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PHENOTYPIC CATEGORIZATION OF RESISTANCE AGAINST INFECTION BY TWO *BEGOMOVIRUS* (SLCCNV AND ToLCNDV) SPECIES IN *Cucurbita moschata* Duchesne

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ABSTRACT

Squash leaf curl China virus and tomato leaf curl New Delhi virus are the two most prevalent *begomovirus* species in India, causing 100 per cent crop losses in pumpkin. The present study was envisaged to develop a disease rating scale for SLCCNV, ToLCNDV, and their mixed infection in *Cucurbita moschata*. The disease severity of both viruses was assessed at different stages *viz*., nursery, 45 days after sowing (DAS)and 75 DAS to identify the best stage and scale for selecting resistant genotypes to be used in breeding programmes. Based on disease reaction, 10 genotypes were classified into four phenotypic classes *viz*., resistant (1), moderately resistant (2), moderately susceptible (1) and susceptible (6) against these viruses. PVR-1343 showed resistant disease reaction, whereas P-1327 and P-6777 were moderately resistant. Disease expression of all the genotypes changed from nursery stage to 45 DAS along with quantum jump in their coefficient of infection values. However, the same disease reaction was exhibited by all the genotypes from 45 to 75 DAS. Thus, the disease rating scale at 45 DAS is appropriate and effective in terms of resources and time for identifying resistant genotypes in pumpkin. The genotypes expressed similar resistance level with PVR-1343 depicting resistance against both viruses alone and in mixed infection. It can be further utilized in hybridization programmes to develop resistant hybrids and/or to transfer disease resistance into well adapted *C. moschata* varieties.

Keywords: Artificial screening, Phenotyping, Pumpkin, Squash leaf curl China virus, Tomato leaf curl New Delhi virus

Pumpkin (*Cucurbita moschata* Duchesne) is a member of the *Cucurbitaceae* family and has chromosome number 2n=40. It is one of the most important vegetable crops grown in the tropical, subtropical, and temperate regions of the world (Saez *et al.,* 2021). India is the world's second (5.50MT) largest producer of pumpkin after China (8.42MT; Anonymous, 2019). From past two decades due to climate change, viral diseases have erupted on a large scale across crops (Martín-Hernandez and Pico, 2021) and could be the major reason for decline in pumpkin production in India. Among viruses, *begomovirus* belonging to *Geminiviridae* family is the most common and prevalent in India. Three bipartite species of geminivirus infecting *C. moschata* have been reported from different parts of India *viz*., *squash leaf curl China virus* (SLCCNV, India: Coimbatore: Pumpkin; Muniyappa *et al.,* 2003), *tomato leaf curl New Delhi virus* (ToLCNDV, India: New Delhi: Pumpkin; Maruthi *et al.,*2007) and *tomato leaf curl Palampur virus* (ToLCPMV, India: Varanasi: Pumpkin; Jaiswal *et al.,* 2011). These viruses are prevalent from July to September in Northern India (NI) while, from February to June in Southern India (SI) (Muniyappa *et al.,* 2003; Singh *et al*., 2009) which resulted in *begomovirus* epidemic in SI during 2004 followed by NI during 2007 (Muniyappa *et al.,* 2003; Singh *et al*., 2009; Raj *et al*., 2012).

In Punjab, yellow vein mosaic virus disease (YVMVD) caused by SLCCNV was first reported in 2009 (Sharma and Kang, 2009), while ToLCNDV and mixed infection of both viruses were observed recently (unpublished data). Both the viruses have been reported to cause cent per cent crop loss during rainy season, thereby restricting pumpkin cultivation to spring season only (Sharma *et al*., 2012, Dhatt *et al*., 2020). The typical symptoms of both the viruses consist of yellow vein, leaf curling, puckering of interveinal areas, rolling, thickening, and swelling of veins, shorter internodes, leaf clustering at the apical region and severe stunting of diseased plant.

