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RESEARCH ARTICLE

Identification of novel resistant sources and inheritance of ToLCNDV resistance in cucumber (*Cucumis sativus* L.) germplasm

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Abstract

Tomato leaf curl New Delhi virus (ToLCNDV) is a major constraint in cucumber (*Cucumis sativus* L.) cultivation, causing yield losses ranging from 17.6 to 99.4%. Although resistant sources have been identified in some cucurbits, no commercial ToLCNDV-resistant cucumber variety is presently available. Fifty indigenous cucumber genotypes were evaluated under natural epiphytotic conditions during *kharif* 2021 and 2022, followed by artificial screening of 17 promising genotypes in 2023–2024 using both whitefly and *Agrobacterium*-mediated inoculation. Quantitative PCR-based virus titre estimation was undertaken in six representative genotypes. A significant year × genotype × time interaction revealed variable disease progression patterns. Genotypes DC-70, DC-61, DC-91, DC-45 and WBC-17 consistently exhibited resistance (VI < 25), whereas DPaC-21, DC-769, DC-773, SI-749 and Pusa Parthenocarpic Cucumber-6 were highly susceptible (VI > 75) across years. Cluster analysis, supported by principal component analysis and K-means clustering, grouped the genotypes into four distinct clusters. Three genotypes (DC-70, DC-60 and DC-91) maintained resistance across both natural and artificial screening conditions. qPCR results validated phenotypic reactions. Inheritance analysis from a cross between resistant (DC-70) and susceptible (DC-773) genotypes revealed the segregating ratio 3:1 in F_2 and 1:1 in BC_1P_1 populations, confirming the genetic control by a single recessive gene. The identified resistant genotypes constitute valuable genetic resources for breeding ToLCNDV-resistant cucumber cultivars.

Keywords: Cucumber, ToLCNDV, Vulnerability index, Host plant resistance, Inheritance.

Introduction

Cucumber (*Cucumis sativus L.*), a most widely cultivated cucurbit species, originated in India, where a substantial genetic diversity existed among indigenous germplasm lines (Whitaker and Davis 1962). Its wild progenitor, *C. sativus* var. *hardwickii*, still grows in the Himalayan foothills. It is consumed fresh (salad), pickled, and utilized in cosmetic industries, valued for its cooling properties and nutritional content -rich in potassium and vitamins C, K, and A (Rolnik and Olas 2020). Globally, cucumber is extensively cultivated in China, India, Turkey, Iran, Japan, Europe and the USA. In 2023, global cucumber and gherkin production reached 94.72 million metric tons, with China contributing 81.66% (FAO 2023). India is a major producer and exporter, ranking second among cucurbits with 139,000 hectares under cultivation and production of 1.99 million metric tons during 2024–2025 (DA & FW 2025).

In India, cucumber is primarily grown during the spring-summer and rainy (*kharif*) seasons (Vennkataravanappa et al. 2019). However, the *kharif* cucumber cultivation is highly vulnerable to begomoviral infections, particularly

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Tomato leaf curl New Delhi Virus (ToLCNDV), which causes severe leaf curl disease (Venkataravanappa et al. 2019; Cai et al. 2023). ToLCNDV (*Begomovirus solanum delhiense*, family Geminiviridae) is a whitefly-transmitted bipartite begomovirus with ssDNA genomes (~2.7 kb) and can cause a serious threat to kharif-season cucumber cultivation in both greenhouse and open-field cultivation, especially in northern India (Varma and Malathi 2003; Venkataravanappa et al. 2019; Cai et al. 2023). It was first reported in tomato in Northern India in 1995 (Chakraborty 2009). It has since spread across the Indian subcontinent and Southeast Asia, infecting a wide range of solanaceae crops and cucurbit crops (Fortes et al. 2016). ToLCNDV now affects cucurbit crops in atleast 16 distinct countries, causing yield losses ranging from 17.6% to 99.4%, with early infection causing complete crop failure (Cai et al. 2023; Goundar et al. 2024). Recognizing its global threat, the European Plant Protection Organization (EPPO) included ToLCNDV in its alert list from 2017-2020, identifying cucumber as a major host (EPPO 2025).

Among the different approaches followed for ToLCNDV management, conventional control measures, such as whitefly control through pesticides, are often expensive and increasingly ineffective due to pesticide resistance in whitefly (Rojas et al. 2018; Tatineni and Hein 2023). Therefore, breeding for genetic resistance remains the most sustainable and long-term approach (Majid et al. 2023). However, resistance breeding of ToLCNDV in cucumber is still in its early stages. Since cucumber originated in India, its diverse wild and semi-cultivated germplasm represents valuable reservoirs for resistance genes. Field screening for ToLCNDV is often hampered by environmental variability and mixed infections (Koeda et al. 2017). To overcome challenges, controlled artificial screening, such as whitefly-mediated and agro-inoculation with infectious clone procedures, offers more precise, efficient and rapid evaluation of resistance in a limited space against conventional labour-intensive and time-consuming methods under field conditions. Additionally, understanding the genetic basis of resistance and the extent of gene actions involved in ToLCNDV resistance is crucial for developing effective breeding strategies. To identify the resistance sources of cucumber genotypes for ToLCNDV, the present study aimed at screening Indian cucumber genotypes under natural epiphytotic, whitefly-mediated inoculation and *Agrobacterium*-mediated inoculation via infectious clones of DNA-A, DNA-B, and beta satellite of ToLCNDV, quantification of virus copy number of a few selected genotypes using quantitative real-time PCR (qPCR), and determining the mode of inheritance of ToLCNDV resistance in cucumber.

Materials and methods

Fifty Indian cucumber germplasms, including cultivated commercial cultivars, namely, Pusa Uday, Pusa Long Green, Pusa Barkha, Pusa Parthenocarpic Cucumber-6 and other breeding lines, were evaluated at the experimental field of Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, during *kharif* seasons of 2021 and 2022. Artificial screening of 17 promising genotypes was carried out in 2023–2024 using both whitefly and *Agrobacterium*-mediated inoculation. All genotypes were maintained as inbreds through selfing at the experimental field (Table 1).

Screening under the epiphytotic natural conditions

The field experimentation was conducted with fifty cucumber genotypes, planted in a randomized complete block design (RCBD) with three replications, each genotype at a spacing of 1.2 m × 60 cm. Recommended cultivation practices were followed, avoiding insecticide application to maintain whitefly populations for natural ToLCNDV infection. The disease symptom on the topmost leaf was assessed on five plants per genotype in each of three replications, with 15 plants scored per genotype at 15-day intervals from 30 to 60 days after sowing (DAS) in each season by visual evaluation using the 0-4 scale of Sáez et al. (2021), as described later. Randomly selected plants underwent PCR amplification using ToLCNDV-specific coat protein primers, AG155F (5′-GCCGATGAACAGAAAACCC-3′) and AG158R (5′-TCACACAGAATCGCTTCCC-3′), for confirmation of the existence of ToLCNDV during the field experiment.

Screening for ToLCNDV under artificial conditions

Out of 50 cucumber genotypes, 17 selected genotypes (5 resistant, 4 each of moderately resistant, moderately susceptible and susceptible categories) were evaluated during 2023-2024 under artificial conditions. Fifteen plants per genotype (5 plants per genotype per replication, with three replications) were screened using viruliferous white flies and *Agrobacterium*-mediated inoculation via infectious clones of ToLCNDV DNA-A, DNA-B, and beta-satellite, as described later.

Whitefly-mediated inoculation

A virus-free stock of whiteflies (*Bemisia tabaci*, Genn.; Hemiptera, Aleyrodidae) was reared on healthy cucumber (variety Barsati) under controlled conditions (28 ± 2°C, 60 ± 10% RH, 16 h light-8 h dark photoperiod) at the Advance Centre for Plant Virology, ICAR-IARI, New Delhi. Seventeen cucumber genotypes were screened using whitefly inoculation. Fifteen seeds per cucumber genotype (at least five plants per genotype per replication, with three replications) were directly sown in a plastic pot/ tray filled with coco peat. The aviruliferous whiteflies from pure culture were allowed to feed on ToLCNDV-infected cucumber plants (maintained under greenhouse conditions) for 24 hours. Ten to twelve virulent whiteflies were then allowed access to each testing ten-day-old healthy cucumber seedlings (two true-leaf stage) for 48 hrs in a mylar cage. Whitefly inoculation was continued at one-day intervals up to 22 days after sowing (DAS). Then, inoculated plants were sprayed with imidacloprid 0.05% and maintained for symptom expression. Disease severity was scored visually using a 0 to 4 scale (Sáez et al. 2021). ToLCNDV infection was confirmed at 28 dpi via PCR using ToLCNDV-specific primers (AG155 and AG158) (Roy et al. 2021). Quantitative PCR-based diagnosis was performed on six top-performing cucumber genotypes to assess viral load.

Agrobacterium-mediated inoculation using an infectious clone

Agrobacterium tumefaciens strains EHA105 cultures harbouring the recombinant plasmid of agroinfectious clones of DNA-A, DNA-B, and beta satellite of ToLCNDV were grown in 2 mL of Luria-Bertani (LB) broth with antibiotics (30 mg/mL rifampicin and 50 mg/mL of kanamycin) at 28°C, 200 rpm for 48 hours. Then 2 mL of this primary culture was transferred to 50 mL of LB with the same antibiotics and grown at 28°C in a shaker overnight to produce a secondary culture. After adding 100nM acetosyringone, the culture was incubated overnight. The bacterial pellet of three clones was resuspended in 100 µl LB separately, mixed in 1:1:1 ratio, and inoculated into the cotyledon leaf stage of seven-day-old healthy cucumber seedlings using needle puncture at the petiole-leaf junction. *A. tumefaciens* strains EHA105 cultures harbouring the vector pCAMBIA2300 (Abcam, Boston, MA, USA) were used for mock inoculation. Seventeen genotypes (5 best resistant genotypes and four genotypes from the other three disease reaction groups based on both years' field screening performance) were screened via artificial virus inoculations. A total of 15 plants per genotype (five plants per replication, with three replications) were agro-inoculated with ToLCNDV infectious clones. Thereafter, agro-inoculated and control plants of each genotype were grown in an insect-proof growth chamber with a photoperiod of 16h/8h (day and night), temperature (28 ± 2°C), relative humidity (60%), and maintained for symptom appearance upto 30 dpi. The agro-inoculated plants were observed regularly and scored for symptoms upto 30 dpi on the 0-4 scale (Sáez et al. 2021), described later. The uppermost leaves from inoculated and control plants of each tested cucumber genotype were collected at 7, 14, and 21 dpi to detect ToLCNDV using AG155F/AG158R primers.

