Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Variability in indigenous Pakistani tomato lines and worldwide reference collection for *Tomato Mosaic Virus* (ToMV) and *Tomato Yellow Leaf Curl Virus* (TYLCV) infection

Variabilidade em linhagens indígenas de tomate do Paquistão e coleção de referência global para infecção por vírus do mosaico do tomateiro (ToMV) e vírus da folha amarela do tomate (TYLCV)

I. Hussain[a*], T. Farooq[a], S. A. Khan[a], N. Ali[a], M. Waris[b], A. Jalal[c], S. L. Nielsen[d] and S. Ali[c,e*]

[a]University of Haripur, Haripur, Pakistan
[b]Balochistan Agriculture College, Department of Plant Pathology, Quetta, Pakistan
[c]The University of Agriculture, Institute of Biotechnology and Genetic Engineering, Peshawar, Pakistan
[d]Aarhus University, Department of Agroecology, Slagelse, Denmark
[e]Hazara University, Deparment of Agriculture, Mansehra, Pakistan

Abstract

Local and exotic germplasm of tomato remains a major source for genetic improvement. Assessment of such lines for biotic stresses particularly viral diseases are the most important criteria for selection in Pakistan, where *Tomato Yellow Leaf Curl Virus* (TYLCV) and *Tomato Mosaic Virus* (ToMV) are the major diseases/viruses. A set of 40 accessions (including indigenous Pakistani lines and exotic germplasm from Europe, the United States, and Asia) were evaluated for their resistance/infection response to ToMV with artificial inoculation under greenhouse conditions. Infection response was quantified through disease scoring and DAS-ELISA test (for ToMV). A subset of 24 lines, was further screened for TYLCV using disease scoring and TAS-ELISA. The tested lines showed significant variability for resistance to ToMV. Only one accession (Acc-17878) was resistant to the ToMV whereas seven accessions i.e. Acc-17890, AVR-261, CLN-312, AVR-321, EUR-333, CLN-352, and CLN-362 expressed resistance to TYLCV. Correlation between phenotypic evaluation was confirmed by the ELISA results in both diseases, although both tools complemented to assess the viral infection status. In future, tomato breeding programs must consider breeding for ToMV and TYLCV resistance (using identified germplasm in our study) so as to deliver virus resistant tomato varieties.

Keywords: *Lycopersicon esculentum*, ELISA, artificial inoculation, disease severity, indigenous and exotic lines.

RESUMO

O germoplasma local e exótico do tomate continua sendo uma importante fonte de melhoramento genético. A avaliação de linhagens para estresses bióticos, particularmente as doenças virais, é o critério mais importantes para seleção no Paquistão, onde o vírus da folha amarela do tomate (TYLCV) e o vírus do mosaico do tomateiro (ToMV) são as principais doenças/vírus. Um conjunto de 40 acessos (incluindo linhagens indígenas do Paquistão e germoplasma exótico da Europa, dos Estados Unidos e da Ásia) foi avaliado quanto à resistência/resposta à infecção ao ToMV com inoculação artificial em casa de vegetação. A resposta à infecção foi quantificada por meio de pontuação da doença e de teste DAS-ELISA (para ToMV). Um subconjunto de 24 linhas foi posteriormente rastreado para TYLCV usando pontuação de doença e TAS-ELISA. As linhas testadas apresentaram variabilidade significativa para resistência ao ToMV. Apenas um acesso (Acc-17878) foi resistente ao ToMV, enquanto sete acessos (Acc-17890, AVR-261, CLN-312, AVR-321, EUR-333, CLN-352 e CLN-362) expressaram resistência ao TYLCV. A correlação entre a avaliação fenotípica foi confirmada pelos resultados do ELISA nas duas doenças, embora ambas as ferramentas tenham se complementado para avaliar o estado da infecção viral. No futuro, os programas de melhoramento de tomate devem considerar aperfeiçoamentos para resistência ao ToMV e TYLCV (usando germoplasma identificado em nosso estudo) de modo a fornecer variedades de tomate resistentes a vírus.

Palavras-chave: *Lycopersicon esculentum*, ELISA, inoculação artificial, severidade da doença, linhagens indígenas e exóticas.

*E -mail: izhar@uoh.edu.pk; bioscientist122@yahoo.com
Received: June 25, 2021 – Accepted: November 08, 2021

1. Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the major vegetable crops of Pakistan and India, being part of many dishes those are widely prepared around the sub-continent. Tomato was first introduced into Indian sub-continent by British colonists at the commencement of 19th century, with the first report about tomato farming in India in 1832, while in Punjab (Pakistan), it was first reported in 1916 in the book "Plants of the Punjab" (McCue, 1952). However, tomato production remains low in Pakistan, largely due to poor genetic material and lack of locally developed and adapted varieties. Genetic improvement of tomato has been the main priority of breeding programs in tomato growing areas (Agong et al., 2001). The improved varieties of any crop remain at risk to diseases due to the continuous adaptation and evolution of pathogens to the host resistance (Brown, 2015; Vallavieille-Pope et al., 2012). Tomato in Indian sub-continent is affected by many diseases, particularly those caused by viruses. Among viral diseases, the ones caused by *Tomato Mosaic Virus* (ToMV) and *Tomato Yellow Leaf Curl Virus* (TYLCV) are economically important and the most devastating (Arooj et al., 2017; Imran et al., 2013).

Tomato mosaic virus is a tobamovirus, a stable and RNA-virus with worldwide distribution (Hollings and Huttinga, 1976). The virion consists of capsid, which is un-enveloped and the nucleocapsid is elongated with helically symmetry. The typical symptoms of tomato mosaic include mosaic and curling of leaves and uneven fruit ripening. The virus is transmitted through mechanical methods, grafting and seed (Büchen-Osmond, 2003). Severe infection could lead to 25% loss, depending on the environment and host genotypes (Imran et al., 2013). This disease along with TYLCV represents a big risk to tomato production throughout the country (Imran et al., 2013).

Tomato Yellow Leaf Curl Virus is a Geminivirus, also associated with huge economic loss in tomato crops (Cohen and Harpaz, 1964; Czosnek et al., 1988). TYLCV species have been assigned to the Genus Begomovirus, based on the molecular diagnostic data. TYLCV is a monopartite virus with geminate, quasi- isometric particles of 20-30nm (A. et al., 1990). The disease caused by TYLCV has variable symptoms with severe stunted growth along with reduced leaf size and deformation (upward cupping) and discoloration. Intensity of the disease and thus expression of the symptoms could be variable as influenced by the time of infection, plant growth stage and prevailing environmental conditions. The disease could lead to 100% losses under the climatic conditions prevalent in the warm temperate and tropical zones.