The begomoviruses are transmitted by whitefly (*Bemisia tabaci* Gen.). A single viruliferous whitefly causes 21.67 per cent infection while, 15 whiteflies per plant lead to cent per cent infection (Muniyappa *et al*., 2003). Therefore, the most effective and economical method to control viral diseases is to cultivate resistant varieties. In this context, breeding against begomovirus(es) resistance began at Punjab

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Agricultural University (PAU) by screening the available germplasm under natural and artificial epiphytotic conditions. Viruses have their own symptoms and their aggressiveness and expression which vary with crop and their growth stages. Therefore, to score disease reaction of SLCCNV, ToLCNDV and their mixed infection effectively, this study was envisaged to devise a disease rating scale for pumpkin at different growth stages *viz*., nursery stage (4-6 leaf stage), 45 days after sowing (DAS), and at 75 DAS.

MATERIALS AND METHODS

Plant material

The experimental material consisted of ten diverse pumpkin genotypes (P-61, P-62, P-64, P-65, P-78, P-133, P-370, P-1327, P-6777 and PVR-1343). These genotypes were subjected to whitefly mediated artificially screening against SLCCNV, ToLCNDV and mixed infection (MI) of both viruses during rainy season 2017.

Artificial screening

Whitefly (Bemisia tabaci)

Non-viruliferous whitefly (*Bemisia tabaci* Asia-II strain) culture maintained on cotton plants in the insect-proof cages at Department of Vegetable Science, PAU Ludhiana, Punjab, India was used to inoculate healthy pumpkin seedlings.

Virus inoculum

The inoculum of PCR {SLCCNVF/SLCCNVR (unpublished data) and CRNDv30/CRNDc1181 (Reddy *et al.,* 2005)} confirmed SLCCNV, ToLCNDV and mixed infection (MI) maintained in three separate insect proof cages on susceptible pumpkin cultivar Punjab Samrat was used to study the response of 10 genotypes against each virus.

Artificial inoculation of genotypes against SLCCNV, ToLCNDV and mixed infection

Nursery of 10 pumpkin genotypes to be screened was raised in plug trays filled with cocopeat, vermiculite and perlite mixture in 3:1:1 proportion. The non-viruliferous whiteflies maintained on cotton plants were collected with an aspirator in bottles. These non-viruliferous whiteflies were released on infected Punjab Samrat plants raised in respective cages for maintenance of virus inoculum and viruliferous vector. This procedure of releasing non-viruliferous whiteflies on infected inoculum was repeated for one week. When sufficient whiteflies were maintained in respective cages, plug trays with healthy seedlings at two true leaf stage of all 10 genotypes were placed inside the cages. Further for screening of plants at 45 and 75 DAS, 20 seedlings per genotype raised in polybags filled with soil and FYM (1:1) were kept in each cage. Virus infected plants (inoculum) were shaken 2-3 times a day for uniform spread of virus by whiteflies. Data on symptom severity grade ranging from 0 to 4 was recorded at 4-5 true leaf stage in nursery (Table 1), 45 DAS (Table 2) and 75 DAS (Table 3) on individual plants in all the genotypes. Each severity grade was assigned a response value as given in Table 1, 2 and 3. Disease incidence (DI) was calculated by dividing number of diseased plants to the total number of plants. DI was then multiplied by response value to get coefficient of infection (CI). Based on CI, each genotype of *C. moschata* was assigned a specific disease reaction (DR; Table 1, 2 and 3).

RESULTS AND DISCUSSION

Disease reaction against SLCCNV

Based on coefficient of infection (CI) at nursery stage, only one genotype, PVR-1343 was observed as highly resistant (HR), while the genotypes *viz*., P-6777 and P-1327 exhibited resistant (R) and moderately resistant (MR) reaction with CI values of 5.00 and 8.75, respectively. The genotypes *viz*., P-370 and P-133 with CI values of 28.75 and 36.25, respectively were ranked moderately susceptible (MS). The other five genotypes (P-61, P-62, P-64, P-65, and P-78) showed a susceptible (S) disease reaction with CI ≥57.50 (Table 4). At 45 DAS (Table 2), similar trend was recorded in nursery stage screening for all genotypes except PVR-1343 and P-6777, whose DR changed from HR to R, R to MR respectively (Table 4). However, CI values showed quantum jump for each category. The genotypes categorized as susceptible during nursery stage/45 DAS maintained their disease reaction even at 75 DAS with highest CI value (100). CI value also increased for MS and MR genotypes, whereas for resistant genotype *viz*., PVR-1343, it declined from 3.75 to 2.50 (Table 4).

