Pak. J. Bot., 40(6): 2669-2681, 2008.

TRANSMISSION AND HOST RANGE STUDIES OF PAKISTANI ISOLATE OF CHILLI VEINAL MOTTLE VIRUS

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Abstract

Chilli pepper (*Capsicum frutescens* L.), being the most important remunerative vegetable of Pakistan is susceptible to a wide range of viruses which are the major constraints in its production resulting to heavy crop losses. Among these, *Chilli veinal mottle* Potyvirus (ChiVMV) is the major prevalent virus with an incidence range of 50% that reduce yield by 50% worldwide. Transmission and host range studies under glasshouse conditions revealed that ChiVMV Pakistani isolate is transmitted mechanically, through aphid vector (*Aphis gossypii*) and grafting to chilli pepper and tobacco but not through seed. Among 44 host plants tested, 5 different plant species (*Nicotiana tabacum cv. Samsun, Nicotiana glutinosa, Nicotiana occidentalis, Chenopodium quinoa, Solanum nigrum, Datura metel and Physalis floridana)* induced characteristic systemic mottling symptoms within 7 to 14 days of inoculation. The rest of the hosts remained asymptomatic and were DAS-ELISA negative.

Introduction

Vegetables are high value crops and often provide excellent income-generating opportunities to small farmers (Nono-womdim, 2001). Chilli (*Capsicum frutescens*) is among the world's most popular vegetable of *solanaceous* family after potato and tomato and is being used mainly as spices and condiments (Berke, 2002). On worldwide basis chilli pepper is grown on estimated areas of 1.9 million hectares (Pyou *et al*, 1980; Martelli & Quacquarelli, 1982; Jamil, 2001). In Pakistan, it is a significant cash crop occupying 19% of the total area under vegetable cultivation mainly concentrated in Sindh (46917 ha) and Punjab (6400 ha), Baluchistan (2082 ha) and on a small scale in NWFP (392 ha). During 2003-4, the total cropped area under chilli cultivation was 55791 ha with the production of 96394 tones with an average national yield of 1.73 tones per hectare (Anon., 2003-4).

Several abiotic and biotic stresses affect the productivity of chilli pepper crop worldwide. More than 45-65 viruses have been reported infecting crop worldwide (Green & Kim, 1994; Anon., 2001). Among pathogenic diseases, viruses are the most devastating agents of chilli pepper, causing serious losses by reducing both fruit quality and quantity (Kang *et al*., 1973; Lockhart & Fischer, 1974; Villalon, 1975; Ong *et al.*, 1980; Yoon *et al.*, 1989; Chew & Ong, 1990). Viruses produce various types of disease syndrome like mosaic, mottling, leaf distortion, vein etching, yellowing, stunting and narrowing of leaves (Green, 1991; Hameed *et al.*, 1995; Anon., 2001). *Chilli veinal mottle virus* (ChiVMV) is the major virus infecting chilli pepper (Anon., 2000) reducing yield losses upto 50% (Joshi & Dubey, 1973; Ong *et al*., 1980).

In Pakistan some viral diseases were initially identified in solanaceous crops on the basis of symptoms expression only but no work has been reported for the identification of viruses since 1960s (Kamal & Moghal, 1968). Later viruses infecting chilli pepper, were

identified as *Chilli veinal mottle virus* (ChiVMV), *Cucumber mosaic virus* (CMV), *Potato virus E* (PVE), *Pepper mild mottle virus* (PMMV) and *Pepper veinal mottle virus* (PVMV) from different provinces of Pakistan (Hameed *et al.*, 1995). The present study was designed to investigate transmission and host range of Pakistani isolate of ChiVMV in order to put an effort to manage this virus by exploring its biological properties.

Materials and Methods

Transmission studies: In order to investigate the transmissibility of ChiVMV, the following transmission methods were tried.

Sources of virus inoculum: A local isolate of ChiVMV was obtained from chilli pepper field of District Chakwal, Punjab Province, found only positive for ChiVMV through DAS-ELISA among four chilli viruses tested (ChiVMV, CMV, TMV and PVY). The plant was maintained under glasshouse conditions for further experimentation.

