Pak. J. Bot., 43(3): 1707-1711, 2011.

REACTION OF EXOTIC AND INDIGENOUS *CAPSICUM* GENOTYPES AGAINST PAKISTANI ISOLATES OF CHILI VEINAL MOTTLE VIRUS

HUSSAIN SHAH*, TAHIRA YASMIN, MUHAMMAD FAHIM, SHAHID HAMEED, IRFAN-UL-HAQUE[1], MUHAMMAD MUNIR[2] AND KHALIL AHMED KHANZADA[3]

Plant Virology Lab, Crop Diseases Research Program, National Agricultural Research Centre, Islamabad, Pakistan
[1]Department of Plant Pathology, University of Arid Agriculture, Rawalpindi, Pakistan
[2]Plant Sciences Division, PARC, Islamabad, Pakistan
[3]Crop Diseases Research Institute, University of Karachi, Karachi, Pakistan
**Corresponding author E-mail: hussain_shah1@hotmail.com*

Abstract

Viral diseases are considered to be the major limiting factor in chili pepper production. *Chili veinal mottle virus* (ChiVMV) is one of the important viruses, which decrease yield by 50%. Screening of 32 (exotic and indigenous) chili pepper germplasm against ChiVMV through symptomatology and serology (DAS-ELISA) under glasshouse conditions showed that all local cultivars (12) except Rawala and Gola Peshawari, are susceptible to ChiVMV, however, Asian Vegetable Research Center (AVRDC) lines CV-1, CV-2, CV-3, CV-7. CV-11 and CV-12 were found highly resistant to both Sindh and Punjab isolates under controlled conditions.

Introduction

Chili pepper (*Capsicum* sp.) is an important vegetable crop grown worldwide. Due to wide application and high economic value, chilli ranges 30,000 to 40,000 tones annually on International Trade (Knott & Deanon, 1967; Khoso, 1982; Qunchu, 1993; Lukmana, 1995). In Pakistan, chili pepper is an important vegetable and has a tremendous export potential due to its demand in the international market and its non-perishable nature on drying. Among vegetable crops, chilies occupy the largest area followed by potato and onion. The crop covers nearly 19% of the total cropped area under vegetables including potato and condiments; it adds as much as 151 million rupees per annum to GNP and valued 3871 million rupees at current factor cost during 1997-98 (Anon., Agriculture Stat. of Pakistan, 1997-98). Sindh province is the major growing area (66.72%) and half of the total cropped area under chili in the country.

Among pathogenic diseases, more than 45 viruses have been reported infecting chili pepper worldwide (Green & Kim, 1991). The main viral diseases infecting chili pepper crop in Pakistan and particularly in Sindh province are leaf curl virus and *Chili veinal mottle virus* (ChiVMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY) and *Potato virus X* (PVX) (Hameed *et al*., 1995). ChiVMV and CMV have been found the most economically important viruses with an incidence of 40% in Pakistan as well as in some other countries (Hameed *et al*., 1995; Ong *et al*., 1980; Josh & Dubey, 1973). During chili diseases monitoring in 1998-99 ChiVMV was found the most prevalent virus with increase incidence of 19.6% as compared to previous surveys and monitoring in Pakistan. In view of this scenario, an effort was made to screen out available chili pepper germplasm through most reliable, authentic and convenient approach i.e. serology (ELISA) under glasshouse conditions so that breeders could get resistant material to incorporate resistance gene in highly susceptible cultivars as well for farmers to improve chili pepper yield.

Materials and Methods

Source of seeds: Seeds of chili pepper germplasm were obtained from Asian Vegetable Research Center (AVRDC) Taiwan under LCVRTP Project (Phase II), Horticultural Research Institute (HRI), NARC, Sindh Agriculture Department, Mirpurkhas, Sindh and Agriculture Research Station Mingora (N), Swat, NWFP and were used for screening purpose.

