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RESEARCH ARTICLE :

Evaluation of tomato hybrids for resistance to leaf curl virus

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SUMMARY : Tomato leaf curl virus disease (TLCVD) is a serious problem in cultivation and production of tomato in India. The disease is widespread in tomato during the summer season in South India and autumn in North India. In South India, the incidence of TLCVD in susceptible cultivars increases rapidly from 27 to 90 per cent causing yield losses exceeding 90 per cent.The objective of this study was to screen the two newly synthesized tomato F_1 hybrids *viz.*, CLN 2123A X HN_2 and HN_2 X CLN 2123A to find out the leaf curl virus resistance along with their parents and check varieties/hybrids. The experiment was conducted both under natural epiphytotic condition as well as glass house condition through whitefly mediated inoculation. The experiment was laid out in a Randomized Block Design and replicated thrice. The results revealed that the newly synthesized hybrids CLN 2123A X HN_2 and HN_2 X CLN 2123A registered low level of percent of disease infection under artificial and natural epiphytotic conditions and these varieties showed high values of defense enzymes *viz.*, peroxidase and poly phenol oxidase both under artificial and natural epiphytotic conditions. The same hybrids also registered higher plant height, number of branches per plant, number of fruits per plant and yield per plant under field condition inferred that these two synthesized hybrids are tolerant tomato leaf curl virus. These two newly synthesized hybrids were on par with tomato leaf curl virus resistant check hybrid Lakshmi for tomato leaf curl virus disease incidence.

KEY WORDS :
Tomato, TLC VD, Tolerance, Peroxidase, Poly phenol oxidase

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BACKGROUND AND OBJECTIVES

Tomato (*Solanum lycopersicum* L.) is a solanaceous vegetable crop, occupies prime position among different fruit vegetables grown in the world due to its wider adaptability both in open field and protected conditions. However, successful cultivation of tomato is being limited by affecting several pest and diseases (Singh *et al.*, 1999). Among the several pests, attacking tomato crop, whitefly (*Bemisia tabaci* Genn.) is the major pest, which not only cause damage by direct feeding but also transmits the leaf curl virus disease. Tomato leaf curl virus diseases (TLCVD) have seriously hampered the cultivation and production of tomato in India (Pico *et al.*, 1998). This disease is characterized by curling of leaves, reduction

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in leaf size with uneven surface, excessive branching with stunted growth (Muniyappa *et al*., 2000). The disease is widespread in tomato during the summer season in South India (Reddy and Kumar, 2004) and autumn in North India (Ratul and Bordoli, 1998). In South India, the incidence of TLCVD in susceptible cultivars increases rapidly from 27 to 90 per cent causing yield losses exceeding 90 per cent (Devaraj *et al*., 2005). The maximum and minimum temperature for high TLCVD incidence is 28.7°C to 30.8°C for day time and night time temperature between 15.1°C to 22.3°C. However, the incidence of tomato leaf curl virus can be reduced to some extent with the application of insecticides. The repeated uses of such chemicals are expensive, labour intensive and associated with many ecological hazards. This emphasized the need for alternative strategies to control tomato leaf curl virus. During the past 20 years, there has been considerable effort to develop Tomato leaf curl virus resistant cultivars. However, they are not completely resistant to TLCVD (Table A), therefore wild *Lycopersicon* species have been screened for virus resistance in India (Mala andVadivel, 1999; Hanson *et al*., 2000; Kalloo and Banerjee, 2000; Gomez *et al*., 2004; Shahnaz and Krishnakumar, 2004) and Israel (Pico*et al*., 1998). Nevertheless, progress in breeding for TLCVD resistance has been slow (Lapidot *et al*., 1997). Because of the complex genetics of resistance, which probably explain why the cultivars and breeding lines are not as resistant as wild species. Hence, development of resistant/tolerant cultivars to pest and diseases would be a boon to the farmers to grow tomato organically. With this view the present investigation was carried out to analyze the performance of tomato hybrids for resistance to leaf curl virus with the following objectives.

– Evaluation of tomato hybrids and their parents for resistance to TLCVD under natural field condition as well as pot culture

– Biological response of tomato hybrids and their parents for resistance to TLCVD.

RESOURCES AND METHODS

Tomato genotypes :

The experimental materials consisted of nine genotypes including two newly synthesized F_1 hybrids *viz*., CLN 2123A X HN_2, HN_2 X CLN 2123A, their parents CLN 2123A and HN_2, leaf curl virus resistant check Lakshmi, $COTH_2$, susceptible check HisarLalit, LCR2 and CO 3.