Quantification of ToLCNDV via qPCR

The viral load in whitefly-inoculated and agro-inoculated cucumber plants was quantified using real-time quantitative PCR (qPCR). Field-grown plants under natural epiphytotic conditions were not subjected to qPCR due to the possibility of mixed infections with other related begomoviruses in the open field, and that could interfere with ToLCNDV quantification. Six cucumber genotypes, including two resistant genotypes (DC-70 and DC-61), one moderately resistant (Pusa Barkha), one susceptible genotype (DC-773), and two susceptible checks (Pusa Uday and DPaC-21), identified through natural epiphytotic screening and artificial screening, were assessed for viral titer at 30 dpi. The coat protein-specific primers AG155F (5′-GCCGATGAACAGAAAACCC-3′) and AG158R (5′-TCACACAGAATCGCTTCCC-3′) were used for q-PCR (Roy et al. 2021). For qPCR, an equal amount of genomic DNA (10 ng/µL) was taken as a template, with three biological replicates per sample. Each 10 µL qPCR reaction was performed in triplicate (technical replicates) and contained 5 µL of 2X PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 1 µL of each primer (AG155F, AG158R), and 3 µL of nuclease-free water. The coat protein-specific primers (AG155F/AG158R) were used for amplification of a ~190 bp fragment of the coat protein gene of ToLCNDV DNA-A. Reactions were run on a Bio-Rad CFX96 Real-Time PCR system (Hercules, California, USA) with the following conditions: 1 cycle at 95°C for 5 minutes, 1 cycle at 95°C for 30 sec, followed by 40 cycles of 60°C for 1 minute and 65°C for 5 seconds, 5 seconds at 55°C, again followed by a melting curve. Virus copy number per ng of nucleic acid was calculated using Ct value by comparing the standard curve. Further, to compare virus copy number in whitefly-inoculated and agro-inoculated cucumber genotypes, a bar diagram was generated using R software.

Development of population and screening under natural epiphytotic conditions

The most resistant cucumber genotype, DC-70 and the most susceptible genotype, DC-773, identified through natural epiphytotic and artificial screening, were selected for genetic inheritance study of ToLCNDV in cucumber. To generate F_1s, susceptible genotype DC-773 (female parent) was crossed with resistant genotype DC-70 (as male parent). Further resulting F_1 progenies were crossed with each parent separately (BC_1P_1: backcrossed with susceptible parent DC-773; BC_1P_2: backcrossed with resistant parent DC-70), and F_1 selfed to produce F_2 population. In the field, 156 plants of F_2 populations of (DC-773 × DC-70), 50 plants of BC_1P_1 (F_1 × DC-773), and 44 plants each of BC_1P_2 (F_1 × DC-70) populations were screened with the same scoring method as described later.

Symptom scoring and estimation of disease variables

Based on ToLCNDV symptom severity scale in cucumber given by Sáez et al. (2021), we developed five-point phenotypic scale measured for the topmost leaf of cucumber plants, where 0 = no symptoms, 1=very mild curling (up to 25% leaf surface), 2= moderate curling, puckering (26–50% leaf surface), 3= severe curling, puckering (50–75% leaf surface) and 4= very severe symptoms, stunted plant or dead plant (>75% leaf surface). The vulnerability index (VI) value was calculated for each genotype at each time interval for a better comparison of resistance among the genotypes by using 0 to 4 score scale, as mentioned above. Under natural epiphytotic screening, scoring was done at 30, 45, and 60 DAS. While under artificial conditions, scoring was done at 7, 14, 21, and 28 dpi. The vulnerability Index (VI) of a specific genotype was calculated for each time interval using the following formula (Sáez et al. 2021):

$$\text{Vulnerability Index (VI)} = \left(\frac{(0\text{no} + 1\text{n}1 + 2\text{n}2 + 3\text{n}3 + 4\text{n}4)}{\text{nt}(\text{nc} - 1)}\right) \times 100 \qquad (1)$$

Where, n_0, n_1, n_2…n_5 = Number of plants in score 0, 1, 2…4, respectively; nt = Total number of plants; nc = Total number of categories.

The area under the disease progress curve (AUDPC) was calculated to understand the disease intensity over time, following the formula of the trapezoidal rule (Madden et al. 2007).

$$AUDPC = \sum_{i=1}^{n-1} \frac{(Y_i + Y_{i+1})}{2} \times (t_{i+1} - t_i) \qquad (2)$$

Where, Y_i is the vulnerability index (VI) at the *i-th* observation, t_i is the time (days after sowing or days post inoculation) at the *i-th* observation, and n is the total number of observations. The relative AUDPC (rAUDPC) was expressed as a proportion of the maximum theoretical value of AUDPC, following the method described by Feng et al. (2018). An Excel-based calculator was utilized to estimate both AUDPC and rAUDPC (Simko, 2021). Depending on VI, genotypes were categorized into five categories, namely, Immune (VI=0); Resistant (VI=1-25); Moderately Resistant (VI=26-50); Moderately Susceptible (VI=51-75); Susceptible (VI=76-100) (Islam et al. 2011; Kaur et al. 2021).

Statistical analysis

The pooled ANOVA assessed the effects of genotype, year, and their interaction on the vulnerability index across 50 cucumber genotypes over 2021–2022 using the agricolae package in R 4.2.2 (R Core Team 2022; de Mendiburu 2021) within RStudio (RStudio Team 2022). The mean vulnerability index per genotype was used for analysis. Tukey's HSD at $\alpha = 0.05$ compared genotype means within each year. All graphs were produced with ggplot2 (Wickham 2016) and correlation plots were generated using the Performance Analytics package (Peterson and Carl 2020).

Inheritance analysis of ToLCNDV resistance

The most resistant cucumber genotype (DC-70, as male parent) and the most susceptible cucumber genotypes (DC-773, as female parent), identified from field screening and artificial screening, were used to study the inheritance of ToLCNDV resistance. DC-773 (P_1) was crossed with DC-70 (P_2) to generate F_1, which were backcrossed to both parents to obtain BC_1P_1 and BC_1P_2. F_1 plants were also selfed to produce F_2 population. These six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) were evaluated under natural epiphytotic conditions. Individual plants of each generation were scored for disease reaction against ToLCNDV using a scale (0–4), and grouped as resistant (scores 0–1) or susceptible (scores 2–4). The segregation data were subjected to chi-square analysis to test the goodness of fit to classical Mendelian ratios. The calculation was performed manually using Microsoft Excel based on the formula:

$$\chi 2 = \left(\frac{\Sigma(\text{Oi} - \text{Ei})2}{\text{Ei}}\right) \qquad (3)$$

where O_i= Observed number of plants and E_i= Expected number (Panse and Sukatme 1985). This analysis validated the genetic basis of resistance by testing the conformity of observed data to theoretical inheritance models.

Results

Analysis of variance

A three-way ANOVA revealed highly significant effects (p <0.001) of genotype, year, and their interaction: genotype-by-year on disease variables under natural epiphytotic conditions. Additionally, significant interactions were observed for year × replication ($p < 0.001$), year × time ($p < 0.05$), and genotype × time (p <0.001). Moreover, a highly significant three-way interaction between year, genotype, and time (F=1.78, p <0.001) indicates that the disease reaction of cucumber genotypes varies dynamically across both years.

Descriptive statistics

Year-wise analysis of disease variables across fifty cucumber genotypes revealed considerable variability under natural epiphytotic conditions. In *kharif* 2021, the vulnerability index ranged from 1.67 to 95.00 (Supplementary Table S1), with a mean of 25.77 and a range of 93.33. The variation in vulnerability index was supported by a standard deviation of 23.01 and a variance of 529.39. The distribution exhibited positive skewness (1.28) and a kurtosis value of 0.77, indicating a right-tailed distribution with a relatively flat peak. In *kharif,* 2022, the VI ranged from 3.33 to 91.67, with a mean of 27.87 and a range of 88.33, while the standard deviation and variance were 21.15 and 447.28, respectively (Supplementary Table S2). Similar to 2021, the distribution of vulnerability index in 2022 was positively skewed (1.25) with a kurtosis of 0.89, reflecting a moderately peaked distribution with an extended right tail.

Field screening under natural epiphytotic conditions

The disease reactions of 50 cucumber genotypes against ToLCNDV during *kharif* seasons of 2021 and 2022 are summarized in Table 2. Out of 50 genotypes screened, 5 genotypes namely, DC-70, DC-61, DC-91, DC-45, and WBC-17 were grouped as resistant with a vulnerability index ranging from 8.89 to 22.78 with mean VI of 16.00 in *kharif* 2021, while, another one genotype DC-77 added with these existing resistant genotypes and resistant group showed vulnerability index ranging from 15.56 to 24.44 with mean VI of 19.26 during *kharif* 2022 (Table 2, Fig. 1A). Thirty genotypes with vulnerability index of 27.22–50.56 (mean VI =40.32) were classified into the moderately resistant group in *kharif*-2021 and 23 genotypes with vulnerability index ranging from 29.18–48.89 (mean VI =43.15) during *kharif*-2022 (Table 2). Eight genotypes were grouped as moderately susceptible with vulnerability index varying from 51.11 to 75.00 in *Kharif* 2021 and 14 genotypes were classified as moderately susceptible (VI= 52.22–70.00) during *kharif* 2022 (Fig. 1B). The remaining seven genotypes (Pusa Parthenocarpic Cucumber-6, DPaC-21, DC-769, DC-773, Sl-749, DPaC-41, and DPaC-59) were categorized as susceptible with vulnerability index value of 77.22–97.22 in 2021 (Table 2). During 2022, seven genotypes, namely, Pusa Parthenocarpic Cucumber-6, DPaC-21, DC-769, DC-773, Sl-749, Sl-746, and DPaC-41 showed susceptible disease reaction with vulnerability index ranging from 77.78–95.56 (Table 2, Fig. 1B). Disease progress based on vulnerability index of resistant genotypes was very low in three-time interval (30, 45, and 60 DAS) as compared

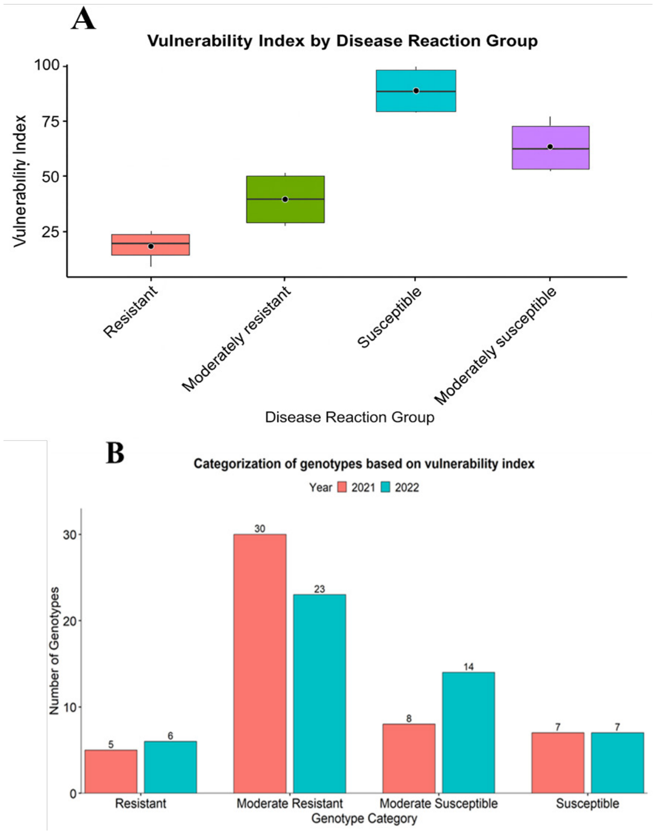

Fig. 1. Year-wise comparative vulnerability index (VI) in each disease reaction group. (A) Range, mean value of VI in each disease reaction group.(B) Categorization of genotypes based on VI