Transmission of the virus occurs through whitefly (*Bemisia tabaci*), where the virus infects and circulate within the insect vector (Cohen and Nitzany, 1966). The worldwide spread of the insect vector is considered to be associated with the increased incidence of TYLCV into Europe (Bellows Junior et al., 1994). In Europe, the disease/virus has been reported in major tomato growing regions of France, Spain, Portugal and Italy. At the global scale the disease is prevalent in North African and Sub-Saharan countries, Australia, South America and Asia (Mabvakure et al., 2016). The disease is quite frequent in the tomato growing countries of Asia, including Pakistan. Tomato crop faced severe infestation of TYLCV in Sindh province during 2012-13 in Pakistan. During the menace, tomato production in the province reduced up to approximately 19%, which significantly affected yield by devastating the planted crops on 22.5 thousand hectares alone in Sindh province. Thus, management of the two viral diseases are inevitable to attain a sustainable high yield of tomato in Pakistan.

Like many other horticultural crops, tomato diseases could be managed through various measures including cultural practices, phytosanitary chemicals, and genetic resistance (Hovmøller and Henriksen, 2008). However, considering the human health and environmental aspects of agrochemical use, coupled with the labor, time and resource investments in cultural management, development and deployment of genetic resistance is favorable option, particularly in developing countries like Pakistan (Ali et al., 2007; Hovmøller and Henriksen, 2008; Iqbal et al., 2020; Kutcher et al., 2018). In tomato, the use of genetic resistance to combat viral disease has been advocated (Segbefia et al., 2018), however, development and deployment of resistant varieties require regular assessment of germplasm with viral diseases to identify sources of durable resistance.

Numerous studies have been undertaken to assess diversity for morphological and yield potential in indigenous tomato germplasm (Iqbal et al., 2014; Ullah et al., 2016), the reaction of the indigenous Pakistani accessions to ToMV and TYLCV under artificial inoculation, in relation with the exotic lines introduced from various parts of the world is limited. Therefore, it is imperative to assess the reaction of the indigenous Pakistani accessions as well as exotic ones to ToMV and TYLCV under artificial inoculation and the controlled environmental conditions. These lines could represent variable sources of resistance and needs to be assessed under both field and greenhouse conditions.

The present study was conducted to assess the resistance status in a set of indigenous and exotic tomato accessions to ToMV and TYLCV, with artificial inoculation under greenhouse conditions. Our objectives were to assess: i). the status of ToMV and TYLCV resistance in tomato accessions, ii). the relationship of diversity in relative resistance status with the origin of these accessions, and iii). the correlation of phenotypic assessment with enzyme-linked immunosorbent assay (ELISA) based characterization. In this paper we describe the resistance status against ToMV and TYLCV in tomato accessions from various geographical origins.

2. Materials and Methods

2.1. Germplasm selection

A set of 40 indigenous accessions were selected for the study, including accessions collected from Pakistan (obtained from Plant Genetic Resource Institute (PGRI), Islamabad, Pakistan) and exotic varieties from Federal Seed Certification & Registration Department (FSC & RD), Islamabad, Pakistan (Table 1). Three local types were

Table 1. Details of tomato accessions screened against tomato mosaic virus (ToMV) and tomato yellow leaf curl virus (TYLCV).

No.	Category	Country	Accession	Viral screening	Source
1	Indigenous line	Pakistan	Acc-06232	ToMV	PGRI, Pakistan
2	Indigenous line	Pakistan	Acc-10572	ToMV	PGRI, Pakistan
3	Indigenous line	Pakistan	Acc-10587	ToMV& TYLCV	PGRI, Pakistan
4	Indigenous line	Pakistan	Acc-17867	ToMV& TYLCV	PGRI, Pakistan
5	Indigenous line	Pakistan	Acc-17870	ToMV& TYLCV	PGRI, Pakistan
6	Indigenous line	Pakistan	Acc-17872	ToMV	PGRI, Pakistan
7	Indigenous line	Pakistan	Acc-17874	ToMV	PGRI, Pakistan
8	Indigenous line	Pakistan	Acc-17877	ToMV& TYLCV	PGRI, Pakistan
9	Indigenous line	Pakistan	Acc-17878	ToMV	PGRI, Pakistan
10	Indigenous line	Pakistan	Acc-17879	ToMV	PGRI, Pakistan
11	Indigenous line	Pakistan	Acc-17882	ToMV	PGRI, Pakistan
12	Indigenous line	Pakistan	Acc-17883	ToMV& TYLCV	PGRI, Pakistan
13	Indigenous line	Pakistan	Acc-17889	ToMV& TYLCV	PGRI, Pakistan
14	Indigenous line	Pakistan	Acc-17890	ToMV	PGRI, Pakistan
15	Indigenous line	Pakistan	Acc-19288	ToMV& TYLCV	PGRI, Pakistan
16	Indigenous line	Pakistan	Acc-19289	ToMV& TYLCV	PGRI, Pakistan
17	Indigenous line	Pakistan	Acc-19290	ToMV& TYLCV	PGRI, Pakistan
18	Indigenous line	Pakistan	Acc-19893	ToMV	PGRI, Pakistan
19	Indigenous line	Pakistan	Acc-19912	ToMV	PGRI, Pakistan
20	Indigenous line	Pakistan	STM-1	ToMV	Collected, Pakistan
21	Indigenous line	Pakistan	STM-2	ToMV& TYLCV	Collected, Pakistan
22	Indigenous line	Pakistan	STM-3	ToMV	Collected, Pakistan
23	Taiwan, AVRDC,	China	AVR-201	ToMV& TYLCV	FSC&RD, Pakistan
24	Taiwan, AVRDC,	China	AVR-211	ToMV& TYLCV	FSC&RD, Pakistan
25	Taiwan, AVRDC,	China	AVR-241	ToMV& TYLCV	FSC&RD, Pakistan
26	Taiwan, AVRDC,	China	AVR-251	ToMV& TYLCV	FSC&RD, Pakistan
27	Taiwan, AVRDC,	China	AVR-261	ToMV	FSC&RD, Pakistan
28	Taiwan, AVRDC,	China	AVR-321	ToMV	FSC&RD, Pakistan
29	Taiwan, AVRDC,	China	AVR-341	ToMV	FSC&RD, Pakistan
30	USA	USA	CLN-222	ToMV	FSC&RD, Pakistan
31	USA	USA	CLN-232	ToMV	FSC&RD, Pakistan
32	USA	USA	CLN-272	ToMV	FSC&RD, Pakistan
33	USA	USA	CLN-282	ToMV& TYLCV	FSC&RD, Pakistan
34	USA	USA	CLN-292	ToMV& TYLCV	FSC&RD, Pakistan
35	USA	USA	CLN-312	ToMV	FSC&RD, Pakistan
36	USA	USA	CLN-352	ToMV	FSC&RD, Pakistan
37	USA	USA	CLN-362	ToMV	FSC&RD, Pakistan
38	Europe	Netherlands	EUR-303	ToMV	FSC&RD, Pakistan
39	Europe	Netherlands	EUR-333	ToMV	FSC&RD, Pakistan
40	Commercial hybrid	Netherlands	Sahel	ToMV	Syngenta

