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VIRUS TITER AS A DISEASE RESISTANCE INDICATOR IN TOMATO

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Abstract

Tomato leaf curl new Delhi virus, a geminivirus with a wide host range is a contagious pathogen of tomato, which also infects many other crops and weeds. Whitefly (*Bemisia tabaci* Gennadius), a polyphagous vector, is the agent responsible for its spread on a large scale. The pathogen is responsible for a major reduction in the yield of tomato. Ten commercial cultivars of tomato plant were selected to evaluate the effect of virus titer on crop yield. The yield potential along with other traits of these cultivars was assessed on the basis of symptom development, and virus DNA accumulation. The relationship between the virus titer, symptom severity, and agro-economic traits were established. The present results explain that the high level of virus accumulation in plant tissue results in the development of severe symptoms and leads to a major reduction in yield in case of susceptible cultivars, but this is not true for the cultivars showing intermediate resistance. The virus DNA remains low and approximately constant in resistant cultivars and has minimal effect on the yield and health of tomato.

Introduction

Tomato (*Solanum lycopersicum* L.) is universally treated as a vegetable, and is extensively grown as an annual plant. Tomato plants are herbaceous, annual to perennial, sexually (but occasionally asexually) propagated (Kimura and Sinha 2008). According to FAO survey in 2015, Pakistan produced 574052 tons of tomato from an area of 58196 ha (9.86 t/ha) during the year 2013. Tomato is susceptible to approximately 200 diseases (Aktar *et al*. 2016) caused by pests like fungi, bacteria and viruses. Many viruses also attack tomato crop and cause great economic losses. More than 20 viruses are known to infect tomato around the world and these losses reach up to 20-90%. Some of the most devastating diseases of tomato are attributed to viruses belonging to the family *Geminiviridae* (Picó *et al*. 1996, Varma and Malathi 2003, Nowakowska *et al*. 2014).

Among all of the reported diseases, Tomato leaf curl new dehli virus (ToLCV), a geminivirus (Geminiviridae: subgroup - III) is the most devastating pathogen of cultivated tomato (Reddy *et al*. 2005, Borah and Dasgupta 2012) commonly occurring in Pakistan. It can affect the plant at any growth stage and is responsible for approximately 70% yield loss in tomato grown in February - May (Tahir *et al*. 2012). If the infection occurs within the first four weeks of the transplantation, the yield losses may exceed 90 per cent.

ToLCV disease is characterized by the twisting, and curling of leaves followed by reduction in leaf size. The diseased plants look pale and stunted due to shortening of intermodal length with more lateral branches resulting in a bushy appearance. The whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) has been found to be the vector of the virus (Sastry and Zitter 2014). The vector life period is shortest during May-September being 11 - 14 days as against 43 - 83 days during the cold months of December - February (Mann 2011). Injurious strains of *B. tabaci* have appeared

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and spread from their home ranges to many other countries during the last 15 years. These strains have high fecundity and are more efficient as virus vectors than the indigenous population. In California, the newly appeared 'B' biotype of *B. tabaci* on the winter vegetable crop caused an estimated US $ 200 million losses in yield and continues to cause a loss of the US $ 130 million each year (Ramos *et al*., 2018). Tomato leaf curl disease is more serious in summer in the South and North part of Pakistan, but a high proportion of disease has also been observed in other seasons due to continuous and overlapping cultivation of tomato.

The host range of ToLCV and its vector *B. tabaci* is wide and can infect many annual and perennial plant species. They act as reservoirs throughout the year for both the vector and the disease (Muniyappa *et al*. 2000, Borah and Dasgupta 2012). The incidence of ToLCV is a major yield limiting factor because tomato plants of all ages are susceptible to the agent and show disease symptoms at 2 - 3 weeks after infection. Yield loss can be reduced by integrated disease management strategies like checking the vector population by using barriers, trap crops and application of systematic insecticides (Borah and Dasgupta 2012). However, coat protein mediated resistance by introducing the viral coat protein gene in crop plants is a consistent control against infection (Shin *et al*. 2002). Cloning and sequencing of the coat protein gene of the pathogen is a reliable method of knowing the existing variability among the virus isolates. The variability among the virus isolates and vector is the main reason for the breakdown of resistance (Pink *et al*. 1992, García-Arenal *et al*. 2001).

