Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS

Plant Pathology Journal

ISSN 1812-5387
DOI: 10.3923/ppj.2020.211.220

Research Article
Confirmation of Radish Isolate of *Turnip mosaic* virus in India Through Biological and Serological Evidences

[1]Shelly Kapoor, [1]Anil Handa, [2]John A. Walsh and [1]Rajnish Sharma

[1]Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India
[2]School of Life Science, University of Warwick, UK

Abstract

Background and Objective: Oilseed brassica are one of the most exploited agricultural commodities in International trade with diversified use in human and animal consumption besides their potential use in producing green energy in the form of biofuels. *Turnip mosaic* virus is one of the limiting factors for declining oil content in brassica. The present studies were therefore conducted to confirm the presence of this important virus in brassica through biological and serological assays. **Materials and Methods:** A total of 518 samples collected from 84 locations spanning across 5 states and 1 union territory from symptomatic plants were collected and assayed in DAS-ELISA using *Turnip mosaic virus* (TuMV) specific polyclonal antiserum. Biological and serological host range of the virus isolate was established and different varieties/breeding lines of oilseed brassica were screened for developing a resistance panel against TuMV. **Results:** *Turnip mosaic* virus incidence ranged between 0.6-8.3% in oilseed brassica and 0.2-17.6% in crucifer vegetables. *Turnip mosaic* virus was recorded in very high concentration from radish as indicated by the optical density values. Mustard variety Tender Green was established as the best propagative host of Indian radish isolate of *Turnip mosaic* virus. Out of 32 varieties/breeding lines of oilseed brassica collected from different sources in India, 25 varieties/lines were found to be susceptible to *Turnip mosaic* virus under glasshouse conditions and DAS-ELISA further confirmed these findings. **Conclusion:** A radish isolate of *Turnip mosaic* virus has been identified on the basis of biological and serological assays and results obtained for screening of brassica germplasm against *Turnip mosaic* virus are expected to help in ascertaining the sources of resistance against this virus.

Key words: Oilseed brassica, *Turnip mosaic* virus, DAS-ELISA, biological assays, germplasm screening

Citation: Kapoor, S., A. Handa, J.A. Walsh and R. Sharma, 2020. Confirmation of radish isolate of *Turnip mosaic virus* in India through biological and serological evidences. Plant Pathol. J., 19: 211-220.

Corresponding Author: Shelly Kapoor, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mustard rape is a major oil yielding crop throughout the world with India contributing about 11.3% of oilseed brassica and ranking third after Canada and China in its production besides oilseed brassica also occupies first place for edible oil in the country with a share of 23 % in overall production[1]. Though India is a major producer of oilseed brassica, it still imports edible oils as mustard rape yields have stagnated despite the release of elite cultivars as the crop faces many production challenges particularly from the abiotic stresses like drought, high temperature, salinity besides biotic stresses from bacterial and fungal diseases[2] to *Turnip mosaic* virus[3]. Plants representing the family *Brassicaceae* are susceptible to many viruses and *Turnip mosaic* virus (TuMV), a member of the genus *Potyvirus*, has the widest host range among all viruses infecting brassica[4]. The virus is of worldwide occurrence including the temperate and tropical regions of all continents[5,6] and it ranks second only to *Cucumber mosaic* virus for infecting vegetables[7].

Biological indexing is probably the best informative and basic method used in plant virology for detecting certain viruses. The utility of biological indexing lies in the fact that it forms the very basis of establishing diagnostic host range of a number of viruses and differentiating between plant viruses in mixed infections[8]. Subsequent development of enzyme-linked immunosorbent assay (ELISA) as an efficient and rapid detection technique for diagnosis of plant viruses established itself as an imperative and relevant necessity as biological indexing is a time consuming technique. More often than not, it is virtually impossible to diagnose plant viruses on the basis of symptoms on diagnostic hosts as the symptoms often vary depending on the interaction between the plant variety and the strain of the virus[9]. The symptoms are therefore inconclusive at times and could result from a synergistic effect of mixed infection by two or more viruses. The use of indicator hosts in bioassays is an indispensable tool since original symptoms still play a major role in diagnosis[6,10].

A number of laboratory techniques are available to the researchers for the diagnosis of plant viruses. However serology particularly ELISA, has gained popularity over other techniques owing to its high specificity, rapidity and precision[11]. The strength of ELISA lies in its capability for final identification of viruses decisively and establishing the relationship between different viruses and their strains.