PGRI = Plant Genetic Resource Institute, Islamabad, Pakistan; AVRDC = Asian Vegetable Research and Development Centre, Taiwan (China); FSC&RD = Federal Seed Certification & Registration Department, Islamabad, Pakistan.

collected from Swabi district of Khyber Pakhtunkhwa province by the authors and one commercial hybrid from the Netherlands (named Sahel in Pakistan), used as check, was obtained from Syngenta® International. All 40 lines were tested for susceptibility to ToMV, while a subset of 24 accessions were further tested with TYLCV under greenhouse conditions and with ELISA test to identify their virus resistance level.

2.2. Screening with ToMV

To assess the response of the tomato accessions to the viral infection under controlled environmental conditions, all 40 tomato accessions were grown under greenhouse conditions and were artificially inoculated. The seed coat was disinfected for the ToMV by treatment with 1% NaOCl for five minutes, transferred to 0.1N HCl for 10 min, and washed eight times with distilled water. All the plants were tested for viral infection before inoculation with Double Antibody Sandwich-Enzyme-linked Immune-Sorbent Assay (DAS-ELISA) test to further confirm their virus free status, using the LOEWE test kit. On the very next day, leaf inoculation was performed by rubbing infected plant leaf extract and carborundum powder on the leaves, which were properly tagged. Four plants per accession were seeded in the greenhouse under controlled environment in completely randomized design (CRD). Two pre-tested ToMV free plants were included in each experiment as negative control, while the known susceptible line ToMV-99-01 was included as positive control. Both visual scoring and DAS- ELISA test were used to assess the viral infection.

Infection was recorded 2-3 weeks after inoculation, based on symptoms as follows, local symptoms, systematic symptoms, leaf chlorosis, leaf necrosis, ring spots, leaf bubbling, vein clearing, and stunting. Leaf samples from each accession for DAS-ELISA test were collected separately to avoid contamination from other accessions. The ELISA procedures were performed in accordance with the protocols of the firms. Sample buffer was added to each sample separately and crushed to a uniform leaf extract for DAS-ELISA test. This color development was evaluated visually as well as measured in a spectrophotometer at 405 nm after 1-2 h. The same procedure for DAS-ELISA test was followed at 10^{th}, 12^{th} and 16^{th} leaf stages. Scoring was also made based on symptomatic expression of the disease on a 0-4 scale.

2.3. Screening for TYLCV

A subset of 24 accessions were screened to assess their level of resistance to TYLCV in controlled environment in greenhouse at the Department of Agroecology, Flakebjerg, Research Institute of Aarhus University, Denmark. The 24 accessions were selected as they showed resistance to ToMV. The experiment was conducted in Complete Randomized Design (CRD) with two replications (one pot with one plant). During the experiment in December no outdoor populations of White Fly (*Bemisia tabaci*), the vector of the disease (Cohen and Harpaz, 1964), was present in Denmark, and mechanical inoculation is usually not successful (Avgelis et al., 2001); therefore, grafting healthy plants cuttings on infected rootstock was used for inoculation (Kashina et al., 2007b). Disinfected seed of 24 accessions were sown, where at the second permanent leaf stage pre-grafting samples from two plants of each accession were taken and tested, using Triple Antibody Sandwich-Enzyme-linked Immune-Sorbent Assay (TAS-ELISA), using the DSMZ test kit. After pre-grafting sampling, grafting was practiced on the TYLCV infected rootstock material of all 24 accessions as previously described (Kashina et al., 2007a). Longitudinal italic cuts from two sides were made on the root stock infected with TYLCV to form a V-shape. Same type of cut was made on scion from each accession, using sterile razor blade. The scions were then inserted in root stock V-shape cut in such a way that there was no space between the exposed tissues and have close contact with each other. Budding tape was used for the union to hold the scion and graft (Figure 1). The grafted plants were maintained in glasshouse at 20-30ºC and 72.4% relative humidity, for 5 weeks to ensure graft success, viral incidence and symptoms severity on each accession (Friedmann et al., 1998). After successful grafting and clearly visible symptoms, TAS-ELISA test was carried out to measure the resistance and susceptibility levels of all 24 accessions. Scoring was also made based on symptomatic expression of the disease on a 0-4 scale.

2.4. Interpretation of the ELISA test and data analyses

The ELISA test was recorded positive when the absorbance value of the sample was equal or greater than two times the absorbance of the healthy control. The phenotypic data was analyzed in MS Excel® and the R-statistical environment (Fox and Leanage, 2016).

3. Results

Significant variability was observed among the genotypes for their response to ToMV and TYLC infection. ToMV infection response was not correlated with TYLCV infection, under the tested conditions.

3.1. Progress of ToMV infection over time using DAS-ELISA test

Comparison of lines from different geographical origin revealed that the progress of ToMV over time varied significantly at different leaf stages i.e., Leaf 2, Leaf 6, Leaf 12 and Leaf 14, as assessed through DAS-ELISA test (Figure 2). Results indicated significant differences in overall mean values. Mean values for all 40 accessions ranged from 1.2 (Acc-17878) to 8.3 optical density (OD) (Acc-17870) with a total difference of 7.1 OD, followed by Acc-17883 (3.3 OD) at the minimum and Acc-10587 (7.7 OD) at the maximum (Figure 2).