Nursery raising: Forty seeds each (40 seeds pot) of thirty two (32) *Capsicum* germplasm (20 viz., CV-1, CV-2, CV-3, CV-5, CV-6, CV-7, CV-8, CV-10, CV-11, CV-12, CV-21, M-1-2, PBC-534, PBC-386, 4-14-299 (99), ELPASO, K-A-2, Korean, Huag Sithon, & Tabasco and twelve local genotypes viz., NARC-4, Red chili, Red top, Sanum, Swat Local, Ghotki, BSS-269, Loungi, Sufi, Rawala, Choo and Gola Peshawar were sown and raised in sterilized soil mixture under glass house conditions. The seeds were sown in round bottom clay pots in sterilized soil mixture composed of peat, clay and sand, mixed in equal ratio of 1:1:1 under green house conditions. At 2-3 leaf stage the seedlings were transplanted to plastic pots (one seedling per pot). To enhance vegetative growth, urea was applied 1% solution. In order to check the health status of the raised seedlings, Enzyme-linked immunosorbent assay (ELISA) was performed against ChiVMV, TMV, CMV, and PVY, in each test plant.

Virus inoculation: After physiological establishment, about 30 seedlings of each cultivar/line were mechanically inoculated with ChiVMV local isolate separately. ChiVMV infected leaf tissue was grounded (1:2 w/v) in a sterilized pre-cold mortar and pestle in chilled 0.05M K-phosphate buffer, pH 7.0 containing sodium sulfite (Na_2SO_3) and sieved through muslin cloth as described by Noordam (1973) and Hill (1984). The mechanical inoculations were carried out according to the protocol described by Noordam (1973).

Host response: Phenotypic data of host reaction was recorded in terms of symptom manifestation following mechanical inoculation on plants of each cultivar/lines, placed under green house conditions four weeks post inoculation. The host reaction was recorded according to disease rating scale of Reddy *et al.,* (2001) with some modifications.

Virus detection: Direct ELISA (DAS-ELISA) was performed following the method of Clark & Adam (1977) of all seedlings of each chili pepper genotypes for detection of ChiVMV. Asymptomatic and ELISA negative plants were decapitated and the new sprouted leaves (three leaves) of each plant were re-inoculated. Second ELISA was performed four weeks post inoculation. The cultivar/lines were rated as HR (Highly Resistance, 0-10% infection; R (Resistance, 11-20%); MR. (Moderately Resistance, 21-30%, MS. (Moderately Susceptible, 31-40%); S (susceptible, >60%), based on accumulative data of host response and ELISA values.

Results and Discussion

The lines CV-1, CV-2, CV-3, CV-7, CV-11 and CV-12, have not shown any symptoms and were found virus free after testing with DAS-ELISA against Punjab isolate. They were categorizes at HR. In this group there was exception of Gola Peshawar that showed 6.7% infection and found positive by ELISA. The Rawala plants showed 11.1% infection and were also ELISA positive and fall under resistant group. The remaining plant species falls under MR to susceptible group. Similar pattern was obtained against Punjab isolate. The highly resistant group was same. Only Gola Peshawari showed 17% infection rate and falls under resistant (R) group. Another exception was CV-5, CV-6, CV-10, Ghotki, BSS-269, and ELAPSO, which showed resistance.

The detailed results of *Capsicum* germplasm screening against ChiVMV Sindh isolate are presented in Table 1. The lines/genotypes CV-1, CV-2, CV-3, CV-7, CV-11, and CV-12 did not show any symptoms and were ELISA negative and were under high resistant group. The reaction showed by Gola Peshawari (6.7%) and Rawala plants (11%) showed mild vein mottling symptoms and were ELISA positive. Third group termed moderately resistant (MR) include genotypes CV-5, CV-6, CV-10, Ghotki, BSS-269 & ELPASO where 26-40% plant become infected showing mild vein mottling and were ELISA positive. Fourth group termed as moderately susceptible (MS reaction) include CV-21, PBC-534, PBC 386, Swat Local (Fig. 1), Soofi, Korean and 4-14-299 (99) as 41-60% plants produced mild vein mottling (MVMo) symptom and were also ELISA positive. The fifth group termed as susceptible (S) includes CV-8, M-1-2, Loungi, Sanam (Fig. 2), NARC-4, Tabasco (Fig. 3), Choo (Fig. 4), Red chili, Re top, K-A-2 and Huag Sithon as more than 60 percent plants of these genotypes manifested severe vein mottling (SVMo) symptoms and all the symptomatic plants were ELISA positive.

