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Validation of ToLCNDV resistant germplasm in melon (*Cucumis melo*) and cucumber (*Cucumis sativus*) through challenged inoculation

Pragya Ranjan*, Amalendu Ghosh[1], Shiksha Bhandari[1], J K Ranjan[1], Pavan Kumar Malav, Anjula Pandey, R K Gautam and G P Singh

ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India

ABSTRACT

The study was carried out to validate the Tomato Leaf Curl New Delhi Virus (ToLCNDV) resistance in four promising accessions of melon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.) accessions, *viz*. IC629818-A, IC629818-B, IC629821, and IC410617 along with checks PI 124111, Pusa Uday and Punjab Naveen through artificial inoculation at glasshouse facility of ICAR-IARI, New Delhi, during 2024-25. These lines were identified through field screening against ToLCNDV during the *kharif* seasons of 2021–2024 at the ICAR-NBPGR, New Delhi. Under artificial inoculation in the glasshouse, accessions IC629818-A and IC629818-B exhibited no visible symptoms of ToLCNDV and recorded a vulnerability index of 0. PCR amplification had further confirmed the absence of viral infection in these lines. This is perhaps the first report wherein any melon germplasm showed immune/highly resistant reaction to ToLCNDV. The cucumber genotype IC410617 had little symptom (VI=4.5) with no amplification of viral coat protein and thus categorized as resistant. These lines show potential for their utilization as novel sources of resistance for breeding of ToLCNDV-resistant cucumber and melon cultivars.

Key words: ToLCNDV, Resistant germplasm, Cucumber, Melon, Vulnerability index, Artificial screening, Challenged inoculation

Cucumber (*Cucumis sativus* L.) and melons (*Cucumis melo* L.) constitute an important group of Cucurbitaceous vegetables cultivated for their nutritional benefits. Cucumber cultivation during the rainy season in Northern India is becoming a challenge due to the spread of Tomato Leaf Curl New Delhi Virus (ToLCNDV), which compels the farmers to increase insecticide applications per harvest, thereby posing serious threats to human health and the environment. Moreover, in many countries, this virus has presented a serious threat to melon crops (Juárez *et al*., 2014). Huge crop yield loss has been reported worldwide due to infection of plant viruses that are predicted to cost over $30 billion a year (Rabadán *et al*., 2023). These consequences highlight how crucial it is to create and carry out efficient virus-resistance breeding programmes for melons and other crops. The primary method of controlling this virus is by using pesticides to regulate its vectors, which poses a serious risk to both human health and the environment. The best strategy to combat with diseases and pests is genetic resistance, which necessitates identification of resistance sources from the gene pool to use in development of resistant varieties. Keeping this in view, the present investigation was planned to validate and identify ToLCNDV resistant genotypes in cucumber and melon through artificial screening.

Based on the disease response of 48 cucumber and melon genotypes to ToLCNDV under field conditions during 2023–24 (data not presented), seven genotypes, including the most resistant ones along with three checks, were selected for validation of resistance through challenge inoculation using viruliferous whiteflies. A virus-free stock of whiteflies (*Bamacia tabaci*) was reared on healthy cucumber plants in controlled environment (28 ± 2°C, 60 ± 10% RH, 16 hr light-8 hr dark photoperiod) in whitefly rearing chamber at the Advance Centre for Plant Virology, ICAR-Indian Agricultural Research Institute, New Delhi during March 2024. A culture of ToLCNDV was maintained on tomato plant (accessions no: MW399221) grown under controlled conditions in an insect-proof greenhouse.

Thirty seedlings of each genotype were sown in plastic pots, and 15 days-old seedlings were used for inoculation. Whiteflies were allowed an acquisition access to ToLCNDV-infected tomato plants maintained under greenhouse condition for 24 hr. Ten adult virulent whiteflies were then allowed for access to each accession at the second true-leaf stage for 48 hrs within mylar cages. Symptoms on the upper leaves of each genotypes were recorded by visual evaluation using the following scale (Islam *et al*., 2010), where 0= no symptoms; 1= mild symptoms on young leaves covering >10% of leaf area; 2= mosaic on young leaves covering >25% of leaf area symptoms; 3= mosaic on young leaves covering >50% of leaf area symptoms and 4=mosaic on young leaves covering >75% of leaf area symptoms. For better comparison of

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Corresponding author : ruchi_105@rediffmail.com

[1] ICAR-Indian Agricultural Research Institute, Pusa, New Delhi 110 012, India

resistance among the genotypes, vulnerability index (V) values were calculated using the formula as given below.

Vulnerability Index (VI)= $(0n_o+1n_1+2n_2+3n_3+4n_4 + 5n_5)/nt(nc-1) \times 100$

where, n_0, n_1, n_2...n_5 = number of plants in score 0, 1, 2...5 respectively; nt = total number of plants; nc = total number of categories. On the basis of the vulnerability indices, the genotypes were categorized into five categories, VI=0 immune; VI= 1-25, resistant; VI= 26-50, moderately resistant; VI= 51-75, moderately susceptible; VI= 76-100, susceptible (Islam *et al*., 2010).

Total DNA was isolated at 4th week after inoculation (Doyle and Doyle, 1990; Murray and Thompson, 1980) from all the tested genotypes, and PCR amplification was conducted using ToLCNDV-specific primer pair AG155F-AG158R (Roy *et al*., 2021) for ToLCNDV. The symptoms in glasshouse-grown plants started 1st week after inoculation with minute yellow spots; later, they progressed to yellow mosaic and curling and covered the entire leaf very quickly within 3 weeks in susceptible genotypes.