European accessions EUR-303 and EUR-333 had almost similar level of ToMV infection (Figure 2). Pre-inoculation at two leaf stage by DAS-ELISA test, resulted values of 0.92 OD and 0.97 OD for both accessions EUR-303 and EUR-333, which was less than two (<2), confirming absence for ToMV. Post-inoculation at sixth leaf stage, resulted high value of 8.0 OD for accession EUR-303 and then slightly reduced to 5.3 OD at 14^{th} leaf stage, but still it was in the range of

Figure 1. Tomato plants used for graft-mediated viral transmission and subsequent screening analysis.

positive ToMV infection. Similar post-inoculation results were shown by EUR-333 but with a little bit difference from EUR-303 at 12th leaf stage; whereas, DAS-ELISA test result value 7.0 OD greater than the previous value of 6.4 OD at sixth leaf stage, again a slight decrease at 14th leaf stage with value 5.7 OD. All the results in case of accession EUR-333 were positive for ToMV. Indigenous accessions showed significant differences at different leaf stages as well as in overall mean performance, during screening against ToMV, using DAS-ELISA test (Figure 2). Mean values of indigenous accessions ranged from 1.2 (Acc-17878) to 8.3 OD (Acc-17870) with a total difference of 7.1 OD, followed by Acc-17883 (3.3 OD) at the minimum and Acc-10587 (7.7 OD) at the maximum.

At two leaf stage, all the indigenous accessions showed absence for ToMV. Only accession Acc-17878 was found with low OD values at all leaf stages with a mean value of 1.2 OD, confirming a lack of the virus. The remaining indigenous genotypes were all positive, but two distinct patterns of the disease were observed. Accessions Acc-17867, Acc-17872, Acc-17874, Acc-17879, Acc-17882, Acc-19912, Sahel, STM-1, and STM-3 showed significant increase in disease severity from second permanent leaf stage after inoculation to 12th leaf stage and a slight decrease at 14th leaf stage (Figure 3). The remaining indigenous accessions i.e., Acc-06232, Acc-10572, Acc-10587, Acc17870, Acc-17877, Acc-17878, Acc-17883, Acc-17889, Acc-17890, Acc-19288, Acc-19289, Acc-19290, Acc-19893, and STM-2 disease severity was high at sixth leaf stage and then decreased significantly at 12th and 14th leaf stage. All the Taiwan accessions were positive for ToMV at different leaf stages except at two permanent leaf stage. A unique pattern of disease incidence was observed for AVR-341, where up to sixth leaf stage the OD value was 1.1, considered to lack any viral infection. The infection increased at 12th leaf stage (7.3 OD), but significant decline was recorded at 14th leaf stage. Accessions AVR-201, AVR-241, and AVR-251 showed relatively stable expression for disease incidence.

Accessions CLN-222, CLN-232, CLN-272, CLN-282, CLN-292, CLN-312, CLN-352, and CLN-362 from the United

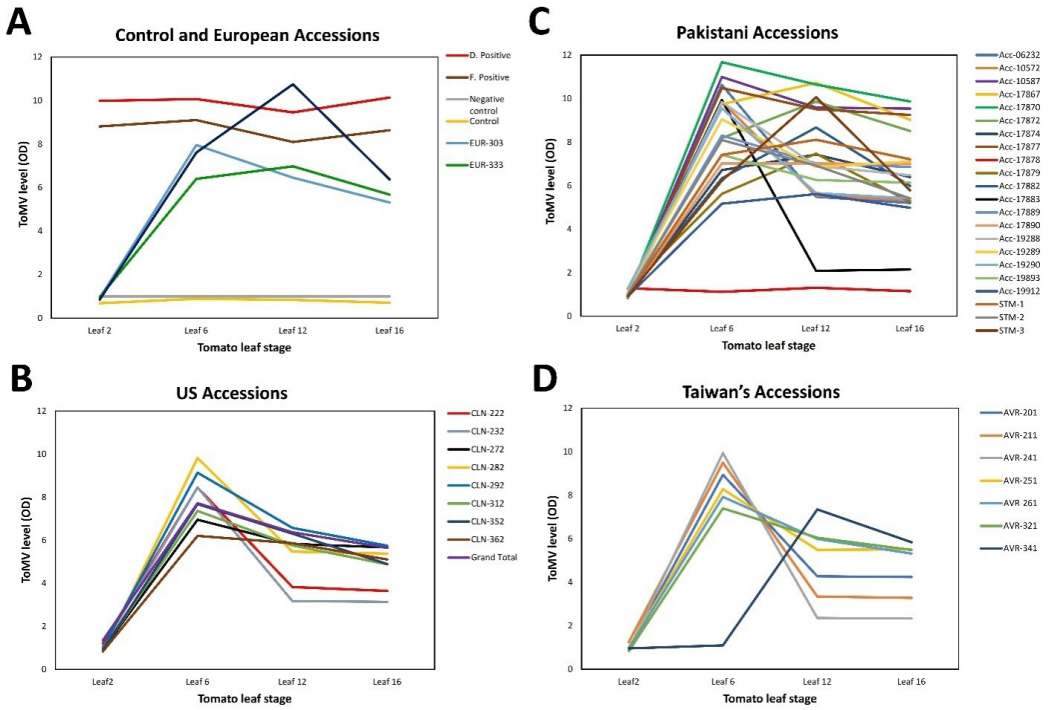

Figure 2. Screening results with ToMV at different leaf stages in tomato accessions. A. Positive and negative controls and European accessions, B. US accessions, C. Pakistani accessions, D. Taiwan's accessions.

States showed positive result for ToMV, with high infection at 6th leaf stage, with the maximum and the minimum values of 9.8 and 6.2 OD for the accessions CLN-282 and CLN-362, respectively. All the US accessions showed significant decline in disease incidence at 12th and 14th leaf stage (Figure 2).

3.2. Symptomatic ToMV infection and its correlation with DAS-ELISA results

Considering the overall parameters i.e., ToMV symptoms, disease severity index, and percentage of infection, none of the accessions was found asymptomatic. Only accession Acc-17878 was resistant with no clear symptoms (Figure 2A), disease severity index of 1 and disease infection ranging 1-10%. None of the accessions showed sparse light-yellow spots, which comes under disease severity index two ranging infection from 11-20% with moderate resistance (MR) of the host. Acc-17883 expressed clear visible yellow patches on leaves, leaf bubbling, and a little bit chlorosis was observed which fulfils disease severity index three, falls in the range 20-30% of infection showing moderate susceptibility (MS) of host reaction (Figure 3A).