Disease reaction against ToLCNDV

Based on phenotypic observations, ToLCNDV resulted in more severe and aggressive symptoms than SLCCNV. The susceptible genotypes under SLCCNV exhibited similar response for ToLCNDV with CI value ≥58.75. The disease response of P-370 (MS) remained same for ToLCNDV at nursery stage, 45 DAS, and 75 DAS, respectively. While, P-133 shifted from MS disease reaction at nursery/45 DAS to susceptible at 75 DAS. The MR genotypes for SLCCNV (P-1327 and P-6777) also exhibited MR disease reaction for ToLCNDV at 75 DAS. PVR-1343 was invariably found resistant against this virus with a CI value of 2.5 (Table 5).

Table 1. Phenotypic classes of SLCCNV, ToLCNDV and their mixed infection (MI) for artificial screening of nursery in *C. moschata*

SLCCNV		ToLCNDV		MI		SSG	Response Value	CI	DR
Symptom	Phenotype	Symptom	Phenotype	Symptom	Phenotype				
No visual symptom		No visual symptom		No visual symptom		0	0	0-1.24	Highly Resistant (HR)
Chlorotic spots on 1-2 true leaves		Puckering on 1-2 true leaves		Mild puckering and minute chlorotic spots on 1-2 true leaves		1	0.25	1.25-6.25	Resistant (R)
Starting of yellow vein covering upto 25 % area of 1-2 true leaves		Curling of 1-2 true leaves and size reduction of newly emerging leaf		Mild puckering, minute chlorotic spots/yellow veins on 1-2 true leaves with severe reduction and curling of newly emerging leaf		2	0.50	6.26-25	Moderately Resistant (MR)
Yellow vein covering 25-50 % area of 2-3 true leaves		Curling and puckering of 1-2 true leaves and distortion of petiole and veins of new leaves with size reduction		Yellow veins of >2 true leaves with severe reduction and curling of newly emerging leaf		3	0.75	25.1-56.25	Moderately Susceptible (MS)
Yellow vein covering >50 % area of all true leaves		Curling and puckering of all true leaves and severe reduction of leaf size		Yellow veins with severe reduction and curling of all leaves		4	1.00	>56.25	Susceptible (S)

SSG: Symptom severity grade, CI: Coefficient of infection, DR: Disease reaction

Table 2. Phenotypic classes of SLCCNV, ToLCNDV and their mixed infection for artificial screening at 45DAS in *C. moschata*

SLCCNV		ToLCNDV		MI		SSG	Response Value	CI	DR
Symptom	Phenotype	Symptom	Phenotype	Symptom	Phenotype				
No visual symptom		No visual symptom		No visual symptom		0	0	0-1.24	Highly Resistant (HR)
0 to 5% leaf area showing chlorotic spot of pin head size on the older leaves		Mild curling on top 1-2 leaves		0 to 5% leaf area showing chlorotic spot of pin head size on the young leaves with mild puckering		1	0.25	1.25-6.25	Resistant (R)
6 to 40% leaf area showing yellow vein on top 2-3 leaves		Curling and puckering of top 2-4 leaves with size reduction		6 to 40% leaf area showing curling of top 2-3 leaves along with yellowing of vein		2	0.50	6.26-25	Moderately Resistant (MR)
41 to 75% leaf area showing yellow vein on top 3-4 leaves and stunted growth		Curling and puckering of 5-6 leaves and distortion of petiole and veins of infected leaves with size reduction		41 to 75% leaf area showing puckering /curling along with yellow vein on top 3-4 leaves with stunted growth		3	0.75	25.1-56.25	Moderately Susceptible (MS)
> 75% leaf area or all the leaves showing yellow vein with stunted growth		All the leaves showing curling and puckering with severe stunted growth		All the leaves showing curling with yellow vein and severe stunted growth		4	1.00	>56.25	Susceptible (S)

Table 3. Phenotypic classes of SLCCNV, ToLCNDV and their mixed infection for artificial screening at 75DAS in *C. moschata*

