

Biochemical factors for TLCV tolerance in tomato genotypes

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ABSTRACT

An experiment was conducted during 2000 – 05 to evaluate 25 tomato (*Lycopersicon esculentum* Mill.) genotypes for resistance to tomato leaf curl virus. The percent disease incidence and coefficient of infection in the genotypes was highest in early autumn (planting in last week of August) followed by spring-summer (planting in first week of February) and autumn-winter (planting in second week of October) seasons suggesting significant influence of environment on symptom expression apart from white fly population. The three moderately resistant lines emerged from the study viz., 'H-24', 'Agata' and 'EC-321425' showing very low coefficient of infection (2-6), were crossed with 5 highly susceptible testers with very high coefficient of infection (above 21), namely 'Punjab Chhuhara', 'Pusa Ruby', 'Ratan', 'Hisar Arun' and 'Patharkutchi' in line x tester mating design. The hybrids recorded a range of intermediate disease reaction and it varied widely in 2 seasons suggesting polygenic nature of disease resistance. Higher total phenol content in the leaves estimated at 80 days growth stage appeared to have determined the resistance actively in the host. Exploitable level of disease resistance could be achieved in very few hybrids involving moderately resistant x susceptible cross.

Key Words: Genotypes, bio-chemical, tolerance, resistance.

Tomato leaf curl and tomato yellow leaf curl virus, a heterogeneous complex of white fly vectored Gemini virus is a serious production constraint of tomato (*Lycopersicon esculentum* Mill.) in Asia, the Middle east and the Americas. Resistance is determined by combination of biochemical defense mechanisms, inhibition of long distance virus movement (Michelson *et al.*, 1994). Keeping the importance of leaf curl virus, the present research programmes have been framed to identify resistance source and determine the biochemical factors for host resistance.

MATERIALS AND METHODS

Six consecutive field level screenings of 25 tomato genotypes were carried out during 2000 – 2005 in randomized block design with 3 replications at Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. In three seasons, i.e early autumn (last week of August to January) with 27.5 to 32.9°C range of day temperature and 10.4 to 25.1°C range of night temperature, the average day/ night being 29.7°/18.6°C, autumn-winter season (second week of October to February) with 22.5 to 31.9°C range of day temperature and 8.4 to 22.4°C range of night temperature, the average day/night being 27.6°/15.1°C and spring-summer season (first week of February to May) with 27.3 to 42.3°C range of day temperature and 13.8 to 22.9°C night temperature, the average day/night temperature being 34.5°/19.2°C. Tomato leaf curl virus infected tomato plants of a highly susceptible local cultivar 'Patharkutchi' was maintained around the evaluation field to maintain sufficient virus inoculums. No insecticide was administered. Ten seedlings of 30 days age were transplanted in each replication in single row at 60 cm x 45 cm spacing. First appearance of disease symptom was recorded from each of the 10

plants per replication and average days after transplanting for the expression of disease symptom was noted. Assessment on the reaction of the genotypes to tomato leaf curl virus was oriented to three disease reaction parameters as per Kalloo and Banerjee (2000), viz per cent disease incidence (number of diseased plants/total number of plants x 100), per cent disease index (sum of numerical ratings/ (highest grade of ratings x total number of plants) x 100 and coefficient of infection. The coefficient of infection was calculated by multiplying the percentage disease incidence by the response value assigned to each severity grade (Kalloo and Banerjee, 2000).

On the basis of lowest coefficient of infection value over 6 screenings (2-6), 'H-24', 'Agata' and 'EC-321425' emerged as moderately resistant genotypes. These genotypes were used as lines and crossed with 5 highly susceptible testers revealing mean coefficient of infection above 21, viz. 'Punjab Chhuhara', 'Pusa Ruby', 'Hisar Arun', 'Ratan' and 'Patharkutchi' in line x tester mating design. The hybrids along with their parents were evaluated in both early autumn and autumn-winter seasons in the same manner as adopted for screening trials. A random leaf samples were collected in early autumn season 80 days after transplanting for estimation of ascorbic acid and total phenol contents following Sadasivam and Manicham (1996) and Bray and Thorpe (1954), respectively. We fixed the sampling date 80 days after planting because after this growth stage no new disease symptoms occurred in both tolerant and susceptible genotypes. The three disease reaction parameters viz, percent disease incidence, percent disease index and coefficient of infection recorded in the hybrids and the parental lines in early

autumn season were correlated with the ascorbic acid and total phenol contents of the leaves.