Disease symptoms such as leaf and vein chlorosis followed by necrosis were observed on accessions EUR-303, EUR-333, AVR-201, Acc-06232, Acc-10572, AVR-211, Acc-17874, Acc-17879, AVR-241, Acc-17882, Acc-17889, AVR-251, Acc- 17890, Acc-19288, AVR-261, Acc-19290, Acc-19893, AVR-321, Acc-19912, STM-1, AVR-341, STM-2, STM-3, CLN-222, CLN-232, CLN-272, CLN-282, CLN-292, CLN-312, CLN-352, and CLN-362 with percent infection in the range of 31- 50%, which have disease severity index four and susceptible (S) host reaction.

Accessions Acc-10587, Acc-17867, Acc-17870, Acc-17872, Acc-17877, and Sahel were susceptible, which was also confirmed through the high value of dry positive and fresh positive. Severe chlorosis and necrosis, stunted plant growth and young leaves deformation with more than 50% infection, which fulfilled category five of the disease severity index was observed for the accessions (Figure 2A). An overall concordance was observed between the results obtained through phenotyping and DAS-ELISA results (Figure 3B).

3.3. Assessment of TYLCV infection in tomato accessions

Compared to ToMV, the infection of TYLCV was low on many tomato genotypes (Figure 4A). No visible symptoms for TYLCV infection were observed for accessions Acc-17890, AVR-261, CLN-312, AVR-321, EUR-333, CLN-352, and CLN-362, and had normal growth and leaf size. These symptoms-based results were further confirmed by TAS-ELISA test, which showed lack of infection for these aforementioned accessions with values of 0.132, 0.123, 0.142, 0.127, 0.120, 0.121 and 0.132 OD respectively. These accessions were placed in the category of zero (asymptomatic) or resistant to TYLCV.

Among others, eight accessions showed slight infections with mild symptoms (with interveinal chlorosis on apical leaves only) on accessions Acc-06232, Acc-17872, Acc-17874, Acc-19893, EUR-303, AVR-341,

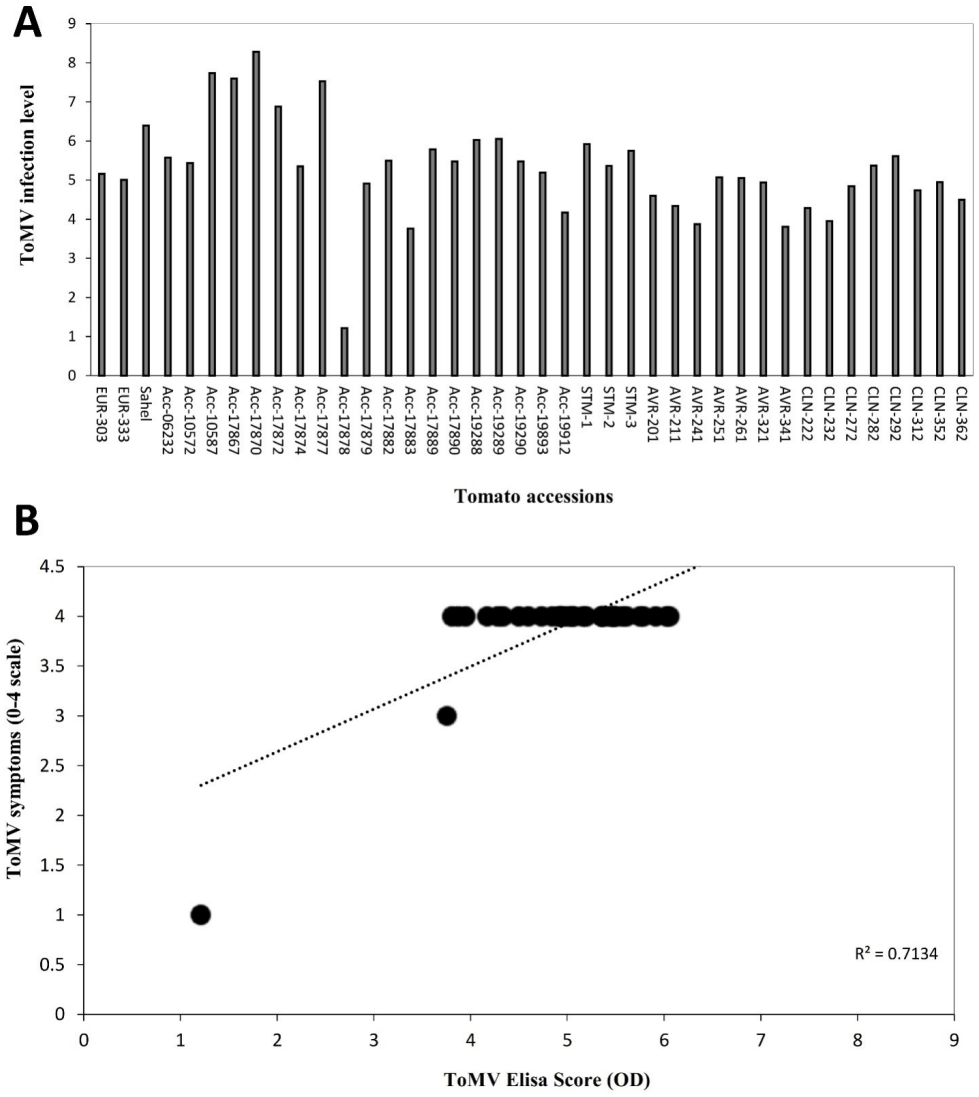

Figure 3. Screening of 40 tomato accessions with ToMV. A. response of all the accessions; B. correlations between ELISA score and phenotypic rating.

STM-1, and STM-3 with TAS-ELISA average absorbance values between 0.231 to 0.346 respectively at 405 OD. Accessions Acc-10572 and CLN-272 showed moderate symptoms TYLCV after 60 days of grafting on infected root stock.