Though the disease has been known for a quite long period with a good amount of literature on various aspects of the disease, the long term strategies and additional creative approaches are needed now and then to reduce the losses currently sustained from ToLCV disease and information regarding the extent of the seasonal incidence of disease, epidemiology and type of vector / biotype of *B. tabaci* is scanty/meager in the tomato growing areas of Punjab. Therefore, the present study was planned and carried out to draw the attention of scientists to these objectives.

Materials and Methods

This experiment was conducted in the two consecutive years (2011 and 2012) at different locations of Punjab. Every set of experiment was replicated thrice following RCBD. Ten commercial varieties of tomato named as Tomato F_1 pound (V_1), Fayoum F_1 hybrid (V_2), Super special (V_3), Rio Grande (V_4), Tomato Fayoum (V_5), Tomato Remus (V_6), Tomato 1359 (V_7), Tomato Romaking (V_8), Raja (V_9) and Money Maker (V_{10}) were used. Seeds were collected from the Federal Seed Certification and Registration Department (FSCRD), Lahore, Pakistan.

The experimental field was well prepared by different agronomic operations to ensure the complete removal of weeds. Manure and fertilizer were added in the field at the rate of 10 ton/ha cow dung, urea, TSP and MOP was applied @ 550, 450 and 250 kg/ha, respectively. Plants were seeded in March in earthen pots under the greenhouse and transplanted in May.

Whiteflies were reared in the cages on cotton plants. These were given 36 hrs acquisition access period to Tomato leaf curlnew dehli virus (ToLCNDV) infected *S. lycopersicum.* These were then released on the tomato nursery at the 3rd leaf stage to ensure 100% infection at 50 whiteflies/plant. Control plants (non-inoculated) were not exposed to whiteflies. The inoculated plants were kept in the insect proof greenhouse for 14 days, and then transplanted in the field. Plant to plant and row to row distance was maintained at 40 and 100 cm, respectively. The plants were irrigated fortnightly with canal water. Insecticide sprays were applied to plants at 15 days interval throughout the experiment to control whiteflies (Verlaan *et al.* 2013).

Plants were harvested twice during the single season. Only mature red fruits were collected in the 1st harvest. While, both mature red and immature green fruits were collected in 2nd harvest which was done 15 days after the 1st harvest. The parameters like number of fruits, yield plant, individual fruit weight, and yield/ha were also studied. Data were recorded fortnightly for each parameter and analyzed statistically by ANOVA following DMRT (Hruschka 2017) using computer based software Statistix® 10.

Two hundred µg of leaf tissues were used to extract DNA by Cetyl tri-methyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Degenerative primers were used in PCR for preliminary identification of begomo virus infection (Rojas *et al*. 1993) as described previously (Fazeli *et al.* 2009). The presence of DNA-A genome components was detected using Tend (5' GGT ACC TAA GGA CCT GGG TTA TAG 3'), ToNDR (5' GGT ACC TGG ATA TGC TAG GTG TTA TAG 3') and CPF (5' ATG (C/A/T)(G/C)(G/C/A) AAG CG(A/T) (C/A)C(G/C) (A/C)GA TAT 3'), CPR (5' TTA ATT (T/G/C)(C/G/A)(A/T/C) (A/T/G)A(C/T) (A/T/C) (G/C) (C/A/T) (A/G)TC ATA (G/A)AA (A/G)TA 3'). Viral DNA in each band of the PCR product was quantified using Gel Analyzer software developed by Dr Istavan Lazar (Version 2010a). Standard values were provided to the software. The background level used as nil value for each measurement.

Results and Discussion

ToLCV usually infects the tomato crop resulting in severe yield reduction throughout the world/subcontinent. The loss observed in tomato grown field in February - May due to ToLCV attack was approximately 70% (Borah and Dasgupta 2012). However, yield loss exceeds 90%, when infection occurs within four weeks after transplanting in the field (Reddy *et al*. 2005). Here we report the results of ten selected commercial germplasms of tomato in which two cultivars V_6 and V_8 exhibited high resistance against ToLCNDV were reported. Level of resistance was assessed in the field trial by comparing the yield potential of healthy and infected cultivars.