RESULTS AND DISCUSSION

The perusal of data on percent disease incidence, percent disease index (PDI) and coefficient of infection (CI) recorded in the 25 genotypes over 6 screenings clearly suggested significant moderate level of tomato leaf curl virus resistance in 'H-24', 'Agata' and 'EC-321523' compared to the average of 22 susceptible genotypes (Table 1). All the earlier findings (Kalloo and Banerjee, 2000; Raghupati and Narayanaswamy, 2000; Maruthi *et al.*, 2003) also suggested 'H-24' as moderately resistant variety. Hanson *et al.* (2000) could locate the resistant alleles in chromosome 11 of 'H-24' which was introgressed from *Lycopersicon hirsutum*.

The disease expression and its severity were highest in early–autumn season followed by spring-summer and autumn-winter season (Table 1). These results clearly showed that in addition to genetic make up of the plant, season and time of infection played a crucial role in phenotypic expression of the disease which agreed well to the results of the earlier experiments (Sastry and Singh, 1973; Pico *et al.*, 1996; Vidavsky *et al.*, 1998).

In the moderately resistant genotypes delayed expression of disease symptom (Table 1), low disease incidence and mild expression of disease symptom as judged by coefficient of infection (Table 1) operated for the manifestation of disease resistance. In corroboration to the present findings, Ansari *et al.* (2006) also recorded that time taken for a cultivar to exhibit the first appearance of symptom was directly correlated with symptom severity and in the susceptible cultivars exhibiting severe symptoms, the time taken for first appearance of symptom was less (10 – 20 days after inoculation). It was recorded that resistance to tomato yellow leaf curl virus consisting in attenuation and delay in time of symptom development was correlated with reduction in virus accumulation in the host plant (Lapidot *et al.*, 2001; Rubio *et al.*, 2003; Perez de Castro *et al.*, 2005). Tomato leaf curl virus is a ssDNA plant virus, a member of the family Geminiviridae of the genus Begomovirus and it replicates in the host cell (Gafni, 2003). Delay in symptom expression and lack of disease severity in the plant were the chief resistance manifestation of the host which might be due to significant delay in accumulation of viral DNA inside the plant and inhibition of long distance virus movement (Rom *et al.*, 1993; Michelson *et al.*, 1994) because all tomato cultivars and wild *Lycopersicon* species excepting *L. chilense* LA1969 support propagation and accumulation of various amounts of virus, although some wild *Lycopersicon* accessions

are symptomless (Zakai *et al.*, 1990; Vidavsky *et al.*, 1998).

Analysis of the mean values for ascorbic acid and total phenol content in the parents and hybrids with respect to the expected theoretical mean of the hybrids (Table 3) suggested that over dominance of higher ascorbic acid content and partial dominance of higher total phenol content were operative in the control of these two biochemical parameters.

Perusal of data (Table 4) clearly indicated that both ascorbic acid and total phenol content in the leaves had negative correspondence with PDI and CI in the line x tester population. So both ascorbic acid and total phenol contents in the leaves deserve due attention as a biochemical parameter in tomato leaf curl virus resistance breeding programme of tomato.

REFERENCES

- Ansari, N. A., Srivastava, G., Srivastava, A. K., Pandey, A., Shukla, D., Chaturvedi, R. C., Shukla, S., Yadav, A. K. and Tewari, J. P. 2006. Effect of temperature on the incidence of tomato leaf curl disease in some tomato varieties in Eastern Uttar Pradesh. *J. Living World* **13**: 41-46.
- Banerjee, M. K. and Kalloo, G. 1987. Sources and inheritance of resistance to leaf curl virus in *Lycopersicon*. *Theoretical Applied Genet.* **73**: 707-10.
- Boiteux, L. S., Oliveira, V. R., Silva, C. H., Makishima, N., Inoue Nagata, A. K., de Fonseca, M. E. N., de Giordano, L. B. 2007. Reaction of tomato hybrids carrying the Ty-1 locus to Brazilian bipartite Begomovirus species. *Horticultura Brasileira* **25**: 20-23
- Bray H G, and Thorpe W V. 1954. Analysis of phenolic compounds of interest in metabolism. *Methods of Biochemistry Analysis* **1**: 27-52.
- Chandra Shekara, A. C., Kulkarni, R. S., and Muniyappa, V. 2003. Triple test cross analysis of tomato leaf curl virus resistance in tomato (*Lycopersicon esculentum* Mill.). *Indian J. Genet. Pl. Breed.* **63**: 185-86.
- Gafni, Y. 2003. Tomato yellow leaf curl virus, the intracellular dynamics of a plant DNA virus. *Molecular Plant Patho.* **4**: 9-15.
- Hanson, P. M., Bernacchi, D., Green, S., Tanksley, S. D., Muniyappa, V., Padmaja, A. S., Chen, H. M., Kuo, G., Fang, D., and Chen, J. T. 2000. Mapping a wild tomato introgression associated with tomato yellow leaf curl virus resistance in a cultivated tomato line. *J. Ameri. Soci. Hort. Sci.* **125**: 15-20.