Turnip mosaic virus infects brassica including rapeseed and crucifer vegetables around the globe but a limited knowledge is available on this virus in the states of Himachal Pradesh in India. Mustard is the only edible oilseed crop which holds significant potential towards augmenting the total oilseed production by improving its productivity through hybrid adoption, value addition and disease and pest resistance breeding in an integrated manner[1]. Screening of brassica germplasm is an important strategy used for identifying sources of resistance to diseases particularly TuMV[4]. The findings of these studies will help to unravel the dynamics of TuMV in brassica with particular reference to the development of resistance panel against Indian radish isolate of TuMV. The purpose of the present study was to investigate the diversity of TuMV in brassica and crucifer vegetables and uncover the widespread nature of this devastating virus in India.

MATERIALS AND METHODS

Study area: Field surveys were conducted at 84 locations during 2018-2019 in the Indian states of Himachal Pradesh, Punjab, Haryana, Rajasthan and Meghalaya and Union Territory of Jammu and Kashmir.

Surveys: Field surveys were conducted to record the presence and prevalence of *Turnip mosaic* virus in oilseed brassica and crucifer vegetable crops including mustard, rapeseed, cauliflower, cabbage, radish, turnip, broccoli, kale, knol khol, bok choy, Brussels sprout, lettuce and Chinese cabbage. In each field, ten plants were selected along two diagonals for sampling and a total of 518 plants were marked on the basis of visual symptoms and leaves from infected plants were collected and maintained in the glasshouse for further biological and serological studies. Incidence counts were made during active growing stage of the crop on at least 100 plants by choosing 4-5 locations in the field at random and observations on the number of healthy and diseased plants were recorded. The % disease incidence was calculated by using the following formula[12]:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Serological detection: Alkaline phosphatase (ALP) based double antibody sandwich ELISA (DAS-ELISA) commercial kits from BIOREBA AG, Switzerland were used for serological detection of TuMV in the collected samples of brassica oilseed crops and crucifer vegetables as per the instructions of the manufacturer. The absorbance value for each sample was read at 405 nm to record the O.D. in MicroScan Plate Reader MS5608A (ECIL, India). Microtiter plates were kept in dark at room temperature in a humid box for 15-60 min till the

development of yellow colour. The reaction was stopped by adding 50 µL of 3 M NaOH to each well, if desired. The results obtained in ELISA tests were interpreted and the samples with O.D. values twice the mean values of healthy control samples were considered to be infected with *Turnip mosaic* virus[13].

Maintenance of isolates and host range studies: The collected isolate which tested positive in DAS-ELISA was mechanically sap transmitted to *Chenopodium quinoa*, *C. amranticolor*, *C. album*, *Nicotiana tabacum* var. White burley, *N. glutinosa*, *Nicandra physalodes*, *Datura metel* and *D. festuosa*. Young plants with 4-5 leaves were inoculated using 0.01 M phosphate buffer (pH-7.2) and kept under observation in a glasshouse for a period of 4 weeks at 25°C. Prior to the establishment of host range, the isolate was subjected to three successive single local lesion transfers on *C. amranticolor.* As a standard practice, the isolate was maintained on mustard variety Tender Green (Ed Hume Seeds Inc., Kent, UK), a highly susceptible variety exhibiting typical symptoms of TuMV infection. At least three test plants of each species or cultivar belonging to the family *Cruciferae*, *Chenopodiaceae*, *Brassicaseae* and *Solanaceae* were inoculated. Four weeks after inoculation with test samples, back inoculations were made to confirm the presence of virus. All inoculated plants including those that did not exhibit any visible symptoms were assayed by DAS-ELISA.

Germplasm screening: Seeds of thirty two different varieties/breeding lines of oilseed brassica procured from different research institutes of India including IARI Regional Station Kullu, research stations of CSKHPKV Palampur, Dr YS Parmar University of Horticulture and Forestry, Solan in Himachal Pradesh, PAU, Ludhiana (Punjab), CCSHAU, Hisar (Haryana) besides commercial varieties from the states of Himachal Pradesh and Rajasthan were inoculated with TuMV isolates maintained under insect-proof glasshouse of the Department of Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry Solan. The inoculated plants were observed for the appearance of visual symptoms and the results were further confirmed by DAS-ELISA.

RESULTS

Incidence of TuMV: Extensive surveys conducted to record the occurrence and distribution of TuMV in brassica oilseed and crucifer vegetable at eighty five locations spread across five major brassica and crucifer vegetables growing states and one Union Territory of India resulted in the collection of 518 samples from symptomatic plants. Oilseed brassica was surveyed in all five states and union territory whereas only two states namely Himachal Pradesh and Meghalaya were surveyed for crucifer vegetables as these two states grow crucifer vegetables on commercial scale because of cooler climate. In the present studies, TuMV was found to be prevalent in all the locations surveyed and maximum incidence of TuMV was recorded in Himachal Pradesh with an average disease incidence of 12.5 % across 53 locations surveyed (Table 1). Average incidence of TuMV in Punjab, Haryana, Rajasthan, Jammu and Kashmir and Meghalaya was observed to be 6.72, 5.87, 4.65, 3.29 and 2.18 %, respectively.

