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Differential Pathogenicity amongst the Isolates of Tomato Leaf Curl Virus from Gujarat and Screening of the Most Potent Virus

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Abstract—*Tomato leaf curl virus (TLCV) has been identified as one of the most devastating pathogen causing tomato leaf curl disease (TLCD) in India. In the tomato growing regions of four agro climatic zones of Gujarat, plants were showing severe symptoms of TLCD such as vein clearing, dwarfing, puckering and curling of leaves, excessive branching, dropping of flowers and poor fruit setting. Worldwide about, 146 viruses belonging to 33 genera are reported to infect tomato. The genus Begomovirus contains more than 200 species and belongs to the taxonomic family Geminiviridae. Tomato crops are particularly susceptible to more than 50 different species of the Begomovirus genus, and among them to a group of species, responsible for Tomato yellow leaf curl disease. From India 21 strains of TLCV have been reported each showing different symptoms. Eighteen isolates of the virus from four different agro climatic zones of Gujarat were analyzed based on the types of symptoms and identified to be seven different strains. Each of the isolates were analyzed for their pathogenicity on eight different tomato varieties of tomato. Growth parameters such as plant height, percentage of leaves curled, number of branches per plant, number of trichomes/cm^2, number of clusters per plant, number of flowers per plant, delaying in flowers, physiological parameter such as chlorophyll content (mg%) were evaluated at a weekly interval. The total yield loss was calculated for each variety infected with each of the viral samples. The variation in virulence amongst the collected viral samples was determined and the most potent virus was identified.*

Keyword*: TLCD, TLCV isolates, Gujarat, Growth parameters, Yield loss study*

1. INTRODUCTION

Tomato (Solanum lycopersicum) belongs to the nightshade family Solanum lycopersicum is the 2nd most important vegetable crop in the world in terms of consumption. India ranks third in tomato production. In Gujarat, tomato is grown throughout the year and the contributes to 7% of the total production of the country. Although, the contribution of Gujarat has increased to 8.2% from 2010 to 2013 however, the productivity is still very low compared to other states (vegetable statistics technical bulletin).

Geminiviruses are the second largest family of plants infecting viruses comprising a group of single stranded circular DNA with twinned isometric particles, subdivided into 7 genera. Among them a begomoviruses, the largest group transmitted by vector whitefly causing devastating disease of economic importance. Further, they are classified into monopartite and bipartite. Tomato leaf curl disease has emerged as the major hindrance for tomato cultivation in India (Chakraborty, S. et al.).

The incidence of Tomato leaf curl viruses, in the symptomatic tomato plant species evaluated was up to 100%, in the field survey of the different agro climatic regions of Gujarat suggested that tomato leaf curl diseases as most devastating pathogen and climatic conditions as well as cultural practices play a vital role in infection (Shelat M). Tomato yellow leaf curl disease (TLCD) is the most severe loss causing agent characterized by dwarfing of plants, leaves curling, wobbling, chlorosis, reduction in the number of trichomes, reduction in the number of branches, number of clusters, leaves deformation, excessive branching, purpling of veins, dropping of flowers, reduction in number of fruits, reduction in size of fruits are the significant characteristics of TLCV. Variation may occur due to host gene resistance, viral recombination and agro climatic conditions.

The present study was done to establish the diversity of Tomato leaf curl virus infecting tomato plants from four different agro climatic zones of Gujarat.

2. MATERIALS AND METHODS

2.1 Collection of viral samples:

Eighteen isolates of the virus from four different agro climatic zones of Gujarat were analyzed based on the types of symptoms and identified to be seven different strains. Young leaves showing variation in symptoms were collected. Diseased plant samples were collected and used as a source of virus inoculum.

2.2 Isolation of viruses:

Isolation of the virus was done with modification in the technique of Honda et.al and saps were stored at low temperature.