Table 1: Tomato leaf curl virus disease (TLCV) reaction parameters in moderately resistant and susceptible genotypes over six evaluations and mean percent disease index in three seasons

Genotype	Percent disease incidence	Percent disease index	Coefficient of infection
Moderately resistant			
H-24	15.42	13.43	1.42
Agata	20.90	20.19	4.13
EC-321425	22.75	18.72	3.06
Susceptible			
Av. of 22 genotypes	52.41	41.34	25.88

Genotype/season	Mean percent disease index in three seasons		
	Early autumn	Autumn-winter	Spring-summer
H-24	15.55 (52.53)	10.62 (67.61)	14.11 (42.48)
Agata	19.21 (42.73)	16.12 (59.55)	25.32 (39.08)
EC-321425	20.52 (43.56)	15.48 (58.53)	19.27 (47.08)
Av. of 22 genotypes	44.68 (24.25)	36.78 (28.71)	40.57 (25.68)

Days after planting for first visual symptom development in parenthesis

Table 2: Ascorbic acid and total phenol content in the leaves (mg / 100g fresh) of the Line x Tester population in early autumn season

Parental /F ₁ population	Leaf constituents		Parental /F ₁ population	Leaf constituents	
	Ascorbic acid	Total phenol		Ascorbic acid	Total phenol
H24	24.80	33.97	H24 x Ratan	27.20	27.69
Agata	15.60	32.90	H24 x PK	25.60	27.66
EC 321425 (EC)	16.80	28.01	Agata x PC	15.73	23.35
Punjab Chhuhara (PC)	13.81	18.46	Agata x PR	15.80	23.61
Pusa Ruby (PR)	14.80	27.52	Agata x HA	14.45	24.51
Hisar Arun (HA)	14.40	13.61	Agata x Ratan	15.60	24.99
Ratan	12.80	20.56	Agata x PK	14.13	23.73
Patharkutchi (PK)	13.60	17.28	EC x PC	19.40	24.61
H24 x PC	25.64	25.50	EC x PR	21.60	25.55
H24 x PR	24.10	27.14	EC x HA	17.20	23.17
H24 x HA	26.90	23.72	EC x Ratan	19.60	21.95
			EC x PK	17.80	22.68

SE Ascorbic acid = ±0.28; SE Phenol = ± 0.17.

Table 3: Mean values of the parents and hybrids and significance of mean difference.

Mean and variance of the genetic population	Ascorbic acid content in leaf (mg/100 g fresh weight)	Total phenol content in leaf (mg/100g fresh weight)
Line (resistant)	18.15	29.19
Variance	14.97	22.75
Tester (susceptible)	14.28	19.88
Variance	0.88	20.07
Significance of mean difference	t= 1.96	t= 2.99*
Hybrid (observed)	19.17	22.98
Variance	18.74	10.62
Hybrid (theoretical)	16.21	24.53
Significance of mean difference from that of line	t= 0.47	t= 2.49*
Significance of mean difference from that of tester	t= 4.64*	t= 1.45

