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SCREENING FOR RESISTANCE TO CUCUMBER MOSAIC CUCUMOVIRUS IN CHILLI PEPPER

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ABSTRACT

Cucumber mosaic cucumovirus (CMV) is destructive pathogen with widest host range, infecting more than 100 botanical families comprising more than 500 genera and 1300 plant species. Chilli pepper is a significant cash crop of Pakistan among vegetable grown. The identification of genetic resistance to CMV in Pakistan (CMV chilli isolate) in chilli pepper is of economic importance. Thus, 40 Chilli pepper genotypes, both local and imported, were evaluated by mechanical/ manual virus inoculation and resistance to CMV chilli isolate was examined by visual observations and enzyme-linked immunosorbent assay (DAS-ELISA). On the basis of 0-5 disease rating scale and ELISA, nine genotypes viz., C-2, CV-2, CV-5, BSS-269, PGRI, M-2001, CM-2001, M-97 and CP-328 were remained free of infection and catalogued as highly resistant. Rest of the genotypes exhibited characteristic symptoms like mosaic, mottling, leaf curling and reduced leaf size depending upon tested genotypes. Among these genotypes, five were categorized as resistant, seven as moderately resistant, eight as moderately susceptible and 11 as susceptible. These resistant and moderate resistant genotypes could be used by farmers in cultivation under integrated production systems and by breeders in developing new chilli pepper hybrid resistant genotypes to CMV.

Key words: Chilli pepper, *Cucumber mosaic virus*, resistant, ELISA.

INTRODUCTION

Chilli pepper (*Capsicum annum* L.) is one of the most important members of solanaceous vegetables grown in Pakistan and ranked at third position after potato and tomato (Iqbal *et al*., 2012). Chilli contains more vitamins C than any other vegetable crop (Dexiang, 1994). Among the various factors limiting to chilli production in Pakistan, viruses appear to be significant production constraints. Among these viruses, *Cucumber mosaic virus* (CMV) causes severe/ economic yield reduction in chillies. Doolittle (1916) and Jagger (1916) first described *Cucumber mosaic virus* (CMV) and the virus was assigned to the *Cucumovirus* group as the type member. CMV is tri-partite, single-stranded, +ive sense RNA virus. Intrinsically RNA viruses are heterogeneous and to a certain extent their heterogeneous nature is because of error-prone nature of RNA replication (Ding *et al*., 1995; Domingo and Holland, 1994). CMV has a very broad host range of wild and cultivated plants, with more than 1300 known hosts including some monocotyledons and a great number of dicotyledons (Chen *et al*., 2006). Tomlinson (1978) described CMV as the most economically important virus in cowpea, celery, cucurbits, pepper, lettuce and tomato. Other researchers (Palukaitis *et al*., 1992; Gafny *et al*., 1996, Davis *et al*., 1996, Latham *et al*., 1999) reported that Banana, Pasture legumes, Kava and ornamentals are also affected by CMV. CMV is easily transmitted by mechanical inoculation of plant sap and naturally transmitted (non-persistently) by 80 aphid species (Palukaitis and Garcia-Arenal, 2003).

Use of disease resistant crop varieties is regarded as an economical and durable method for controlling plant diseases, especially those caused by viruses. Recently the role of mineral metabolism and total soluble phenols in imparting resistance/susceptibility against viral diseases of plants has also been manifested (Ashfaq *et al*., 2014). A good deal of research work has been directed to identify resistant sources under diverse environmental conditions and continuing screening of available genotypes and new germplasm, which constitutes the basis of this work has been suggested by several research workers (Bashir *et al*., 2005; Ashfaq *et al*., 2007; Ashfaq *et al*., 2008; Ashfaq *et al*., 2014). Therefore, to evaluate and catalogue sources of CMV resistant genotypes, forty local and exotic chilli pepper genotypes were screened by mechanical inoculation. The level of resistance to CMV accumulation in chilli pepper leaf tissues was evaluated using a combination of visual symptom observations and enzyme-linked immunosorbent assay (ELISA).

MATERIAL AND METHODS

Virus source and maintenance: A Pakistani isolate of chilli pepper infecting CMV (CMV chilli isolate) was used as virus source for mechanical inoculation (Iqbal *et*

al., 2011). The virus was propagated and maintained in *Nicotiana benthamiana* plants.

Plant materials: Forty different *Capsicum* genotypes were obtained from Asian Vegetable Research and Development Center (AVRDC) Taiwan, Vegetable Research Program, Horticultural Research Institute (HRI), NARC and Mexico (Table 1). Twenty seeds of each genotype were sown in small clay pots that contained a 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 *Capsicum* seedlings were transplanted to plastic pots (2 seedlings per pot) and 1% urea solution was applied to each pot to enhance the vegetative growth.

