Indian J. Plant Genet. Resour. 31(3): 243-250 (2018)
DOI 10.5958/0976-1926.2018.00028.1

RESEARCH ARTICLE

Evaluation of Hot Pepper Germplasm for Multiple Disease Resistance against Root Knot Nematode and Viruses

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(Received: 25 February 2017; Revised: 20 February 2018; Accepted: 15 June 2018)

One hundred forty genotypes of hot pepper were screened for resistance against root knot nematode *Meloidogyne incognita* and two viruses *viz*, *Tomato leaf curl Joydebpur virus* (ToLCJV) and *Pepper mottle virus* (PepMov) prevalent under Punjab conditions. Artificial screening was done separately against each pathogen under controlled conditions. In case of *Tomato leaf curl Joydebpur virus* the resistant lines were further confirmed for presence of the virus by subjecting their DNA to PCR amplification using universal begomovirus specific AV/AC primers. For *Pepper mottle virus,* resistant lines were subjected to double antibody sandwich ELISA (DAS-ELISA) against *Pepper mottle virus* (PepMoV) specific antibodies. The results showed that for root knot nematode, *M. incognita* single genotype, PP 9950-5197-0107-7058 exhibited resistant reaction and four genotypes S-343, SL 472, SAS-39 and CH-27 exhibited moderately resistant reaction. For *Tomato leaf curl Joydebpur virus*, four genotypes *viz*., NSS-2, S-343, SL 473 and CH-27 were regarded as moderately resistant whereas, for *Pepper mottle virus,* eight genotypes, *viz*., VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2, SL 472 gave negative ELISA reaction after sap inoculation with *Pepper mottle virus* and were thus regarded resistant to the virus. Further by comparing the screening results for individual pathogen it was observed that four genotypes *viz.,* SL 472, NSS-2, S-343 and PP 9950-5197-0107-7058 were found to have potential for multiple disease resistance to these three pathogens.

Key Words: Screening, Resistance, Root knot nematode, Viruses.

Introduction

Among the five recognized cultivated species of genus *Capsicum, Capsicum annuum* L. is the dominant species all over the world over for its pungent (chilli or hot pepper) and non-pungent (sweet pepper) fruits (Bosland and Votava, 2000). In India, hot-pepper is cultivated as an important commercial crop for vegetable, spice and industrial (capsaicin and oleoresin) purpose (Kumar and Rai, 2005).Root knot nematodes and viruses are among the major factors limiting the pepper cultivation in the country. Root knot nematodes belonging to the genus *Meloidogyne* are the important pest infecting pepper, worldwide (Khan and Haider, 1991). Several species of root knot nematode (*Meloidogyne incognita, M. javanica, M. hapla* and *M. arenaria*) are infecting peppers (Theis and Fery, 2002) among these, *M. incognita* and *M. javanica* have worldwide distribution and occur in varied warm-temperate & tropical climates and agro-ecosystems. Jain *et al*., 2007 reported economic losses amounting to approximately Rs. 210 million due to damage caused by root knot nematode in hot peppers. In spite of direct damage these nematodes also predispose the plants to other soil borne pathogens like bacteria and fungi by leaching plant nutrients into the soil.

Among viruses, whitefly transmitted begomoviruses causing leaf curl disease (syn. Pepper leaf curl disease) are the most destructive in terms of incidence and yield losses (Muniyappa and Veeresh, 1984). Leaf curl disease results in curling and cupping of leaves resulting in stunting of plants with either no or few undersized fruits leading to huge economic losses to the farmers. Early infection of the virus, on young plants results in flower bud abscission before attaining full size. The occurrence of *Tomato leaf curl Joydebpur virus, Tomato leaf curl New Delhi virus* and *Chilli leaf curl India virus* causing leaf curl disease in hot pepper has been reported in India (Senanayake *et al.,* 2006; Shih *et al.,* 2007). Apart from begomoviruses, some potyviruses are also causing significant damage to pepper crop globally. *Pepper mottle virus* (PepMoV) an aphid-transmitted virus belonging to potyvirus group is reported to infect *Capsicum* species (Shukla *et al.,* 1994). The virus cause mottling and

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puckering of leaves and along with misshapen leaves and fruits (Kim *et al.,* 2009). It was first reported in *Capsicum annuum* from Florida, USA (Zitter, 1972) and later on from different parts of the world. Earlier, in 1979 Sandhu and Chohan also reported mottle disease of chilli from India. Recently, the association of *Pepper mottle virus* (PepMoV) causing mottling disease has been confirmed from India (Kaur *et al.,* 2014).