* Significant at P= 0.05

- Kaloo, G. and Banerjee, M. K. 2000. Moderately leaf curl resistant variety of tomato (*Lycopersicon esculentum* Mill). *Veg. Sci.* **27**: 117-20.
- Kempthorne, O. 1957. *An Introduction to Genetic Statistics*. John Wiley & Sons. New York
- Michelson, I., Zamir, D., and Czosnek, H. 1994. Accumulation and translocation of tomato yellow leaf curl virus (TYLCV) in *Lycopersicon esculentum* breeding line containing the *L. chilense* TYLCV tolerance gene *Ty-1*. *Phytopatho.* **84**: 928-33.
- Lapidot, M., Friedmann, M., Pilowsky, M., Ben Joseph, R., and Cohen, S. 2001. Effect of host plant resistance to tomato yellow leaf curl virus (TYLCV) on virus acquisition and transmission by its whitefly vector. *Phytopatho.* **91**: 1209-13.
- Lapidot, M., Goldray, O., Ben Joseph, R., Cohen, S., Friedmann, M., Shlomo, A., Nahon, S., Chen, L. and Pilowsky, M. 2000. Breeding tomatoes for resistance to tomato yellow leaf curl begomovirus. *Bull. OEPP* **30**: 317-21.
- Maruthi, M. N., Muniyappa, V., Green, S. K., Colvin, J. and Hanson, P. 2003. Resistance of tomato and sweet-pepper genotypes to Tomato leaf curl Bangalore virus and its vector *Bemisia tabaci*. *Inter. J. Pest Management* **49**: 297-303.
- Perez, de., Castro, A., Diez, M. J. and Nuez, F. 2005. Evaluation of breeding tomato lines partially resistant to tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus derived from *Lycopersicon chilense*. *Canadian J. Pl. Patho.* **27**: 268-75.
- Nainar, P. and Pappiah, C. M. 2002. Inheritance of resistance to tomato leaf curl virus disease (TLCV) in the segregating generations of eight crosses of tomato (*Lycopersicon esculentum* Mill.). *South Indian Hort.* **50**: 72-77.
- Pilowsky, M., and Cohen, S. 1990. Tolerance to tomato yellow leaf curl virus derived from *Lycopersicon peruvianum*. *Pl. Disease* **74**: 248-50.
- Pico, B., Diez, M. J. and Nunez, F. 1996. Viral disease causes the greatest economic losses to the tomato crop. III. The tomato yellow leaf curl virus – A Review. *Scientia Hort.* **67**: 151- 96.
- Ragupathi, N. and Narayanaswamy, P. 2000. Screening of tomato germplasm to tomato leaf curl virus (TLCV) disease. *Madras Agril. J.* **87**: 715-17.
- Rom, M., Antignus, Y., Gidoni, D., Pilowsky, M. and Cohen, S. 1993. Accumulation of tomato yellow leaf curl virus DNA in tolerant and susceptible tomato lines. *Pl. Disease* **77**: 253-57.
- Rubio, L., Herrero, J. R., Sarrio, J., Moreno, P. and Guerri, J. . 2003. A new approach to evaluate relative resistance and tolerance of tomato cultivars to begomoviruses causing the tomato yellow leaf curl disease in Spain. *Pl. Patho.* **52**: 763-69.
- Sadasivam, S. and Manickam, A. 1996. *Biochemical Methods*. New Age International Pvt. Ltd., Tamil Nadu.
- Sastry, K. S. M. and Singh, S. J. 1973. Assessment of losses in tomato leaf curl virus. *Indian J. Mycology Pl. Patho.* **3**: 50-54.
- Vidavsky, F., Leviatov, S., Milo, J., Rabinowitch, H. D., Kedar, N. and Czosnek, H. 1998. Response to tolerant breeding line of tomato, *Lycopersicon esculentum*, originating from three different sources (*L. peruvianum*, *L. pimpinellifolium* and *L. chilense*) to early controlled inoculation by tomato yellow leaf curl virus (TYLCV). *Plant Breeding* **117**: 165-69.
- Zakai, Y., Navot, N., Zeidan, M., Kedar, N., Rabinowitch, H., Czosnek, H. and Zamir, D. 1990. Screening of *Lycopersicon* accessions for resistance to tomato yellow leaf curl virus: presence of viral DNA and symptom development. *Pl. Disease* **75**: 279-81.

Table 4: Correlation coefficient of disease reaction (PDI and CI) with leaf biochemical constituents (ascorbic acid and total phenol contents).

Character		Percent Disease Index (PDI)	Coefficient of Infection (CI)	Ascorbic acid (mg/100 g)
Coefficient of infection (CI)	G	0.450		
	P	0.445**		
Ascorbic acid (mg/100 g)	G	- 0.400	- 0.682	
	P	- 0.323*	- 0.557**	
Total phenol (µg/100 g)	G	- 0.417	- 0.609	0.997
	P	- 0.304*	- 0.500**	0.959**

*Significant in 1% level of significance

**Significant in 5% level of significance

G = Genotypic correlation coefficient

P = Phenotypic correlation coefficient