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Screening of Exotic Pepper Lines Against Local Isolate of Chili Veinal Mottle Potyvirus

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Abstract: To identify source of resistance against the most important chili virus viz. chili veinal mottle virus (CVMV), eleven exotic chili lines (CV-1, CV-2, CV-3, CV-5, CV-6, CV-7, CV-8, CV-10, CV-11, CV-12, & CV-21) were screened in controlled conditions. The isolated virus was collected from Sindh province and maintained on *Nicotiana tabacum* cv. *Samsun* by successive mechanical inoculations confirmed by double antibody sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) at each step. The seedlings were raised in sterilized soil mixture of peat, clay and sand. Plants were mechanically inoculated (1:4 w/v) at 3-4 leaf stage and the new shoots were tested through DAS-ELISA two weeks post inoculation. The ELISA negative plants were decapitated and the new sprouting were re-inoculated and ELISA was performed two weeks after inoculation. Six lines (CV-1, CV-2, CV-3, CV-7, CV-11 & CV-12) showed no symptoms and were ELISA negative, while four lines (CV-5, CV-6, CV-10, & CV-21) displayed variable reaction resulting to 5, 13, 16 and 22% infection respectively while the susceptible check (CV-8) showed severe mottling with stunted growth (100 % infection) that gave high virus titer (>2). So, asymptomatic and ELISA negative lines showed resistance while the rest are segregating for resistance against CVMV infection.

Key words: Screening, resistance, DAS-ELISA, chili veinal mottle virus (CVMV)

Introduction

Pepper (*Capsicum* spp.) is an important remunerative vegetable and an aid to farmer income. It is covering 19 % area of vegetables grown producing 140.2 thousand tones annually (Anonymous, 1997-98). Among other factors responsible for low yield in Pakistan, diseases of viral nature are of great importance. So far 45 viruses have been reported infecting pepper throughout the world (Green and Kim, 1991). Of these chili veinal mottle virus (CVMV) (Hameed *et al.*, 1995), cucumber mosaic virus (CMV) (Swanepoel and Nel, 1995), tobacco mosaic virus (TMV) (Gorter, 1977) and PVY (Hameed *et al.*, 1995) are mostly associated with pepper crops. Previously CVMV was reported as the most important virus of chili in Pakistan (Hameed *et al.*, 1995). Infection of CVMV at an early growth stage reduces leaf size along with distortion, which produces fewer & smaller fruits. Yield losses of more than 50 % have been reported in Malaysia (Ong *et al.*, 1979 and 1980).

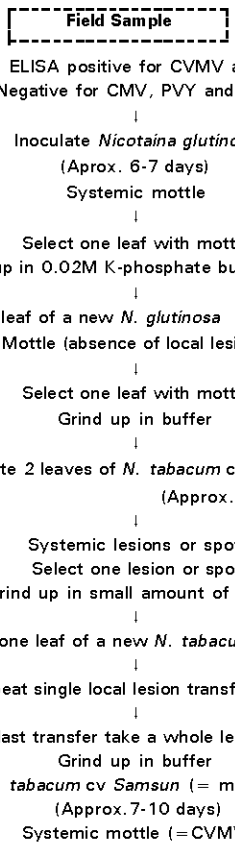
To overcome production losses, different approaches are generally adopted for the management of plant viral diseases. Growth of resistant varieties, if available, is considered to be the best and desirable approach in viral disease management. In Pakistan all the cultivated chilies varieties are susceptible to CVMV. Therefore, the need of the hour is to search for sources of resistance against CVMV, so that losses can be minimized. In pursuance of this goal a collaborative research work with Asian Vegetable Research and Development centre Taiwan, is underway. Some advance lines developed by AVRDC are supplied to collaborating partners, which are then screened against local strains of respective viruses. The study was initiated to screen and identify source of resistance in exotic pepper advance breeding lines against local isolate of CVMV under controlled conditions.

Materials and Methods

Virus source: To obtain CVMV isolate, chili plants showing virus-like symptoms were collected during survey from Sindh province. These samples were tested against CVMV, CMV, TMV and PVY through DAS-ELISA.

Maintenance and purity of the virus isolate: The virus was isolated in pure form using the following scheme of AVRDC Taiwan with some modification, finally maintained and multiplied on *Nicotiana tabacum* Cv. *Samsun* for further screening by successive mechanical inoculation confirmed at each step by DAS-ELISA.