Field experiment :

Field experiment was conducted in the farmer's field at Rayakottai of Dharmapuri district of Tamil Nadu, which was identified as a hot spot for tomato leaf curl virus. The experiment was conducted during summer season (February 2006- July 2006 and February 2007-July 2007). The field experiment was laid out in a Randomized Block Design with three replications. Biometrical observation like plant height (cm), number of branches per plant, per cent of disease infection at 75 DAP, co-efficient of infection at 75 DAP, peroxidase (PO), poly phenol oxidase (PPO) and yield per plant were made from randomly selected ten plants.

Pot experiment :

In the second experiment the same genotypes were subjected to confirmation study for resistance to tomato leaf curl virus under pot culture through artificial inoculation.

Virus source :

The Tomato leaf curl virus isolate used in inoculations was collected from an infected tomato plant (Department of Horticulture, Tamil Nadu Agricutural University) from Coimbatore, India and isolated by two serial single-whitefly passages. The isolate was shown to consist of only one virus by sequencing a number of clones of polymerase chain reaction (PCR) products (unpublished results). The virus was maintained on cv. CO 3 tomato in whitefly- proof cages. Virus was maintained, propagated, and used as inoculum in the trials using whitefly-mediated transmission.

Whitefly culture :

Adult *B. tabaci* were collected from bhendi, *Abelmoschus esculentus*, at the Department of Vegetables crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore and maintained on brinjal (*Solnaum melongena* cv. CO 2) plants in wooden cages (45 × 45 × 30 cm) covered with 40 mesh size nylon net.

Tomato leaf curl virus inoculation :

Seeds of all the nine genotypes were sown in protrays filled with coco peat as media to produce healthy

Table A : A scale for classifying disease reaction to TLCVD

Sr. No.	Symptoms	Symptoms severity grade	Response value	Co-efficient of infection	Reaction
1.	No symptoms	0	0	0-4.9	Highly resistant (HR)
2.	Very mild curling upto 25% leaves	1	0.25	5-9.9	Resistant (R)
3.	Curling and puckering of 51-75% of leaves	2	0.50	10-19.9	Moderately resistant (MR)
4.	Curling and puckering of 51-75% of leaves	3	0.75	20-39.9	Moderately susceptible(MS)
5.	Severe curling and puckering of > 75% of leaves	4	1.00	40-69.9	Susceptible (S)
				70-100	Highly susceptible (HS)

$$\text{Per cent disease infection (PDI)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Co – efficient of Infection (CI) = PDI x CRV

seedlings. Twenty-five days old healthy seedlings were transplanted in ¾ size pot. The pots were filled with steam sterilized soil mixed with an equal amount of coarse sand, which were kept in an insect-proof glasshouse. Whiteflies were released onto Tomato Leaf Curl Virus infected tomato plants for a 24 h virus acquisition access period. Meanwhile, individual tomato seedling was caged in a separate plastic tube (2×10 cm) with a provision (hole) to release whiteflies. About 10–15 viruliferous whiteflies were released into the cage and the hole was plugged with cotton wool to allow a 24 h virus inoculation access period (Muniyappa *et al.*, 1991). To assess the resistance symptom severity grades, designated with numerical values of 0-4 were given on the basis of visual observations. To quantify the disease severity, calculation were made as suggested by (Banerjee and Kalloo, 1987).

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Per se performance of parents, hybrids and check variety/hybrids under field condition :

Results of the field experiment revealed that the parent CLN 2123A (13.88), CLN 2123A X HN_2 (14.84) and HN_2 X CLN 2123A (16.18) registered the lowest values for per cent of disease infection at 75 days after planting. Similarly the genotypes *viz.*, CLN 2123A (6.52), CLN 2123A X HN_2 (6.94) and HN_2 X CLN 2123A (9.09) registered the lowest values for co-efficient of infection at 75 days after planting indicating that these genotypes fell under the category of resistant to leaf cur virus. The leaf curl virus resistant check Lakshmi registered 14.84 as percent of disease infection and 3.70 as co-efficient of infection. Whereas the other genotypes *viz.*, HN_2, COTH2, HisarLalit and CO 3 registered higher values of percent of disease infection and co-efficient of infection at 75 days after planting (Table 1) and fell under susceptible to highly susceptible category. The two test hybrids *viz*., CLN 2123A X HN_2 and HN_2 X CLN 2123A showed tolerance to ToLCV disease might be due to the involvement of the parent CLN 2123A. The parent CLN 2123A is a multiple cross derivative having the blood of *Lycopersicon hirsutum f. glabratum* a wild species resistant to leaf curl virus disease (Ragupathi and Narayanasamy, 2001).