Table 1: Average incidence of *Turnip mosaic* virus in different parts of India

State/union territory	Number of locations	Disease incidence (%)
Himachal Pradesh	53	12.50
Punjab	06	6.72
Rajasthan	08	4.65
Meghalaya	06	2.18
Haryana	04	5.87
Jammu and Kashmir	07	3.29

The detailed data on visual symptoms and disease incidence at 85 locations surveyed is presented in Table 2. A critical analysis of the variation in symptoms revealed that most of the locations had mosaic, puckering and leaf deformation as the most characteristic symptoms in both oilseed brassica and crucifer vegetable crops though other symptoms such as mottling and crinkling were also observed during surveys. It is evident from the data set out in the Table 2 that a maximum incidence of 8.3% was recorded at Abohar (Punjab) in oilseed brassica whereas minimum incidence of 0.6% was recorded at two locations namely Dharja (Sirmour) and Sharabhai (Kullu). In case of crucifer vegetables, a high incidence of 17.6% was recorded at Shamrod (Solan) and a minimum of 0.3% at Sundernagar (Mandi).

DAS-ELISA assays for TuMV: Leaves from symptomatic plants were subjected to DAS-ELISA test to confirm the presence of TuMV in brassica oilseed and crucifer vegetables. The data on O.D. values recorded at 405 nm presented in Table 3 and it is apparent from the data that out of the 13 hosts tested only radish (Shamrod, Himachal Pradesh) yielded positive results with an O.D. value of 0.886 which was more than twice the O.D. value of negative control (0.226). The data clearly indicates that all other hosts though exhibiting symptoms did not test positive in DAS-ELISA against TuMV.

Biological and serological reaction on indicator hosts: Ten indicator hosts including Mustard var. Tender green were mechanically inoculated with radish isolate of TuMV and the

Table 2: Incidence of *Turnip mosaic* virus on oilseed brassica and crucifer vegetable crops

(A) Himachal Pradesh	Symptoms	Disease incidence (%)	
		Oilseed brassica	Crucifer vegetables
Solan			
Deothi	Mosaic, leaf deformation	3.0	9.5
Kuthar	Mosaic, mottle	4.6	7.2
Kandaghat	Leaf deformation, puckering	-	6.8
Jatoli	Mosaic, leaf deformation	3.3	7.4
Nauni	Leaf deformation, puckering	2.0	5.7
Shamrod	Mosaic, leaf deformation	1.7	17.6
Nalagarh	Mosaic, leaf deformation	4.9	0.8
Baddi	Mosaic, leaf deformation	6.2	1.1
Sirmour			
Giripul	Mosaic, mottle	-	3.9
Maryog	Mottle, puckering	-	4.0
Nahan	Mosaic, leaf deformation	-	4.3
Haban	Mosaic, leaf deformation	-	3.9
Pabyana	Mosaic, puckering	-	2.4
Dharja	Crinkling, leaf deformation	0.6	5.3
Shimla			
Phagli	Mosaic, leaf deformation	-	3.9
Tutikandi	Leaf deformation, puckering	-	10.6
Mashobra	Mosaic, mottle	-	4.0
Rampur	Mosaic, leaf deformation	1.5	1.1
Kumarsain	Mosaic, leaf deformation	-	4.8
Theog	Mosaic, puckering	-	7.2
Tikar	Mosaic, mottle	-	2.2
Bilaspur			
Ghumarwin	Leaf deformation, puckering	7.9	0.4
Jhanduta	Mosaic, leaf deformation	5.0	1.2
Bilaspur	Mosaic, leaf deformation	4.9	0.6
Namhol	Mottle, puckering	2.8	3.0
Kangra			
Baijnath	Mosaic	1.7	2.1
Palampur	Mosaic, leaf deformation	4.3	3.7
Jaswan	Mottle, puckering	7.2	0.4
Dharamshala	Leaf deformation, puckering	1.3	1.6
Nagrota Bagwan	Mosaic, leaf deformation	6.1	1.4
Nurpur	Crinkling, leaf deformation	5.5	0.5
Mandi			
Sundernagar	Mosaic, crinkling	4.3	0.3
Nerchowk	Leaf deformation, mottle	5.0	1.2
Dhabban	Mosaic, puckering	4.5	0.7
Sakroha	Mosaic, puckering	3.0	1.3
Chakkar	Mosaic, leaf deformation	4.4	0.2
Aut	Mottle, puckering	0.6	4.3
Kotli	Mosaic, leaf deformation	1.5	3.2
Kullu			
Bajaura	Mottle	3.9	6.4
Jhiri	Leaf deformation, puckering	3.1	5.7
Sainj	Mosaic, leaf deformation	0.4	1.8
Shamshi	Mosaic, leaf deformation	3.3	4.0
Sharabhai	Mosaic, puckering	0.6	3.5
Manali	Mottle, puckering	-	2.0
Raison	Mosaic, leaf deformation	-	4.9
Gadsa	Crinkling, leaf deformation	-	3.1
Banjar	Mottle, puckering	-	3.3
UNA			
Amb	Mosaic, leaf deformation	6.3	-
Chintpurni	Mosaic, puckering	4.4	-
Gagret	Mottle, puckering	7.3	-