Both nematodes and viruses are difficult to manage once they attack the crop. Most of the nematicides are banned due to their hazardous nature. Cultural control like crop rotation is effective but it also has limitation for root knot nematode due to polyphagous nature. Similarly for viruses, one has to manage vectors by applying insecticides, which fails to provide complete protection under field conditions. Host plant resistance is only the safer and economical way for management of these pathogens. Therefore, in the present study, hot pepper germplasm available with the Department of Vegetable Science, PAU, Ludhiana was screened in a systematic manner for resistance against root-knot nematode, *Meloidogne incognita*; begomovirus, *Tomato leaf curl Joydebpur virus* and a Potyvirus, *Pepper mottle virus* prevalent under Punjab conditions so as to identify the resistant source(s) which can be further utilized for resistance breeding purpose.

Materials and Methods

A total one hundred forty genotypes of pepper including improved varieties, inbread lines and hybrids representing two cultivated species *viz.*, *C. annuum* (138) and *C. frutescence* (2) were screened for resistance against root knot nematode (*Meloidogyne incognita*) a begomovirus, (*Tomato leaf curl Joydebpur virus*) and a Potyvirus (*Pepper mottle virus*). Three sets of genotypes were maintained separately for screening against each pathogen mentioned above.

Screening of Germplasm against Root Knot Nematode (Meloidogyne incognita)

Nursery of one hundred forty genotypes along with susceptible checks (Punjab Surkh and Kashi Anmol) was sown in plastic plug trays filled with steam sterilized coco-peat, perlite and vermiculite mixture in 3:1:1 ratio. At 3-4 leaf stage, plants were transplanted in 8 inch diameter pots filled with steam sterilized soil. After one week of transplanting when plants got established properly in the pots, inoculations were done with freshly hatched 2nd stage juveniles of *M. incognita* maintained in pure culture @ 500 juveniles per plant by making holes with the help of a glass rod near the roots of the plant. Four replications were maintained for each line along with susceptible check. Plants were watered regularly and properly maintained. Observations were recorded on root knot index and number of egg masses/ root system after 45 days of inoculations as per (0-5) scale given by Taylor and Sasser,1978.

Screening of Germplasm against Viruses

Same set of 140 genotypes along with local checks (Punjab Surkh and Kashi Anmol) were raised at Vegetable Research Farm, Department of Vegetable Sciences PAU, Ludhiana by following all the recommended cultural practices. No insecticide was sprayed so as to protect the vector population and initial screening for both the viruses was done under natural field conditions. The visual observations on appearance of symptoms were recorded at fortnight interval after transplanting the crop in end February till the month of August. For *Pepper mottle virus* (PepMoV) observations were recorded from the month of March to May and for leaf curl disease observations were recorded during July-August when the virus and vector load was at peak.

Screening of Germplasm against Leaf Curl Disease

Disease symptom severity recorded for each genotype was rated according to 0-5 scale given by Banerjee and Kalloo, 1987. According to the scale, Coefficient of Infection (CI) was calculated by multiplying disease incidence (DI) to response value, all the genotypes were assigned specific disease reaction (Table 1). The coefficient combined the amount of infection and its severity. The reaction grades assigned to each entry were added together for all observations and the mean values were calculated.

Non-viruliferous whitefly culture was maintained on cotton (*Gossipium hirsutum* L.) grown in 12 × 8 cm size plastic pots in insect-proof glass house. The day/ night temperature in the glass house was maintained at 28/20°C and the relative humidity at 70%. Inoculum of *Tomato leaf curl Joydebpur virus* (ToLCJV) was maintained on susceptible hot pepper cv. Kashi Anmol grown in the glass house by frequently inoculating 10-15 day old seedlings using viruliferous whitefly.

Promising entries along with susceptible checks were sown in plug trays filled with cocopeat, vermiculite

Table 1. Severity scale (0-5) for Leaf curl disease (Banerjee and Kalloo, 1987)

Symptom	Symptom severity grade	Response value	Coefficient of infection (CI)	Disease reaction
No visual symptom	0	0	0	Symptomless
0–5% curling of upper leaves	1	0.05	0.1–5	Highly Resistant (HR)
6-25% curling of leaves and swelling of veins	2	0.25	5.1–10	Resistant (R)
26–50% curling puckering and yellowing of leaves and swelling of veins	3	0.50	10.1–20	Moderately Resistant (MR)
51–75% leaf curling and stunted plant growth and blistering of internodes	4	0.75	20.1–40	Moderately Susceptible (MS)
More than 75% curling and deformed small leaves, stunted plant growth with small flowers and no or small fruit set	5	1.00	40.1–70	Susceptible (S)
			>70	Highly susceptible (HS)

and perlite mixture in 2:1:1 proportion. The trays were kept in the insect-proof glasshouse. Adults of non-viruleferous whitefly collected from stock culture were released into the plastic bottle cage containing ToLCJV infected branch and the flies were allowed to feed for 24 h acquisition access period. Ten seedlings from each genotype were inoculated at two-three leaf stage at twice weekly interval using 20 viruliferous whiteflies per plant. Inoculated plants were examined for ToLCJV symptoms expression daily till six weeks. The seedlings were rated according to (0-5) scale given by Banerjee and Kalloo,1987 as mentioned above. Total DNA was extracted from the genotypes showing resistant reaction on visual symptom bases and subjected to PCR amplification using begomovirus specific AV494/ AC1048 primers (Wyatt and Brown, 1996).