Mechanical inoculation: Symptomatic leaves of *Nicotiana benthamiana* inoculated with CMV chilli isolate were harvested and one gram of these leaf tissues used as inoculums and homogenized (1/3 w/v) in 0.05 *M* phosphate buffer, pH 7.2, containing 1% Na_2SO_3. The *Capsicum* plants at the two leaf stage were rub-inoculated with sap extract as described by Ashfaq *et al*. (2010). After inoculation, the plants were rinsed with distilled water to remove superfluous inoculum and kept in an insect free glasshouse (25^{o}C temperature and 70% humidity). The un-inoculated plants (healthy plants) of each test genotype were maintained as control. The symptoms on the host plants were recorded according to disease rating scale (0-5) as used by Shah *et al*., (2011) and genotypes were categorized as HR (Highly Resistant with 0-10% infection), R (Resistant with 11-20% infection), MR (Moderately resistant with 21-30% infection), MS (Moderately susceptible with 31-50% infection), S (Susceptible with >50 infection) on the basis of host reaction.

Serological assay: DAS-ELISA (Double Antibody Sandwich- ELISA) tests were employed (Clark and Adam, 1977; Verma *et al*., 2005) for investigation of virus in leaves of Capsicum genotype after four weeks of inoculation. Polystyrene plates were coated with anti-CMV antibodies (Bioreba AG, Switzerland), diluted 1:200 in coating buffer and incubated overnight at 4 ^{o}C. Sap was extracted by grinding leaves in the extraction buffer in pestle and mortar and then filtered through the double layered muslin cloth. Exactly 200µl of the extracted sap of each sample was then added to the coated polystyrene plate and incubated overnight at 4^{o}C. Alkaline phosphatase-conjugated anti-CMV antibodies (Bioreba AG) were added and incubated overnight at 4 ^{o}C, followed by incubation with *p*-nitrophenyl phosphate (MP Biomedicals, Inc. Ohio, USA) at room temperature for 1 h. The absorbance values (405 nm) were measured with an Automatic ELISA Reader (HER-480 HT Company (Illford) Ltd., UK). Samples were considered positive for CMV infection when the ELISA absorbance value was equal to two times or higher than the average of absorbance value of the healthy tissue as well as negative control. Commercial positive and negative controls (Bioreba) were included in CMV ELISA kit.

RESULTS

Results on reaction of Chilli germplasm consisting of 40 genotypes, both local and imported, against *Cucumber mosaic virus* (CMV) under controlled conditions are given in Table 1. Thirty-one of forty genotypes showed systemic symptoms of CMV including mosaic, mottling, leaf curling, necrosis, upward curling, yellowing and smalling of leaves (Table 1). Individual plants of C-1, C-11, CV-10, CV-21, MI-2, PTY-8, PTY-11, PBC-149, PBC-518, NARC-4 (SAVERNET chilli-pepper genotypes) and GM-2001 (Mexico-chilli pepper genotype) showed mosaic, mottle, leaf curling, necrosis, yellowing and smalling of leaves, and symptoms developed at 10 days post inoculation (dpi) while other genotypes exhibited symptoms between 18 and 24 dpi. All of these eleven genotypes exhibited 57.14-100 % CMV infection on the basis of 0-5 disease rating scale with relatively high titre (> 1.0) detection in the upper symptomatic leaves, so considered all of these susceptible to CMV chilli isolate. Similarly on the basis of disease rating scale (0-5) and ELISA tests, eight genotypes viz., C-4, C-5, PTY-10, PBC-386, and PBC-495 (SAVERNET chilli pepper genotypes), sanam, chilli 0027 and chilli 007 (Pakistan local chilli pepper genotypes) were grouped as moderate susceptible.

On the other hand, the nine genotypes viz., C-2, CV-2, CV-5, BSS-269, PGRI, M-2001, CM-2001, M-97 and CP-328 did not manifest any symptom as well as CMV detection in relatively low titer (<0.25) in the upper leaves and therefore catalogued as highly resistant against CMV. Similarly five genotypes viz., C-10, CV-1, CV-12, PBC-142 and PBC-385 and rest of the seven genotypes viz., C-6, C-7, C-8, CV-7, CV-9(SAVERNET chilli-pepper genotypes), chilli 0013 and swat local (Pakistan local chilli pepper genotypes) were regarded as resistant and moderately resistant, respectively, based on both disease rating scale and ELISA tests (Table 1).

Table 1. Reaction of Capsicum genotypes against CMV under glass house conditions.

