms should be screened from time to time for their relative tolerance/susceptibility against ToLCV and whitefly [5]. Considering this, an investigation was undertaken to identify the tomato germplasm resistance/susceptibility under natural conditions against *B. tabaci* and ToLCV disease.

Materials and Methods

The tomato accessions (population/genotypes/cultivars/lines (50 Nos.)) received from NBPGR (National Bureau of Plant Genetic Resources, New Delhi) along with the variety, Arka Vikas (susceptible check) from IIHR (Indian Institute of Horticultural Research, Bangalore) and PKM1 variety from TNAU, Coimbatore were used in the present for evaluation of resistance against against both the whitefly, *B. tabaci*, vector of ToLCV and the virus, ToLCV causing tomato leaf curl virus disease (ToLCVD) during summer season of 2019 at farmer's holding, Linganayakanpati, Usilampati Taluk, (Coordinates: 9.9651ºN, 77.7885ºE), Madurai district, Tamil Nadu, a hot spot for whitefly, *B. tabaci* and ToLCV incidence.

Raising of seedlings

All the tomato accessions were sown in a raised nursery bed of 1.2 × 0.3 m^2 size. Thirty days old seedlings were transplanted to the main field, on ridges and furrows at a spacing of 60 × 45 cm, well maintained as healthy seedlings with proper irrigation and nutrients as per the package of practices recommended by Tamil Nadu Agricultural University. Insect control alone was excluded to meet out the objective. The genotypes were individually maintained in plot size of 5×4 m^2 using Randomized block design (RBD) with two replicates.

Whitefly incidence severity

Data for the trial was recorded from ten randomly selected plants excluding border to examine adult whitefly population at weekly interval on 37, 44, 51, 58, 65, 72 and 79 days after transplanting (DAT). The adult whitefly count was taken under lower surface of leaves and three leaves from the selected plants in each genotype representing top, middle and bottom leaf canopy were observed. Overall mean whitefly per three leaves was worked out. Data from the assessment were transformed with $\sqrt{x+0.5}$ and subjected to RBD [6]. The mean values were separated using Duncan's Multiple Range Test (DMRT) [7] (P=0.05). Statistical analysis was performed using a one-way analysis of variance (ANOVA) (SAS Institute, 1985).

ToLCV incidence and severity

The natural incidence of ToLCVD was recorded at 100 day after transplanting after appearance of symptom. The symptom severity was recorded on 20 plants of each plot at a 0-4 scale (Table 1) on each genotype following the method described by Banerjee and Kalloo (1987) with certain modifications. Each disease severity grade was also assigned with a response value. The ToLCV infestation was recorded based on diseases symptoms and damage score. Genotypes were categorized based on the Percent disease incidence (PDI) and Coefficient of infection (CI).

Table 1: Disease severity score assigned to tomato accessions for identifying resistance responses to ToLCV [8]

Symptom	Score	Response value (RV)	Coefficient of infection (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 70-100	Susceptible (S) Highly Susceptible (HS)

he Percent disease incidence (PDI) was calculated by dividing number of diseased plants to the total number of plants (20 in each genotype).

$$\text{PDI} = \frac{\text{Number of plant infected}}{\text{Total number of plants oberserved}} \times 100$$

Based on the PDI, the CI) was derived by multiplying PDI and the response value (RV)