Table 1. **A list of genotypes with their origin and characteristics used in the study**

S. No.	Genotype	Source of the genotype	Sex expression
1	AZMC 1	Collection from North-Eastern India	Mono
2	Pusa Uday	Improved variety	Mono
3	DC-70	Breeding line	Mono
4	DC-61	Breeding line	Mono
5	Pusa Long Green	Improved variety	Mono
6	Pusa Barkha	Improved variety	Mono
7	Pusa Parthenocarpic Cucumber-6	Improved variety	Parth
8	Panipat Local	Collection from North India	Mono
9	DC-77	Collection from Eastern India	Mono
10	DPaC--21	Breeding line	Parth
11	DC-769	Collection from North- Eastern India	Mono
12	DC-773	Collection from North-Eastern India	Mono
13	GyCl -15	Breeding line	Gyno
14	GyCl -101	Breeding line	Gyno
15	DC-43	Breeding line	Mono
16	DC-8	Breeding line	Mono
17	GyCl-6	Breeding line	Gyno
18	DC-91	West Bengal	Mono
19	DC-45	West Bengal	Mono
20	WBC-26	Collection from Eastern India	Mono
21	WBC-5	Collection from Eastern India	Mono
22	Sl-749	Collection from North-Eastern India	Mono
23	Sl-746	Collection from North-Eastern India	Mono
24	EOM-400	Exotic line	Mono
25	C-1	Cucumis hardwickii collected from Uttarakhand	Mono
26	DC-63	Collection from Eastern India	Mono
27	DC-71	Collection from Eastern India	Mono
28	DC-107	Collected from Rajasthan	Mono

29	WBC-21	Collection from Eastern India	Mono
30	PI 197086	Collection from Eastern India	Mono
31	DC-40	Breeding line	Mono
32	WBC-13	Collection from Eastern India	Mono
33	WBC-22	Collection from Eastern India	Mono
34	DC-83	Improved variety	Mono
35	DC-48	Natural Variant	Mono
36	DPaC-41	Breeding line	Parth
37	Pahari Barsati	Breeding line	Mono
38	PI 197088	Collection from Eastern India	Mono
39	Japanese Long Green	Commercial variety	Mono
40	WBC-17	Collection from Eastern India	Mono
41	DC-92	Breeding line	Mono
42	GyCl-10	Breeding line	Gyno
43	7026-B-76	Collection from North-Eastern India	Mono
44	PCUC-8	Breeding line	Parth
45	DPaC-59	Breeding line	Gyno
46	Kalyanpur Green	Commercial variety	Mono
47	KSP-301	Collection from North-Eastern India	Mono
48	DC-47	Collection from North India	Mono
49	H-16 (*C. hardwickii*)	Odissa	Mono
50	Sel-3 (*C. melo* var. *agretis*)	Odissa	Andro

Mono = Monoecious, Gyno = Gynoceious, Parth = Parthenocarpic, Andro = Andromonoecious

to the susceptible genotypes during both the years (Supplementary Tables S1 and S2).

Moreover, AUDPC was calculated for all screened cucumber genotypes in the field and its value varied from 262.50 to 2925.00 with a mean AUDPC value of 1440.50 during *kharif* 2021, whereas during the second year (*kharif* 2022), the AUDPC value ranged from 487.50 to 2875.00 with a mean AUDPC value of 1532.00 (Table 2). The lowest value of AUDPC (less than 500) was recorded in resistant genotypes, namely DC-70 (262.50 and 612.50), followed by DC-61 (387.50 and 512.50), and DC-91 (462.50 and 487.50) during both years, respectively. The highest AUDPC (>2500) value was observed in susceptible genotypes, namely DC-773 (2925 and 2875), DPaC-59 (2712.50 and 2612.50), and DC-769 (2575.00 and 2437.50) in both the years 2021 and 2022, respectively. The rAUDPC value ranged from 0.09 to 0.98, with a mean value of 0.48 in 2021, while in 2022, the value of rAUDPC varied from 0.16 to 0.96, with a mean value of 0.51 (Table 2). The resistant genotypes had no symptoms in field conditions (Fig. 2A). Whereas, the typical ToLCNDV symptoms, like upward-downward leaf curling, leaf yellowing, and vein thickening, were found in susceptible genotypes (Fig. 2B) and, in extreme cases, stunting and plant death also occurred.

Clustering of genotypes based on disease reaction

The Elbow method showed that the total within-cluster sum of squares (WSS) declines sharply before stabilizing when k=4, suggesting the optimal number of clusters generated was 4 (Supplementary Fig. 2). The hierarchical clustering (dendrogram) constructed based on disease reaction data grouped the fifty cucumber genotypes into four clusters (Fig. 3A). Among the tested cucumber genotypes, 20% of genotypes (*viz*, 5 genotypes, namely DC-91, DC-70, DC-61, DC-45, and WBC-17) were grouped in cluster I, which also demonstrated lower VI values in field screening during both years (Fig. 3A, Table 3). However, 23 genotypes (46%) were grouped in cluster II (Fig. 3A, Table 3), while 8 (DPaC-41, Pusa Parthenocarpic Cucumber-6, DPaC-21, DC-769, SI-746, SI-749, DPaC-59, and DC-773), accounting 16% of total genotypes, were grouped into cluster III (Fig. 3A; Table 3). The rest of the 14 genotypes accounting for 28% clustered into the same group as presented in cluster IV (Fig. 3A, Table 3).

Principal component analysis

Principal component analysis (PCA) depicted grouping of 50 cucumber genotypes into four distinct groups, consistent with the dendrogram results (Fig. 3B, Supplementary Fig. 2). The PCA biplot revealed a clear separation between resistant and susceptible genotypes, with moderately resistant and moderately susceptible groups positioned in between. The majority of variation was explained by PC1 (99.9%), while PC2 (0.1%) further classified subgroups within clusters. PC1 primarily distinguished major differences among groups, whereas PC2 refined subgroup classification. The integration of PCA with K-means clustering effectively categorized genotypes, placing resistant and susceptible groups at opposite extremes, with moderately resistant and moderately susceptible groups distributed between them. Further the top cucumber genotypes from each disease reaction category were analyzed using a venn diagram to identify common genotypes consistently classified for ToLCNDV response across *kharif* 2021, *kharif* 2022, and PCA analysis (Fig. 4). A higher overlap was observed among resistant (57%) and moderately resistant (61%) genotypes, while moderately susceptible (35%) and susceptible (42%)

Table 2. Mean* values under field screening of cucumber genotypes for resistance against ToLCNDV during *kharif* 2021 and *kharif* 2022

Sl no.	Genotype#	*Kharif*-2021					*Kharif*-2022				
		Mean scoring	Mean VI	Category	AUDPC	rAUDPC	Mean scoring	Mean VI		AUDPC	rAUDPC
1	DC-70	0.36 ± 0.17	8.89 ± 2.42	R	262.50 ± 64.9	0.09	0.76 ± 0.28	18.89 ± 2.42	R	612.50 ± 76.0	0.20
2	DC-61	0.51 ± 0.13	12.78 ± 2.22	R	387.50 ± 62.5	0.13	0.66 ± 0.18	16.67 ± 0.96	R	512.50 ± 33.1	0.17
3	DC-91	0.60 ± 0.30	15.00 ± 0.96	R	462.50 ± 33.0	0.15	0.64 ± 0.17	16.11 ± 0.56	R	487.50 ± 21.7	0.16
4	DC-45	0.82 ± 0.46	20.56 ± 0.56	R	550.00 ± 25.0	0.18	0.96 ± 0.29	23.89 ± 2.00	R	712.50 ± 57.3	0.24
5	WBC-17	0.91 ± 0.19	22.78 ± 2.00	R	712.50 ± 57.3	0.24	0.62 ± 0.18	15.56 ± 0.56	R	487.50 ± 21.7	0.16
6	DC-40	1.09 ± 0.41	27.22 ± 0.56	MR	837.50 ± 33.1	0.28	1.38 ± 0.54	34.44 ± 0.56	MR	1050 ± 21.7	0.35
7	DC-77	1.13 ± 0.50	32.22 ± 1.11	MR	1025.00 ± 45.1	0.34	1.95 ± 0.67	24.44 ± 2.78	R	775.00 ± 100.0	0.26
8	Pusa Barkha	1.13 ± 0.37	28.33 ± 3.47	MR	850.00 ± 102.3	0.28	1.89 ± 0.29	48.89 ± 4.34	MR	1462.50 ± 129.9	0.49
9	EOM-400	1.27 ± 0.63	28.33 ± 1.67	MR	912.50 ± 45.1	0.3	1.73 ± 0.63	47.22 ± 2.94	MR	1437.50 ± 87.5	0.48
10	WBC-26	1.29 ± 0.49	31.67 ± 2.55	MR	900.00 ± 75.0	0.3	0.98 ± 0.44	43.33 ± 0.96	MR	1250.00 ± 25.0	0.42
11	PI-197086	1.31 ± 0.46	32.78 ± 1.47	MR	975.00 ± 43.3	0.33	1.71 ± 0.87	42.78 ± 1.11	MR	1275 ± 37.5	0.43
12	DC-107	1.33 ± 0.77	33.33 ± 0.96	MR	975.00 ± 43.3	0.33	1.51 ± 0.69	37.78 ± 2.00	MR	1150.00 ± 66.1	0.38
13	GyCl-6	1.38 ± 0.32	34.44 ± 2.78	MR	1087.50 ± 94.4	0.36	1.71 ± 0.39	42.78 ± 1.47	MR	1350.00 ± 43.3	0.45
14	DC-71	1.38 ± 0.46	34.44 ± 0.56	MR	1050.00 ± 21.7	0.35	1.67 ± 0.69	41.67 ± 0.96	MR	1250.00 ± 33.1	0.42
15	PI-197088	1.38 ± 0.82	34.44 ± 3.09	MR	975.00 ± 94.4	0.33	1.65 ± 0.79	41.11 ± 3.38	MR	1237.50 ± 129.9	0.41
16	Pusa Long Green	1.42 ± 0.26	35.56 ± 2.94	MR	1087.50 ± 99.2	0.36	1.91 ± 0.53	47.78 ± 0.56	MR	1462.50 ± 21.6	0.49
17	PCUC-8	1.47 ± 0.61	36.67 ± 2.55	MR	1100.00 ± 82.0	0.37	1.71 ± 0.54	42.78 ± 6.41	MR	1275.00 ± 169.1	0.43
18	DC-43	1.51 ± 0.79	37.78 ± 2.94	MR	1075.00 ± 81.9	0.36	1.96 ± 0.61	48.89 ± 2.42	MR	1512.50 ± 76.1	0.5
19	DC-8	1.53 ± 1.15	38.33 ± 5.00	MR	1200 ± 150.0	0.4	1.91 ± 0.70	47.78 ± 1.47	MR	1475.00 ± 45.1	0.49
20	Pahari Barsati	1.64 ± 0.77	41.11 ± 0.56	MR	1250.00 ± 12.5	0.42	1.96 ± 0.84	48.89 ± 2.78	MR	1512.50 ± 100.0	0.5
21	H-16 (*C. hardwickii*)	1.64 ± 0.27	41.11 ± 1.11	MR	1250.00 ± 33.0	0.42	1.96 ± 0.46	48.89 ± 4.01	MR	1450.00 ± 123.1	0.48
22	Kalyanpur Green	2.18 ± 0.73	41.16 ± 5.20	MR	1625.00 ± 100.0	0.54	1.36 ± 0.48	33.89 ± 1.47	MR	1037.50 ± 45.1	0.35
23	DC-47	1.69 ± 0.61	42.22 ± 2.78	MR	1212.50 ± 100.0	0.4	1.91 ± 0.64	47.78 ± 2.94	MR	1437.50 ± 97.6	0.48
24	DC-63	1.78 ± 0.91	44.44 ± 2.78	MR	1362.50 ± 87.5	0.45	1.89 ± 0.95	47.22 ± 2.22	MR	1437.50 ± 90.1	0.48
25	DC-48	1.82 ± 0.77	45.56 ± 1.11	MR	1350.00 ± 21.6	0.45	1.94 ± 0.70	48.33 ± 3.47	MR	1475.00 ± 111.1	0.49
26	WBC-22	1.87 ± 0.66	46.67 ± 5.00	MR	1400.00 ± 162.5	0.47	2.22 ± 0.67	55.56 ± 2.42	MS	1687.50 ± 86.6	0.56
27	Japanese Long Green	1.87 ± 0.66	46.67 ± 2.89	MR	1375.00 ± 97.6	0.46	1.62 ± 0.64	40.56 ± 2.78	MR	1187.50 ± 87.5	0.4