Pepper genotypes	Total plants tested.	Infected plants.	ELISA reading for CMV	CMV% Infection	Type of symptoms observed	Remarks
SAVERNET chilli-pepper genotypes						
C-1	18	12	1.253	66.66	M, m, LC	S
C-2	12	0	0.125	0	NS	HR
C-4	13	5	0.688	38.46	M, LC	MS
C-5	14	5	0.602	35.71	M, LC	MS
C-6	16	4	0.593	25	M, m	MR
C-7	12	3	0.593	25	M, UC	MR
C-8	8	2	0.593	25	M, LC	MR
C-10	18	3	0.322	16.66	M	R
C-11	15	15	1.236	100	M, Y,N, SL	S
CV-1	14	2	0.225	14.28	M	R
CV-2	14	0	0.232	0	NS	HR
CV-5	16	0	0.232	0	NS	HR
CV-7	15	4	0.600	26.66	M, m	MR
CV-9	15	3	0.502	20	M	MR
CV-10	18	11	1.084	61.11	M, SL	S
CV-12	17	2	0.2252	14.28	M	R
CV-21	16	9	1.085	56.25	M, LC	S
MI-2	20	12	1.053	60	M,m,LL	S
PTY-8	16	10	1.251	62	M, LC	S
PTY-10	16	6	0.556	37.50	M,UL	MS
PTY-11	14	8	1.245	57.14	M, LC	S
PBC-386	18	6	0.489	33.33	M,m	MS
PBC-495	15	5	0.565	33.33	M,m	MS
PBC-142	14	4	0.265	28.57	M	R
PBC-518	16	10	1.223	62.5	M,m, SL	S
PBC-149	13	13	2.333	100	m, LC, Y	S
BSS-269	12	0	0.232	0	NS	HR
NARC-4	15	9	1.069	60	M, m, SL	S
PGRI	12	0	0.232	0	NS	HR
Mexico- Chilli pepper genotypes						
M-2001	15	0	0.26	0	NS	HR
GM-2001	14	14	1.23	100	M, Y, SL	S
CM-2001	10	0	0.167	0	NS	HR
M-97	13	0	0.125	0	NS	HR
CP-328	11	0	0.049	0	NS	HR
Pakistan Local Chilli pepper genotypes						
Sanam	15	7	0.753	46.66 %	M, LC	MS
Chili 0027	12	4	0.602	33.33 %	M, UC	MS
Chili 0013	16	4	0.439	25 %	M	MR
Chili 007	13	5	0.688	38.46 %	M, UL	MS
Swat local	18	5	0.45	27.78 %	M,	MR
PBC – 385	19	4	0.39	21.05 %	M	R

M= mosaic, m= mottle, LC= leaf curling, UC= upward curling, Y= yellowing, SL= smalling of leaves,
NS= no symptom, N= necrosis

DISCUSSION

In view of ubiquitous nature of CMV disease, 40 chilli genotypes were evaluated against CMV under green house conditions. The genotypes were classified into five reaction groups based upon % infected plants and ELISA test. These were: highly resistant, resistant, moderately resistant, moderately susceptible and

susceptible. The mean percentage genotypes falling in the categories were: 22.50, 12.50, 17.50, 20.0 and 27.50 respectively. It is apparent from the above results that all local genotypes were susceptible to CMV infection except PBC-385 that showed high resistant response to CMV and all Mexican genotypes viz., M-2001, CM-2001, M-97, and CP-328 were remained highly resistant to CMV infection except GM-2001. However, Asian Vegetable Research and Development Center lines i.e. C-2, CV-2, CV-5, BSS-269 and PGRI were resistant to CMV, where as other genotypes showed susceptibility to CMV when inoculated under glasshouse conditions. These results are in agreement with Rashid *et al*. (2007) who did not observe any infection by ELISA in C-1, C-2, C-5, C-7,C-9, C-11except the pepper lines C-4, C-8,C-9 and local check which did show positive reaction to CMV while in the present study the lines C-6, C-7, C-8, CV-7, CV-9 exhibited moderately resistant reaction whereas the genotypes, C-4, C-5, PTY-10, PBC-386, and PBC-495 showed moderately susceptible reaction. Only two genotypes viz., C-7 and C-8 showed different response of reaction and this might be due to disease escape because the Rashid *et al*. (2007) made their study under natural conditions.

In Pakistan no systematic work has been conducted to determine the yield losses due to viral diseases on chilli crop. CMV is one of the major pepper viruses recorded in world elsewhere including Pakistan (Iqbal *et al*., 2012; Green and Kim, 1991). But under the field condition, it is difficult to predict the existence of virus species because of the complex nature of the viruses infection i.e., more than two viruses occur in combination e.g. TMV, PVY, ChiVMV and so on (Green and Kim, 1991; Shah *et al*., 2001). As *Cucumber mosaic virus* is one of the major virus that is known to have broad host range so it is not easy to control it. Usually the conventional measures like cross protection, eradication of infected plants, crop rotation, use of virus free plants and use of chemicals against vectors has been practiced since a long time to control or manage the plant viral diseases (Boss, 2000; Hull, 2014). Anyhow, use of resistant varieties is considered as an economical and durable method for controlling viral diseases and therefore, management of viral diseases has always been focused on control of insect-vector and use of resistant varieties. The present findings suggest that the genotypes showing resistance to CMV local isolate should be need to be maintained for further studies for locating resistance sources under field conditions and for genetic manipulations and breeding purpose. One main problem in germplasm evaluation is that some genotypes found resistance at one location turn out to be susceptible at another place (Ashfaq *et al*., 2007), therefore environmental-genotype interaction should also be studied for durable resistance (Ashfaq *et al*., 2008) in future.

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