2.3 Study of symptom and yield variation:

Healthy tomato seedlings (15 days old) were cultivated in nursery bags and kept in an insect proof net house. Tomato seedlings were mechanically inoculated with TLCV saps at a concentration of 420micrograms/plant. (Allam et.al) and development of symptoms were recorded. Growth parameters such as plant height, percentage of leaves curled, number of branches per plant, number of trichomes/cm2, number of clusters per plant, number of flowers per plant, delaying in flowers, physiological parameter such as chlorophyll content (mg%) were evaluated at a weekly interval. The total yield loss was calculated for each variety infected with each of the viral samples. Non inoculated plants for each of the eight varieties served as controls. The experiment was carried out in triplicates.

2.4 Classification of viruses based on the symptom severity:

Symptom severity was recorded according to the disease severity scale, where, Scale 0 = Mild viral strains, Scale 1 = moderately severe viral strains, Scale 2 = severe viral strain, Scale 3 = potent viral strain, Scale 4 = most potent viral strain.

Tomato plant samples from each healthy and infected with TLCV isolate were observed weekly to determine growth characteristics mentioned above.

2.5 Statistical analysis:

The experiment was set up in a completely randomized design. The mean values of growth parameters were calculated from three replicates and all other mean values in the study were calculated from three replicates.

3. RESULTS

3.1 Collection of viral samples:

Seven isolates out of eighteen isolates collected from four agro climatic zones were diverse in their symptoms and selected for the study.

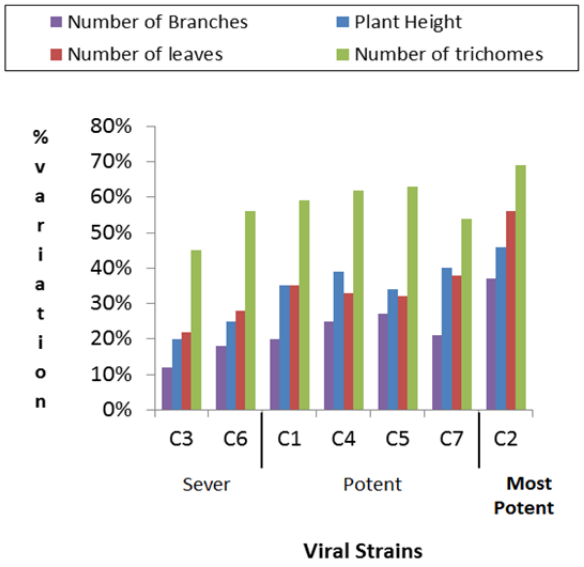
3.2 Isolation of viruses:

Virus concentration in the purification process was checked by measuring absorbance at 240 nm and set to a final concentration of 60mg/ml.

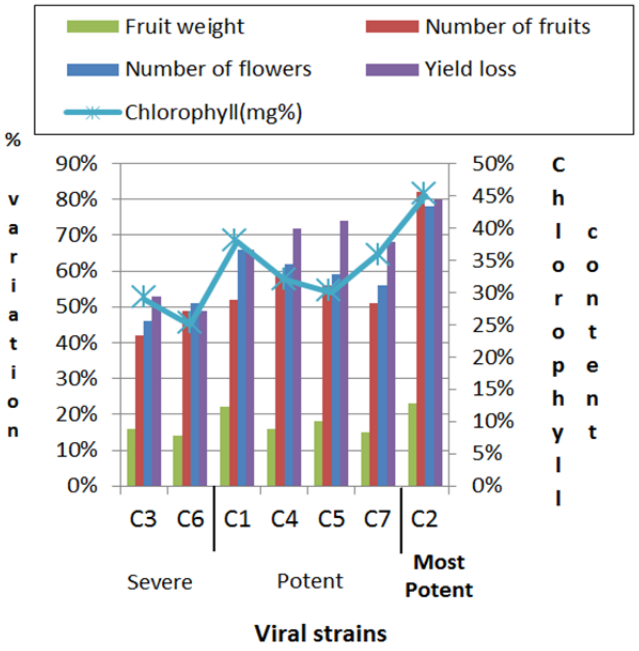
3.3 Study of symptom and yield variation:

The first symptoms of infection such as wobbling, yellowing and curling appeared on the uppermost younger leaves.