$$\text{CI} = \text{PDI} \times \text{RV}$$

Results and Discussion

Screening for whitefly resistance under field conditions

It is known that certain varieties or hybrids or strains of crops are attacked less by insects than other because of natural resistance. In cultural practices, currently employed to minimize the losses caused by whitefly, growing of resistant varieties/hybrids against the whitefly is the most important tool used in the whitefly management, which is a practice without any additional cost to the growers. In the present investigation, tomato accessions (52 nos- 50 germplasms with checks) were evaluated in field under natural ToLCVD incidence condition and the results are tabulated (Tables 2 and 3) and summarized (Table 4 and Fig 1). The symptoms started by upward and downward curling of leaves in infected plants. Infected plants remained stunted and became yellowish in color and had less fruit formation with progression of growth and age. Whitefly population during the testing ranged from 0.24 to 3.73 mean numbers per 3 leaves. As observed, the least preferred genotype was EC-520078 (0.24 adults/3 leaves) followed by EC-631364 (0.26 adults/3 leaves). While, EC-165700 (3.41 adults/3 leaves) was the most preferred genotype followed by EC-160885 (3.40 adults/3 leaves) and EC-538156 (3.40 adults/3 leaves). The susceptible check Arka vikas had the highest mean population of whitefly (3.73 adults/3 leaves) (F= 7.21; df=51: Pr > F = <.0001) (Table 3). The present study is in accordance with results of [9] who reported that whitefly population in Arka vikas and PKM-1 as (1 adults/3 leaves) and (0.2 adults/3 leaves) respectively.

Screening for ToLCV resistance under field condition

The exploration of identified resistant source(s) is of highest significance for the management of diseases of crop plants, especially for the virus diseases in the context of lack of curative approaches for viral infection. It has been reported that the extent of yield loss due to ToLCV infection under field condition is directly proportional to earliness of initiation of virus infection [10]. The results from the field trial for ToLCV resistance in tomato germplasm are tabulated in (Table 3). Among the germplasm evaluated for ToLCV resistance the range of infection is found to be from 7.88 - 96 percent. The accession EC-520078 was observed to have the least percent disease infection (7.88) and the highest PDI was observed in EC-620372 (94.25). Susceptible check used in the study had recorded a PDI of 96 (Table 4).

In the present study, the resistance reaction observed share a common platform with Rajsri and Vijayalakshmi (2013), who had reported that the tomato accessions Punjab Chhuhara, Arka Vikas, Arka Meghali, Arka Saurabh and Arka Alok exhibited susceptible (100% disease incidence) reactions to ToLCV. Many researchers reported that wild tomato accessions such as *Lycopersicon pimpinellifolium* (A-1921) and *Lycopersicon hirsutum* f. sp. *glabratum* (B-6013) as resistant sources for ToLCV [8, 11, 12] and they had recorded Arka Vikas as a susceptible cultivar. Similar findings were given for Arka vikas by [13] as a susceptible variety with 76.67 PDI. Based on the coefficient of infection, [14] screened nine tomato genotypes (*viz.*, EC-520049 (*S. chmielewskii*), EC-520058, EC-520060 and EC-520061 (*S. habrochaites*), EC-520070, EC-520071, EC-520077, EC-520079 (*S. pimpinellifolium*) and H-88-78-1(*S. lycopersicum*; a derivative of *S. habrochaites* f. *glabratum*) and were found to be highly resistant.

The results are further supported by [15], who screened thirty five tomato genotypes including wild accessions for its resistance/susceptible reaction against tomato leaf curl disease in field condition during summer cropping season 2015. Among the screened thirty five genotypes seven genotypes Vaibhav, EC-541109, EC-168283, IIHR2372, IIHR1970, IIHR2200 and LA2805 showed higher resistance against ToLCV without producing any symptoms of leaf curl disease. Genotype EC-165751 showed resistant reaction and two genotypes *viz.*, Nandi and EC-620545 showed moderately resistant reaction to ToLCV, twelve genotypes showed moderately susceptible reaction, seven genotypes showed susceptible reaction and six genotypes were found highly susceptible to ToLCV.

In the current investigation, notable resistance for both the vector whitefly and the virus, ToLCV were registered in the germplasms accessions EC-520078, EC- 631364, EC-315477 and EC- 620389. Further exploration of theses accessions at laboratory level could yield notable results to assess the type of resistance and their mechanisms whether for the vector only or to the virus alone or both of them put together. The resistance mechanism in some of the wild tomato species had been reported to be associated with the presence of exudates from trichome glands on the leaf surface, in which whiteflies become entrapped [16]. Nevertheless, further studies are required to characterize the impact of insect feeding behavior of *B. tabaci* on the reported resistant plants.