Mechanical Transmission: The nursery of indicator plants (*Nicotiana tabacum cv. Samsun, White Burley, K-399, Nicotiana glutinosa, Capsicum annum* and *C. frutescens)* was raised at first in green house conditions. The seeds were obtained from CIP Peru, AVRDC Taiwan, and Vegetable Research Programme, Horticultural Research Institute (HRI), NARC, Islamabad. At 2-3 leaf stage the seedlings were transplanted to plastic pots. To enhance vegetative growth, urea was applied at the rate of 1% in solution form. In order to check the health status of the raised seedlings, ELISA was performed against ChiVMV, *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY). Inoculum was prepared by grinding ChiVMV infected leaves (w/v) in 0.05M K-phosphate buffer, pH 7.0 containing Sodium sulfite (Na_2SO_3) following the methods of Noordam (1973) and Hill (1984). Fully expanded leaves of tobacco and chilli plants were inoculated following the method of Noordam (1973). About 10 plants of each cultivar were inoculated including negative control plants, which were treated in the same way with buffer only. The tested plants were observed daily for symptom development in the glasshouse. The presence of ChiVMV was confirmed through Double antibody sandwitch ELISA (DAS-ELISA) two weeks post inoculation.

Grafting transmission: Wedge grafting was used to test the transmissibility of ChiVMV. ELISA-confirmed virus infected plants (Tobacco and Chilli pepper) were selected as a donor or scion. Similarly healthy plants of the same species and age were chosen as recipient or rootstock. The scion from the infected plants was taken and a wedge was made with a sterile sharp scalpel knife by removing cambium tissue. A slanting cut of about 5mm was made in the rootstocks of healthy plant species and wedge shaped cut on scions were inserted. Parafilm was tightly wrapped around the insertion portion. Similarly healthy plants were auto-grafted as negative check. The grafted plants were covered in moist polyethylene bags, well irrigated and placed in shade for one week in insect-proof cages under growth room conditions till union established. Systemic insecticide (Temik, Aldicarb group) was applied @ ca. 50 grains per pot to discourage insect feeding. After complete union formation, the plants were shifted to glasshouse and were observed for symptoms development. About five plants of each host (tobacco cv. *Samsun* and bell pepper cv. California Wonder) were grafted. DAS-ELISA was carried out for ChiVMV confirmation four weeks post grafting.

Vector transmission: The aviruliferous and viruliferous aphid vectors were collected from chilli pepper plants grown at Vegetable Programme, NARC and propagated for the study. These were later reared and maintained on healthy chilli pepper plants in insect proof perspex cages under growth room conditions at temperature of 10-22°C and photoperiod of 12 hours. Aphids were gently picked up one by one with painter brush from colony collected and released on healthy chilli pepper plants at 3-4 leaf stage in insect-proof cages. After three weeks of rearing, colonies of the aphids were disturbed so that they withdraw their stylet from the phloem tissue. The aphids were starved for one hour and were transferred to infected detached leaves in a separate Petri dish allowing acquisition-feeding period of 2-3 minutes. A total of 100 adult aphids were released on ChiVMV infected chilli and tobacco plant to acquire the virus. Five to ten viruliferous aphids were allowed transmission feeding period of one hour on three sets of test plants (chilli & tobacco) in insect-proof perspex glass cages (36.25 x 36.25 x 56.25 cm). After one hour all aphids vector were killed physically as well as sprayed with insecticide Karate (*Lambda cyhalohtrine*) @ 0.1%. Granules of systemic insecticide, Temik, were also applied to each pot. The plants were observed for symptoms appearance daily. After three weeks of inoculation, symptoms were noted and ELISA was performed to confirm ChiVMV presence.

Seed transmission: Fully mature fruits from ELISA confirmed ChiVMV infected chilli plants were harvested and stored for a month in shade place to dry. Seeds were collected from the fruits and stored at room temperature for a month to break dormancy. Twenty-five seeds were then sown in sterilized conditions. At 3-4 leaf stages, ELISA was performed of all the germinated seedlings for ChiVMV presence. Similarly one lot of the same seeds was tested serologically as described by Bashir & Hassan (1998). Infectivity assay was also performed by inoculating the seed extract onto seedlings of indicator plants i.e. *Chenopodium amaranticolor* and tobacco cv. Samsun. The inoculated plants were kept in the growth room at 18-25°C under a photoperiod of 12 hours for one month and observed daily for symptoms development. The extract of seeds was observed under EM. To investigate whether ChiVMV infect the seed, chilli and tobacco seedlings were also inoculated with seed extract at 2-3 leaf stage in insect-proof perspex cage and were observed for symptom development. Before and after the flowering stage, ELISA of plant parts (seed, sepal, petal, androecium and gynoecium) was performed.