Screening of Germplasm against Pepper Mottle Virus

Preliminary screening for the virus was done under natural field conditions from the month of March to May as mentioned above. *Pepper mottle virus* symptom and severity was recorded following the 0-5 severity scale given by Mughal and Khan, 2001 with minor modification (Table 2).

The genotypes found resistant under field conditions were further confirmed by artificial screening. The *Pepper mottle virus* inoculum was maintained on susceptible line SL465 by sap inoculations to young healthy seedlings after every 15 days and maintained under insect proof conditions. The inoculum was prepared by grinding infected leaves in a grinder with phosphate buffer (0.01M).Ten plants for each genotype sown under insect proof conditions were sap inoculated with leaf rub method at 2-3 true leaf stage. After inoculation, leaves were washed with double distilled water to avoid any injury by carborandum powder and to remove excess inoculum. The inoculated plants were kept in an insect proof cage and observed regularly for symptom appearance up to five weeks. The disease incidence and severity was recorded on visual basis. The severity of plants was categorized as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible.

The genotypes showing resistant reaction were further subjected to double antibody sandwich ELISA (DAS-ELISA) against *Pepper mottle virus* (PepMoV) specific antibodies (procured from Agdia, Elkhart, USA) as per manufacturers procedure. The colour changes were read visually and photo metrically with ELISA Reader (Tecan, Austria) at 405 nm. Readings of ELISA plates were taken by ELISA reader at OD value of 405nm. ELISA reaction was rated as negative, mild positive, positive and strong positive according to scale given by Moury *et al.,* 2005.

Table 2. Modified 0-5 severity scale for *Pepper mottle virus* (Mughal and Khan, 2001)

Symptoms	Severity grade	Disease reaction
No visible symptoms	0	HR
Mild mottling on the upper leaves	1	R
Banding of vein on few leaves, Mosaic initiation on all leaves	2	MR
Distinct mosaic and vein-banding symptoms on all leaves	3	MS
Severe mosaic and vein-banding along with narrowing of leaves, and misshapen leaf lamina	4	S
Severe mosaic and vein-banding, misshapen leaves and fruits, defoliation, small number of fruits	5	HS

Results and Discussion

Screening of Pepper Germplasm against Root Knot Nematode (Meloidogyne incognita)

Out of total 140 genotypes evaluated, one genotype (PP 9950-5197-0107-7058) was found resistant to root-knot nematode (*M. incognita*) with root gall/egg mass index 1.0 (Table 3). The roots of this genotype were free from egg masses. Four genotypes *viz.,* S-343, SL 472, SAS-39 and CH-27 exhibited moderately resistant reaction with 3-10 galls per root system and showing root gall index ranging from 1.25 to 2.0. These genotypes also showed lesser number of egg masses per root system as compared to the susceptible checks, Punjab Surkh and Kashi Anmol. Among others, 67 genotypes showed moderately susceptible and 67 showed susceptible reaction to root knot nematode. Genotype VR 526 was found highly susceptible to root-knot nematode with root gall index 4.5 (Table 3).

Resistance to *M. incognita, M. javanica* and *M. arenaria* had been identified in several *Capsicum* germplsm sources, including *C. annuum* L., *C. chacoense* L., *C. chinense* Jacq., and *C. frutescens* L. (Di Vito *et al.,* 1992; Theis and Fery, 2002). The resistance to root-knot nematode species that are considered important from economic stand point such as *M. incognita*, *M. javanica* and *M. arenaria* have been associated with at least nine dominant genes (*N, Me1, Me2, Me3, Me4, Me5, Me6, Me7, Mech1 e Mech2*) in pepper that are considered independently acting on gene-for-gene basis (Djian-Caporalino *et al.,* 2001; Wang and Bosland, 2006; Fazari *et al.,* 2012). Some of these genes (*Me1, Me3, Me7*) are considered thermostable and effective against a wide range of *Meloidogyne* spp., including *M. incognita, M. arenaria* and *M. javanica* (Djian-Caporalino *et al.,* 1999). The genotypes showing resistant and moderately resistant reaction to RKN (*M. incognita*) could be further investigated using molecular markers for the presence of resistance genes for exploitation in resistance breeding programmes.