28	Sel-3 (*C. melo* var. *agretis*)	1.89 ± 0.54	47.22 ± 1.47	MR	1450.00 ± 25.0	0.48	1.49 ± 0.41	37.22 ± 1.11	MR	1137.50 ± 45.1	0.38
29	KSP-301	2.15 ± 0.67	47.86 ± 4.66	MR	1612.5 ± 57.8	0.54	1.29 ± 0.40	29.18 ± 1.72	MR	962.50 ± 108.9	0.32
30	C-1	1.93 ± 0.48	48.33 ± 2.55	MR	1437.50 ± 81.9	0.48	2.51 ± 0.49	62.78 ± 2.42	MS	1862.50 ± 76.0	0.62
31	WBC-5	2.00 ± 0.83	50.00 ± 3.33	MR	1537.50 ± 112.5	0.51	2.11 ± 0.79	52.78 ± 1.11	MS	1612.50 ± 37.5	0.54
32	DC-92	2.00 ± 0.81	50.00 ± 0.96	MR	1475.00 ± 33.1	0.49	2.13 ± 0.58	53.33 ± 1.67	MS	1600.00 ± 45.1	0.53
33	WBC-13	2.02 ± 0.69	50.56 ± 1.47	MR	1512.50 ± 50.0	0.5	2.15 ± 0.62	52.22 ± 1.47	MS	1600.00 ± 45.0	0.53
34	GyCl-101	2.02 ± 0.60	50.56 ± 0.56	MR	1500.00 ± 37.5	0.5	2.20 ± 0.60	53.89 ± 1.47	MS	1650.00 ± 43.4	0.55
35	WBC-21	2.02 ± 0.87	50.56 ± 0.56	MR	1537.50 ± 21.7	0.51	2.09 ± 0.77	55.00 ± 0.96	MS	1662.50 ± 33.0	0.55
36	DC -83	2.05 ± 0.60	51.11 ± 4.34	MS	1537.50 ± 129.9	0.51	2.13 ± 0.61	53.33 ± 1.67	MS	1600 ± 45.0	0.53
37	7026-B-76	2.04 ± 0.93	51.11 ± 1.47	MS	1500.00 ± 43.3	0.5	2.18 ± 0.71	54.44 ± 1.47	MS	1662.50 ± 66.1	0.55
38	GyCl-10	2.18 ± 0.42	54.44 ± 3.09	MS	1587.50 ± 82.0	0.53	2.60 ± 0.48	65.00 ± 1.92	MS	1937.50 ± 54.5	0.65
39	GyCl-15	2.60 ± 2.60	65.00 ± 2.55	MS	1937.50 ± 82.0	0.65	2.47 ± 0.51	61.67 ± 3.47	MS	1875.00 ± 99.2	0.63
40	Panipat Local	2.64 ± 0.49	66.11 ± 3.09	MS	2012.50 ± 87.50	0.67	2.20 ± 0.65	55.00 ± 2.55	MS	1650.00 ± 78.0	0.55
41	AZMC-1	2.55 ± 0.46	66.11 ± 7.47	MS	1975.00 ± 225.4	0.66	2.33 ± 0.43	58.33 ± 6.01	MS	1725.00 ± 198.4	0.58
42	Pusa Uday	2.24 ± 1.12	69.44 ± 1.11	MS	2062.50 ± 37.5	0.69	2.80 ± 0.60	70.00 ± 2.89	MS	2087.50 ± 97.6	0.7
43	SI-746	3.00 ± 0.25	75.00 ± 1.67	MS	2237.50 ± 50.0	0.75	3.24 ± 0.25	81.11 ± 1.11	S	2412.50 ± 33.7	0.8
44	SI-749	3.09 ± 0.36	77.22 ± 1.11	S	2312.50 ± 33.1	0.77	3.22 ± 0.51	80.56 ± 2.42	S	2437.50 ± 86.6	0.81
45	DPaC-21	3.13 ± 3.13	78.33 ± 2.55	S	2400.00 ± 78.1	0.8	3.35 ± 0.33	83.89 ± 1.11	S	2512.50 ± 37.5	0.84
46	DPaC-41	3.18 ± 0.30	79.45 ± 4.94	S	2412.50 ± 152.1	0.8	3.22 ± 0.35	80.56 ± 2.78	S	2462.50 ± 54.5	0.82
47	DPaC-59	3.58 ± 0.26	79.77 ± 10.228	S	2712.50 ± 33.1	0.9	3.46 ± 0.29	86.67 ± 0.96	S	2612.50 ± 45.1	0.87
48	Pusa Parthenocarpic Cucumber-6	3.35 ± 0.35	83.89 ± 1.47	S	2525.00 ± 45.1	0.84	3.11 ± 0.48	77.78 ± 1.47	S	2337.50 ± 33.1	0.78
49	DC-769	3.40 ± 0.32	85.00 ± 3.37	S	2575.00 ± 108.9	0.86	3.15 ± 0.48	78.89 ± 4.94	S	2437.50 ± 141.1	0.81
50	DC-773	3.89 ± 0.04	97.22 ± 2.00	S	2925.00 ± 57.2	0.98	3.82 ± 0.08	95.56 ± 2.00	S	2875.00 ± 50.0	0.96
	Mean	1.90	47.37		1440.50		2.03	50.66		1532.00	

*Mean VI= Mean (± SE) of vulnerability index values at30 DAS, 45 DAS and 60 DAS; AUDPC= Area under disease progress curve at 60 DAS; R= Resistant; MR= Moderately resistant; MS= Moderately susceptible; S= Susceptible

[#] Genotypes names mentioned based on lower disease variables to higher disease variable pattern

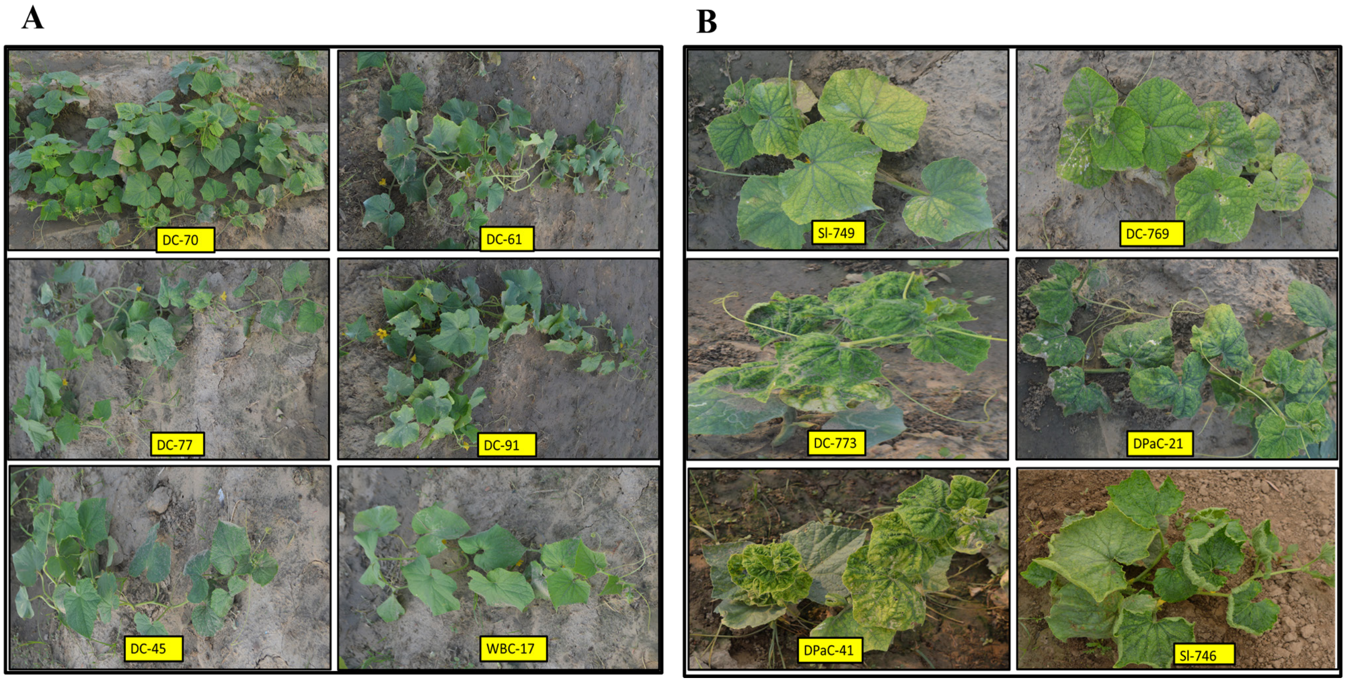

Fig. 2. **Disease reaction of cucumber genotypes under natural epiphytotic conditions during kharif-2021. (A) Resistant genotypes.(B) Susceptible genotypes**

groups showed lower consistency, indicating greater variability in these groups.