Biometric traits *viz.*, plant height, the parent CLN 2123A (118.86 cm), the newly synthesized hybrids CLN 2123A X HN_2 (127.30 cm), HN_2 X CLN 2123A (127.86 cm), COTH2 (122.30 cm) and Lakshmi (134.03 cm) recorded higher values than other genotypes. Higher plant height observed by these genotypes might be due to tolerance nature of these genotypes to tomato leaf curl virus. The two newly synthesized hybrids CLN 2123A X HN_2 and HN_2 X CLN 2123A and check hybrid COTH2 registered higher plant height values even under high temperature might be due to the involvement of the parent CLN 2123A. This genotype includes one heat tolerant line and *Lycopersicon hirsutum* blood in its development, which resulted in higher plant height. While the other genotypes *viz.*, HN_2, LCR_2, HisarLalit and CO 3 recorded lower values for plant height might be due to higher incidence of tomato leaf curl virus and higher temperature during the growing period. Similarly, The same genotypes CLN 2123A X HN_2 and HN_2 X CLN 2123A also recorded higher values for number of branches per plant (13.73 and 14.20)and number of fruits per plant (49.09 and 53.07) (Table 2). The resistant check

hybrid Lakshmi recorded 12.10 numbers of branches and 41.47 numbers of fruits per plant. For the most important economic character yield per plant the two synthesized hybrids CLN 2123A X HN_2 (2867.06 g) and HN_2 X CLN 2123A (3296.86 g) excelled both the parents. It was interesting to note that the reciprocal cross excelled all the genotypes of the present study including leaf curl resistant check Lakshmi (2815.52 g) a private hybrid. Higher yield recorded by these two hybrids over parents and check hybrids might have due to increased plant height, number of branches per plant and number of fruits per plant as well as tolerance to tomato leaf curl virus disease. These results are in accordance with the findings of Banerjee and Kalloo (2000).

Per se performance of parents, hybrids and check variety/hybrids under pot culture :

Mean values of parents, hybrids and check variety/ hybrids for per cent of disease infection and co-efficient of infection after whitefly mediated inoculation (Table 2) showed that the lowest values for per cent of disease of infection and co-efficient of infection (13.25 and 4.26) were registered by the parent CLN 2123 A when compared to other parent HN_2 (56.69 and 76.66). Among the hybrids evaluated the direct cross CLN 2123 A X HN_2 recorded the lowest per cent disease infection as 9.84 when compared to reciprocal cross HN_2 X CLN 2123 A (15.33). However the two synthesized hybrids recorded similar value of 8.66 as co-efficient of infection. Incase of check variety/hybrids, the TLCVD resistant check Lakshmi recorded lower values of 15.66 as per cent of disease infection and 8.89 as Co-efficient of infection. The results indicated that these two hybrids are on par with the leaf curl resistant check Lakshmi for leaf curl virus incidence and fell under resistant category. The another check $COTH_2$ registered a percent of disease infection value of 22.66 and co-efficient of infection value of 18.75 and fell under moderately resistant

Table 1 : *Per se* performance of tomato genotypes for TLCV under field condition

Genotypes	Plant height (cm)	No. of branches /plant	Number of fruits / plant	Per cent of disease infection 75 DAP	Co-efficient of infection 75 DAP	Fruit weight (g)	Yield /plant (g)
CLN 2123A	118.86	8.06	41.88	13.88	6.52	40.00	2141.98
HN_2	94.33	6.76	22.88	45.00	72.50	56.88	1459.73
CLN 2123A x HN_2	127.30	13.73	49.09	14.84	6.94	53.70	2867.06
HN_2 x CLN 2123A	127.86	14.20	53.07	16.18	9.09	50.44	3296.86
LCR2	77.86	6.20	19.48	80.00	91.25	61.45	1335.74
COTH2	122.30	11.23	42.26	21.66	32.50	51.31	2455.05
Hisar Lalit	110.06	12.20	41.47	44.03	30.56	50.42	2402.88
Lakshmi	134.03	12.10	41.86	14.84	3.70	61.51	2815.52
CO3	72.36	5.93	20.18	52.93	92.63	53.46	1177.66
S.E. $\pm$	4.09	0.83	2.23	2.21	3.09	9.76	183.89
C.D. (P=0.05)	8.07	0.23	4.74	4.70	6.56	20.69	389.92