Screening of Germplasm against Viruses

Screening of Germplasm against Leaf Curl Disease

Out of total 140 genotypes screened, fifteen genotypes (EC-532386, PP0237-7508, PC-410, CH-21312, LR 325, S-343, NSS-2, 09/CHIVAR-4, VS-524, SD 463, SL 473, PC-6, PP 0537-7541, CC-148 and CH-27) were found symptomless with coefficient of infection (CI) = 0.0. One genotype (SL 466) was found highly resistant with CI=5.0 and five genotypes (JL 282, PLS-1, AC-101, SL-8E-1and IS-268) were found resistant with CI ranging from 6.25-1.0 under field conditions. Besides, twenty four genotypes were found moderately resistant (with CI ranging from 10.70-20.0), fifty genotypes moderately susceptible (with CI from 21.40-40.0), thirty nine susceptible (with CI from 41.63-66.0) and six highly susceptible (CI = 75.0) to leaf curl disease.

Table 3. Screening of pepper germplasm to root-knot nematode, *M. incognita*

Reaction / No. of genotypes	Root gall/Egg mass index (0-5) scale	Genotypes/lines
Resistant (1)	0.1-1.0	PP 9950-5197-0107-7058.
Moderately Resistant (4)	1.1-2.0	S-343, SL 472, SAS -39, CH-27.
Moderately Susceptible (67)	2.1-3.0	MSC-31-4-1, VR521, IS 264, IS 265, ML-345, Pepsi-8-1,VR-36, EC-532390, Selection 44, TM 481, AC-103, IS-266, VS-528, SS-470, MSFL, PLS-1, SHHP-4884, MS-12-2, IS-267, MS-341, LS 324, PC-410, ATG, CH-21312, DL161, 2011/CHIVAR-7, 10/CHIVAR-4 (Yellow), IS-263, S-2530, PC-408, line 1-6-4, CH-21412(Orange), LR 325, PG-1-1, 2011/CHIVAR-1, NSS-2, 2011/CHIVAR-9, PLS-14, PLS-5, PLS-3-1, FZ-202, PC-1, VS-524, PLS-2, Perennial, PLS-12, JCA-283, UY 502, G-4, LLS, 10/CHIVAR-6, PLS 5-2,SR 467, PLS-15, IS 269, CH-21311, ACC-06-01, JCA-288, MSFL-4-2, PP 0537-7541, SL-474, VS-530, IS-261, GS-221,Ujwala KAU, Vellayani Atutlya, CARI-1.
Susceptible (67)	3.1-4.0	MSFL-4-3-1, MSFL-1-2, SL465, PP409, PP 402, PP 91-7195-1, SL 469, MSFL-5-2-1, MSC-31-6-1B, VR 527, EC-532386, PP 0437-7510, M-344, AC-102, CH-21511(orange), Saurian-2010, BCC-1, SHHP-404, JL 282, US 501, VS 522, PLS-3, AC-101, IS-262, CH 21411, SL 471, CH-2221, PLS-13, Surajmukhi, Dev Long, CH-1, C-142, MSC-31-10-1, YL 581,IS-263, S-2539, PL-411, SL-8E-1, ML 342, PLS-10, IS-268, 09/CHIVAR-4, SD-463, L-326,VS-529, Punjab Tej, SL 473, CS 147, PC-6, SL461, 09/CHIVAR-6, SL 466, IS-270, Annugraha, BL-121, CC-148, CAG-43, CAG-29, CAG-24, CART-2, CARI-5, CARI-4, CAG-3,CARI-3, S-217621, Punjab Surkh (Susceptible Check), Kashi Anmol (Susceptible Check).
Highly Susceptible (1)	4.1-5.0	VR 526.

The resistance exhibited by some genotypes under field conditions cannot be inferred as true resistance but they might have escaped the infection. In order to establish their nature of resistance, further confirmation was done by artificial inoculation.

Screening of Genotypes against Tomato Leaf Curl Joydebpur Virus under Artificial Conditions

The genotypes showing symptomless (EC-532386, PP0237-7508, PC-410, CH-21312, LR 325, S-343, NSS-2, 09/CHIVAR-4, VS-524, SD 463, SL 473, PC-6, PP 0537-7541, CC-148,CH-27), resistant (JL 282, PLS-1, AC-101, SL-8E-1, IS-268) and highly resistant (SL 466) reaction during field screening were subjected to artificial screening against most prevalent begomovirus (*Tomato leaf curl Joydebpur virus*) causing leaf curl disease in pepper under Punjab conditions.