The resistant nature of accessions to the vector are possibly linked to the presence of glandular trichomes on leaf surface which contributes to the resistance by entrapping the whiteflies when they are encountered with contact and possibly known to change their feeding nature [17-21]. Type IV and VI trichomes of glandular nature in *Solanum* spp., are known to have a high level of resistance directly by entrapping and indirectly by releasing secondary metabolites [18, 22-24]. The resistance of similar nature between trichome type and whitefly resistance are widely reported in *S. galapagense* [19, 25], and *S. habrochaites* [17].

The best performed germplasms at field screening could possibly have significant wax concentration and the presence of cuticular waxes make whitefly attachment difficult to plant surfaces and act as physical barrier which limits the entry of pathogens, and also acts as a basin of signals to trigger the plant defense responses. The cuticular waxes act as first line of defense against whiteflies and thus the leaf curl virus. The crops with natural defensive strategies with longer trichomes, inorganic salts with increased concentration of cuticular waxes, stays as an armor against whiteflies, leaf curl virus and other pathogens widely [26-28].

Resistance may also be due to the lack of feeding by the vector or avoidance by the vector therefore, accessions displaying natural resistance against the transmitting vector may not be resistant to its transmittable virus. In real resistance can be determined by introducing these resistant accessions in controlled inoculation conditions were the vector and virus are in quantifying numbers [29] and therefore the resistant lines reported in the present investigation may need additional laboratory studies before making incorporation into resistance breeding strategies.

Conclusion

The field screening of fifty tomato germplasms against whitefly and ToLCV recorded that the accession EC-520078 followed by EC-631364, EC-315477 and EC-620389 had the highest resistance against whitefly and ToLCV as compared to the other accessions this favours the opportunities for further resistance breeding varieties and genetic studies.