Scheme for the isolation of CVMV from field sample



Plant material: Ten advanced breeding lines of pepper (CV-1, CV-2, CV-3, CV-5, CV-6, CV-7, CV 10, CV-11, CV-12 and CV-21) were tested for resistance against CVMV in control conditions. Line CV-8 was included as the susceptible check. The seeds of these lines were obtained through a coordinated joint project "Leaf curl virus resistance of tomato and pepper (LCVRTP)" from South Asian Vegetable Research Network (SAVERNET, Phase-II), AVRDC, Taiwan. Seeds were sown in a standardized soil mixture of peat, clay and sand mixed in equal proportion in plastic pots. The seedlings were then transferred to individual pots and placed in containment where temperature (20-25°C) and light period (12 hr) were maintained artificially.

Mechanical Inoculation: The CVMV inoculum was prepared by crushing freshly harvested leaves from propagative *N. tabacum* cv. *Samsun*. The tissues (1:4 w/v) were homogenized in 0.02M K-phosphate buffer, pH 7.0 containing 0.01% sodium sulfite and passed through muslin cloth. The sap was rubbed on Carborudum dusted young top leaves (1-2) of all seedlings of each line and washed with distilled water after three minutes to avoid plant phyto-toxicity. The plants were observed daily for symptom development for two weeks.

DAS-ELISA: Fourteen days post inoculation, DAS-ELISA (E-1) was performed. ELISA plates (Fastec Brand) were coated with CVMV specific IgG diluted 1000 fold in coating buffer (15.9g Na_2CO_3, 2.93g $NaHCO_3$ per liter of distilled water), pH 9.6. Later added 100 µl/well, incubated at 35°C for 2-3 hrs in moist conditions and washed thrice with three minutes interval in phosphate buffer saline (1.19g Na_2HPO_4, 0.2g KH_2PO_4, 0.2g KCl, 8.0g NaCl per liter of distilled water) containing 0.05% Tween-20 (PBS-T). Samples were prepared by crushing (1:10 w/v) freshly harvested top leaves in extraction buffer (PBS-T containing 2% Polyvinylpyrrolidone, MW 2400, PBS-T-PVP), pH 7.4, loaded (100µl/well) incubated overnight at 4°C and washed. Reference blank, negative and positive controls were also included. CVMV conjugated IgG diluted 1000 fold in conjugate buffer (PBS-T-PVP containing 0.002% egg albumin), pH 7.4, added 100µl/well and incubated as before followed by washing. Substrate (p-nitrophenyl-phosphate, Sigma brand, in 10% Diethanolamine in distilled water pH adjusted with HCl to pH 9.8) was added 150 µl/well @ 0.6mg/ml and incubated at room temperature for an hour at least. The reaction strength was measured at 405 nm as well as rated visually as - = no reaction, + = weak reaction, ++ = definite reaction, +++ = very strong reaction.

Re-Inoculation: Asymptomatic and E-1 negative plants were decapitated, re-inoculated at new sprouting stage and ELISA (E-2) was performed as before after two weeks of inoculation. Plants were observed daily for symptom development and virus concentration was determined with ELISA reader.

Results and Discussion

The screening results showed that there are two types of genotypes; one group that produced mild to severe mottling with light yellowing and vein banding 7-10 days post inoculation at 2-3-leaf stage (CV-5, CV-6, CV-10, & CV-21) while the other remained asymptomatic (CV-1, CV-2, CV-3, CV-7, CV-11 & CV-12). Lines producing symptoms were ELISA positive and gave high values (>2) at 405 nm while asymptomatic were ELISA (E-1) negative (Table 1). The first group can be termed as susceptible while the other can be regarded as highly resistant/tolerant. The susceptible check (CV-8) produced characteristic CVMV symptoms like vein banding, severe mottling, over all stunted growth with highest virus concentration (>2). Upon re-inoculation of symptomless and ELISA negative plants (to confirm whether they are susceptible or resistant/tolerant) more plants of the susceptible lines (CV-5, CV-6, CV-10, & CV-21) became infected with CVMV, giving variable virus titer (Table 2). Similar incubation period (5-10 days) for symptom development have also been reported by other workers (Siriwong *et al.*, 1995; Ong *et al.*, 1979). Increase in number of infected plants of the same lines can be attributed as they might have escaped initial inoculation and became infected upon re-inoculation. Plants with severe symptoms show a linear co-relation between symptom severity and virus concentration. Lines opposed CVMV 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/tolerant.