It was observed that in susceptible check Kashi Anmol and genotypes, PLS-1, SL-8E-1, VS-524, Chilli colletion-4 and Surajmukhi symptoms started appearing on 18th day after inoculation and up to the 26thday after inoculation, all the genotypes were showing symptoms (Table 4). However, there was difference in symptom severity among the

Table 4. Screening of pepper genotypes against *Tomato leaf curl Joydebpur virus* under artificial conditions

S.No.	Genotype	Symptom appearance (Days after inoculations)	Reaction after Artificial Inoculation					
			Type of Symptoms	Severity Grade 0-5 scale	Disease Incidence (%)	Response Value	Coefficient of Infection	Disease Reaction
1	NSS-2	26	Mild curling of leaves	2.0	75.0	0.25	18.7	MR
2	S-343	22	Mild puckering no curling of leaves	2.0	100	0.25	25.0	MR
3	SL 473	23	Puckering without curling	2.0	70.0	0.25	17.5	MR
4	CH- 27	26	Mild curling of leaves	2.0	100	0.25	25.0	MR
5	LR 325	24	Mild curling of leaves and vein thickening	2.0	100	0.25	25.0	MR
6	EC-532386	22	Mild puckering, yellowing and curling of leaf margins	3.0	50.0	0.5	25.0	MS
7	PP0237-7508	23	Puckering, yellowing and curling	3.0	75.0	0.5	37.5	MS
8	PLS-1	18	Mild curling, twisting of leaf with swelling of veins	2.0	100	0.25	25.0	MS
9	AC-101	20	Curling of leaf margins with vein thickening	2.0	100	0.25	25.0	MS
10	PP 0537-7541	22	Puckering and downward curling of leaf margins	2.0	100	0.25	25.0	MS
11	Surajmukhi	18	Mild puckering and curling of leaf margins	3.0	50.0	0.5	25.0	MS
12	09/CHIVAR-4	23	Mild puckering and curling of leaves	2.0	100	0.25	25.0	MS
13	SD 463	20	Puckering and downward curling of leaf margins	2.0	100	0.25	25.0	MS
14	JL 282	20	Curling of leaves and vein swellings	2.0	100	0.25	25.0	MS
15	SL-8E-1	18	Pronounced puckering, curling and crinkling of leaves	3.0	100	0.5	50.0	S
16	IS-268	24	Mild puckering and curling of leaves	3.0	100	0.5	50.0	S
17	VS-524	18	Pronounced puckering and curling and twisting of leaf	3.0	88.0	0.5	44.0	S
18	SL 466	20	Puckering and downward curling of leaf margins	3.0	100	0.50	50.0	S
19	Chilli Collection-4	18	Pronounced puckering, curling and crinkling of leaves	3.0	100	0.50	50.0	S
20	PC-410	20	Puckering and downward curling of leaf margins	3.0	100	0.5	50.0	S
21	CH-21312	20	Downward curling of leaf margins	3.0	100	0.5	50.0	S
22	PC-6	20	Puckering and downward curling of leaf margins	3.0	100	0.5	50.0	S
23	KashiAnmol (Susceptible check)	18	Pronounced puckering, curling and crinkling of leaves, stunting of plants	5.0	100	1.0	100	HS
24	Punjab Surkh (Susceptible check)	20	Puckering, curling of leaves with thickening of veins	4.0	100	0.7	75.0	HS

genotypes. Five genotypes *viz.,* NSS-2, S-343, SL 473, CH-27 and LR 325 showed only mild puckering of leaves even 22 days after inoculation with CI ranging from 17.5 to 20.0. These genotypes were therefore, regarded as moderately resistant. The other genotypes which were symptomless (EC-532386, PP0237-7508, 09/CHIVAR-4, VS-524, SD463, CC-148, PP0537-7541, PC-410, CH-21312, PC-6, LR 325) highly resistant (SL 466) and resistant (JL 282, IS-268, Sel8-E, PLS-1, AC-101) under field conditions showed pronounced puckering, curling and blistering of leaves after artificial inoculations and were therefore rated as moderately susceptible and susceptible, respectively. The susceptible checks, Kashi Anmol and Punjab Surkh showed typical leaf curl type symptoms with pronounced puckering, curling and crinkling of leaves along with vein thickenings. The DNA of artificially inoculated lines giving resistant reaction when subjected to PCR amplification, showed presence of virus by amplifying ~570 bp band with begomovirus specific AV/AC primers. But the apparent symptoms vary with different genotypes which show that there is some resistant mechanism working in the genotypes NSS-2, S-343, SL 473, CH-27 and LR 325.