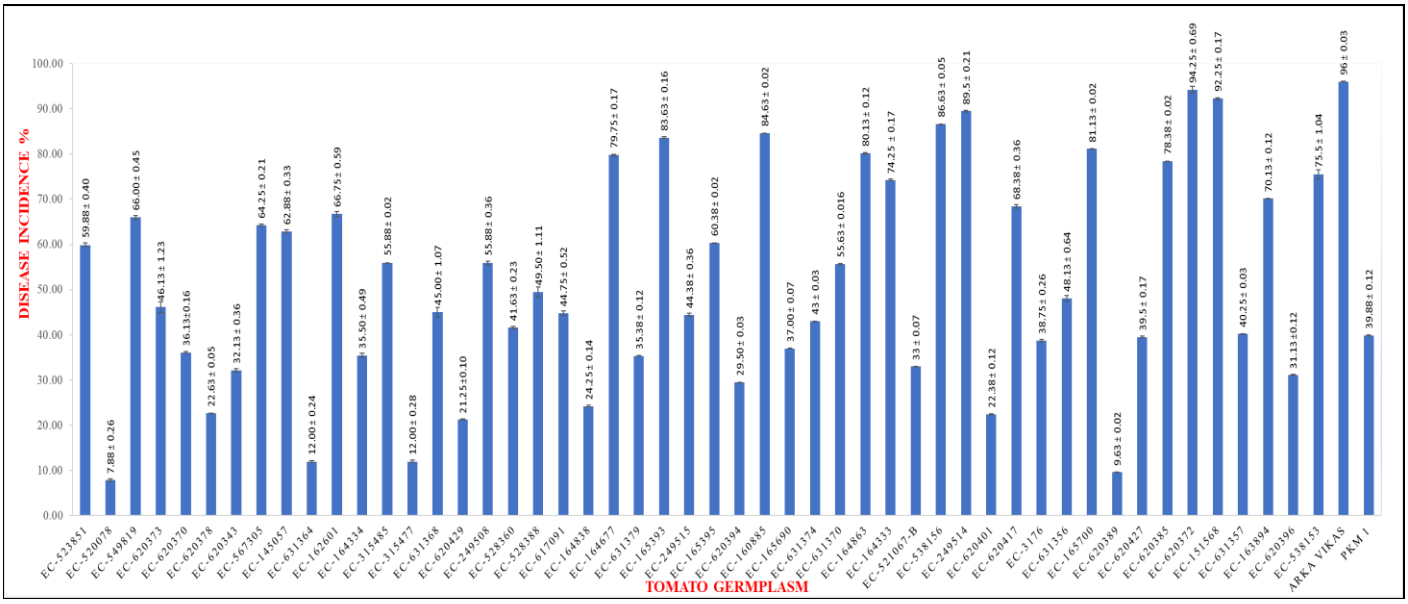

Fig 1: Natural screening of Tomato genotypes for ToLCV disease incidence

Table 2: Reactions of tomato accessions in field screening against whitefly, *B. tabaci* at Linganayakanpati, Usilampatti Taluk, Madurai district, Tamil Nadu during summer, 2019

Tomato accession	Whitefly count per trifoliate leaf*	Tomato accession	Whitefly count per trifoliate leaf*
EC-523851	2.59 (1.61)^{q}	EC-620394	1.30 (1.14)^{i}
EC-520078	0.24 (0.49)^{a}	EC-160885	3.40 (1.84)^{xy}
EC-549819	2.64 (1.63)^{q}	EC-165690	1.30 (1.14)^{i}
EC-620373	2.01 (1.42)^{m}	EC-631374	2.19 (1.48)^{o}
EC-620370	1.39 (1.18)^{j}	EC-631370	2.31 (1.52)^{p}
EC-620378	0.81 (0.90)^{f}	EC-164863	3.30 (1.82)^{vw}
EC-620343	1.14 (1.07)^{h}	EC-164333	2.80 (1.67)st
EC-567305	2.76 (1.66)^{rs}	EC-521067-B	1.50 (1.22)^{l}
EC-145057	2.71 (1.65)^{r}	EC-538156	3.40 (1.84)^{xy}
EC-631364	0.26 (0.51)^{b}	EC-249514	3.36 (1.83)^{wxy}
EC-162601	2.64 (1.63)^{q}	EC-620401	0.77 (0.88)^{e}
EC-164334	1.29 (1.13)^{i}	EC-620417	2.81 (1.68)^{t}
EC-315485	2.03 (1.42)^{m}	EC-3176	1.51 (1.23)^{l}
EC-315477	0.27 (0.52)^{b}	EC-631356	2.27 (1.51)^{p}
EC-631368	2.10 (1.45)^{n}	EC-165700	3.41 (1.85)^{y}
EC-620429	0.89 (0.94)^{g}	EC-620389	0.31 (0.56)^{c}
EC-249508	2.13 (1.46)^{n}	EC-620427	1.46 (1.21)^{k}
EC-528360	2.19 (1.48)^{o}	EC-620385	2.70 (1.64)^{r}
EC-528388	2.11 (1.45)^{n}	EC-620372	3.34 (1.83)^{wx}
EC-617091	2.13 (1.46)^{n}	EC-151568	3.33 (1.82)^{wv}
EC-164838	0.63 (0.79)^{d}	EC-631357	2.30 (1.52)^{p}
EC-164677	3.27 (1.81)^{v}	EC-163894	2.64 (1.63)^{q}
EC-631379	1.30 (1.14)^{i}	EC-620396	1.44 (1.20)^{k}
EC-165393	3.36 (1.83)^{wxy}	EC-538153	2.91 (1.71)^{u}
EC-249515	2.30 (1.52)^{p}	Arka Vikas	3.73 (1.93)^{z}
EC-165395	2.89 (1.70)^{u}	PKM 1	1.53 (1.24)^{l}
SEd	0.1500	CD (.05)	0.3012

Mean of two replications per trifoliate leaf*
The mean values were separated using Duncan's Multiple Range Test (DMRT) (Duncan, 1951) (*P*=0.05).
Values in parantheses are square root transformed.