Table 2: Continue

Gobind Sagar	Mosaic, leaf deformation	5.8	-
Una	Mottle, puckering	7.2	-
Mehatpur	Leaf deformation, mosaic	6.1	-
(B) Punjab			
Khanna	Mottle, mosaic	5.0	-
Nangal	Mosaic, leaf deformation	6.7	-
Abohar	Mosaic, puckering	8.3	-
Bathinda	Mottle, puckering	5.4	-
Pathankot	Mosaic, leaf deformation	4.9	-
Ludhiana	Puckering, leaf deformation	6.0	-
(C) Rajasthan			
Bikaner	Crinkling, mosaic	4.5	-
Jaisalmer	Mosaic, leaf deformation	7.0	-
Sri Ganganagar	Mottle, puckering	5.2	-
Hanumangarh	Mosaic, leaf deformation	6.3	-
Mahajan	Mosaic, leaf deformation	5.8	-
Udana	Mottle, puckering	4.7	-
Khara	Mosaic	6.6	-
Gajner	Mosaic	4.9	-
(D) Haryana			
Kurukshetra	Mottle, puckering	3.6	-
Hisar	Leaf deformation, puckering	4.7	-
Sonipat	Mosaic, leaf deformation	3.2	-
Jind	Mosaic, puckering	5.0	-
(E) Jammu and Kashmir			
Agor	Mottle, leaf deformation	4.1	-
Baran	Mosaic	3.9	-
Chak Burah	Mosaic, puckering	4.7	-
Darap	Mosaic, leaf deformation	3.1	-
Handwal	Mottle, puckering	2.9	-
Rajpora	Mosaic, leaf deformation	4.3	-
Tikri Dayalan	Puckering, mosaic	2.5	-
(F) Meghalaya			
Nongpoh	Mottle, mosaic	5.6	0.8
Umsning	Mosaic	4.9	1.3
Jowai	Mosaic, leaf deformation	6.2	1.5
Shillong	Mottle, puckering	4.4	2.7
Mawsynram	Mosaic, crickling	4.2	0.8
Umwai	Leaf deformation, mosaic	7.1	-

Table 3: DAS-ELISA detection of *Turnip mosaic* virus in oilseed brassica and crucifer vegetable

Host	Location	Symptoms	O.D. A_{405}nm
Mustard	Ludhiana, Punjab	Mosaic	0.309 (-ve)
Radish	Shamrod, HP	Mosaic	0.886 (+ve)
Cauliflower	Katrain, HP	Mottle	0.118 (-ve)
Cabbage	Kullu, HP	Puckering	0.225 (-ve)
Bak Choy	Nauni, HP	Mosaic	0.310 (-ve)
Knol Khol	Handwal, J and K	Leaf deformation	0.212 (-ve)
Turnip	Nauni, HP	Crinkling	0.340 (-ve)
Broccoli	Palampur, HP	Mosaic	0.298 (-ve)
Chinese Cabbage	Nalagarh, HP	Mottle	0.137 (-ve)
Brussel Sprouts	Theog, HP	Puckering	0.210 (-ve)
Lettuce	Solan, HP	Mosaic	0.270 (-ve)
Rape seed	Gajner, Rajasthan	Mottle, crinkling	0.243 (-ve)
Positive control			1.105 (+ve)
Negative Control			0.226 (-ve)

HP: Himachal Pradesh, J and K: Jammu and Kashmir, O.D.: Optical density, A_{405nm}: Absorbance at 405 nm

data on symptoms and O.D. values at 405 nm in DAS-ELISA assays is presented in Table 4. The data reveals that mechanical transmission of TuMV resulted in the development of systemic infection in Mustard var. Tender Green whereas *Chenopodium amaranticolor* and *C. quinoa* (Fig. 1) developed local chlorotic lesions 8-12 days post inoculation turning necrotic after 4 weeks of inoculation however, *C. album* did not develop any symptoms. The remaining