In India, a number of hot pepper lines resistant to leaf curl virus has been reported by different workers. Pepper cultivars like Perennial, BG-1, Lorai, and Punjab Lal are reported as important multiple disease resistant lines (Thakur *et al.,* 1987; Singh and Singh, 1989). The symptomless reaction of genotypes under field conditions can either be attributed to slow multiplication of virus which may be one of the probable reasons of resistance to leaf curl disease or may be simply due to escape (Banerjee and Kalloo, 1987). The resistance by escape could be because of a non-preference of whitefly under field conditions to a particular genotype planted along with several other genotypes or such lines may not be suitable for whitefly multiplication but suitable for virus multiplication and *vice-versa*. Hence, the field reaction of genotypes to particular organism may not be considered for having true resistance. However, if some genotype(s) show consistently low disease incidence under field conditions followed by artificial inoculation these genotypes might have some economic value. Even if viral DNA is detected using PCR assays these genotypes appear to be a good source of resistance for use in breeding programmes.

Screening of Genotypes against Pepper Mottle Virus

For *pepper mottle virus*, out of total 140 genotypes, 19 were found highly resistant, 11 resistant, 42 moderately resistant, 51 moderately susceptible, 16 susceptible and one genotype was found to be highly susceptible to the virus under natural field conditions. For further confirmation of resistance, a total of 49 lines comprising 19 genotypes showing highly resistant reaction, 11 resistant, 15 moderately resistant, 3 moderately susceptible and one highly susceptible reaction during natural field screening were tested under artificial conditions by sap inoculation.

It was observed that most of the genotypes exhibiting highly resistant and resistant reaction during field screening were found susceptible or moderately susceptible under artificial conditions (Table 5). Ten genotypes (VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2, SL 472,Surajmukhi and MSFL-1-2) showed resistant reaction, nine genotypes (IS 269, VR521, BCC-1, EC-532390, Punjab Tej, IS-267, LR 325, S-343 and CH 27) showed moderately resistant reaction, seventeen genotypes (MSFL 4-3-1, DL161, ML-345, PC-408,CH-21411, MSC 31-10-1, PLS-14, SL-8E-1, AC 103, CH-2221, Line 1-6-4, PLS-13, Dev long, YL 581, PLS-3-1, Kashi Anmol and Punjab Surkh) showed moderately susceptible reaction, and ten genotypes (PP 402, MSC 31-4-1, SS-470, SHHP-4884, M-344, PG 1-1, CC-148, CART-2, CARI-1 and JCA-283) showed susceptible reaction. Three genotypes *viz*., CH-1, CARI-4 and SL465 showed highly susceptible reaction under artificial screening.

On confirmation of lines showing resistance during artificial screening by ELISA, it was observed that out of ten genotypes showing resistant reaction to PepMoV, eight genotypes (VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2 and SL 472) gave negative ELISA reaction. While two genotypes *viz*., Surajmukhi and MSFL-1-2, showed mild positive (+) and positive (++) reaction with ELISA, respectively (Table 5). Among the genotypes showing moderately resistant reaction five genotypes *viz*., BCC-1, IS 269, VR521, S-343, CH-27 showed mild positive (+) reaction with ELISA whereas four genotypes *viz*., EC-532390, Punjab Tej, IS-267, LR 325 gave positive (++) reaction with ELISA. Similarly, lines showing moderately susceptible and susceptible reaction during artificial screening showed mild positive to strong positive reaction with ELISA. The genotypes CARI-4, SL465 and CH-1 showing highly susceptible reaction during artificial reaction gave strong positive (+++) reaction with ELISA.

Table 5. Screening of pepper germplasm against *Pepper mottle virus* under artificial conditions

S No.	Disease Reaction/ No. of genotypes	Severity Grade (0-5) Scale	Line /Genotype	ELISA Reaction*
1	Resistant (8)	1	VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266SS-470, NSS-2, SL 472.	-
2	Resistant (1)	1	Surajmukhi	+
3	Resistant (1)	1	MSFL-1-2.	++
4	Moderately Resistant (5)	2	BCC-1, IS 269, VR521,S-343, CH-27.	+
5	Moderately Resistant (4)	2	EC-532390, Punjab Tej, IS-267, LR 325, PP 9950-5197-0107-7058	++
6	Moderately Susceptible (11)	3	DL-161, ML-345, PC-408, MSC-31-10-1, PLS-14,SL-8E-1,AC-103, CH-221 (Orange), Line 1-6-4, Dev Long, PLS-3-1.	+
7	Moderately Susceptible (5)	3	MSFL-4-3-1,PLS-13, YL 581, KashiAnmol (Susceptible Check), Punjab Surkh (Susceptible Check).	++
8	Moderately Susceptible (1)	4	CH-21411	+++
9	Susceptible (4)	4	SHHP-4884, CC-148, CART-2, JCA-283.	+
10	Susceptible (2)	4	MSC-31-4-1,M-344.	++
11	Susceptible (4)	4	PG-1-1, PP 402, SS-470, CARI-1.	+++
12	Highly Susceptible (3)	5	CARI-4, SL465, CH-1.	+++