Disease response of selected cucumber genotypes under artificial screening conditions

The disease reactions of 17 selected cucumber genotypes to ToLCNDV under artificial screening conditions (whitefly-mediated and *Agrobacterium*-mediated inoculation) during 2023-2024 are summarized in Supplementary Tables S3 and S4. Out of 17 genotypes screened under both whitefly-mediated inoculation and *Agrobacterium*-mediated inoculation, none of the genotypes showed an immune reaction. The genotypes, DC-70, DC-61 and DC-91, showed resistant response in both artificial screening methods with vulnerability index (VI) values ranging from 17.50 to 18.33 in whitefly-mediated inoculation and 9.58 to 14.17 in *Agrobacterium*-mediated inoculation (Table 4). Three genotypes, Sel-3 (*Cucumis melo* var. *agretis*), WBC-17, and

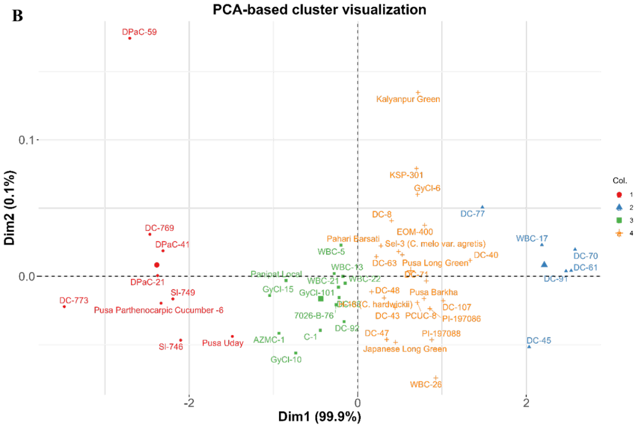

Fig. 3. **Cluster and Principal Component Analysis (PCA) of 50 cucumber genotypes based on disease response to ToLCNDV under natural epiphytotic conditions. PCA, biplot with K-means clustering illustrating genotype groupings (Cluster I:** Blue coloured, Cluster II: Orange coloured, Cluster III: Red coloured, Cluster IV: Green coloured)

Table 3. **The hierarchical clustering of 50 cucumber genotypes based on disease variables**

Cluster	No. of genotypes	List of genotypes
Cluster I	5	WBC-17, DC-45, DC-61, DC-70, and DC-91
Cluster II	23	DC-71, Pusa Barkha, EOM-400,PCUC-8,KSP-301, GyCl-6,Kalyanpur Green,PI-197088,PI- 197086,WBC-26, DC-107, DC-40, DC-77, Pahari Barsati,DC-63, DC-48, H-16 (C. *hardwickii*),DC-47,Japanese Long Green,DC-43,DC-8, Sel 3 (*C. melo* var. *agretis*), andPusa Long Green
Cluster III	8	DPaC-41, Pusa Parthenocarpic Cucumber-6, DPaC-21, DC-769,SI-769, SI-749, DPaC-59, and DC-773
Cluster IV	14	Panipat Local,AZMC -1,GyCl-15,GyCl-10,Pusa Uday,DC-83, GyCl-101, WBC-5, 7026-B-76, WBC-21,WBC-22,WBC-13, DC-92, and C-`1

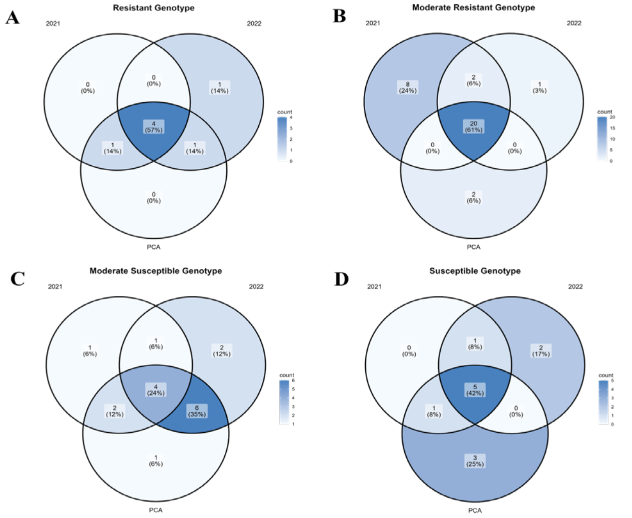

Fig. 4. Venn diagram showing cucumber germplasms overlapping for ToLCNDV disease reaction across *kharif* 2021 and *kharif* 2022 and PCA analysis: (A) Resistant genotypes. (B) Moderately resistant genotypes. (C) Moderately susceptible genotypes. (D) Susceptible genotypes

WBC-26, were moderately resistant, while four genotypes *viz*. Pusa Uday, Pusa Barkha, H-16 (*Cucumis hardwickii*), and DC-45 were moderately susceptible (VI 57.50–61.67) in whitefly-mediated inoculation. Remaining genotypes, DPaC-21, DC-769, DC-773, GyCl-15, WBC-5, Sl-749 and GyCl-10 were classified as susceptible (VI=76.25 to 85.00) in the whitefly screening method (Table 4). In *Agrobacterium*-mediated inoculation, six genotypes, namely, Pusa Barkha, WBC-26, WBC-17, GyCl-15, Sel-3 (*Cucumis melo* var. *agretis*), and H-16 (*Cucumis hardwickii*) were classified as moderately susceptible with VI value ranging from 57.08 to 77.92. However, no genotypes were grouped as moderately resistant and the remaining genotypes, namely, DC-45, Pusa Uday, GyCl-10, WBC-5, Sl-749, DC-773, DPaC-21, and DC-769, were susceptible (VI = 75.83–90.00) in *Agrobacterium*-mediated inoculation (Table 4).

Correlation of VI and AUDPC across different screening methods

The correlation among the quantitative variables (VI and AUDPC) of disease response against ToLCNDV was estimated based on Pearson's correlation at the significance level of $p < 0.01$. The inter-relationship between each pair of variables was represented using a gg corrplot (Fig. 5). A strong positive correlation was found between VI and AUDPC under natural epiphytotic conditions (r = 0.86, $p < 0.001$), whitefly-mediated inoculation (r = 0.89, $p < 0.001$), and *Agrobacterium*-mediated inoculation (r = 0.88, $p < 0.001$). VI value of selected 16 genotypes under natural epiphytotic conditions was significantly correlated with VI of both artificial screening methods, such as whitefly-mediated inoculation and *Agrobacterium*-mediated inoculation, with a correlation coefficient (at $p < 0.001$) value of 0.87 and 0.80, respectively. Disease incident results of both artificial screening methods, such as whitefly-mediated inoculation and *Agrobacterium*-mediated inoculation, were also positively correlated with a correlation coefficient of 0.89 ($p < 0.001$).

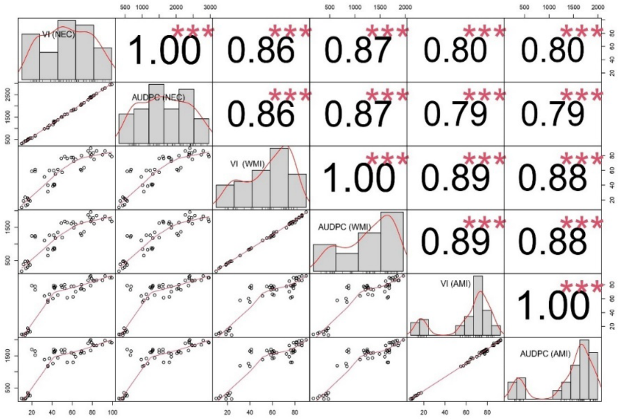

Fig. 5. Corrplot based on Pearson's correlation coefficient showing interrelationships among quantitative variables (VI & AUDPC) under natural epiphytotic conditions (NEC), whitefly-mediated inoculation (WMI), and *Agrobacterium*-mediated inoculation (AMI). Signifiance: ***P <0.001; **P <0.01; *P <0.05

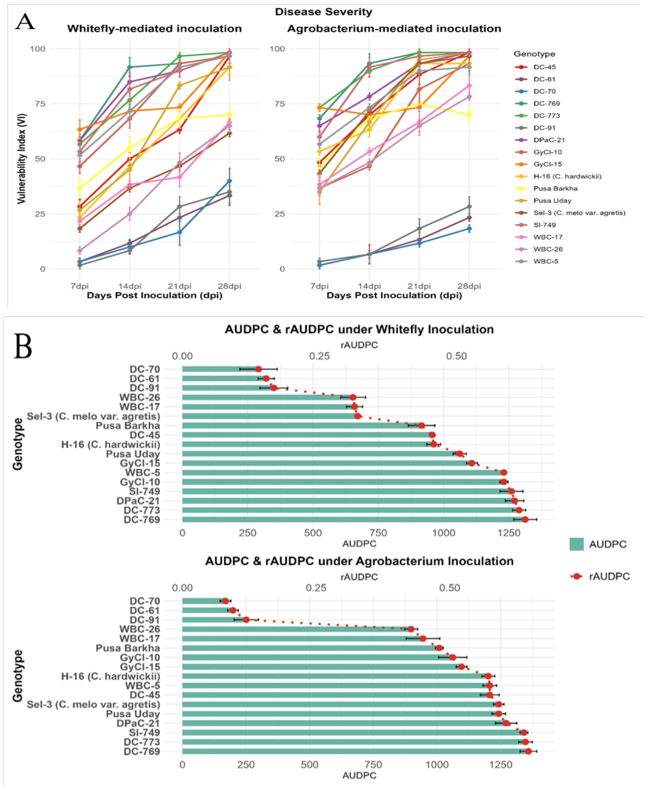

Fig. 6. Comparative disease progression in 17 genotypes under whitefly-mediated and *Agrobacterium*-mediated inoculation. (A) Disease progress curve based on Vulnerability index (VI). (B) Area under disease progress curve(AUDPC) and relative AUDPC (rAUDPC)

Table 4. **Mean* values of 17 genotypes for ToLCNDV resistance under artificial inoculated conditions**