TLCV infection decreased leaf number in all the varieties. While the leaf numbers were nearly equal before inoculation, leaf number per plant was significantly reduced ranging from 22% to 28% for severe viral strains, 32% to 35% for the potent viral strains and 56% of the most potent viral strains (Fig1 A).



(a)



(b)

Fig. 1: Effect of Severe, Potent and Most Potent viral strains on the Tomato plants. (A) Effect of *TLCV* on number of branches, plant height, number of leaves and number of trichomes of Tomato plants (B) Effect of TLCV on number of flowers, number of fruits, fruit weight, % yield loss and chlorophyll content of Tomato plants

Reduction in plant height was a prominent symptom of *TLCV*. All the varieties were showing a reduction in plant height. The most potent viral strain caused maximum reduction in plant height with a reduction of around 46%. This was followed by reduction ranging from 34 - 40% by the potent viral strains, whereas, 20 – 25% by the severe viral strains (Fig1 A).

Results showed that variation in the number of trichomes for the most potent viral strain C2 to be 69% (Fig1 A).

The difference in the number of branches, varied from 37% for C2 viral strain, where as for potent viral strains, it was ranging from 20% to 27 % and in case of severe viral strains the variation was 12% and 18% compared to the uninoculated plants (Fig1 A).

Chlorophyll Content was also found to decrease in all the tomato varieties infected with *TLCV*. Maximum variation was observed as reduction by 45% due to infection of the most potent viral strain (Fig1 A).

3.4 Classification of viruses based on the symptom severity:

Viral isolates were classified under five different scales based on their growth parameters and yield parameters according to (Table 1).

Table 1: Classification of TLCV according to their Growth parameters and Yield Parameters

Scale	Scale 0	Scale 1	Scale 2	Scale 3	Scale 4
Viral strains	-	-	C3, C6	C1, C4, C5	C2

Scale 0 = Mild, Scale 1 = Moderately Susceptible, Scale 2 = Severe, Scale 3 = Potent, Scale 4 = Most Potent

C1, C4 and C5 were found to fall under Scale 3. C3 and C6 strains were found to fall under scale 2. One viral strain C2 was classified under Scale 4 and therefore considered the most potent virus.

4. DISCUSSION

Our study shows that tomato leaf curl virus significantly influenced growth parameters and yield parameters of tomato plants. Most of the growth parameters such as plant height, percentage leaflets curled, total number of leaves per plant, number of branches/plant, number of clusters/branch, chlorophyll content [mg%], number of trichomes (cm2), puckering, purpling of veins, clearing of veins were significantly influenced with preceeding virus infection. Our results are in agreement with those obtained by Czosnek et.al (1997).

Tajul, M. I. (2011) found that the TLCV alters phytochemical constituents in tomato fruits. In the present work, TLCV infection showed decreases in chlorophyll contents in infected tomato plants. It is well known that photosynthetic activity is directly related supply of energy and defense against infection reduction in leaf number, total leaf area due to curling and lower chlorophyll content influence the overall growth of these plants resulting in shorter and weaker plants

Our study supports the results that flower abscission was ranging from 46% to 78% for severe to the most potent viral strain, thus few fruits were produced. This result is similar to the results of the study of Al-Musa in which majority (up to 90%) of flowers abscise after infection. (1982).

Yield losses caused by TLCV vary between 50% and 80%, depending on the strain of the virus, the tomato cultivar and the growth stage at which plants became infected. Previous reports suggested yield loss to be related to the severity of the symptoms (Bos, 1967 and 1976). Our study supports these reports with the most potent viral strain showing maximum symptom severity. The severity of the virus is directly correlated to yield loss with less severe viral strains showing low yield loss.

5. CONCLUSION

In conclusion, it appears that TLCV infection causes a reduction in growth, and photosynthetic pigment. The variation in the yield parameter suggests that the yield loss may vary according to the potency of the viral strain tested. In general, the C2 Viral strain showed more symptom severity than other viral strains. So, C2 viral isolate was considered as the most potent viral strain across four different agroclimatic zones of gujarat .

6. ACKNOWLEDGEMENTS

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