Table 3: Response of tomato accessions to Tomato leaf curl disease caused by ToLCV under field condition at Linganayakanpati, Usilampati, Madurai district, Tamil Nadu

Tomato accession	PDI	CI	Disease reaction/ Category*	Tomato accession	PDI	CI	Disease reaction/ Category*
EC-523851	59.88	41.54	S	EC-620394	29.50	11.06	MR
EC-520078	7.88	1.58	HR	EC-160885	84.63	78.81	HS
EC-549819	66.00	49.91	S	EC-165690	37.00	17.81	MR
EC-620373	46.13	26.23	MS	EC-631374	43	22.84	MS
EC-620370	36.13	17.16	MR	EC-631370	55.63	36.50	MS
EC-620378	22.63	7.64	R	EC-164863	80.13	73.61	HS

EC-620343	32.13	13.65	MR	EC-164333	74.25	62.65	S
EC-567305	64.25	47.38	S	EC-521067-B	33	14.23	MR
EC-145057	62.88	45.19	S	EC-538156	86.63	80.13	HS
EC-631364	12.00	2.93	HR	EC-249514	89.5	85.03	HS
EC-162601	66.75	50.48	S	EC-620401	22.38	7.13	R
EC-164334	35.50	16.20	MR	EC-620417	68.38	53.85	S
EC-315485	55.88	38.06	MS	EC-3176	38.75	17.92	MR
EC-315477	12.00	2.48	HR	EC-631356	48.13	28.27	MS
EC-631368	45.00	25.03	MS	EC-165700	81.13	75.04	HS
EC-620429	21.25	6.64	R	EC-620389	9.63	2.05	HR
EC-249508	55.88	37.37	MS	EC-620427	39.5	18.76	MR
EC-528360	41.63	21.85	MS	EC-620385	78.38	68.58	S
EC-528388	49.50	30.32	MS	EC-620372	94.25	91.30	HS
EC-617091	44.75	24.89	MS	EC-151568	92.25	85.91	HS
EC-164838	24.25	8.18	R	EC-631357	40.25	20.13	MS
EC-164677	79.75	72.27	HS	EC-163894	70.13	56.54	S
EC-631379	35.38	15.92	MR	EC-620396	31.13	12.06	MR
EC-165393	83.63	78.40	HS	EC-538153	75.5	65.12	S
EC-249515	44.38	24.13	MS	Arka vikas	96	93.60	HS
EC-165395	60.38	43.02	S	PKM 1	39.88	18.44	MR

* PDI- Percent disease incidence, CI- Coefficient of the infection, HR – Highly Resistant, R-Resistant MR- Moderately Resistant, MS-Moderately Susceptible,
S-Susceptible HS- Highly susceptible.

Table 4: Categorization of tomato genotypes based on the incidence of whitefly and resistance reaction to ToLCV (0-4 Scale)

Disease scale	Reaction	Mean number of whitefly/3 leaves	ToLCV Incidence (%)	Total number of genotypes	Tomato accessions
0-4	HR	0.24-0.31	7.88-12.00	4	EC-520078, EC-631364, EC-315477, EC-620389
5-9	R	0.63-0.89	21.25-24.25	4	EC-620378, EC-620429, EC-164838, EC-620401
10-19	MR	1.14-1.53	29.50-39.88	11	EC-620370, EC-620343, EC-164334, EC-631379, EC-620394, EC-165690, EC-521067-B, EC-3176, EC-620427, EC-620396, PKM 1
20-39	MS	2.01-2.31	40.25-55.88	12	EC-620373, EC-315485, EC-631368, EC-249508, EC-528360, EC-528388, EC-617091, EC-249515, EC-631374, EC-631370, EC-631356, EC-631357
40-69	S	2.59-2.91	59.88-78.38	11	EC-523851, EC-549819, EC-567305, EC-145057, EC-162601, EC-165395, EC-164333, EC-620417, EC-620385, EC-163894, EC-538153
70-100	HS	3.27-3.73	79.75-96	10	EC-164677, EC-165393, EC-160885, EC-164863, EC-538156, EC-249514, EC-165700, EC-620372, EC-151568, Arka Vikas

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