Fig. 1: Local lesions on *Chenopodium amaranticolor* and chlorotic spots on *Chenopodium quinoa*

Fig. 2: Mustard var. Tender green expressing TuMV symptoms

indicator hosts representing three genera namely *Nicotiana*, *Datura* and *Nicandra* did not produced any symptom. DAS-ELISA was performed to confirm the findings of biological assays and the data clearly supported these findings as indicated by very high O.D. value of 1.422 against negative control (0.279) and *C. amranticolor* and *C. quinoa* also tested positive with O.D. values of 0.930 and 1.008, respectively (Table 4).

Maintenance host: Mustard var. Tender Green was mechanically transmitted for maintaining the radish TuMV isolate and the inoculated plants developed prominent symptoms in the form of mosaic, green vein banding, blistering, puckering and severe leaf deformation under glasshouse conditions (Fig. 2). The symptoms started appearing as diffused mottling 10-12 days after inoculation and developed blisters and puckering 4 weeks after inoculations.

Resistance panel against TuMV: Thirty two entries (varieties/breeding lines) of oilseed brassica were evaluated under insect-proof glasshouse conditions to ascertain their

Fig. 3: Susceptible reaction of oilseed brassica accessions to TuMV

Table 4: Serological reaction of indicator hosts to Radish isolate of *Turnip mosaic* virus

Host	Symptoms	O.D. A_{405}nm
Mustard var. Tender green	Mosaic, mottle, blisters	1.422 (+ve)
Chenopodium album	No symptom	0.485 (-ve)
Chenopodium amaranticolor	Chlorotic local lesions	0.930 (+ve)
Chenopodium quinoa	Chlorotic local lesions	1.008 (+ve)
Nicotiana tabacum var. White Burley	No symptom	0.322 (-ve)
Nicotiana benthamiana	No symptom	0.138 (-ve)
Nicotiana glutinosa	No symptom	0.265 (-ve)
Datura metel	No symptom	0.408 (-ve)
Datura stramonium	No symptom	0.180 (-ve)
Nicandra physallodes	No symptom	0.264 (-ve)
Positive control		1.129 (+ve)
Negative control		0.279 (-ve)

status with regard to resistance or susceptibility against radish isolate of TuMV. The basic objective of the study was to identify sources of resistance for the development of resistance panel. The results obtained are presented in Table 5. A critical analysis of the data indicates that twenty five out of thirty two accessions exhibited mosaic, mottle, blisters, puckering and leaf deformation (Fig. 3). The findings of mechanical transmission were substantiated by O.D. values recorded in DAS-ELISA assays as all twenty five entries that produced symptoms also tested positive with O.D. values at least two times higher than that of negative control (0.213). Seven accessions testing negative against radish isolate of TuMV can be included in the resistance panel. Out of these seven accessions, breeding line RGN229 had the least O.D. value of 0.112 closely followed by PC-6 with an O.D. value of 0.139. All other entries that did not develop symptoms also recorded very low O.D. values.

Table 5: Screening of brassica Germplasm against *Turnip mosaic* virus using DAS-ELISA