Cultivars were screened for symptom development during the period of summer as this season favors the disease development. The recorded average temperature of the experimental station was around 30°C. There were fundamental differences in the onset and the degree of progression of symptoms in the selected cultivars (Sastry and Zitter 2014). In this study, cultivars V_1 and V_{10} were the first to show vein clearing, reduction in leaf size, stunted growth, deformation of leaflets (Fig. 1A, E), inward and outward curling and puckering of leaflets (Fig. 1B, D, C) and were declared susceptible. The plants having a mild susceptibility (V_2, V_5 and V_7) were next to produce curling and mosaic like symptoms on leaves (Fig. 1E). However, these varieties produced mild symptoms than those of susceptible varieties, whereas V_4 and V_9 varieties displayed varying levels of symptoms, mostly mild, while a few plants were asymptomatic overall and strong symptoms were very uncommon in these cultivars. Cultivar V_3 produced no yellowing and curling of leaves, but the inoculated plants showed stunted growth. In contrast V_6 and V_8 cultivars were absolutely symptomless and did not show stunting when compared with the healthy plants.

A major reduction in production cannot be based on a single parameter. The selected germplasms were evaluated for the degree of yield loss/ha caused by viral infection compared with healthy plants, as well as for the reduction in individual fruit weight, fruits/plant which ultimately caused yield loss (Varma and Malathi 2003). The experimental field conditions were favorable to pathogen for causing virulence *i.e.*, high inoculation pressure and inoculation at early growth stages. Symptoms were assessed throughout the summer season, whenever they were exhibited by plants susceptible cultivars V_1 and V_{10} produced extremely low yield as compared to inoculated resistant cultivars V_6 and V_8. These cultivars have the potential to produce growing and setting

fruit even under extreme virus inoculation conditions. V_6 and V_8 cultivars exhibited the highest level of resistance to ToLCNDV, inoculated plants barely exhibited the symptoms and suffered only 22 to 30% yield losses when compared with the healthy plants. Minor damage was observed in fruit/plant and individual fruit weight (Table 1, Fig. 2).

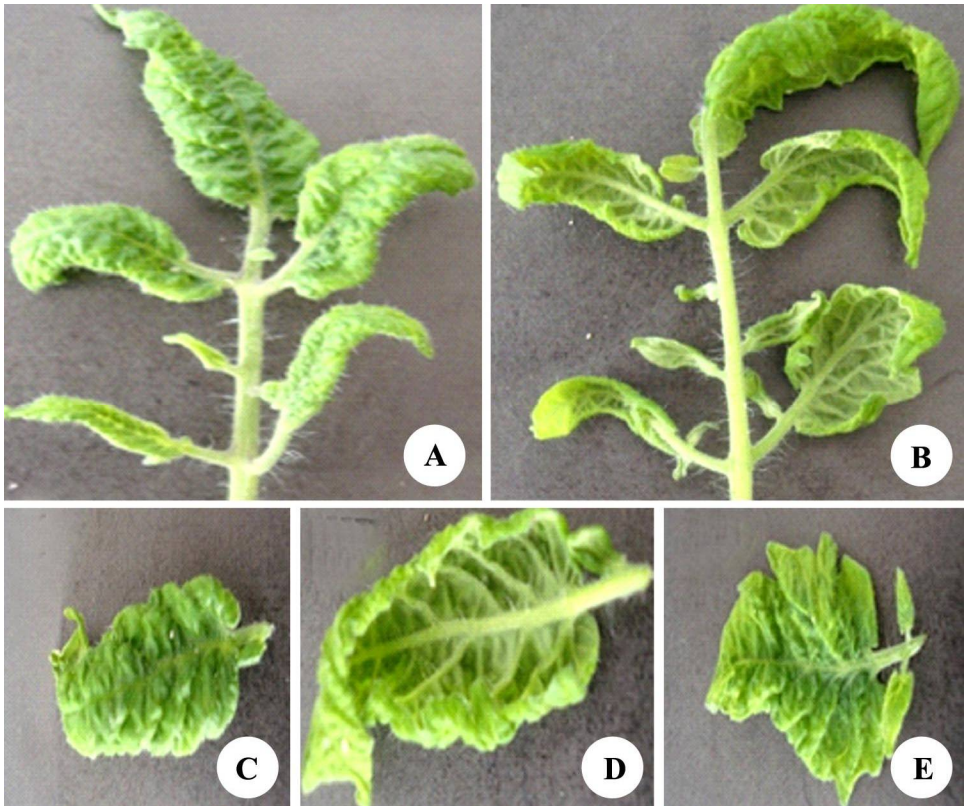

Fig. 1. Symptoms observed on tomato plants infected with ToLCNDV show stunted growth, deformed leaf lets, reduction of leaf size (A), inward and outward curling (B, D), puckering of leafs (C), mosaic and vein clearing (E).