Table 2 : *Per se* performance of tomato genotypes for TLCV under pot culture

Varieties	Per cent of disease infection	Co-efficient of infection	Fruit weight	PO (Changes in OD min^{-1} g^{-1} of leaves)	PPO (Changes in OD min^{-1} g^{-1} of leaves)
CLN 2123A	9.84	4.26	45.70	0.507	0.328
HN_2	56.69	76.66	57.90	0.465	0.262
CLN 2123A x HN_2	13.25	8.66	54.60	0.665	0.392
HN_2 x CLN 2123A	15.33	8.86	56.10	0.690	0.395
LCR2	55.66	78.30	58.60	0.446	0.225
COTH2	22.66	18.75	52.50	0.654	0.486
HisarLalit	53.33	77.54	46.40	0.506	0.252
Lakshmi	15.66	8.89	61.30	0.694	0.396
CO3	58.33	79.33	53.00	0.354	0.142
S.E.$\pm$	0.86	1.46	0.25	0.0055	0.0016
C.D. (P=0.05)	1.82	3.10	0.55	0.0111	0.003

category. While the other genotypes *viz.*, HN_2 (56.69 and 76.66), LCR2 (55.66 and 78.30), HisarLalit (53.33 and 77.54) and CO 3 (58.33 and 79.33) registered higher values of percent of disease infection and co-efficient of infection and fell under susceptible category. These results are in accordance with the findings of Pico *et al.* (2001).Enzyme activities revealed that the tomato leaf curl disease tolerance genotypes *viz.*, CLN 2123A (0.507 and 0.328 changes in OD per minute per g. of leaves), CLN 2123A X HN_2 (0.665 and 0.392 changes in OD per minute per g. of leaves), HN_2 X CLN 2123 A (0.690 and 0.395 changes in OD per minute per g. of leaves), $COTH_2$ (0.654 and 0.486 changes in OD per minute per g. of leaves) and Lakshmi (0.694 and 0.396 changes in OD per minute per g. of leaves) recorded higher peroxidase and polyphenol oxidase activities. These results also proved the resistance/tolerance behaviour of these genotypes to leaf curl virus disease.

Peroxidase activity (changes in OD per minute per g. of leaves) :

The results of peroxidase activity (Table 3) in the white fly mediated plants of parents, hybrids and check variety/hybrids showed that peroxidase activity increased after artificial inoculation and continued to show increase in its activity till 96 hours. After that there was a slight decrease in the activity of peroxidase. Between the two hybrids tested the reciprocal cross HN_2 X CLN 2123 A recorded the highest peroxidase activity value of 0.690 in the pooled mean. It was closely followed by the direct cross CLN 2123A X HN_2 (0.665OD per minute per g. of leaves). Among the parents CLN 2123A registered the highest peroxidase activity of 0.507OD per minute per g. of leaves. With respect to check variety/hybrids the TLCVD resistant check Lakshmi showed the highest peroxidase activity of 0.694 OD per minute per g of leaveswhile the susceptible check CO 3 registered the

Table 3 : Peroxidase activity (changes in OD per minutes per gram of leaves)

Genotypes	Inoculated							
	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours	Mean	Control
CLN 2123A	0.342	0.489	0.525	0.567	0.576	0.540	0.507	0.312
HN_2	0.234	0.443	0.521	0.531	0.536	0.522	0.465	0.210
CLN 2123A x HN_2	0.392	0.550	0.682	0.767	0.808	0.793	0.665	0.340
HN_2 x CLN 2123A	0.454	0.596	0.688	0.780	0.821	0.801	0.690	0.410
LCR2	0.236	0.360	0.452	0.531	0.587	0.510	0.446	0.220
COTH2	0.393	0.530	0.620	0.767	0.821	0.793	0.654	0.356
Hisar Lalit	0.287	0.410	0.530	0.587	0.618	0.601	0.506	0.243
Lakshmi	0.455	0.590	0.710	0.501	0.808	0.802	0.694	0.420
CO3	0.200	0.320	0.352	0.412	0.431	0.411	0.354	0.190
S.E.±	0.0055	0.005	0.0059	0.006	0.006	0.0057	0.0055	0.0049
C.D. (P=0.05)	0.0111	0.011	0.012	0.012	0.012	0.011	0.0111	0.009