Based on symptoms, accessions Acc-17878, Acc-17879, Acc-17882, Acc-19912, CLN-222, and CLN-232 showed severe level of symptoms thus expressing susceptible host response. The TAS-ELISA results values were 6.4, 7.7, 7.0, 7.3, 7.9 and 7.0 OD for accessions Acc-17878, Acc-17879, Acc-17882, Acc-19912, CLN-222, and CLN-232, respectively, which also showed that disease infestation was severe. Only one genotype i.e. Sahel out of 24, showed very severe symptoms as well as very high absorbance value of 14.92 OD, indicating no resistance and high susceptibility (Figure 4B).

4. Discussion

Our study is the first one to report variability in Pakistan's indigenous tomato accessions for their response to ToMV and TYLC infections, in comparison with worldwide representative accessions. Although there was a relative variability in terms of infection for accessions from various geographical origins, only one accession was highly resistant to ToMV and seven to TYLCV. The work elucidated the variable response of the tested tomato accessions to ToMV and TYLC and that the infection to ToMV was not directly correlated with TYLCV infection, which could be expected as the two viruses are different and different loci controlling resistance could be involved. The progression of disease at various leaf stages revealed an increase in the disease on developing new leaves,

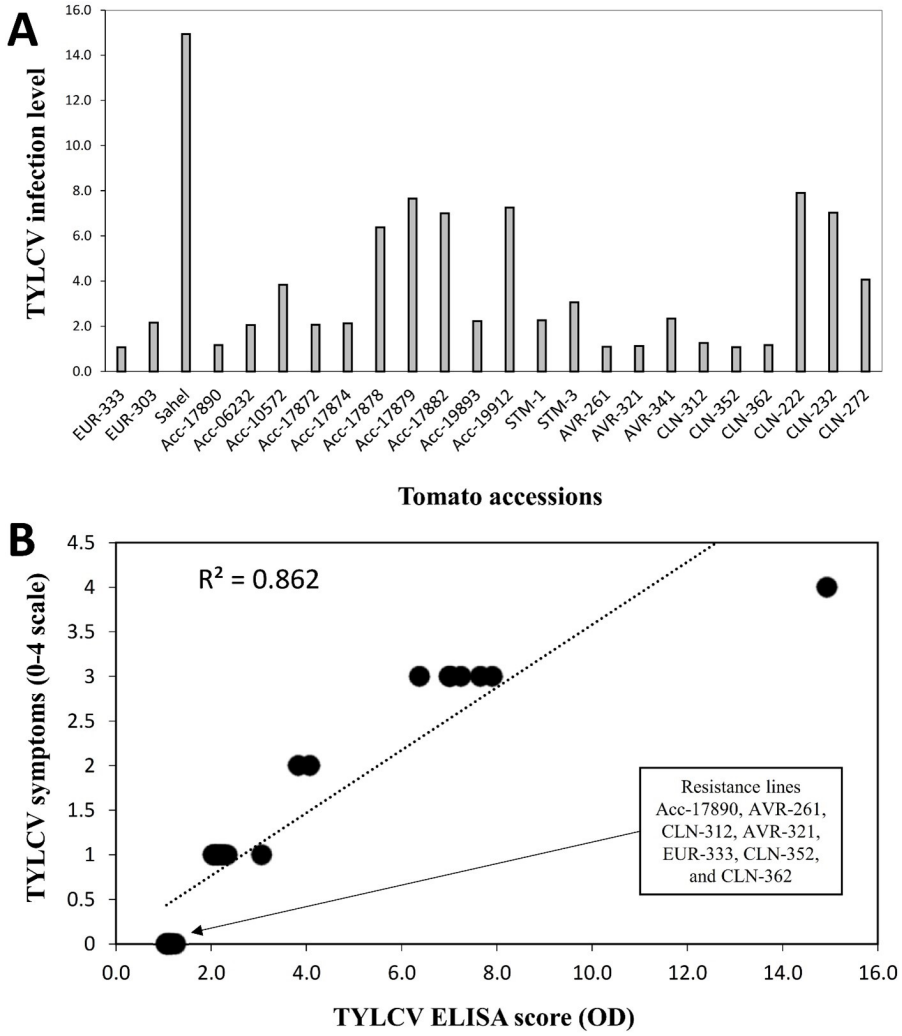

Figure 4. Screening of 24 tomato accessions with TYLCV. A. response of all the accessions 24 tomato accessions; B. correlations between ELISA score and phenotypic rating.

suggesting that the disease was more sever on developing younger leaves. The variation in genetics could further be exploited for tomato genetic improvement (by using the resistant accessions for gene mapping, marker-assisted selection in breeding programs) in Pakistan, as suggested in previous studies (Osei et al., 2012; Qaryouti et al., 2003). The information is valuable for breeders as most of the previous studies emphasized on assessment of the diversity for morphological and yield potential in indigenous tomato germplasm (Iqbal et al., 2014; Ullah et al., 2016); the current work will be complementing previously published studies on these accessions. Utility and life span of tomato varieties is increased if the pre-approval testing consider information from assessment of the resistance to viral infections in tomato accessions.

Resistance and infection response to viral diseases have been assessed through both phenotypic scoring and ELISA based screening, where the former is subject to quantitative bias (subjective) by the scorer, while ELISA is a relatively sensitive technique (objective) and is widely used for preliminary identification and quantification of viruses in plants (Segbefia et al., 2018; Silva et al., 2010). An overall consensus was revealed between phenotypic and ELISA based scoring, which was in concordance with the previous results (Segbefia et al., 2018). Normal growth and leaf size in tomato accessions against TYLCV was observed when the values were less than the double of the value considered for absence of virus (0.226), which we also considered as a value for resistance against TYLCV as reported by Segbefia et al. (2018) for genotype Hyb-2 showing least value of 0.1860 (Zakay et al., 1991; Zhang and Klessig, 1998). Sample selection remains critical for serological detection of TYLCV, compared to ToMV, where fresh and young laves have more viral load, compared to older plant parts. The main advantage is the fact that once the test is established, it is relatively simple to perform and analyze

the data resulting in rapid deployment and quarantine screening (Silva et al., 2010; Yan et al., 2018). It will enable to protect the crop against tomato viral diseases to protect yield and quality losses in a long run.

Disease management is essential to achieve higher yield and to protect yield loss through a sustainable disease management strategy including cultural practices, phytosanitary measures, and genetic resistance (Hovmøller and Henriksen, 2008; Kutcher et al., 2018). Genetic resistance remains, however, the most important considering the environmental impact and costliness of chemical control and labor demanding nature of cultural practices (Ali et al., 2007; Ullah et al., 2019). In tomato, the use of genetic resistance to control viral disease have been advocated in numerous studies (Segbefia et al., 2018; Ullah et al., 2019) and the assessment of infection status from the current study would reflect on the resistance of accessions, which is a prerequisite for the development and deployment of resistance in varieties.