Susceptible plants rarely showed normal growth, however some plants bore fruits but the weight, number and yield of fruits/plant, and yield/ha were significantly lower as compared to healthy plants (Ji *et al*. 2007). In the present study, fruits produced on infected V_5 cultivar were one third of that of the healthy plants both in yield/plant as well as in yield/ha (Table 1, Fig. 2). A comparison of inoculated plants with the control in terms of per plant and per hectare yield showed that cultivar V_8 followed by V_6 performed much better than other cultivars tested for resistance. The infected plants produced one third to one fourth yield per hectare as compared to healthy plants (Table 1, Fig. 2D).

Fargette *el al.* (1996) used serological study to categorize resistance level in geminiviruses (ToLCV) and found a relationship between the amount of virus and level of resistance in plant; they recommended that virus resistance can be evaluated by serological methods. Cultivar V_8 exhibited the best level of resistance, measured in terms of fruit weight, fruits/plant, yield/plant

Table 1. Effect of ToLCNDV against different yield parameters of tomato plant. Means followed by the same letter are not statistically significant ($p < 0.05$). Capital letters separate the means of control plants and small letters separate the means of inoculated plant.

Sl. No.	Cultivar/ variety	Fruits/plant		Ind. fruit wt. (g)		Yield (kg/plant)		Yield (t/ha)	
		Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
V_1	Raja	13.67D	8.50d	55.00D	43.00c	0.75E	0.37d	9.77D	4.75d
V_2	Fayoum F_1 hybrid	14.33D	8.80d	72.00BC	66.00b	1.03DE	0.58cd	13.41CD	7.55cd
V_3	Super special	26.10AB	8.10d	80.00B	75.00a	2.09AB	0.61c	27.14A	7.90c
V_4	Rio grand	19.00C	12.87c	96.77A	60.00b	1.84B	0.77bc	23.90A	10.04bc
V_5	Tomato fayoum	13.98D	8.10d	80.33B	67.13ab	1.12D	0.54cd	14.60C	7.07cd
V_6	Tomato remus	23.30BC	18.55b	61.00CD	47.10c	1.42C	0.87b	18.48B	11.36b
V_7	Tomato 1359	20.14C	13.05c	66.00C	41.35c	1.33CD	0.54cd	17.28BC	7.02cd
V_8	Tomato romaking	28.67A	24.58a	74.43BC	67.10ab	2.13A	1.65a	27.74A	21.44a
V_9	Tomato F_1 pound	25.16B	15.55bc	60.00CD	47.00c	1.51C	0.73bc	19.62B	9.50bc
V_{10}	Money maker	22.10BC	11.13d	55.00D	40.00c	1.22CD	0.45cd	15.80BC	5.79cd

and yield/ha when compared with non-inoculated plants (Table 1, Fig. 2). All other cultivars suffered significant loss in studied parameters when compared with healthy plants. V_2 and V_3 showed non-significant difference in fruit weight, but a significant difference was observed in fruits/plant (Table 1, Fig. 2A, B). V_1 and V_{10} cultivars were most affected in terms of the development of symptoms and reduction in the agro-economic traits as compared to healthy plants (Table 1, Fig. 2).