Host range studies: Seedlings of 44 host species available at Plant Virology Lab., or obtained from AVRDC, HRI/Crop Sciences Research Institute (CSRI), NARC, were raised as described earlier. Some weed species of chilli pepper crop were also included. The raised seedlings were mechanically inoculated with virus inoculum as described earlier. The host species tested include tobacco species (K-399, *Nicotiana glutinosa), Datura stramonium, D. metel, Physalis floridana*, Spinach (*Spinacia oleracea, Chenopodiaceae*), *Chenopodium alba, Chenopodium quinoa*, black pepper (*Solanum nigrum, Piperaceae*), tomato (*Lycopersicon esculentum* Mill., *Solanaceae*), potato (*Solanum tuberosum* L. Solanaceae), Mash (*Vigna radiata),* Mung *(Vigna mungo*), Egg plant (*Solanum melongena* L. Solanaceae), Bitter gourd (*Luffa cylindrica, Luffa octangulata*), Sponge gourd, pumpkin and squashes (*Cucurbita* spp. *L., Cucurbitaceae*), *Gomphrena globosa, Alocasia* sp., Cucumber (*Cucumis sativus* cv. Vorgebigst), *Cucurbita pepo*, Bottle gourd (Ghiya Kado), Cotton (*Gossypium hirsutum, Malvaceae*), *Portulaca oleracea* (Kulfa), *Cyprus rotundus* (Deela), *Echinochloa* sp., *Amarnthus viridis, Dactyloctenium* sp., Soybean (*Glycine max* [L.} Merrill, *Leguminosae*),

Sunflower (*Helianthus annuus L, Compositae*), Etsit (*Trianthema pentandra*), Signal grass, Horse grass (*Setaria* sp.), Okra (*Hibiscus esculentus L., Malvaceae*), Radish (*Raphanus sativus L. Cruciferae*, Gool moli & Aam moli), Corriander and Fenugreek (*Trigonella foenugraecum*, local name Mathee). Plants were observed daily for symptom development and DAS-ELISA was performed after 3-4 weeks post inoculation.

Results and Discussion

Transmission studies

Mechanical transmission: ChiVMV was transmitted mechanically to chilli and tobacco plants showing typical systemic mottling symptoms. Inoculation at 2-3 leaf stage gave better results than inoculation to older leaves stage as symptoms started appearing on the subsequent emerging leaves. Likewise, potassium phosphate buffer (0.05M, pH 7.0) gave similar results as sodium buffer of the same molarity and pH for inoculation. There are reports that leaf homogenization in phosphate buffer greatly enhance infectivity (Yarwood 1952; Fulton, 1964). However, Matthews (1981) reported that this buffer system has some deleterious effect on other viruses during extraction. To maintain the stability and infectivity of virus the reducing agent, Sodium sulfite, was added to the extraction buffer to prevent oxidation that inactivates ChiVMV infectivity in sap homogenate during extraction. Similar properties of this salt have been reported by Bawden, (1954), Walkey (1985) and (Hill, 1984).

Graft transmission: ChiVMV was successfully transmitted through wedge grafting onto chilli and tobacco from their respective donor hosts (Fig. 1). Typical systemic mottling symptoms manifested by the grafted tobacco and chilli plants and virus presence was further confirmed by DAS-ELISA. These results are similar to those reported by Ong *et al.*, (1979) and Lal & Singh (1988).

Fig. 1. Successful wedge grafting of infected ChiVMV scion (infected tobacco shoot) on to healthy tobacco plant (cv. Samsun).

Fig. 2. Local lesion on inoculated leaves of *Nicotiana* tabacum cv. Samsum.

Fig. 3. Tobacco (*Nicotiana tabacum* cv. White Burley) manifested systemic mottling.

Fig. 4. Mottling symptoms on successive leaves of *Nicotiana glutinosa* after inoculation.

Fig. 5. Circular local lesion symptoms on inoculated on leaves of *Chenopodium quinoa* two weeks post inoculation.

Fig. 6. Mosaic symptoms on *Solanum nigrum* 7-14 days post inoculation

Fig. 7. Vein banding symptoms of ChiVMV on *Nicotiana occidentalis*.