*Scale for depicting ELISA results (Moury *et al.*,2005)

OD at 405 nm	Response	Symbol
Same as negative control	Negative	-
2-5 times higher than negative control	Mild positive	+
5-10 times higher than negative control	Positive	++
10-15 times higher than negative control	Strong positive	+++

Earlier, *Capsicum annuum* L. cv. Avelar was shown to have a monogenic, recessively inherited factor for resistance to *Pepper mottle potyvirus* (PepMoV) (Zitter and Cook, 1973; Guerini and Murphy, 1999). In India, not much information is available regarding hot pepper resistance to PepMoV. However, resistance to potyvirus viz., *Chilli veinal mottle virus* and *Pepper veinal mottle virus* was identified at AVRDC in two accessions of *C. annuum*, 'Perennial HDV' and 'PSP-11', which were originally of Indian origin (Anonymous, 1990; 1991).

Discussion

Hot pepper is one of the important spice and vegetable crop of India with high export potential. Problem of root-knot nematodes and viruses limits the pepper cultivation in many tracts of India particularly hilly and northern plains of the country. Due to the lack of effective management strategies, growing of resistant sources is the most economical strategy for management of these diseases. Identification of resistant source(s) and their utilization in resistant breeding programme are thus imperative to manage these pathogens in the long run. Thus, the present study was carried out keeping this objective in mind. The results showed that out of total 140 pepper genotypes evaluated against root knot nematode (*M. incognita*), *Tomato leaf curl Joydebpur virus* and *Pepper mottle virus*, one genotype (PP 9950-5197-0107-7058) was found resistant and four genotypes (S-343, SL 472, SAS-39 and CH-27) moderately resistant to *M. incognita*. For viruses, four genotypes *viz.*, NSS-2, S-343, SL 473 and CH-27 were found promising against *Tomato leaf curl Joydebpur virus* and eight genotypes (VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2, SL 472) were found resistant against *Pepper mottle virus.* In addition to resistance against individual pathogens, four genotypes *viz.*, SL 472, NSS-2, S-343 and PP 9950-5197-0107-7058 were found promising for multiple disease resistance against these pathogens. On comparing together the individual screening results against each pathogen it was found that genotype 'S-343' showed moderately resistant reaction to both the viruses and root-knot nematode, SL 472 was found resistant to PepMoV and moderately resistant to RKN, NSS-2 was observed resistant to PepMoV and moderately resistant to *Tomato leaf curl Joydebpur virus* and genotype PP 9950-5197-0107-7058 was resistant to *M. incognita* and moderately resistant to PepMoV. Earlier, Pegard *et al.*, 2005 also reported disease resistance in *Capsicum annuum* against more than one pathogen.They found that the line CM334, being used by breeders as a source of resistance to

Phytophthora species and potyviruses, entirely suppresses reproduction of the root-knot nematode (*Meloidogyne* species). Thus, the promising genotypes identified in the present study can be exploited as source of resistance in breeding programme against these pathogens.

References

Anonymous (1990) *Progress Report.* Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Anonymous (1991) *Progress Report*. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Banerjee MK and G Kalloo (1987) Sources and inheritance of resistance to *Leaf curl virus* in *Lycopersicon*. *Theor. Appl. Genet.* **73**: 707-710.

Bosland PW and EJ Votava (2000) *Pepper: Vegetable and Spice Capsicum*. CABI Publishing CAB International, Wallingford /UK.

Di Vito M, V Cianciotta and G Zaccheo (1992) Yield of susceptible and resistant pepper in micro plots infested with *Meloidogyne incognita*. *Nematropica* **22**: 1-6.

Djian-Caporalino C, L Pijarowski , A Januel, V Lefebvre, T Phally, A Palloix, A Dalmasso and P Abad (1999) A spectrum of resistance to root-knot nematodes (*Meloidogyne* species) in sweet pepper (*Capsicum annuum* L.) and inheritance of heat-stable resistance in the PM687 line derived from PI 322719. *Theor. Appl. Genet.* **99**: 496-502.

Djian-Caporalino C, L Pijarowski, A Fazari, M Samson, L Gaveau, CO'Byrne, V Lefebvre, C Caranta, A Palloix and P Abad (2001) High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci *Me3* and *Me4* conferring heat-stable resistance to root-knot nematode (*Meloidogyne* species). *Theor. Appl. Genet.***103**: 592-600.