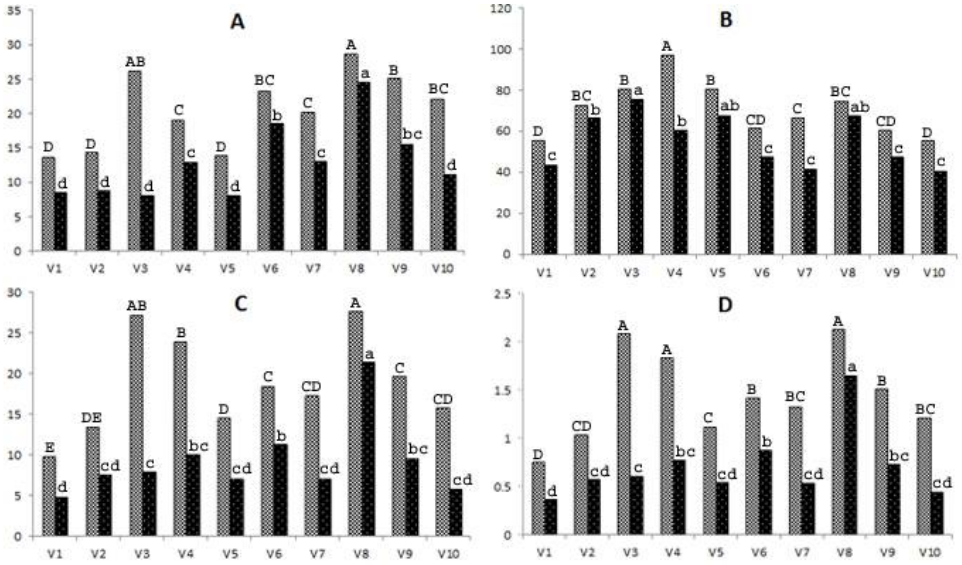

Fig. 2. Number of fruits/plant (A), individual fruit weight in gram (B), yield/plant in km (C) and yield/hectare in ton (D) was shown for Tomato F_1 pound (V_1), Fayoum F_1 hybrid (V_2), Super special (V_3), Rio Grand (V_4), Tomato Fayoum (V_5), Tomato Remus(V_6), Tomato 1359 (V_7), Tomato Romaking (V_8), Raja (V_9) and Money maker (V_{10}). Means followed by the same letter are not statistically significant ($p < 0.05$). Capital letters separate the means of healthy plants and small letters separate the means of infected plants.

Under extreme infection, V_6 and V_8 cultivars were able to grow well and produced 80% (V_6), 86% (V_8) of number of fruits, 77 (V_6), 90% (V_8) of individual fruit weight as compared with healthy control plants. Cultivar V_6 exhibited a high level of resistance but lower than cultivar V_8. Inoculated plants of cultivar V_6 exhibited more damage in terms of individual fruit weight and fruits/plant which resulted in a reduction in yield. Inoculated plants of other cultivars showed variable losses in yield like 52% (V_1), 44% (V_2), 71% (V_3), 58% (V_4), 52% (V_5), 59.4% (V_7), 52% (V_9) and 63.45 (V_{10}).

Ji *et al*. (2007) used the viral DNA concentration present in infected plants tissue for screening of *S. lycopersicon* accessions against viral inoculums. In the present study it was also found that positive relationship in level of resistance, as a proof by comparative loss of yield and viral DNA concentration. This correlation was not valid in case of intermediate resistance, because viral DNA was less in cultivar V_3 and V_7 than in cultivar V_5 and V_9 plants, however, cultivar V_5 and V_9 expressed more resistance than cultivar V_3 and V_7 in terms of fruits/plant, individual fruit weight, yield/plant and yield/ha (Tables 1, 2. Fig. 3). Moreover, the yield of cultivar V_2, V_5, V_7 and V_{10} were of the same level and differ non-significantly, but their viral DNA level was not same in any one of the cultivars suggesting that a reduction in virus titer is not only the factor that determine the level of resistance.