The reaction of these *Capsicum* germplasm against Punjab isolate has been summarized in Table 2. The genotypes which gave HR score were CV-1, CV-2, CV-3, CV-7, CV-11 and CV-12, resistant (R) score were CV-5, CV-6 and Gola Peshawari, moderately resistant (MR); CV-10, BSS 269, ELPASO, Ghotki, moderately susceptible (MS); CV-21, PBC 534, PBC 386, Soofi, Swat Local, Korean, Huag Sithon, 4-14-299 (99) and Rawala, susceptible (S); CV-8 (100 percent infection rate), M-1-2, Loungi, Sanam, NARC-4, Choo, Tabasco, Red chili, Red Top and K-A-2.

It is apparent from above results that in either case genotype gave highly resistant reaction to Sindh and Punjab isolates and are same except Gola Peshawari that gave highly resistant (HR) reaction against Sindh isolate but resistant (R) reaction to Punjab isolate. One genotype (Rawala) scored resistant reaction against Sindh isolate but moderately susceptible reaction against Punjab isolate. The rest of genotypes reacted in similar fashion and gave similar reaction score against both isolates (Tables 1 & 2). The incubation period (14-21 days) for symptom development was same and symptoms show a linear co-relation between symptom severity and virus concentration (Siriwong *et al*., 1995; Ong *et al*., 1979). Lines resisted ChiVMV infection by exposing to first primary inoculation hitherto remained asymptomatic upon secondary inoculation and ELISA did not detect any latent infection and thus termed highly resistant. Similar results about resistance have been reported earlier by AVRDC (1990a; 1990b). Similar reaction of these AVRDC lines has been also reported by Ariyaratne & Weeraratne (2001), Khalid (2001) and Reddy *et al.,* (2001). C-2 and CV-10 gave resistant reaction whereas the remaining AVRDC lines were found susceptible (Joshi & Shrestha, 2001). Chew & Ong (1990) obtained similar results after screening exotic pepper germplasm by sap inoculation in Malaysia and reported that a pair of recessive genes confers resistance to genotypes against ChiVMV infection. Yoo (1988) reported that out of 27 entries of peppers screened against ChiVMV, "Passion" and HAD 832 showed 20% and 17% infection rate against isolate from Taiwan, Republic of China.

The use of conventional phytosanitary practices is often inefficient against potyviruses as they spread rapidly in the field through non-persistent transmission by aphids, therefore resistant cultivars remain the most economical and reliable method of control. The CV-1 line (Perennial) showed HR reaction to Sindh isolate whereas resistant to Punjab isolate under glasshouse conditions. Moury *et al*., (2005) reported characterization of as few as three ChiVMV isolates with five pepper genotypes revealing pathogenicity differences and suggested that much variability exists within ChiVMV. The genotype 'Perennial' showed resistance to East African and Asian ChiVMV isolates. The Perennial could be used to breed pepper cultivars resistant to ChiVMV in Pakistan. However, more isolates of ChiVMV should be evaluated for pathogenicity on 'Perennial'. Caranta & Palliox (1996) reported that both common and specific genetic factors are involved in polygenic resistance of pepper to several potyviruses and concluded that absolute resistance to ChiVMV is conferred by pepper line 'Perennial'. They further reported that in the double haploid (DH) progeny from the F1 of a cross 'Perennial' by Yolo Wonder, resistance to ChiVMV was conferred by two independent genes, one with a clear dominant effect. Thus, the polygenic resistance of 'Perennial' to the virus is both due to polyvalent genetic factors i.e., factor that apparently interact with several viruses and strain-specific genetic factors.

Caranta *et al.,* (1997) reported that the resistance of Perennial to potyvirus E was shown to be conferred by four additives and two epistatic qualitative trait loci (QTL). But it is not yet clear that whether the same QTLs are effective against ChiVMV isolates (Moury *et al.*, 2005). 'Perennial' is the only known pepper genotype with broad resistance corresponding to the geographic distribution and phylogenetic grouping of ChiVMV isolates. In spite of the relatively small number of isolates tested, an explanation could be that acquisition or loss of pathogenicity toward Perennial was more ancestral than the loss of pathogenicity toward isolates (CM344 or DH801). The six genotypes (CV-1, CV-2, CV-3, CV-7, CV-11 & CV-12) seem homozygous for both the gene ($pvr2^2$ and pvr6 alleles) tested negative for ChiVMV in DAS-ELISA as reported by Moury *et al.,* (2005).