Arya melon, IC629818-A and IC629818-B, showed no symptom of yellowing and curling of leaves (VI=0) while the check, Pusa Uday and Arya IC629821, had mild symptoms and thus assessed as moderately resistant (VI=25.75-49.81) (Table 1). Cucumber accession IC410617, exhibiting a vulnerability index (VI) of only 4.5%, was identified as resistant to ToLCNDV, whereas the check variety Punjab Naveen was classified as moderately susceptible (VI = 64.75). These findings are in agreement with the field study of Pandey *et al.* (2022), which reported that most Arya melon accessions were resistant to ToLCNDV.

Table 1. Response of cucumber and melon genotypes screened for resistance against ToLCNDV through whitefly mediated challenged inoculation

Genotype	Species	Vulnerability Index %	Disease reaction
IC410617	*Cucumis sativus* L.	4.50	R
Arya IC629821	*Cucumis melo* ssp *melo* var. *alwarensis*	31.14	MR
PI 124111	*Cucumis melo* ssp *melo*	25.75	MR
Punjab Naveen	*Cucumis sativus* L.	64.75	MS
Pusa Uday	*Cucumis sativus* L.	49.81	MR
Arya IC629818-B	*Cucumis melo* ssp *melo* var. *alwarensis*	0.00	I
Arya IC629818-A	*Cucumis melo* ssp *melo* var. *alwarensis*	0.00	I
	CD@ P < 0.05	5.33	
	SE(m)	1.81	
	C.V. %	5.41	

The artificially inoculated plants, when subjected to PCR amplification with coat protein primers, did not show any amplification in Arya IC629818-A, IC629818-B, and cucumber IC410617, confirming the absence of viral genomes (Fig. 1). Though Arya IC629821 showed some symptoms in glasshouse-grown plants which placed it in moderately resistant category but in PCR study, no band was observed, revealing that very low virus load is present (Fig. 1). Check variety Punjab Naveen showed a clear-cut band of higher intensity showing presence of viral coat protein in good concentration.

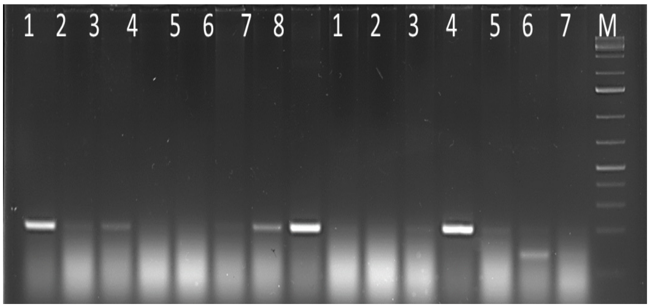

Fig. 1 PCR profiles of susceptible and resistant accessions grown under artificial epiphytotic conditions, sampled and checked for the presence of ToLCNDV using ToLCNDV coat protein-specific primer pairs. Primers used – AG155/ AG158, Expected amplicon- ~200 bp, Lane 1: IC629818-A; Lane 2: IC629818-B; Lane 3: PI 124111; Lane 4:Punjab Naveen; Lane 5:Pusa Uday; Lane 6: IC629821; Lane 7: IC410617; Lane M: 1000 bp Marker

The PI 124111 showed moderately resistant reaction in greenhouse with faint/low intensity of band (Fig 1.) Lower band intensity in a PCR reaction indicates a lower concentration of DNA. This might be possible that viral coat protein was able to multiply but the concentration was not enough to produce any prominent band in the PCR profile or express more symptoms in plants. That is why plants were categorised as moderately resistant on the basis visual symptoms. Previously PI 124111 has been reported to show partial resistance to ToLCNDV (Romay *et al.,* 2019). Five Indian melon genotypes, belonging to subsp. *agrestis* (Naudin) Pangalo, were reported to exhibit resistance to the Spanish isolate of ToLCNDV (López et al., 2015). The wild gene pool has also been utilized for introgression of ToLCNDV resistance in tomato (Singh and Rai, 2023).

Candidate genes for high level of resistance against ToLCNDV were identified from wild melon germplasm (Saez *et al.,* 2017) and recently new source of resistance from (*C. melo* var. *callosus)* and *C. melo* var. *momordica* have been reported from Indian genepool (Padmanabha *et al.,* 2023). This shows the potential of diversity present in cucumber and melons coming from Indian gene centre. However, this is perhaps the first report where a germplasm is showing immune reaction against ToLCNDV disease in melons. Arya melon collected from Alwar district of Rajasthan is a unique type of melon used for salad purpose (Pandey *et al*., 2020). This can serve as a potential source of resistance to other melon types which are crossable with each other. In cucumber, only a few sources of resistance are reported. In our previous study, IC410617 showed field resistance to ToLCNDV (Ranjan *et al*., 2015; Pandey *et al*., 2022). Identification of IC410617 as a novel source of resistance to ToLCNDV in cucumber is very important for successful cucumber cultivation during *kharif* season in northern India.

CONCLUSION

Thus, we report new sources of resistance to ToLCNDV in cucumber and melon. The field resistance of cucumber accession IC410617 and melon accessions Arya IC629818-A and IC629818-B against ToLCNDV has been validated through artificial screening under controlled conditions.The artificially inoculated plants of IC629818-A and IC629818-B genotypes had no symptoms as well as no viral DNA in their PCR profiles. Cucumber IC410617 had very little symptom but no viral DNA in the PCR profile. This is perhaps the first report documenting a melon landrace exhibiting immunity to ToLCNDV. Since melons are inter-crossable within the species, the genes conferring ToLCNDV resistance can be readily introgressed into commercial melon cultivars, facilitating the development of virus-resistant varieties.

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