Local and exotic germplasm of tomato remains a major source for genetic improvement. Assessment of such lines for biotic stresses particularly viral diseases are the most important criteria for selection in Pakistan, where TYLCV and ToMV are the major diseases. Future tomato breeding programs must consider breeding for ToMV and TYLCV resistance so as to deliver high yielding tomato varieties. This becomes even more important in the context of changing pathogen population due to recombination (Arooj et al., 2017) and invasions (Brar et al., 2018), not only at regional but at worldwide scale (Ali et al., 2014).

5. Conclusion

Our work reports on variability response to ToMV and TYLC infections in Pakistan's indigenous tomato accessions in comparison with worldwide representative accessions. Based on the variable response of the tested tomato accessions to ToMV and TYLC, we conclude that the infection to ToMV was not directly correlated with TYLCV infection, which could be expected as the two viruses are un-related and different loci could be involved in resistance against these diseases. The available variability should be useful for tomato genetic improvement to deliver high yielding resistant tomato varieties.

Acknowledgements

The work was financially supported by supported by the International Research Support Program (IRSP) of the Higher Education Commission, Pakistan and Aarhus University, Denmark. We are highly thankful to Gurcharn Singh Brar for his valuable comments on the write up of the manuscript.

References

AGONG, G., SCHITTENHELM, S. and FRIEDT, W., 2001. Genotypic variation of Kenyan tomato (*Lycopersicon esculentum* L.) germplasm. *Journal of Food Technology in Africa*, vol. 6, no. 1, pp. 13-17. http://dx.doi.org/10.4314/jfta.v6i1.19277.

ALI, S., GLADIEUX, P., LECONTE, M., GAUTIER, A., JUSTESEN, A.F., HOVMØLLER, M.S., ENJALBERT, J. and VALLAVIEILLE-POPE, C., 2014. Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen *Puccinia striiformis* f.sp. *tritici*. *PLoS Pathogens*, vol. 10, no. 1, pp. e1003903. http://dx.doi.org/10.1371/journal.ppat.1003903. PMid:24465211.

ALI, S., SHAH, S.J.A. and IBRAHIM, M., 2007. Assessment of wheat breeding lines for slow yellow rusting (*Puccinia striiformis* West. *tritici*). *Pakistan Journal of Biological Sciences*, vol. 10, no. 19, pp. 3440-3444. http://dx.doi.org/10.3923/pjbs.2007.3440.3444. PMid:19090166.

AROOJ, S., IFTIKHAR, Y., KAMRAN, M., ULLAH, M., MUBEEN, M., SHAKEEL, Q., ZEERAK, N. and BILQEES, I., 2017. Management of tomato leaf curl virus through non-chemicals in relation to environmental factors. *Pakistan Journal of Phytopathology*, vol. 29, no. 1, pp. 41-46. http://dx.doi.org/10.33866/phytopathol.029.01.0324.

AVGELIS, A.D., RODITAKIS, N., DOVAS, C.I., KATIS, N.I., VARVERI, C., VASSILAKOS, N. and BEM, F., 2001. First report of tomato yellow leaf curl virus on tomato crops in Greece. *Plant Disease*, vol. 85, no. 6, pp. 678. http://dx.doi.org/10.1094/PDIS.2001.85.6.678C. PMid:30823040.

BELLOWS JUNIOR, T.S., PERRING, T., GILL, R. and HEADRICK, D., 1994. Description of a species of Bemisia (Homoptera: aleyrodidae). *Annals of the Entomological Society of America*, vol. 87, no. 2, pp. 195-206. http://dx.doi.org/10.1093/aesa/87.2.195.

BRAR, G.S., ALI, S., QUTOB, D., AMBROSE, S., LOU, K., MACLACHLAN, R., POZNIAK, C.J., FU, Y.-B., SHARPE, A.G. and KUTCHER, H.R., 2018. Genome re-sequencing and simple sequence repeat markers reveal the existence of divergent lineages in the Canadian Puccinia striiformis f. sp. tritici population with extensive DNA methylation. *Environmental Microbiology*, vol. 20, no. 4, pp. 1498-1515. http://dx.doi.org/10.1111/1462-2920.14067. PMid:29411480.

BROWN, J.K., 2015. Durable resistance of crops to disease: a Darwinian perspective. *Annual Review of Phytopathology*, vol. 53, no. 1, pp. 513-539. http://dx.doi.org/10.1146/annurev-phyto-102313-045914. PMid:26077539.

BÜCHEN-OSMOND, C., 2003. The universal virus database ICTVdB. *Computing in Science & Engineering*, vol. 5, no. 3, pp. 16-25. http://dx.doi.org/10.1109/MCISE.2003.1196303.

COHEN, S. and HARPAZ, I., 1964. Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (Bemisiatabacigennadius). *Entomologia Experimentalis et Applicata*, vol. 7, no. 2, pp. 155-166. http://dx.doi.org/10.1111/j.1570-7458.1964.tb02435.x.

COHEN, S. and NITZANY, F., 1966. Transmission and host range of the tomato yellow leaf curl virus. *Phytopathology*, vol. 56, pp. 1127-1131.

CZOSNEK, H., BER, R., ANTIGNUS, Y., COHEN, S., NAVOT, N. and ZAMIR, D., 1988. Isolation of tomato yellow leaf curl virus, a geminivirus. *Phytopathology*, vol. 78, no. 5, pp. 508-512. http://dx.doi.org/10.1094/Phyto-78-508.

FOX, J. and LEANAGE, A., 2016. R and the Journal of Statistical Software. *Journal of Statistical Software*, vol. 73, no. 2, pp. 1-13. http://dx.doi.org/10.18637/jss.v073.i02.

FRIEDMANN, M., LAPIDOT, M., COHEN, S. and PILOWSKY, M., 1998. A novel source of resistance to tomato yellow leaf curl virus exhibiting a symptomless reaction to viral infection. *Journal of the American Society for Horticultural Science*, vol. 123, no. 6, pp. 1004-1007. http://dx.doi.org/10.21273/JASHS.123.6.1004.