In Pakistan, yield losses due to viral diseases on hot pepper have not been determined previously. However, ChiVMV and CMV are two major pepper viruses recorded in Capsicum in Pakistan with highest incidence (Hameed *et al*., 1995). But in the field, existence of virus species could not be predicted as viruses occur in combination with other viruses i.e. TMV & PVY also (Shah & Khalid, 1999; Hameed *et al*., 1995). So a variety with monogenic resistance may not defend against other viruses. Although some preliminary work on screening of pepper cultivars/lines against ChiVMV has been reported so far in Pakistan (Khalid, 2001), and sources of resistance to PVY and TMV have been identified earlier (Anon., 1992). Management of viral diseases has always been focused on control of insect-vector and the use of resistant varieties. The present findings suggest that the lines showing resistance to both local isolates (Sindh and Punjab) of ChiVMV should be included in the national breeding program to improve the existing pepper germplasm. This might help breeders in incorporating the resistant genes into indigenous pepper genotypes to evolve mono/polygenic pepper varieties against major viruses. To develop a new variety of pepper, besides using modern technology, the conventional breeding is still a good option to choose. This picture of resistance will become clearer if these lines are evaluated for ChiVMV resistance and other agronomic characters under field conditions.

Fig. 1. The mottling symptoms on cv. Swat Local 2-3 weeks post inoculation.

Fig. 2. Vein mottling symptoms on variety Sanam developed 2-3 weeks post inoculation.

Table 1. Reaction of *Capsicum* germplasm against Sindh isolate of ChiVMV under glasshouse conditions.

Chili pepper Germplasm	No. of plants inoculated	No. of plants infected		% Infection	Type of symptoms manifested	Remarks
		Visual symptoms	ELISA results			
CV-1	30	0	0	0	NS	HR
CV-2	29	0	0	0	NS	HR
CV-3	30	0	0	0	NS	HR
CV-5	27	8	8	29.6	MVMo	MR
CV-6	28	10	10	35.7	MVMo	MR
CV-7	29	0	0	0	NS	HR
CV-8	27	27	27	100	SVMo	S
CV-10	29	8	8	27.5	MVMo	MR
CV-11	26	0	0	0	NS	HR
CV-12	27	0	0	0	NS	HR
CV-21	27	15	15	55.5	MVMo	M.S
M-1-2	27	19	15	70.4	SVMo	S
PBC 534	30	14	14	46.7	MVMo	MS
PBC 386	29	17	17	58.6	MVMo	MS
Loungi	29	22	22	75.9	SVMo	S
Soofi	27	13	17	48.1	MMo	MS
Sanam	28	19	19	67.9	SVMo	S
Ghotki	30	9	9	30	MVMo	MR
BSS-269	28	7	7	25	MVMo	MR
ELPASO	27	9	9	33.3	MVMo	MR
NARC-4	28	21	21	75	MVMo	S
Swat Local	29	17	17	58.6	SVMo	MS
Choo	30	19	19	63.3	SVMo	S
Tabasco	27	23	23	65.2	MVMo	S
Red chili	28	17	17	60.7	MVMo	S
Red top	29	19	19	65.5	SVMo	S
K-A-2	28	17	17	60.7	SVMo	S
Korean	28	13	13	46.4	MVMo	MS
Huag Sithon	28	20	20	71.4	SVMo	S
4-14-299 (99)	29	15	15	51.7	MVMo	MS
Rawala	27	3	3	11.1	MVMo	R
Gola Peshawar	30	2	2	6.7	MVMo	HR

NS= No symptoms, M.Vmo= Mild vein mottling; S.V.Mo= Severe vein mottling, H.R= Highly resistance, 0-10% infection, R= Resistance, 11-20%, MR= Moderately resistance, 21-30% 0, MS= Moderately susceptible, 31-40%), S= Susceptible, >60%

Table 2. Reaction of *Capsicum* germplasm against Punjab isolate of ChiVMV under glasshouse conditions.