Variety/Breeding line	Location	Symptoms	O.D. A_{405} nm
MCN-19-25	CSKHPKV, Palampur	Mosaic, blisters	0.886 (+ve)
MCN-19-26	CSKHPKV, Palampur	Mosaic, leaf deformation	0.765 (+ve)
MCN-19-27	CSKHPKV, Palampur	Blisters, leaf deformation	0.709 (+ve)
MCN-19-28	CSKHPKV, Palampur	Mosaic, blisters	0.728 (+ve)
MCN-19-29	CSKHPKV, Palampur	Mosaic, leaf deformation	0.590 (+ve)
MCN-19-30	CSKHPKV, Palampur	Mosaic,	0.836 (+ve)
MCN-19-31	CSKHPKV, Palampur	Mosaic, blisters	0.678 (+ve)
MCN-19-32	CSKHPKV, Palampur	Leaf deformation	0.597 (+ve)
MCN-19-33	CSKHPKV, Palampur	Mosaic, leaf deformation	0.732 (+ve)
MCN-19-34	CSKHPKV, Palampur	Blisters, leaf deformation	0.682 (+ve)
MCN-19-35	CSKHPKV, Palampur	Mosaic	0.599 (+ve)
PBR-357	PAU, Ludhiana	Mosaic, leaf deformation	0.743 (+ve)
RGN229	Rajasthan	No symptom	0.112 (-ve)
RLC-3	PAU, Ludhiana	Blisters, mosaic	0.960 (+ve)
PBR-97	PAU, Ludhiana	Mosaic, puckering	0.840 (+ve)
PC-6	PAU, Ludhiana	No symptom	0.139 (-ve)
RCC-4	CSKHPKV, Palampur	Blisters, leaf deformation	1.107 (+ve)
RH-749	CCSHAU, Hisar	Mosaic, leaf deformation	0.976 (+ve)
RH-725	CCSHAU, Hisar	Mosaic, leaf deformation	0.963 (+ve)
RH-30	CCSHAU, Hisar	Mottle, mosaic	0.703 (+ve)
Anmol No.1	Rajasthan	Mosaic, crinkling	0.583 (+ve)
Parasmani-8	Rajasthan	No symptom	0.201 (-ve)
Kamdhenu-2	Rajasthan	No symptom	0.159 (-ve)
Saloni	Rajasthan	Mottle, leaf deformation	0.985 (+ve)
Kamdhenu-1	Rajasthan	Mosaic	0.863 (+ve)
HPN-3	Rajasthan	No symptom	0.175 (-ve)
GSC-7	PAU, Ludhiana	No symptom	0.185 (-ve)
GSC-6	PAU, Ludhiana	Mosaic, leaf deformation	0.695 (+ve)
AKMS-8026	CSKHPKV, Palampur	Blisters, mosaic	0.986 (+ve)
NCN-17-4-AKGS-8141	CSKHPKV, Palampur	Blisters, mottle	0.873 (+ve)
TL-17	PAU, Ludhiana	No symptom	0.219 (-ve)
ONK 1	Himachal Pradesh	Mosaic, leaf deformation	0.637 (+ve)
Positive Control			1.229
Negative Control			0.213

CSKHPKV: Chaudhary Sarveen Kumar Himachal Pradesh Krishi Vishvavidyalaya, CCSHAU: Chaudhary Charan Singh Haryana Agricultural University, PAU: Punjab Agricultural University

DISCUSSION

The prominent symptoms observed on oilseed brassica and crucifer vegetables during surveys conducted for TuMV were severe mosaic, mottling, blister formation, puckering, crinkling and leaf deformation. *Turnip mosaic* virus is widely known to induce varied symptoms on different hosts including oilseed brassica and crucifers[14]. Observations of present studies were in conjunction with the findings of many other workers who have also reported different symptoms on many hosts infected with TuMV[15-17]. Average incidence of TuMV based on visual symptoms ranged between 2.18-12.5% during surveys. Visual symptoms in brassica and crucifer crops have been used by many workers from different parts of the world for recording the incidence of TuMV. Surveys conducted for four viruses including TuMV in 5 natural populations of *Brassica oleracea* in Dorset, UK found a high TuMV incidence of 43%[18]. Studies conducted in Iran similar to the present studies reported varying level of incidence of TuMV in brassica and crucifer vegetables[19]. However, in yet another study in Southern England did not encounter TuMV in wild *Brassica rapa* ssp. Sylvestris[20].

DAS-ELISA assays detected TuMV in radish only and not in any other crucifer vegetable and brassica oilseed crops. These results go in line with the findings of a number of workers who have efficiently used DAS-ELISA for detecting the presence of TuMV in oilseed brassica, radish and lettuce grown in Saudi Arabia, India, Ukraine, UK, Turkey and many other countries[21-24]. The results of the present studies failed to detect TuMV in crucifer vegetables and brassica which are in contrary to the finding of a study conducted in Turkey that reported the detection of TuMV in Brussels sprout, cabbage and wild mustard besides radish[25].

The studies have revealed the *C. quinoa* and *C. amaranticolor* are good indicator hosts for biological assays of radish isolate of TuMV under study. Similar results have

been reported in *C. amaranticolor*[26] and *C. quinoa*[27] against TuMV. The findings of the present studies are also supported by a study conducted on wild European orchids wherein *C. amaranticolor* and *C. quinoa* developed chlorotic spots upon inoculation with TuMV but not on *N. tabacum* var. White Burley[6]. Mustard var. Tender Green was observed to be a useful propagative host for maintaining the virus isolate under study and produced typical symptoms of TuMV infection. These observations are in conjunction with a report indicating that Mustard var. Tender Green is a susceptible host to both TuMV UKI and JPNI isolates[28].

Efforts to ascertain sources of resistance to TuMV in oilseed brassica resulted in identifying seven breeding lines/varieties that can be used for developing resistance panel. Studies conducted by various research groups have also used oilseed brassica and crucifer vegetable germplasm for screening in quest of sources of resistance against TuMV and have succeeded in identifying the same for use in future breeding programmes[14,29-30].