S. No.	Genotype[#]	Whitefly-mediated inoculation					Agrobacterium-mediated inoculation				
		Mean Score	Vulnerability index	Category	AUDPC	rAUDPC	Average score	Vulnerability index	Category	AUDPC	rAUDPC
1	DC-70	0.7 ± 0.64	17.50 ± 4.59	R	291.67 ± 71.68	0.14	0.38 ± 0.14	9.58 ± 1.99	R	169.17 ± 21.03	0.08
2	DC-61	0.72 ± 0.26	17.92 ± 3.61	R	320.83 ± 30.87	0.15	0.55 ± 0.28	11.25 ± 2.76	R	198.33 ± 21.03	0.09
3	DC-91	0.73 ± 0.32	18.33 ± 4.41	R	350.00 ± 53.46	0.17	0.63 ± 0.29	14.17 ± 3.30	R	250.83 ± 47.75	0.12
4	WBC-26	1.47 ± 0.50	36.67 ± 6.67	MR	653.33 ± 47.75	0.31	2.95 ± 1.18	73.75 ± 7.84	MS	1242.50 ± 26.73	0.59
5	Sel-3 (*C. melo* var *agretis*)	1.63 ± 0.36	40.83 ± 4.80	MR	670.83 ± 5.83	0.32	2.28 ± 0.37	57.08 ± 4.90	MS	898.33 ± 25.43	0.43
6	WBC-17	1.68 ± 0.37	42.08 ± 4.98	MR	659.17 ± 30.87	0.31	2.58 ± 0.54	64.58 ± 7.32	MS	1061.67 ± 55.65	0.51
7	Pusa Barkha	2.30 ± 0.31	57.50 ± 4.42	MS	915.83 ± 50.85	0.44	2.42 ± 0.38	60.42 ± 5.42	MS	945.00 ± 66.25	0.45
8	H-16 (*C. hardwickii*)	2.30 ± 0.59	57.50 ± 7.72	MS	962.50 ± 26.73	0.46	2.60 ± 0.25	65.00 ± 3.48	MS	1009.17 ± 15.43	0.48
9	DC-45	2.38 ± 0.57	59.58 ± 7.57	MS	956.67 ± 5.83	0.46	3.03 ± 0.41	75.83 ± 5.46	S	1201.67 ± 25.43	0.57
10	Pusa Uday	2.47 ± 0.62	61.67 ± 8.31	MS	1061.67 ± 25.43	0.51	3.05 ± 0.44	76.25 ± 5.88	S	1207.50 ± 36.43	0.58
11	GyCl-10	3.05 ± 0.47	76.25 ± 6.25	S	1230.83 ± 15.43	0.59	3.05 ± 0.49	76.25 ± 6.46	S	1242.50 ± 20.21	0.59
12	GyCl-15	3.07 ± 0.30	76.67 ± 4.14	S	1108.33 ± 21.03	0.53	2.54 ± 0.64	77.92 ± 4.41	MS	1207.50 ± 26.73	0.58
13	WBC 5	2.60 ± 0.69	77.92 ± 5.49	S	1230.83 ± 5.83	0.59	3.13 ± 0.25	78.33 ± 3.39	S	1096.67 ± 21.03	0.52
14	SL-749	3.23 ± 0.39	80.83 ± 5.46	S	1260.00 ± 44.04	0.60	3.35 ± 0.30	83.75 ± 4.13	S	1271.67 ± 42.06	0.61
15	DC-773	3.28 ± 0.78	82.08 ± 5.27	S	1289.17 ± 25.43	0.61	3.47 ± 0.36	86.67 ± 4.86	S	1341.67 ± 15.43	0.64
16	DPaC-21	3.32 ± 0.35	82.92 ± 4.62	S	1271.67 ± 35.48	0.61	3.58 ± 0.29	89.58 ± 3.91	S	1359.17 ± 32.48	0.65
17	DC-769	3.40 ± 0.36	85.00 ± 4.85	S	1312.50 ± 44.04	0.63	3.60 ± 0.24	90.00 ± 3.20	S	1347.50 ± 26.73	0.64

*Mean VI= Mean of vulnerability index values at 30 DAS, 45 DAS, and 60 DAS; AUDPC= Area under disease progress curve at 60 DAS;rAUDPC= Relative AUDPC; R=Resistant; MR= Moderate resistant; MS= Moderately susceptible; S= Susceptible

[#] Genotypes names mentioned based on lower disease variables to higher disease variable pattern.

Table 5. Chi-square analysis for the inheritance of ToLCNDV resistance in cucumber

Generation/Progenies		Categories	Observed	Expected	Best fit Mendelian ratio	Chi-squarevalue	p-value
P1	20	Susceptible	20				
P2	20	Resistant	20				
F1	25	Susceptible	25				
F2	156	Susceptible	127	117	3:1	3.41	0.064**
		Resistant	29	39			
BC1P1	44	Susceptible	44	44	1:0	-	-
		Resistant	0	0		-	-
BC1P2	50	Susceptible	22	25	1:1	0.72	0.396**
		Resistant	28	25			

Disease progress in selected genotypes under artificial screening

Among the seventeen cucumber genotypes evaluated using whitefly-mediated inoculation and *Agrobacterium*-mediated inoculation, distinct variations in disease progression were observed from 7 dpi to 28 dpi (Fig. 6). Overall, disease development increased over time in both artificial inoculation methods. Disease progression was slow in three genotypes (DC-70, DC-61, and DC-91), which were classified as resistant (VI <25) (Table 4, Fig. 6A). Notably, a few genotypes (DC-769, DC-773, DPaC-21 and SI-749) showed speedy disease progression, indicating higher susceptibility (Fig. 6). Although disease progression was not consistent in moderately resistant to moderately susceptible genotypes, most showed medium to high levels of disease development under both artificial methods (Fig. 6). Comparatively, slightly higher AUDPC and rAUDPC values were recorded under *Agrobacterium*-mediated inoculation (Fig. 6B). Moreover, relative ranking of resistance and susceptible genotypes followed a largely similar trend across both methods.

Viral accumulation (average copy number) of ToLCNDV under artificial screening

The qPCR study analysis with selected six cucumber genotypes under artificial screening conditions revealed that whitefly-mediated inoculation results in significantly higher viral load compared to *Agrobacterium*-mediated inoculation (Fig. 7). Among the six cucumber genotypes, resistant genotypes DC-70 and DC-61 exhibited the lowest viral loads, with 2.42 × 10^7 and 1.27 × 10^7 copies in whitefly-mediated inoculation, while 3.33 × 10^7 and 3.28 × 10^7 copies in *Agrobacterium*-mediated inoculation. In contrast, the susceptible genotypes, namely, DC-773 and DPaC-21, accumulated the highest viral loads, in whitefly-mediated inoculation (5.08 × 10^9 and 3.58 × 10^9 copies), and in *Agrobacterium*-mediated inoculation (5.96 × 10^8 and 3.84 × 10^8 copies). The moderately resistant genotypes Pusa Barkha and Pusa Uday displayed intermediate virus levels in both methods, with 2.18 × 10^8 (WMI), 1.44 × 10^8 (Agro. Inc.) for Pusa Barkha and 3.41 × 10^8 (WMI), 2.01 × 10^8 (Agro. Inc.) for Pusa Uday.

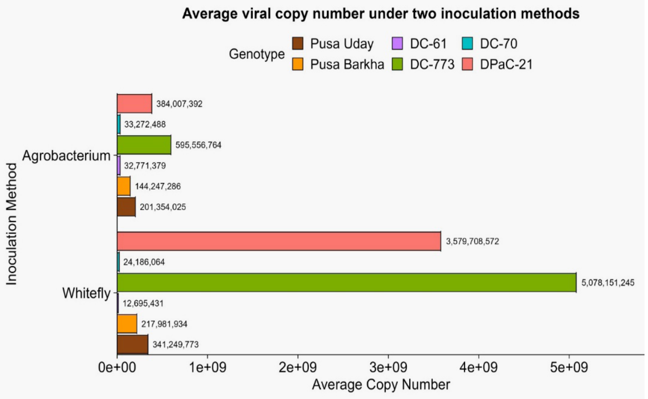

Fig. 7. Comparative viral accumulation (average copy number) of ToLCNDV via whitefly-mediated and *Agrobacterium*-mediated inoculation

Inheritance study of ToLCNDV resistance in Cucumber

A total of 156 F_2 plants from the DC-773 × DC-70 cross were evaluated under natural epiphytotic conditions for ToLCNDV response. Among them, 29 plants were resistant, exhibiting symptomless or mild symptoms (scores 0–1), while 127 plants showed moderate to severe symptoms (scores 2 to 4), fitting a 3:1 Mendelian ratio (susceptible: resistant). The BC_1P_1 (backcross with susceptible parent) plants were susceptible, exhibiting a 1:0 (susceptible: resistant) segregation pattern (Table 5). In contrast, all BC_1P_2 population (backcross progenies with the resistant parent) segregated into 28 resistant and 22 susceptible plants, conforming to a 1:1 ratio (χ^2 = 0.72, p=0.0396).

Discussion

ToLCNDV has emerged as a major constraint to the cucurbit cultivation, including cucumber, particularly when infection occurs during early stages of crop development, leading to complete crop failure (Goundar et al. 2024). Despite the crop's economic importance, no commercially released cucurbit cultivar with confirmed resistance to ToLCNDV is available to date. Resistant breeding offers a promising, economically viable, and durable approach to combat this disease. ToLCNDV resistance has been identified in several cucurbits, although the genetic basis and stability vary across species. In *Cucurbita moschata*, resistance was reported in 'Large Cheese' (PI 604506), and an Indian landrace (PI 381814), both governed by a single recessive gene with a major QTL on chromosome 8 (Sáez et al. 2016, 2017, 2020), whereas a dominant resistance gene has been mapped in the Japanese accession BSUAL-252 to a different locus on the same chromosome (Romero-Masegosa et al. 2021). In melon, resistant agrestis accessions such as Kharbuja, PI 124112, WM9, and PI 282448 have been identified (López et al. 2015; Sáez et al. 2017; Romay et al. 2019). In sponge gourd (*Luffa cylindrica*), resistance was conferred by a single dominant gene in DSG-6, DSG-7, and IIHR accessions (Islam et al. 2010; Kaur et al. 2021). However, resistance in cucumber remains scarce, with only a few Indian accessions (CGN23089, CGN23423, CGN23633) showing resistance under mechanical inoculation (Sáez et al. 2021), and DC-70 and P-85 under both natural and artificial screening (Naveena et al. 2024). Recent mapping studies have identified QTLs on chromosomes 1 and 2, including the fine-mapped *Cy-1* locus in the No. 44 accession, conferring resistance to the European strain (Koeda et al. 2024). Although India, as a primary center of origin for cucumber,possesses rich genetic diversity in wild and semi-cultivated germplasms. However, only a limited number of Indian cucumber lines have been globally explored for resistance evaluation against ToLCNDV. This underscores the need to screen native cucumber genotypes for identifying effective sources of resistance. Therefore, the present study aimed to evaluate a set of fifty cucumber genotypes under natural epiphytotic conditions across two growing seasons (kharif-2021 and kharif-2022). Seventeen promising cucumber genotypes were further validated under artificial screening conditions using both whitefly-mediated inoculation, a method previously employed in resistance screening across various cucurbit crops (Sohrab et al. 2013, López et al. 2015; Sáez et al. 2016, Islam et al. 2011; Kaur et al. 2021), and *Agrobacterium*-mediated inoculation, which has recently been applied in cucumber (Naveena et al. 2024). The disease screening under natural epiphytotic conditions revealed significant genetic variation in disease response, which has been observed in previous screening research for ToLCNDV as well (Islam et al. 2011; Kaur et al. 2021; Padmanabha et al. 2024). Differences in genetic background may be the major cause for the various symptom expression of cucumber genotypes (Naveena et al. 2024). The observed significant variation in disease response under natural field conditions and three-way ANOVA revealed significant interaction of year × genotype × time, highlighting the influence of both genetic and environmental factors on disease progression.