Chili pepper Germplasm	No. of plants inoculated	No. of plants infected		% Infection	Type of symptoms manifested	Remarks
		Visual symptoms	ELISA results			
CV-1	28	2	2	7.1	MVMo	HR
CV-2	27	0	0	0	NS	HR
CV-3	27	0	0	0	NS	HR
CV-5	30	5	5	16.7	MVMo	R
CV-6	30	4	3	13	MVMo	R
CV-7	26	0	0	0	NS	HR
CV-8	25	25	25	100	SVMo	S
CV-10	27	8	8	29.6	MVMo	MR
CV-11	26	0	0	0	NS	HR
CV-12	26	0	0	0	NS	HR
CV-21	25	11	11	44	MVMo	MS
M-1-2	30	21	21	70	SVMo	S
PBC 534	27	11	11	40.7	MVMo	MS
PBC 386	24	12	13	54.2	MVMo	MS
Loungi	27	19	19	70.4	SVMo	S
Soofi	24	11	11	45.8	MMo	MS
Sanam	29	23	23	79.3	SVMo	S
Ghotki	29	11	11	37.9	MVMo	MR
BSS-269	26	9	9	34.6	MVMo	MR
ELPASO	29	11	11	37.9	MVMo	MR
NARC-4	26	18	18	69.2	MVMo	S
Swat Local	29	13	13	44.8	SVMo	MS
Choo	27	21	21	77.8	SVMo	S
Tabasco	27	19	19	70.4	MVMo	S
Red chili	25	16	16	64	MVMo	S
Red top	28	24	24	85.7	SVMo	S
K-A-2	26	18	18	69.2	SVMo	S
Korean	28	15	15	53.6	MVMo	MS
Huag Sithon	28	15	15	53.6	SVMo	MS
4-14-299 (99)	29	15	15	51.7	MVMo	MS
Rawala	29	15	15	51.7	MVMo	MS
Gola Peshawar	23	4	4	17.4	MVM	R

NS= No symptoms, M.Vmo= Mild vein mottling; S.V.Mo= Severe vein mottling, H.R= Highly resistance, 0-10% infection rate, R= Resistance, 11-20%, MR= Moderately resistance, 21-30% 0, MS= Moderately susceptible, 31-40%), S= Susceptible, >60%

Fig. 3. Mottling cum mosaic symptoms on cv. Tabasco developed 2-3 weeks post inoculation.

Fig. 4. Typical vein mottling symptoms on cv. Choo developed 2-3 weeks post inoculation.

References

Anonymous. 1990a. Screening for resistance to PVMV, CVMV and PVY. Asian Vegetable Research and Development Center, Progress Report, 1988: 54-56.

Anonymous. 1990b. Screening of germplasm for resistance to viruses. Asian Vegetable Research and Development Center, Progress Report, 1989: 74-75.

Anonymous. 1997-98. *Agricultural Statistics of Pakistan.* Government of Pakistan, Ministry of Food, Agriculture and Livestock, Economic Wing, Islamabad.

Ariyaratne, I. and W.A.P.G. Weeraratne. 2001. Integrated Disease and Pest Management: Leaf curl and other viruses of tomato and peppers. In: '*Proceedings of the Final Workshop'*, Bangkok, Thailand, 3-8 June 2001.

Bidari, V.B. and H.R. Reddy. 1990. Identification of naturally occurring viruses on commercial cultivars of chilli. *Mysore J. Agric. Sci.,* 24: 45-51.

Brunt, A.A., K.Crabtree and A. Gibbs. 1990. *Viruses of tropical plants*. CAB International, Wallingford, UK, 707 pp.

Burnett, F. 1947. Report on Agriculture in Malaysia for the Year 1946. 85pp.

Caranta, C. and A. Palloix. 1996. Both common and specific genetic factors are involved in polygenic resistance of pepper to several Potyviruses. *Theo. & Appl. Gen.,* 92: 15-20.

Caranta, C., V. Lefebvre and A. Palloix. 1997. Mapping polygenic resistance to potyviruses in pepper: Identification of specific and new broad spectrum resistance factors with quantitative effects. *Molec. Pl-Micr. Inter.,* 10: 872-878.

Chew, B.H. and C.A. Ong. 1990. Genetics and breeding for *Chili veinal mottle virus* and *Cucumber mosaic virus* resistance in hot pepper. *Trop. Gen.,* 20-23.