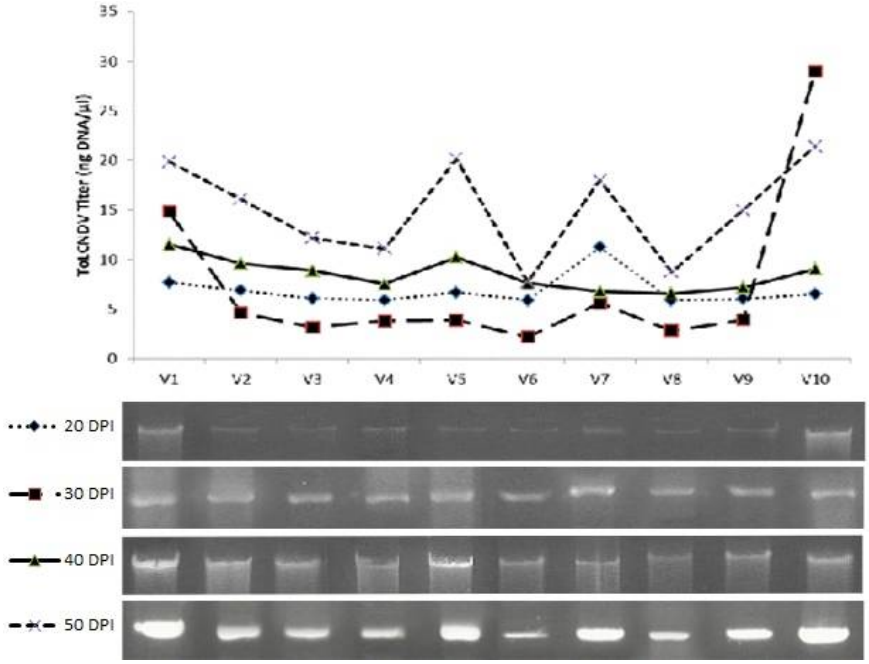

Fig. 3. ToLCNDV titer (ng DNA/µl) in the upper young leafs of different tomato cultivars at 20 days post inoculation (DPI), 30 DPI, 40 DPI and 50 DPI. Viral DNA was quantified by Gel Analyzer software as described in the text. Sampling was done at every 10th day starting at 20 DPI. PCR analysis results was shown below the graph, genomic fragment (~700bp) was amplified using begomovirus universal coat protein primers. Each amplified band at different time interval was shown under their respective cultivars, Tomato F1 pound (V_1), Fayoum F_1 hybrid (V_2), Super special (V_3), RioGrand (V_4), Tomato Fayoum (V_5), Tomato Remus (V_6), Tomato 1359 (V_7), Tomato omaking (V_8), Raja (V_9) and Money maker (V_{10}).

Table 2. ToLCNDVtiter (DNA ng/µl) in the upper young leafs of different tomato cultivars at 20, 30, 40, and 50 DPI.

Sl. No.	Cultivar/ cariety	Virus titer (DNA ng/µl)			
		20 DPI	30 DPI	40 DPI	50 DPI
V_1	Raja	7.716	14.832	11.51	19.88
V_2	Fayoum F_1 hybrid	6.892	4.606	9.6	16.074
V_3	Super special	6.07	3.194	8.89	12.194
V_4	Rio grand	5.896	3.796	7.554	11.104
V_5	Tomato fayoum	6.688	3.88	10.254	20.15
V_6	Tomato remus	5.912	2.214	7.612	7.732
V_7	Tomato 1359	11.26	5.584	6.816	17.986
V_8	Tomato romaking	5.894	2.814	6.562	8.748
V_9	Tomato F_1 pound	6.026	3.928	7.21	14.97
V_{10}	Money maker	6.522	28.98	9.076	21.444

DPI: Days post inoculation.

ToLCNDV DNA concentration in all cultivars was monitored at 20, 30, 40, 50 days after the inoculation. Virus titer was determined using Gel Analyzer software (Heras *et al.*, 2015). Virus titer determined the level of resistance and yield loss in test varieties of tomato under same environmental conditions (Fazeli *et al*. 2009). Resistant cultivars V_6 and V_8 showed a low level of viral DNA in their tissue when compared to other susceptible cultivars (Table 2, Fig. 3). The same results were reported by Wege (2007) for resistant plants in which they observed the positive correlation between symptoms severity and level of virus accumulation. ToLCNDV DNA concentration level peaked two times during the growth period of plants at 30 Days post inoculation (DPI) and 50 DPI in cultivar V_1 and V_{10} (Fig. 1). This is applicable only on susceptible varieties. The virus level in V_6 and V_8 was particularly constant and very low at 30 DPI. This explains the level of resistance in these two cultivars in terms of low virus titer and relatively small loss in yield.

The correlation between the viral DNA concentration and level of resistance held true for resistant varieties. The two best cultivars V_6 and V_8 had the lowest level of viral DNA in their tissues at different stages of growth. In the terms of yield performance, the correlation of improved resistance with low virus content was less applicable in case of moderate level of resistance. Cultivar V_4 at 20 DPI and 40 DPI while cultivar V_7 and V_8 at 40 DPI, had less viral DNA titer than cultivar V_6 and V8 and these cultivars exhibit lower level of resistance as expressed by yield parameters (Tables 1, 2. Figs 2, 3). The present study leads to the statement that viral DNA titer can serve as an indicator for the level of resistance but it cannot be concluded as a sole indicator.

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