SLCCNV		ToLCNDV		MI		SSG	Response Value	CI	DR
Symptom	Phenotype	Symptom	Phenotype	Symptom	Phenotype				
No visual symptom		No visual symptom		No visual symptom		0	0	0-1.24	Highly Resistant (HR)
0 to 5% leaves showing chlorotic spot		0 to 5% leaves showing curling on top 1-2 leaf		0 to 5% leaves showing chlorotic spot with mild puckering		1	0.25	1.25-6.25	Resistant (R)
6 to 40% leaves of vine infected with yellow vein		6 to 40% leaves of vine showing curling with size reduction		6 to 40% leaves of vine showing curling, yellow vein with overall size reduction		2	0.50	6.26-25	Moderately Resistant (MR)
41 to 75% leaves of vine showing yellow vein with stunted growth		41 to 75% leaves of vine showing curling with stunted growth		41 to 75% leaves of vine showing curling, yellow vein and severe stunted growth		3	0.75	25.1-56.25	Moderately Susceptible (MS)
>75% leaves or all the leaves showing yellow vein, or yellow /bleached leaves with stunted growth		All the leaves showing curling with severe stunted growth		All the leaves showing curling, yellow vein with severe stunted growth		4	1.00	>56.25	Susceptible (S)

Table 4. Disease reaction of *C. moschata* genotypes against SLCCNV at different developmental stages

Genotype	SLCCNV at nursery stage				SLCCNV at 45DAS				SLCCNV at 75DAS			
	DI (%)	SSG	CI	DR	DI (%)	SSG	CI	DR	DI (%)	SSG	CI	DR
P-61	95	1,2,3,4	60.00	S	100	3,4	81.25	S	100	4	100.00	S
P-62	90	1,2,3,4	61.25	S	100	3,4	76.25	S	100	4	100.00	S
P-64	90	1,2,3,4	65.00	S	100	1,2,4	87.50	S	100	4	100.00	S
P-65	95	1,2,3,4	57.50	S	100	2,3,4	75.00	S	100	4	100.00	S
P-78	90	1,3,4	58.75	S	95	2,3,4	78.75	S	100	4	100.00	S
P-133	75	1,2,3	36.25	MS	85	1,2,3,4	45.00	MS	100	1,2,3	52.50	MS
P-370	65	1,2,3	28.75	MS	75	1,2,3	41.25	MS	95	1,2,3	47.50	MS
P-1327	35	1	8.75	MR	50	1,2	16.25	MR	70	1,2	21.25	MR
P-6777	20	1	5.00	R	50	1,	12.50	MR	60	1,2	17.50	MR
PVR-1343	0	0	0.00	HR	15	1	3.75	R	10	1	2.50	R

DI: Disease Incidence, SSG: Symptom severity grade, CI: Coefficient of infection, DR: Disease reaction

Disease reaction against mixed infection

The genotypes *viz*., P-61, P-62, P-64, P-65, and P-78 exhibiting susceptible reactions to SLCCNV and ToLCNDV, revealed a similar disease response for mixed infection (MI) with CI ≥60. The P-133 (S) and P-370 (MS), showed the same trend of disease reaction as in ToLCNDV. MR genotype identified under SLCCNV and ToLCNDV were also found MR to mixed infection of SLCCNV and ToLCNDV. The genotype PVR-1343 had a CI value ranging from 0 to 2.5 and showed a resistant disease reaction (Table 6).

Therefore, based on disease reaction against SLCCNV, ToLCNDV and their mixed infection, PVR-1343 can be categorized as resistant, P-1327 and P-6777 as moderately resistant, and P-370 as moderately susceptible. While six genotypes *viz*., P-61, P-62, P-64, P-65, P-78 and P-133 showed the susceptible disease reaction in all cases, except P-133 which is MS for SLCCNV (Table 4, 5 & 6).