HOLLINGS, M. and HUTTINGA, H., 1976. *Tomato mosaic virus (tomato mosaic)*. Wallingford: CABI International.

HOVMØLLER, M.S. and HENRIKSEN, K.E., 2008. *Application of pathogen surveys, disease nurseries and varietal resistance characteristics in an IPM approach for the control of wheat yellow rust. Sustainable disease management in a European context*. Dordrecht: Springer, pp 377-385

IMRAN, M., KHAN, M.A., FIAZ, M., AZEEM, M. and MUSTAFA, M., 2013. Influence of environmental conditions on tomato mosaic virus disease development under natural condition. *Pakistan Journal of Phytopathology*, vol. 25, pp. 117-122.

IQBAL, A., RAMEEZ KHAN, M., ISMAIL, M., KHA, S., JALAL, A., IMTIAZ, M. and ALI, S., 2020. Molecular and field-based characterization of yellow rust resistance in exotic wheat germplasm. *Pakistan Journal of Agricultural Sciences*, vol. 57, pp. 1457-1467.

IQBAL, Q., SALEEM, M., HAMEED, A. and ASGHAR, M., 2014. Assessment of genetic divergence in tomato through agglomerative hierarchical clustering and principal component analysis. *Pakistan Journal of Botany*, vol. 46, pp. 1865-1870.

KASHINA, B.D., MABAGALA, R.B. and MPUNAMI, A.A., 2007a. Serological detection and variability of Tomato yellow leaf curl virus isolates from Tanzania. *Journal of Plant Protection Research*, vol. 47, pp. 367-373.

KASHINA, B.D., MABAGALA, R.B. and MPUNAMI, A.A., 2007b. Transmission properties of Tomato yellow leaf curl virus from Tanzania. *Journal of Plant Protection Research*, vol. 47, pp. 43-51.

KUTCHER, H.R., TURKINGTON, T.K., MCLAREN, D.L., IRVINE, R.B. and BRAR, G.S., 2018. Fungicide and cultivar management of leaf spot diseases of winter wheat in Western Canada. *Plant Disease*, vol. 102, no. 9, pp. 1828-1833. http://dx.doi.org/10.1094/PDIS-12-17-1920-RE. PMid:30125191.

MABVAKURE, B., MARTIN, D.P., KRABERGER, S., CLOETE, L., VAN BRUNSCHOT, S., GEERING, A.D.W., THOMAS, J.E., BANANEJ, K., LETT, J.-M., LEFEUVRE, P., VARSANI, A. and HARKINS, G.W., 2016. Ongoing geographical spread of Tomato yellow leaf curl virus. *Virology*, vol. 498, pp. 257-264. http://dx.doi.org/10.1016/j.virol.2016.08.033. PMid:27619929.

MCCUE, G.A., 1952. The history of the use of the tomato: an annotated bibliography. *Annals of the Missouri Botanical Garden*, vol. 39, no. 4, pp. 289-348. http://dx.doi.org/10.2307/2399094.

OSEI, M., AKROMAH, R., LAMPTEY, J. and QUAIN, M., 2012. Phenotypic and molecular screening of some tomato germplasm for resistance to tomato yellow leaf curl virus disease in GhanaO. *African Journal of Agricultural Research*, vol. 7, pp. 4675-4684.

QARYOUTI, M.M., HURANI, O.M., and MAHADEEN, A.Y., 2003. Susceptibility of Jordanian tomato landraoes to tomato yellow leaf curl virus. *Plant Genetic Resources Newsletter*, vol. 136, pp. 31-33.

SEGBEFIA, M., AHIAKPA, K., QUARTEY, E., APPIAH, A.S., NUNOO, J. and KUSI-ADJEI, R., 2018. Field evaluation of tomato varieties/breeding lines against tomato yellow leaf curl virus disease (TYLCV). *Pertanika. Journal of Tropical Agricultural Science*, vol. 41, pp. 423-440.

SILVA, P., FREITAS, R. and NASCIMENTO, W. 2010. Detection of tomato mosaic virus in tomato seed and treatment by thermotherapy. In: *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on Plant*, 22-27 August 2010, Lisbon, Portugal. Leuven: International Society for Horticultural Science, pp. 303-308

ULLAH, D.M.Z., HASSAN, L., SHAHID, S. and PATWARY, A.K., 2016. Variability and inter relationship studies in tomato (Solanum lycopersicum L.). *Journal of the Bangladesh Agricultural University*, vol. 13, no. 1, pp. 65-69. http://dx.doi.org/10.3329/jbau.v13i1.28716.

ULLAH, N., AKHTAR, K.P., SALEEM, M.Y. and HABIB, M., 2019. Characterization of tomato mosaic virus and search for its resistance in Solanum species. *European Journal of Plant Pathology*, vol. 155, no. 4, pp. 1195-1209. http://dx.doi.org/10.1007/s10658-019-01848-2.

VALLAVIEILLE-POPE, C., ALI, S., LECONTE, M., ENJALBERT, J., DELOS, M. and ROUZET, J., 2012. Virulence dynamics and regional structuring of Puccinia striiformis f. sp. tritici in France between 1984 and 2009. *Plant Disease*, vol. 96, no. 1, pp. 131-140. http://dx.doi.org/10.1094/PDIS-02-11-0078. PMid:30731861.

YAN, Z., PÉREZ-DE-CASTRO, A., DÍEZ, M.J., HUTTON, S.F., VISSER, R.G.F., WOLTERS, A.A., BAI, Y. and LI, J., 2018. Resistance to Tomato Yellow Leaf Curl Virus in Tomato Germplasm. *Frontiers in Plant Science*, vol. 9, pp. 1198. http://dx.doi.org/10.3389/fpls.2018.01198. PMid:30177938.

ZAKAY, Y., NAVOT, N., ZEIDAN, M., KEDAR, N., RABINOWITCH, H., CZOSNEK, H. and ZAMIR, D., 1991. Screening Lycopersicon accessions for resistance to tomato yellow leaf curl virus: presence of viral DNA and symptom development. *Plant Disease*, vol. 75, no. 3, pp. 279-281. http://dx.doi.org/10.1094/PD-75-0279.

ZHANG, S. and KLESSIG, D.F., 1998. Resistance gene N-mediated de novo synthesis and activation of a tobacco mitogen-activated protein kinase by tobacco mosaic virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7433-7438. http://dx.doi.org/10.1073/pnas.95.13.7433. PMid:9636167.