No cucumber genotype exhibited immunity under field conditions, a result consistent with previous screening studies from other cucurbit crops (Sáez et al. 2021; Kaur et al. 2021).However, few cucumber genotypes (e.g., DC-70, DC-61, DC-91, DC-45, and WBC-17) consistently exhibited lower vulnerability indices, suggesting resistance. Notably, a decline in the number of moderately resistant genotypes and a concurrent rise in moderately susceptible genotypes were observed in the second year (*kharif* 2022), possibly due to fluctuating environmental conditions, ever-changing whitefly vector populations in open field, or variability of ToLCNDV inoculum pressure (Kaur et al. 2021; Padmanabha et al. 2024). The annual shifts in genotype responses against TolCNDV reinforce the need for multi-environment testing to identify stable sources of resistance, an approach supported by previous findings in various cucurbits (Islam et al. 2011; Sáez et al. 2021; Kaur et al. 2021; Padmanabha et al. 2024).

Clustering analyses using hierarchical, k-means, and PCA methods categorized 50 cucumber genotypes into four distinct groups, aligned with four disease reaction categories. Notably, resistant and susceptible genotypes were separated, as confirmed by PCA biplot and venn analysis. This consistency of result supports the robustness of genotype classification and highlights the breeding potential of identified cucumber genotypes. However, slight overlaps between intermediate disease reaction groups (moderately resistant and moderately susceptible) indicate that further multi-environment testing is necessary to confirm the stability of disease expression among tested cucumber genotypes. Furthermore, field-based resistance alone may not be ideal for identifying true resistance due to potential disease escape under variable natural conditions. In contrast, artificial screening ensures uniform exposure to a defined viral inoculum under controlled environments, enabling accurate evaluation of genotypic resistance potential (Kaur et al. 2021). Therefore, selected genotypes were further validated using whitefly and *Agrobacterium*-mediated inoculation. Similar methodologies have previously been used in Tomato (Picó et al. 1998), Luffa (Islam et al. 2011; Kaur et al. 2021) and Melon (Padmanabha et al. 2024) for ToLCNDV or related begomoviruses screening.

In the present study, characteristic symptoms such as yellow mosaic symptoms appeared by 10 dpi under artificial screenings as documented in sponge gourd (Islam et al. 2010) and other cucurbits (Sáez et al. 2016). Three genotypes, DC-70, DC-61 and DC-91, exhibited resistance across both artificial screening methods. A strong positive correlation between natural and artificial screening responses confirmed the reliability and consistency across the inoculation methods, as reported in similar studies in tomato and Luffa (Picó et al. 1998; Islam et al. 2011; Kaur et al. 2021). Based on artificial screening, the resistant genotypes (DC-70, DC-61, and DC-91) exhibited lower AUDPC and rAUDPC values, while highly susceptible genotypes like DC-769 and DC-773 showed rapid and severe disease progression. These indicated the importance of integrating multiple screening approaches for robust resistance identification in breeding programs (Kaur et al. 2021).

Moreover, qPCR analysis of selected six cucumber genotypes revealed significantly lower viral loads in resistant cucumber genotypes (DC-70 and DC-61) compared to moderately resistant genotypes (Pusa Barkha and Pusa Uday) and susceptible genotypes (DC-773 and DPaC-21) under both artificial screening methods, The results suggested reduced viral replication and movement in resistant plants is likely due to presence of host resistance genes limiting ToLCNDV accumulation and spread (Kang and Jahn 2005; Koeda et al. 2020; Yamamoto et al. 2021; Padmanabha et al. 2024). The inheritance study further supported the presence of genetic control over resistance. The F_2 population derived from DC-773 × DC-70 cross showed a 3:1 segregation ratio (susceptible: resistant), while BC_1P_1 (backcross with susceptible parent) showed a 1:0 ratio, and BC_1P_2 (backcross with resistant parent) recorded a 1:1 ratio (susceptible: resistant). This segregation ratio concluded that a single recessive gene putatively governs the inheritance of ToLCNDV resistance in cucumber genotypes. However, the monogenetic recessive genetic control for ToLCNDV resistance was earlier reported in pumpkin and cucumber (Sáez et al. 2020; Sáez et al. 2021).

Supplementary material

Supplementary Tables S1 to S4 are provided, which can be accessed at www.isgpb.org

Authors' contribution

Conceptualization of research (SSD, SGK, SM); Designing of the experiments (SSD, SGK, BST, AKS); Contribution of experimental materials (SGK, SSD); Execution of field/lab experiments and data collection (SM, CG, KP, AG); Analysis of data and interpretation (KP, SSD, SKG, SM); Preparation of the manuscript (SSD, SGK, SM, AKS).

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Supplementary Table S1. Vulnerability index of cucumber genotypes at different time interval (30, 45 and 60 DAS) during the *kharif* 2021 season

Genotype	*Kharif* 2021					
	30 DAS		45 DAS		60 DAS	
	Average Scoring	Vulnerability index	Average Scoring	Vulnerability index	Average Scoring	Vulnerability index
DC-70	0.07 ± 0.12	1.67 ± 1.67	0.33 ± 0.12	8.33 ± 1.67	0.67 ± 0.31	16.67 ± 4.41
DC-61	0.27 ± 0.23	6.67 ± 3.33	0.53 ± 0.12	13.33 ± 1.67	0.73 ± 0.12	18.33 ± 1.67
DC-91	0.27 ± 0.12	6.67 ± 1.67	0.67 ± 0.12	16.67 ± 1.67	0.87 ± 0.12	21.67 ± 1.67
DC-45	0.27 ± 0.1	6.67 ± 1.67	0.47 ± 0.12	11.67 ± 1.67	1.73 ± 0.12	43.33 ± 1.67
WBC-17	0.53 ± 0.12	13.33 ± 1.67	1.07 ± 0.12	26.67 ± 1.67	1.13 ± 0.23	28.33 ± 3.33
DC-40	0.33 ± 0.12	8.33 ± 1.67	1.20 ± 0.20	30.00 ± 2.89	1.73 ± 0.12	43.33 ± 1.67
Pusa Barkha	0.27 ± 0.12	6.67 ± 1.67	1.13 ± 0.23	28.33 ± 3.33	2.00 ± 0.40	50.00 ± 5.77
EOM-400	0.40 ± 0.20	10.00 ± 2.89	1.47 ± 0.12	36.67 ± 1.67	1.53 ± 0.12	38.33 ± 1.67
WBC-26	0.33 ± 0.12	8.33 ± 1.67	1.00 ± 0.20	25.00 ± 2.89	2.47 ± 0.31	61.67 ± 4.41
DC-77	0.33 ± 0.31	8.33 ± 4.41	1.60 ± 0.20	40.00 ± 2.89	1.93 ± 0.23	48.33 ± 3.33
PI-197086	0.53 ± 0.12	13.33 ± 1.67	1.27 ± 0.12	31.67 ± 1.67	2.13 ± 0.12	53.33 ± 1.67
DC-107	0.07 ± 0.12	1.67 ± 1.67	1.20 ± 0.20	30.00 ± 2.89	2.73 ± 0.12	68.33 ± 1.67
GyCl-6	0.73 ± 0.12	18.33 ± 1.67	1.67 ± 0.31	41.67 ± 4.41	1.73 ± 0.23	43.33 ± 3.33
DC-71	0.53 ± 0.12	13.33 ± 1.67	1.47 ± 0.12	36.67 ± 1.67	2.13 ± 0.12	53.33 ± 1.67
PI-197088	0.13 ± 0.23	3.33 ± 3.33	1.07 ± 0.23	26.67 ± 3.33	2.93 ± 0.42	73.33 ± 6.01
Pusa Long Green	0.93 ± 0.12	23.33 ± 1.67	1.53 ± 0.31	38.33 ± 4.41	1.80 ± 0.20	45.00 ± 2.89
PCUC-8	0.40 ± 0.35	10.00 ± 5.00	1.47 ± 0.31	36.67 ± 4.41	2.53 ± 0.31	63.33 ± 4.41
DC-43	0.33 ± 0.31	8.33 ± 4.41	1.20 ± 0.20	30.00 ± 2.89	3.00 ± 0.20	75.00 ± 2.89
DC-8	0.27 ± 0.31	6.67 ± 4.41	1.80 ± 0.35	45.00 ± 5.00	2.53 ± 0.42	63.33 ± 6.01
Pahari Barsati	0.27 ± 0.12	6.67 ± 1.67	1.73 ± 0.12	43.33 ± 1.67	2.93 ± 0.12	73.33 ± 1.67
H-16 (*C. hardwickii*)	1.13 ± 0.23	28.33 ± 3.33	1.73 ± 0.12	43.33 ± 1.67	2.07 ± 0.12	51.67 ± 1.67
Kalyanpur Green	0.93 ± 0.31	23.33 ± 4.41	2.13 ± 0.31	53.33 ± 4.41	3.47 ± 0.12	86.67 ± 1.67
DC-47	0.80 ± 0.20	20.00 ± 2.89	1.40 ± 0.35	35.00 ± 5.00	2.87 ± 0.12	71.67 ± 1.67
DC-63	0.13 ± 0.12	3.33 ± 1.67	1.93 ± 0.23	48.33 ± 3.33	3.27 ± 0.31	81.67 ± 4.41
DC-48	0.53 ± 0.12	13.33 ± 1.67	1.73 ± 0.12	43.33 ± 1.67	3.20 ± 0.20	80.00 ± 2.89
WBC-22	0.73 ± 0.42	18.33 ± 6.01	1.87 ± 0.46	46.67 ± 6.67	3.00 ± 0.20	75.00 ± 2.89
Japanese Long Green	0.80 ± 0.20	20.00 ± 2.89	1.73 ± 0.31	43.33 ± 4.41	3.07 ± 0.12	76.67 ± 1.67
Sel-3 (*C. melo* var. *agretis*)	0.87 ± 0.46	21.67 ± 6.67	2.07 ± 0.12	51.67 ± 1.67	2.73 ± 0.23	68.33 ± 3.33
KSP-301	1.00 ± 0.20	25.00 ± 2.89	2.13 ± 0.23	53.33 ± 3.33	3.33 ± 0.23	83.33 ± 3.33
C-1	1.13 ± 0.23	28.33 ± 3.33	1.87 ± 0.23	46.67 ± 3.33	2.80 ± 0.20	70.00 ± 2.89
WBC-5	0.47 ± 0.12	11.67 ± 1.67	2.20 ± 0.35	55.00 ± 5.00	3.33 ± 0.31	83.33 ± 4.41
DC-92	0.67 ± 0.23	16.67 ± 3.33	1.87 ± 0.12	46.67 ± 1.67	3.47 ± 0.12	86.67 ± 1.67
GyCl-101	0.87 ± 0.31	21.67 ± 4.41	1.93 ± 0.31	48.33 ± 4.41	3.27 ± 0.12	81.67 ± 1.67
WBC-21	0.93 ± 0.12	23.33 ± 1.67	2.13 ± 0.12	53.33 ± 1.67	3.00 ± 0.20	75.00 ± 2.89