Biological and Serological evidences have confirmed the presence of TuMV isolate in radish and RT-PCR based molecular studies can further substantiate these findings. A wider resistance panel against TuMV can be developed by screening more accessions in the studies.

CONCLUSION

Oilseed brassica and crucifer vegetable crop growing areas in five states and a Union Territory of India resulted in identifying radish isolate of TuMV on the basis of biological and serological assays. Mustard var. Tender Green was found to be the best propagative host of this isolate. Significant findings of the present studies help in characterizing TuMV isolates representing the diversity in India and knowledge of the relative frequency of different pathotypes of the virus in vegetables and oilseed brassica crop develop a panel of resistant germplasm against Indian isolates of TuMV that can be used to screen brassica germplasm.

SIGNIFICANCE STATEMENT

Oilseed brassica and crucifer vegetables though are commercially important crops throughout the world and recent past has witnessed enormous expansion in the area and production under these crops resulting from an ever-increasing demand, *Turnip mosaic* virus has emerged as a serious threat and hampers commercial cultivation of these crops. Detailed studies on TuMV based on extensive surveys, biological and serological indexing in addition to screening of brassica germplasm conducted in the present study is expected to be of immense use for the further breeding programs which will ultimate help the farming community.

ACKNOWLEDGMENT

The authors sincerely acknowledge Biotechnology and Biological Sciences Research Council (BBSRC), Govt. of UK and Department of Biotechnology, Govt. of India for providing the financial assistance to conduct the research studies under Newton Bhabha PORI Project. The authors also wish to acknowledge Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India for providing infrastructural, laboratory and farm facilities for conducting this research.

REFERENCES

1. Jat, R.S., V.V. Singh, P. Sharma and P.K. Rai, 2019. Oilseed brassica in India: Demand, supply, policy perspective and future potential. OCL., Vol. 26. 10.1051/ocl/2019005.
2. Kayum, M.A., H. Kim, U.J. Nath, J. Park, K.H., Kho, Y. Cho and I. Nou, 2016. Research on biotic and abiotic stress related gene exploration and prediction in *Brassica rapa* and *B. oleracea*: A review. Plant Breed. Biotech., 4: 135-144.
3. Spencer, N.J., N.A. Phiri, S.L. Hughes, A. Mwaniki and S. Simons, 2007. Economic impact of *Turnip mosaic* virus, *Cauliflower mosaic* virus and *Beet mosaic* virus in three kenyan vegetables. Plant Pathol., 56: 317-323.
4. Hughes, S.L., S.K. Green, D.J. Lydiate and J.A. Walsh, 2002. Resistance to *Turnip mosaic* virus in *Brassica rapa* and *B. napus* and the analysis of genetic inheritance in selected lines. Plant Pathol., 51: 567-573.
5. Ohshima, K., Y. Yamaguchi, R. Hirota, T. Hamamoto and K. Tomimura *et al*., 2002. Molecular evolution of *Turnip mosaic* virus: Evidence of host adaptation, genetic recombination and geographical spread. J. Gen. Virol., 83: 1511-1521.
6. Nguyen, H.D., Y. Tomitaka, S.Y.W. Ho, S. Duchene and H.J. Vetten *et al*., 2013. *Turnip mosaic potyvirus* probably first spread to Eurasian *Brassica* crops from wild orchids about 1000 years ago. PLoS ONE, Vol. 8. 10.1371/journal.pone. 0055336.
7. Tomlinson, J.A., 1987. Epidemiology and control of virus diseases of vegetables. Ann. Applied Biol., 110: 661-681.
8. Zherdev, A.V., S.V. Vinogradova, N.A. Byzova, E.V. Porotikova, A.M. Kamionskaya and B.B. Dzantier, 2018. Methods for the diagnosis of grapevine viral infections: A review. Agriculture, Vol. 8. 10.3390/agriculture8120195.