Table 4 : Polyphenol oxidase activity (changes in OD per minutes per gram of leaves)

Genotypes	Inoculated							
	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours	Mean	Control
CLN 2123 A	0.062	0.100	0.313	0.456	0.529	0.510	0.328	0.058
HN_2	0.043	0.060	0.210	0.352	0.489	0.420	0.262	0.031
CLN 2123 A x HN_2	0.092	0.130	0.386	0.532	0.610	0.600	0.392	0.085
HN_2 x CLN 2123 A	0.091	0.120	0.398	0.552	0.611	0.600	0.395	0.087
LCR2	0.031	0.061	0.137	0.312	0.413	0.398	0.225	0.020
COTH2	0.058	0.110	0.386	0.529	0.532	0.502	0.486	0.043
Hisar Lalit	0.048	0.053	0.229	0.341	0.430	0.410	0.252	0.030
Lakshmi	0.063	0.140	0.397	0.568	0.607	0.602	0.396	0.058
CO3	0.038	0.050	0.143	0.210	0.221	0.167	0.142	0.020
S.E.±	0.0014	0.0015	0.0017	0.005	0.0056	0.0054	0.0016	0.0014
C.D. (P=0.05)	0.0029	0.0031	0.0035	0.011	0.012	0.012	0.003	0.0028

lowest peroxidase activity as 0.354OD per minute per g. of leaves. Simultaneously peroxidase activity was estimated in the uninoculated control plants also. There were clear cut differences among the genotypes were observed for this enzyme activity (Table 2). Increased activity of peroxidase enzyme in resistant genotypes was also reported by Sundar *et al*. (1998).

Polyphenol oxidase activity (changes in OD per minute per g of leaves) :

Polyphenol oxidase activity (Table 4) was estimated in the direct as well as reciprocal cross and their parents along with resistant and susceptible check variety/hybrids and the results are presented in the Table 4. The results revealed that there were clear cut differences were observed among the genotypes evaluatedfor polyphenol oxidase activity. Among the hybrids, the reciprocal cross HN_2 X CLN 2123 A recorded the highest polyphenol oxidase activity of 0.395OD per minute per g of leaves and it was closely followed by CLN 2123 A X HN_2 (0.392 OD per minute per g of leaves). In case of parents CLN 2123 A registered the highest polyphenol oxidase activity (0.328OD per minute per g. of leaves) when compared to other parent HN_2 (0.262OD per minute per g. of leaves). The resistant and susceptible check variety/ hybrids evaluated for polyphenol oxidase activity showed that the TLCVD resistant check Lakshmi registered the highest polyphenol oxidase activity of 0.396 OD per minute per g of leaves, while the susceptible check CO 3 recorded the lowest polyphenol oxidase activity of 0.142OD per minute per g. of leaves. The enzyme estimated in the present investigation showed that resistant genotypes possessed higher PPO activity than susceptible genotypes.

Higher perxidase and polyphenol oxidase activity registered by the two newly synthesized hybrids indicates the tolerance nature to leaf curl virus disease. The tolerance/resistance nature of these two hybrids might be due to the involvement of the resistant parent CLN 2123A which one is a multiple cross derivative having the blood of *Lycopersicon hirsutum* a wild species resistant to leaf curl virus. It is also interesting to note that the peroxidase and polyphenol oxidase activities of both the hybrids were on par with the peroxidase and polyphenol oxidase activities of leaf curl virus resistant check Lakshmi.

The results inferred that the two test hybrids CLN 2123 A X HN_2 and HN_2 X CLN 2123 A showed tolerance to tomato leaf curl virus by registering low per cent of disease infection and co-efficient of infection underthe natural open field condition during summer month. Under artificial inoculation through white fly also the same hybrids registered the lowest value for per cent of disease infection and co-efficient of infection proved that these two hybrids are tolerance to tomato leaf curl virus disease. The activity of the defense enzymes *viz*., peroxidase and polyphenol oxidase were also found to be high with the two test hybrids.Hence, it could be concluded that the two newly synthesized tomato hybrids are suitable for growing during summer month with least Tomato Leaf Curl Virus disease incidence and higher yield.

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