Clark, M.F. and A.N. Adams. 1977. Characteristics of microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol*., 34: 475-482.

Green, S.K. and J.S. Kim. 1991. Characteristics and control of viruses infecting peppers: a literature review. *Asian Vegetable Research and Development Centre, Technical Bull No*. 18.

Hameed, S., H. Shah, H. Ali and S. Khalid. 1995. Prevalence of chilli viruses in Pakistan. *Fifth National Congress of Plant Sciences, 28-30 March, NARC, Islamabad*.

Hill, S.A. 1984. *Methods in Plant Virology*, Vol. 1, Published by British Society for Plant Pathology by Blackwell Scientific Publications, ISBN 0-632-00995-0.

Joshi, R.D. and L.N. Dubey. 1973. Assessment of losses due to CMV on chilli. *Sci. & Cult.,* 39: 521-522.

Joshi, S. and S.K. Shrestha. 2001. Integrated Disease and Pest Management: Leaf curl and other viruses of tomato and peppers. *Proceedings of the Final Workshop, Bangkok, Thailand*, 3-8 June 2001.

Khalid, S. 2001. Integrated Disease and Pest Management: Leaf curl and other viruses of tomato and peppers. In: *Proceedings of the Final Workshop, Bangkok, Thailand*, 3-8 June 2001.

Khoso, A.W. 1982. *Crops of Sindh*. Sardar Printing Press, Gadi Khoto, Hyderabad, Sindh, pp. 18-31.

Knott, J.E. and J.R. Deanon. 1967. *Vegetable production in Southeast Asia*. Laguna: University of the Philippines Press, pp. 99-119.

Lukmana, A. 1995. Agriondustri cabaselain untuk keprluan pangan Dalan Agribisnis cabai Penebar Swadaya, Jakarta, hlm: 2-13.

Moury, B.A., C. Palloix, P. Caranta, S. Gognalons, K. Souche, Gebre Selassie and G. Marchoux. 2005. Serological, molecular and pathotype diversity of *Pepper veinal mottle virus* and *Chili veinal mottle virus*. *Phytopath.,* 95: 227-232.

Noordam, D. 1973. Dilution end-point determination In: *Identification of Plant viruses: Methods and Experiments* published by PUDOC, Center for Agricultural Publishing and Documentation, Wageningen, Netherlands.

Ong, C.A., G. Varghese and T.W. Poh. 1980. The effect of C*hili veinal mottle virus* on yield of chili (*Capsicum annuum* L.). *Malaysia Agriculture Research and Development Institute (MARD) Research Bulletin,* 8: 74-79.

Ong, C.A., G. Varghese and W.P. Ting. 1979. Aetiological investigation on a veinal mottle virus of chilli (*Capsicum annuum* L.) newly recorded from peninsular Malaysia, MARDI. *Research Bulletin,* 7: 278-288.

Qunchu, Z. 1993. *Hot pepper varietal trial. Training report*, ARC-AVRDC, Bangkok, Thailand, pp: 170-183.

Reddy, M.K., A.T. Sadashiva, K.M. Reddy, C. Chalam, A.A. Deshpande and A. Chandro. 2001. *Integrated Disease and Pest Management: Leaf curl and other viruses of tomato and peppers*. Proceedings of the Final Workshop, Bangkok, Thailand, 3-8 June 2001.

Shah, H., S. Khalid and I. Ahmad. 2001. Prevalence and distribution of four Pepper viruses in Sindh, Punjab and North West Frontier Province. *J. Biol. Sci.,* 1: 214-217.

Siriwong, P., K. Kittipakorn and M. Ikegami. 1995. Characterization of C*hilli vein banding mottle virus* isolated from pepper in Thailand. *Pl. Pathol.,* 44: 718-727.

Sugiura, M., C.M. Bandaranayake and G.H. Hemachandra. 1975. *Chili virus diseases in Sri Lanka*. Technical Bulletin, Ministry of Agriculture, Forestry and Fisheries, Tropical Agricultural Research Report 8, 62 pp.

Yoo, Y.W. 1988. Screening some *Capsicum* germplasm for resistance to pepper veinal mottle virus and chili veinal mottle virus. *AVRDC, Research International Report*, Taiwan, 10 p.

(Received for publication 30 April 2010)