Fig. 8. Mild vein mottling on *Physalis floridana* 7-14 days post inoculation.

Fig. 9. Severe mottling and rat-tailed symptoms on *Datura metel* 7-14 days post inoculation.

Table 1. List of host species tested by mechanical inoculation under glasshouse conditions for host range studies.

S. No.	Host	# of plant inoculated	Symptoms	ELISA OD Value
1.	*Nicotiana tabacum*		S. Mottling, Necrotic lesion	
	cv. Samsun	5	S. Mottling & Blistering	3.08
	cv. White Burley	5		3.10
	cv. K-399	5		2.87
2.	*Nicotiana glutinosa*	5	S. mosaic	3.4
3.	*Nicotiana occidentalis*	5	Mild Mosaic & vein banding	2.87
4.	*Datura metel*	5	S. Mottling Rat Tail	3.51
5.	*Physalis floridana*	5	S. Mottling	3.02
6.	*Solanum nigrum*	5	Mild Mosaic	2.45
7.	*Ageratum conyzoides*	5	Nil	Negative
8.	*Amanrthus viridis*	5	Nil	Negative
9.	*Arachis hypogaea*	5	Nil	Negative
10.	*Brassica oleracea* var. *capitata*	5	Nil	Negative
11.	*Chenopodium amaranticolor*	5	Nil	Negative
12	*Chenopodium capitatum*	5	Nil	Negative
13	*Chenopodium quinoa*	5	Nil	Negative
14	*Corriander*	5	Nil	Negative
15.	*Cucumis sativus*	5	Nil	Negative
16.	*Cucurbita pepo*	5	Nil	Negative
17.	*Cyprus rotundus*	5	Nil	Negative
18.	*Dactyloctenium aegyptium.*	5	Nil	Negative
19.	*Datura stramonium*	5	Nil	Negative
20.	*Echinochloa crusgali*	5	Nil	Negative
21.	*Glycine max* [L] Merrill	5	Nil	Negative
22.	*Gossypium hirsutum*	5	Nil	Negative
23.	*Helianthus annuus* L.	5	Nil	Negative
24.	*Hibiscus esculentus* L.	5	Nil	Negative
25.	*Setaria* spp. (Horse grass: weed)	5	Nil	Negative
26.	*Luffa acutangula*	5	Nil	Negative
27.	*Luffa cylindrical*	5	Nil	Negative
28.	*Lycopersicon esculentum*	5	Nil	Negative
29.	*Trigonella Foenum graecum* (Mathee: Local name)	5	Nil	Negative
30.	*Ornamental Arvi*	5	Nil	Negative
31.	*Phaseolus vulgaris*	5	Nil	Negative
32.	*Portulaca oleracea* (Kulfa)	5	Nil	Negative
33.	*Raphanus sativus* L.	5	Nil	Negative
34.	*Setaria* spp (Signal grass: Weed)	5	Nil	Negative
35.	*Solanum melongena*	5	Nil	Negative
36.	*Solanum tuberosum*	5	Nil	Negative
37.	*Spinacia oleracea*	5	Nil	Negative
38.	*Tetragonia tetragonioides*	5	Nil	Negative
39.	*Trianthema portulacastrum*	5	Nil	Negative
40.	*Vigna mungo*	5	Nil	Negative
41.	*Vigna radiate*	5	Nil	Negative
42.	*Vigna unguiculata*	5	Nil	Negative
43.	*Zea mays*	5	Nil	Negative
44.	*Zinnia elegans*	5	Nil	Negative

Vector transmission: ChiVMV was successfully transmitted by *Aphis gossypii* (Glov.) to its natural host chilli pepper. The plants induced typical systemic vein mottling symptoms that were positive through DAS-ELISA. Symptoms started appearing two weeks post inoculation. Similar findings have been reported by Ong *et al.*, (1979), Fujisawa *et al*., (1986), Green (1999), Moi (1992) and Prakash *et al*., (2002).

These findings suggest that these insect vectors play major role in the transmission of ChiVMV within the crop population under natural field conditions. Devising management strategies for ChiVMV could be focused on the control of these aphid vectors that are the main source of transmission under farmer's fields. For the control of aphid population in chilli crop, Ong (1984) used reflective mulches that reduced population dynamics of aphids by 87% and reduced ChiVMV infection significantly that in turn increased the yield by 21.7%.