Fazari A, A Palloix, LW Wang, MYAM Hua, S Age-P Alloix, BX Zhang and C Djian-Caporalin (2012) The root-knot nematode resistance N-gene co-localizes in the Me-genes cluster on the pepper (*Capsicum annuum* L.) P9 chromosome. *Plant Breeding* **131**: 665-673.

Guerini MN and JF Murphy (1999) Resistance of *Capsicum annuum* 'Avelar' to pepper mottle potyvirus and alleviation of this resistance by co-infection with Cucumber mosaic cucumovirus are associated with virus movement. *J. Gen. Virol.* **80**: 2785-2792.

Jain RK, KN Mathur and R Singh (2007) Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian J. Nematol.* **37**: 219-221.

Kaur S, SS Kang, A Sharma, S Sharma (2014) First report of *Pepper mottle virus* infecting chilli pepper in India. *New Dis. Rep.* **30**: 14.

Khan MW and SH Haider (1991) Comparative damage potential and reproduction efficiency of *Meloidogyne javanica* and races of *M. incognita* on tomato and eggplant. *Nematologica* **37**: 293-303.

Kim YJ, MG Jonsona, HS Choi, SJ Ko and KH Kim (2009) Molecular characterization of Korean *Pepper mottle virus* isolates and its relationship to symptom variations. *Virus Res.* **144**: 83-88.

Kumar S and M Rai (2005) Chilli in India. *Chilli Pepper Institute Newsletter*. pp 1-3.

Moury B, A Palloix, C Caranta and P Gognalons (2005) Serological, molecular, and patho type diversity of *Pepper veinal mottle virus* and *Chilli veinal mottle virus*. *Phytopathol.* **95**: 227.

Mughal SM and MA Khan (2001) *Disease rating scale for the assessment of disease severity of PVX and PVY to facilitate the researchers and students working on plant viruses.* M.Sc Thesis. Department Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

Muniyappa V and GK Veeresh (1984) *Plant virus diseases transmitted by whiteflies in Karnataka*. Proceedings of the National Academy of Sciences (Animal Science) **93**: 397-406.

Pegard A, G Brizzard, A Fazari, O Soucaze, P Abad and C Djian-Caporalino (2005) Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in *Capsicum annuum*. *Phytopathol.* **95**: 158-165.

Sandhu KS, JS Chohan (1979) Studies on the characterization of the mottle disease of chilli (*Capsicum annuum*) in the Punjab. *Indian J. Mycol. Plant Pathol.* **9**: 177-182.

Senanayake DMJB, B Mandal, S Lodha and A Varma (2006) First report of *Chilli leaf curl virus* affecting chilli in India. *New Dis. Rep.* **13**: 27.

Shih SL, WS Tsai, SK Green and D Singh (2007) First report of *Tomato leaf curl Joydebpur virus* infecting chilli in India. *Plant Path.* **56**: 341.

Shukla DD, CW Ward and AA Brunt (1994) *The Potyviridae*. Wallingford, UK: CAB International.

Singh MJ and J Singh (1989) Mechanism of resistance to Cucumber mosaic virus in chilli pepper (*Capsicum annum* L.) In: *Role of phenols and phonologies*. EUCARPIA VII meeting on genetics and breeding on *Capsicum* and eggplant, Yugoslavia, pp193-203.

Taylor AL and JN Sasser (1978) *Biology, identification and control of root-knot nematodes* (*Meloidogyne species).* Coop Publ. Department of Plant Pathology, North Carolina State Univ. and the US Agency for 1st Div. Raleigh, NC, 111 p.

Thakur MR, J Singh, I Singh and BS Sooch (1987) Punjab La1 – a new multiple disease resistant chilli variety. *Progressive Farming* **23**: 11-16.

Theis JA and RL Fery (2002) Heat stability of resistance to southern root-knot nematode in bell pepper genotypes homozygous and heterozygous for the *N* gene. *J. American Soc. Hort. Sci.* **127**:371-375.

Wang D and PW Bosland (2006) The genes of Capsicum. *Hort. Sci.* **41**: 1169-1187.

Wyatt SD and JK Brown (1996) Detection of geminiviruses in aqueous extracts by polymerase chain reaction. *Phytopathol.* **86:** 1288-1293.

Zitter TA and AA Cook (1973) Inheritance of tolerance to a pepper virus in Florida. *Phytopathol.* **63**: 1211-1212.

Zitter TA (1972) Naturally occurring pepper virus strains in South Florida. *Plant Dis. Rep.* **56**: 586.