The expression and aggressiveness of each virus depends on its virulence therefore, disease rating with same scale against all viruses can be ineffective (Islam *et al.,*2015). Thereby independent scales are being proposed for both the viruses as well as for their mixed infection. Being fast-growing, pumpkin seedlings can be retained in plug trays only for 20-25 days and needs immediate transplanting thereafter. Moreover, the etiology and symptomology of each virus varied with crop stage. On perusal of data, it was seen that disease reaction of R, MR, and MS plants of all genotypes varied from nursery to flowering stage. However, susceptible plants at nursery maintained their reaction over all the crop stages. Therefore, screening of resistant and moderately resistant/susceptible plants should be carried forward from nursery stage, necessitating the development of a scale(s) for advanced crop stages. Disease rating of all genotypes with respect to independent scales at 45 and 75 DAS, showed no

Table 5. Disease reaction of *C. moschata* genotypes against ToLCNDV at different developmental stages

Genotype	ToLCNDV at nursery stage				ToLCNDV at 45DAS				ToLCNDV at 75DAS			
	DI (%)	SSG	CI	DR	DI (%)	SSG	CI	DR	DI (%)	SSG	CI	DR
P-61	90	1,2,3,4	61.25	S	100	2,3,4	95.00	S	100	4	100.00	S
P-62	95	1,2,3,4	67.50	S	100	4	100.00	S	100	4	100.00	S
P-64	90	2,3,4	77.50	S	100	4	100.00	S	100	4	100.00	S
P-65	85	1,2,3,4	58.75	S	100	2,3,4	91.25	S	100	4	100.00	S
P-78	80	1,2,3,4	58.75	S	100	2,4	90.00	S	100	4	100.00	S
P-133	85	1,2,3	33.75	MS	100	1,2,3,4	50.00	MS	100	2,3,4	68.75	S
P-370	75	1,2,3	28.75	MS	100	1,2,3,4	50.00	MS	100	1,2,3,4	53.75	MS
P-1327	30	1	7.50	MR	60	1,2,3	21.25	MR	65	1,2	23.75	MR
P-6777	25	1	6.25	R	45	1,2,3	16.25	MR	55	1,2,3	18.75	MR
PVR-1343	0	0	0	HR	15	1	3.75	R	10	1	2.50	R

Table 6. Disease reaction of *C. moschata* genotypes against MI of SLCCNV and ToLCNDV at different developmental stages

Genotype	MI at nursery stage				MI at 45DAS				MI at 75DAS			
	DI (%)	SSG	CI	DR	DI (%)	SSG	CI	DR	DI (%)	SSG	CI	DR
P-61	90	1,2,3,4	65.00	S	100	3,4	97.50	S	100	4	100.00	S
P-62	95	1,2,3,4	65.00	S	100	4	100.00	S	100	4	100.00	S
P-64	95	2,3,4	76.25	S	100	4	100.00	S	100	4	100.00	S
P-65	90	1,2,3,4	60.00	S	100	2,3,4	88.75	S	100	4	100.00	S
P-78	85	1,2,3,4	62.50	S	100	2,4	92.50	S	100	4	100.00	S
P-133	90	1,2,3	36.25	MS	100	1,2,3,4	43.75	MS	100	2,3,4	66.25	S
P-370	80	1,2,3	33.75	MS	100	1,2,3,4	50.00	MS	100	1,2,3	51.25	MS
P-1327	45	1	11.25	MR	60	1,2	20.00	MR	75	1,2	22.50	MR
P-6777	20	1	5.00	R	45	1,2	15.00	MR	45	1,2	12.50	MR
PVR-1343	0	0	0	HR	10	1	2.50	R	10	1	2.50	R

variation in the disease reaction of resistant/tolerant plants. Thereby ascertaining that the disease rating at 45DAS is appropriate and effective for identification of resistant genotypes in pumpkin. The present screening technique is similar to methodology adopted by Sharma *et al* (2018), while standardizing disease rating scale for screening the plants against chilli leaf curl virus disease (ChiLCVD) in chilli pepper. Thus, in the *Cucurbita* breeding improvement programme, resistant line PVR-1343 can be used to transfer disease resistance in different *Cucurbita* backgrounds in order to develop horticulturally superior resistant cultivars. The proposed resistant scale described here can be used as a standardisation tool for assessing resistance against SLCCNV, ToLCNDV, and mixed infection of both viruses.

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Authors' contribution

Conceptualization and designing of the research work (ASD, AS, MS); Execution of field experiments and data collection (NV, KSG, AS, MS); Analysis of data and interpretation (NV, KSG, AS, MS); Preparation of manuscript (NV, KSG, AS, MS, ASD).

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