9. Haq, Q.M.R., K.M. Srivastava, R.K. Raizada, B.P. Singh, R.K. Jain, A. Mishra and D.D. Shukla, 1994. Biological, serological and coat protein properties of a strain of *Turnip mosaic* virus disease of *Brassica compestris* and *B. juncea* in India. J. Phytopathol., 140: 55-64.
10. Sevik, M.A., 2019. Viruses infecting cool season crops in the northern Turkey. An. Acad. Bras. Ciênc., Vol. 91. 10.1590/0001-3765201920180224.
11. Astier, S., J. Albouy, Y. Maury, C. Robaglia and H. Lecoq, 2008. Principles of plant virology, genome pathogenicity, virus ecology. Plant Pathol., 57: 989-989.
12. Mckinney, H.H., 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. J. Agric. Res., 26: 195-217.
13. Dijkstra, J. and C.P. de Jager, 1998. Standard DAS-ELISA. In: Practical Plant Virology. Dijkstra, J. and C.P. de Jager, Springer Berlin Heidelberg, Springer, ISBN 978-3-642-72030-7 pp: 373-375.
14. Walsh, J.A. and J.A. Tomlinson, 1985. Viruses infecting winter oilseed rape (*Brassica napus* ssp. *oleifera*). Ann. Applied Biol., 107: 485-495.
15. Nyalugwe, E.P., R.A.C. Jones, M.J. Barbetti and M.A. Kehoe, 2015. Biological and molecular variation amongst Australian *Turnip mosaic* virus isolates. Plant Pathol., 64: 1215-1223.
16. Singh, R., A. Banerjee, S.K. Sharma, R. Bhagawati, Baruahs and S.V. Ngachan, 2015. First report of *Turnip mosaic* virus occurrence in cole crops (*Brassica* spp.) from Arunachal Pradesh, India. Virus Dis., 26: 211-213.
17. Park, S., J. Park, T. Park and S. Hong, 2020. First report of *Turnip mosaic* virus infecting *Perilla frutescens* in Korea. J. Plant Pathol., Vol. 102. 10.1007/s42161-020-00536.z.
18. Raybould, A.F., L.C. Maskul, M.L. Edwards, J.I. Cooper and A.J. Gray, 1999. The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. New Phytol., 141: 265-275.
19. Farzadfar, S. and R. Pourrahim, 2014. Characterization of *Turnip mosaic* virus from the Asian-BR population in Iran. J. Phytopathol., 162: 824-828.
20. Pallett, D.W., M.I. Thurston, M.C. Borja, M.L. Edwards and M. Alexander *et al*., 2002. The incidence of viruses in wild *Brassica rapa* ssp. *sylvestris* in southern England. Ann. Appl. Biol., 141: 163-170.
21. Al-Saleh, M.A., I.M. Al-Shahwan, O.A. Abdalla and M.A. Amer, 2009. Identification and coat protein nucleotide sequence of turnip mosaic potyvirus from *Eruca sativa* in Saudi Arabia. Asian J. Plant Pathol., 3: 27-38.
22. Sevik, M.A., 2016. Viruses infecting *Brassica crops* in the black sea region of Turkey. Acta Agric. Scand. Sect. B-Soil Plant Sci., 66: 553-557.
23. Parmar, N., A.K. Thakur, P. Kumar, P.D. Thakur and S.V. Bhardwaj, 2017. Molecular characterization of *Turnip mosaic potyvirus* (TuMV)-infecting radish (*Raphanus sativus* L.) crop in India. 3 Biotech, Vol. 7. 10.1007/s13205-017-1016-y.
24. Shevchenko, O., R. Yasaka, O. Tymchyshyn, T. Shevchenko and K. Ohshima, 2019. Phylogenetic relationships and genetic structure of populations of *Turnip mosaic* virus in Turkey. Eur. J. Plant Pathol., 156: 559-569.
25. Korkmaz, S., Y. Tomitaka, S. Onder and K. Ohshima, 2008. Occurrence and molecular characterization of Turkish isolates of *Turnip mosaic* virus. Plant Pathol., 57: 1155-1162.
26. Green, S.K., 1985. *Turnip Mosaic* virus strains in cruciferous hosts in Taiwan. Plant Dis., 69: 28-31.
27. Tomimura, K., A.J. Gibbs, C.E. Jenner, J.A. Walsh and K. Ohshima, 2003. The phylogeny of *Turnip mosaic* virus, comparison of 38 genomic sequences reveal a Eurasian origin and a recent emergence in east Asia. Mol. Ecol., 12: 2099-2111.
28. Sardaru, P., L. Sinausia, S. Lopez-Gonalez, J. Zindovic, F. Sanchez and F. Ponz, 2018. The apparent non-host resistance of Ethiopian mustard to a radish-infecting strain of *Turnip mosaic* virus is largely determined by the C-terminal region of P3 viral Protein. Mol. Plant Pathol., 19: 1984-1994.
29. Walsh, J.A. and C.E. Jenner, 2002. *Turnip mosaic* virus and the quest for durable resistance. Mol. Plant Pathol., 3: 289-300.
30. Li, G., H. Lu, S. Zhang, S. Zhang and F. Li *et al*., 2019. TuMV management for brassica crops through host resistance: Retrospect and prospects. Plant Pathol., 68: 1035-1044.