Seed transmission: Seedlings raised from infected chilli seed did not show any symptoms under controlled conditions. Similarly chilli (cv. CV-8) and tobacco plants (*cv. Samsun*) did not develop any symptoms when inoculated with extract prepared from seeds of infected chilli plants. No ChiVMV particles were observed under EM directly nor detected in DAS-ELISA. Similar findings have been reported by Ong *et al.*, 1979; Anon., 1993; Nono-Womdim, 2001. There are theories that ssRNA viruses are stable viruses and not seed transmissible (Matthews, 1991) and ChiVMV is no more exception. Floral parts (*sepal, petals* and *pistal*) of infected chilli plants were tested through ELISA, no ChiVMV was detected in the two reproductive parts (*Androecium* and *Gynaecium*) but detected in sepal only. Thus it's concluded that the virus is not transmissible through seed and do not reach the reproductive parts to infect.

So far more than 300 plant viruses have been reported to be seed borne in one or more host species and the number is expected to rise with evolution of viruses and with reports of new viruses' emergence or discoveries (Hampton, 1983). Seed infection plays a major role in both survival and perpetuation of viral pathogens. Seed provides ideal foci of primary infection for the establishment of a disease in a field crop. Results of no seed transmission of ChiVMV are in conformity with findings of Fujisawa *et al*., (1986) and Green (1999).

Host range and symptomatology: Of the 44 different plants tested, 6 host species manifested characteristic systemic mottling symptoms, 7-14 days post inoculation. The hosts that developed systemic mottling on subsequent leaves of inoculation include *Nicotiana tabacum* (cv. *Samsun* (Fig. 2) white Burley (Fig. 3) & K-399, *Nicotiana glutinosa* developed severe vein mottling symptoms (Fig. 4), *Chenopodium quinoa* manifested localized chlorotic local lesion on inoculated leaves (Fig. 5). *Solanum nigrum* displayed mild to severe mosaic symptoms (Fig. 6), *Nicotiana occidentalis* induced mild mosaic symptoms initially but the subsequent leaves were narrower with pronounced vein banding symptoms (Fig. 7). Similarly *Physalis floridana* displayed mild systemic vein mottling (Fig. 8). *Datura metel* manifested severe mottling and rat tailed symptoms (Fig. 9). The symptomatic plants were further confirmed by DAS-ELISA. The rest test plants did not become infected upon repeated inoculations observed till 60 days post inoculation; no latent infection was detected in any plant of each non host through DAS-ELISA (Table 1). Un-inoculated negative control plants did not manifest any symptom and were also ELISA negative.

Siriwong *et al*., (1995) reported that host range of ChiVMV is restricted to Solanaceae family. Present results are inconsistent to those reported by Moury *et al*., (2005) i.e., three isolates of ChiVMV induced systemic mosaic symptoms on *N. occidentalis*, *N. glutinosa* but none infected *Solanum melongena*. Likewise are the findings of Prakash *et al*., (2002) but contradict in some respect i.e. Pakistani isolate did

not infect *Lycopersicon esculentum* while their isolate did infect this host. Brunt *et al*., (1996) reported that *Nicotiana benthamiana* and *N. glutinosa* is diagnostically insusceptible host but our findings show that these host species were susceptible and developed mosaic symptoms and were DAS-ELISA positive. Similar results have also been reported by Ong *et al*., (1979). It became apparent from these observations that Pakistani ChiVMV isolate is different in some respect from the isolates used by Brunt *et al*., (1996) and Ong *et al*., (1979). Due to slight difference in symptoms manifestation on similar or different host, different synonyms have been used for ChiVMV as reported by many authors such as *Pepper vein-banding mosaic virus* (van Regenmortel *et al*., 2000), *Pepper vein banding virus* (Joseph & Savithri, 1999) and *Chilli vein-banding mottle virus* (Siriwong *et al*., 1995).

Brunt (1996) reported that *Gomphrena globosa* and *Nicotiana glutinosa* is diagnostically insusceptible host but in our case these host species became susceptible and developed mosaic symptoms and were DAS-ELISA positive. Similar results have also been reported by Ong *et al*., (1979). It became apparent from these observations that our ChiVMV isolate, designated as ChiVMV-P-Pak, is different from the isolate used by Brunt (1996) and Ong *et al*., (1979).

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(Received for publication 20 June 2008)