From these findings it became obvious that the six lines (CV-1, CV-2, CV-3, CV-7, CV-11 & CV-12) are highly resistant/tolerant to CVMV infection. While the rest are segregating for resistance/tolerance against local isolate of CVMV and displayed variable reactions in E-1 as well as in E-2 i.e., CV-5 (5%), CV-6 (13%), CV-10 (16%) and CV-21 (22%). Similarly a source of resistance against different pepper viruses after screening in glasshouse as well as in the field trials has been reported (Duriat, 1996). The origins of these materials seems to be the same and might be supplied by AVRDC Taiwan or vice versa for testing against Malaysian local CVMV isolate. Chew and 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 CVMV infection.

In Pakistan, yield losses due to viral diseases on hot pepper has not been determined previously. However, CVMV and CMV are two major pepper viruses recorded in Pakistan with highest incidence (Hameed *et al.*, 1995). Among 35 viruses reported so far, these two viruses are also economically

Table 1: Response and ELISA-based (E-1) reaction of pepper lines against CVMV fourteen days after inoculation.

Lines	Response	Disease Severity	# of plants inoculated	# plants positive	O.D. at 405nm
CV-1	-	-	24	-	0.000
CV-2	-	-	29	-	0.000
CV-3	-	-	30	-	0.000
CV-5	Mo.	Mild to severe	28	1+	Above 2
CV-6	Mo.	Mild to severe	27	3+	Above 2
CV-7	-	-	32	-	0.000
CV-8 (S. check)	Mo;Y; Vb; St..	Severe	22	17+	Above 2
CV-10	Mo	Severe	29	4+	Above 2
CV-11	-	-	35	-	0.000
CV-12	-	-	29	-	0.000
CV-21	Mo	Severe	30	5+	Above 2

Mo = Mottling, Y = yellowing, Vb = Vein Banding St. = Stunting - = Sample with no response/reaction
+ = Sample with positive reaction (visual rating: +, ++, +++)

Table 2: Response and reaction (E-2) of asymptomatic-ELISA negative lines after re-inoculation.

Lines	Response/Reaction	# of plants inoculated	# of plants ELISA (+ve)	O.D. at 405nm
CV-1	-	22	-	0.000
CV-2	-	24	-	0.000
CV-3	-	24	-	0.000
CV-5	M. Mo	21	1+	0.851
CV-6	Mod. Mo	20	2+	1= 1.301 1= 1.610
CV-7	-	22	-	0.000
CV-8				
(S. check)	Typical S. Mo	3	3+	Above 2
CV-10	Mod.to S. Mo.	24	6+	1= Above 2 5= 1.645
CV-11	-	21	-	0.000
CV-12	-	22	-	0.000
CV-21	Mod. Mo.	18	4+	2= 1.394 2= 1.297

M. = Mild; Mod. = Moderate; Mo = mottling; S = Severe; - = Sample with no response/reaction
+ = Sample with a positive reaction (visual rating: +, ++, +++)

important in Indonesia, Malaysia and Sri Lanka (Duriat, 1996; Green and Kalloo, 1994). To control these viruses, the most effective way is the use of resistance cultivars. But in the field, existence of virus species could not be predicted as these viruses occur in combination with other viruses i.e., TMV & PVY (Shah and Khalid, 1999). So a variety with monogenic resistance may not defend against other viruses. Although no work on screening of pepper cultivars/lines against CVMV has been reported so far in Pakistan, however, a source of resistance to pepper viruses from different countries has been cataloged in Taiwan (Green and Kim, 1994). Management of viral diseases has always been focused on control of insect-vector as well as the use of resistant varieties. Therefore, many countries are trying to manage the viral diseases by developing resistant varieties through their national breeding program or international collaboration research.

These findings suggest that the lines showing resistance/tolerance to local isolate of CVMV, should be included in the national breeding program to improve the existing pepper germplasm. This might help breeders in identifying and incorporating the resistant gene into indigenous pepper genotypes to evolve mono/polygenic pepper varieties against major viruses. To develop a new variety of pepper, beside using modern technology, the conventional breeding si still a good option to choose. A variety having resistant genes against more than one virus species, would be the best strategy for country like Pakistan. This picture of resistance will become clearer if these lines are evaluated for CVMV resistance and other agronomic characters under field conditions.

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