WBC-13	0.53 ± 0.12	13.33 ± 1.67	2.00 ± 0.20	50.00 ± 2.89	3.53 ± 0.12	88.33 ± 1.67
DC-83	1.00 ± 0.40	25.00 ± 5.77	2.07 ± 0.31	51.67 ± 4.41	3.07 ± 0.23	76.67 ± 3.33
7026-B-76	0.53 ± 0.12	13.33 ± 1.67	1.87 ± 0.12	46.67 ± 1.67	3.73 ± 0.12	93.33 ± 1.67
GYCl-10	1.60 ± 0.20	40.00 ± 2.89	1.93 ± 0.12	48.33 ± 1.67	3.00 ± 0.35	75.00 ± 5.00
GyCl-15	1.60 ± 0.35	40.00 ± 5.00	2.53 ± 0.23	63.33 ± 3.33	3.67 ± 0.23	91.67 ± 3.33
Panipat Local	1.73 ± 0.42	43.33 ± 6.01	2.80 ± 0.20	70.00 ± 2.89	3.40 ± 0.40	85.00 ± 5.77
AZMC-1	1.73 ± 0.49	50.00 ± 10.41	2.60 ± 0.53	65.00 ± 7.64	3.33 ± 0.31	83.33 ± 4.41
Pusa Uday	0.12 ± 1.73	43.33 ± 1.67	2.67 ± 0.12	66.67 ± 1.67	3.93 ± 0.12	98.33 ± 1.67
Sl-746	2.60 ± 0.20	65.00 ± 2.89	2.93 ± 0.12	73.33 ± 1.67	3.47 ± 0.12	86.67 ± 1.67
Sl-749	2.47 ± 0.12	61.67 ± 1.67	3.07 ± 0.12	76.67 ± 1.67	3.73 ± 0.12	93.33 ± 1.67
DPaC-21	2.47 ± 0.12	61.67 ± 1.67	3.40 ± 0.20	85.00 ± 2.89	3.53 ± 0.23	88.33 ± 3.33
DPaC-41	2.60 ± 0.35	65.00 ± 5.00	3.33 ± 0.42	83.33 ± 6.01	3.60 ± 0.35	90.00 ± 5.00
DPaC-59	3.07 ± 0.12	76.67 ± 1.67	3.73 ± 0.12	93.33 ± 1.67	3.93 ± 0.12	98.33 ± 1.67
Pusa Parthenocarpic Cucumber 6	2.73 ± 0.23	68.33 ± 3.33	3.40 ± 0.20	85.00 ± 2.89	3.93 ± 0.12	98.33 ± 1.67
DC-769	2.80 ± 0.35	70.00 ± 5.00	3.53 ± 0.31	88.33 ± 4.41	3.87 ± 0.12	96.67 ± 1.67
DC-773	3.80 ± 0.20	95.00 ± 2.89	3.93 ± 0.12	98.33 ± 1.67	3.93 ± 0.12	98.33 ± 1.67

Supplementary Table S2. **Vulnerability index of cucumber genotypes at different time interval (30, 45 and 60 DAS) during the *kharif* 2022**

Genotype	*Kharif* 2022					
	30 DAS		45 DAS		60 DAS	
	Average scoring	Vulnerability index	Average scoring	Vulnerability index	Average scoring	Vulnerability index
WBC-17	0.27 ± 0.12	6.67 ± 1.67	0.73 ± 0.12	18.33 ± 1.67	0.87 ± 0.12	21.67 ± 1.67
DC-91	0.33 ± 0.12	8.33 ± 1.67	0.67 ± 0.12	16.67 ± 1.67	0.93 ± 0.12	23.33 ± 1.67
DC-61	0.33 ± 0.12	8.33 ± 1.67	0.73 ± 0.12	18.33 ± 1.67	0.93 ± 0.12	23.33 ± 1.67
DC-70	0.20 ± 0.20	5.00 ± 2.89	1.00 ± 0.20	25.00 ± 2.89	1.07 ± 0.12	26.67 ± 1.67
DC-45	0.47 ± 0.31	11.67 ± 4.41	0.93 ± 0.12	23.33 ± 1.67	1.47 ± 0.31	36.67 ± 4.41
DC-77	0.13 ± 0.23	3.33 ± 3.33	1.20 ± 0.35	30.00 ± 5.00	1.6 ± 0.40	40.00 ± 5.77
KSP-301	0.60 ± 0.20	15.00 ± 2.89	1.27 ± 0.31	31.67 ± 4.41	2.00 ± 0.53	50.00 ± 7.64
Kalyanpur Green	0.47 ± 0.12	11.67 ± 1.67	1.47 ± 0.12	36.67 ± 1.67	2.13 ± 0.31	53.33 ± 4.41
DC-40	0.40 ± 0.20	10.00 ± 2.89	1.47 ± 0.12	36.67 ± 1.67	2.27 ± 0.12	56.67 ± 1.67
Sel-3 (C. melo var. agretis)	0.73 ± 0.12	18.33 ± 1.67	1.60 ± 0.20	40.00 ± 2.89	2.13 ± 0.12	53.33 ± 1.67
DC-107	0.27 ± 0.12	6.67 ± 1.67	1.60 ± 0.20	40.00 ± 2.89	2.67 ± 0.12	66.67 ± 1.67
Japanese Long Green	0.60 ± 0.20	15.00 ± 2.89	1.47 ± 0.23	36.67 ± 3.33	2.80 ± 0.20	70.00 ± 2.89
PI-197088	0.27 ± 0.31	6.67 ± 4.41	1.67 ± 0.50	41.67 ± 7.26	3.00 ± 0.35	75.00 ± 5.00
DC-71	0.47 ± 0.23	11.67 ± 3.33	1.67 ± 0.12	41.67 ± 1.67	2.87 ± 0.12	71.67 ± 1.67
PI-197086	0.87 ± 0.12	21.67 ± 1.67	1.67 ± 0.12	41.67 ± 1.67	2.60 ± 0.20	65.00 ± 2.89
GyCl-6	0.93 ± 0.12	23.33 ± 1.67	2.07 ± 0.12	51.67 ± 1.67	2.13 ± 0.31	53.33 ± 4.41
PCUC-8	0.80 ± 0.53	20.00 ± 7.64	1.67 ± 0.23	41.67 ± 3.33	2.67 ± 0.61	66.67 ± 8.82
WBC-26	0.8 ± 0.2	20.00 ± 2.89	1.47 ± 0.12	36.67 ± 1.67	2.93 ± 0.31	73.33 ± 4.41

EOM-400	1.33 ± 0.31	33.33 ± 4.41	2.00 ± 0.20	50.00 ± 2.89	2.33 ± 0.12	58.33 ± 1.67
DC-63	0.20 ± 0.20	5.00 ± 2.89	2.00 ± 0.40	50.00 ± 5.77	3.47 ± 0.12	86.67 ± 1.67
Pusa Long Green	0.93 ± 0.12	23.33 ± 1.67	2.07 ± 0.12	51.67 ± 1.67	2.73 ± 0.12	68.33 ± 1.67
DC-8	0.6 ± 0.2	15.00 ± 2.89	2.13 ± 0.12	53.33 ± 1.67	3.00 ± 0.20	75.00 ± 2.89
DC-47	0.80 ± 0.20	20.00 ± 2.89	1.93 ± 0.31	48.33 ± 4.41	3.00 ± 0.20	75.00 ± 2.89
DC-48	0.67 ± 0.12	16.67 ± 1.67	2.07 ± 0.31	51.67 ± 4.41	3.07 ± 0.31	76.67 ± 4.41
Pusa Barkha	0.80 ± .40	20.00 ± 5.77	1.93 ± 0.31	48.33 ± 4.41	3.13 ± 0.23	78.33 ± 3.33
H-16 (C. hardwickii)	1.20 ± 0.40	30.00 ± 5.77	1.87 ± 0.31	46.67 ± 4.41	2.80 ± 0.20	70.00 ± 2.89
Pahari Barsati	0.40 ± 0.20	10.00 ± 2.89	2.20 ± 0.35	55.00 ± 5.00	3.27 ± 0.42	81.67 ± 6.01
DC-43	0.8 ± 0.2	20.00 ± 2.89	2.20 ± 0.20	55.00 ± 2.89	2.87 ± 0.12	71.67 ± 1.67
WBC-13	0.67 ± 0.23	16.67 ± 3.33	2.27 ± 0.12	56.67 ± 1.67	3.33 ± 0.31	83.33 ± 4.41
WBC-5	0.67 ± 0.12	16.67 ± 1.67	2.27 ± 0.12	56.67 ± 1.67	3.40 ± 0.20	85.00 ± 2.89
DC-83	1.07 ± 0.12	26.67 ± 1.67	2.13 ± 0.12	53.33 ± 1.67	3.20 ± 0.20	80.00 ± 2.89
DC-92	1.13 ± 0.12	28.33 ± 1.67	2.13 ± 0.12	53.33 ± 1.67	3.13 ± 0.23	78.33 ± 3.33
GyCl-101	1 ± 0.2	25.00 ± 2.89	2.33 ± 0.12	58.33 ± 1.67	3.13 ± 0.12	78.33 ± 1.67
7026-B-76	0.87 ± 0.12	21.67 ± 1.67	2.33 ± 0.31	58.33 ± 4.41	3.33 ± 0.12	83.33 ± 1.67
Panipat Local	1.07 ± 0.12	26.67 ± 0.51	2.20 ± 0.20	55.00 ± 2.89	3.33 ± 0.31	83.33 ± 4.41
WBC-21	1.13 ± 0.12	28.33 ± 1.67	2.27 ± 0.12	56.67 ± 1.67	3.20 ± 0.20	80.00 ± 2.89
WBC-22	1.00 ± 0.2	25.00 ± 2.89	2.33 ± 0.31	58.33 ± 4.41	3.33 ± 0.12	83.33 ± 1.67
AZMC-1	1.67 ± 0.42	41.67 ± 6.01	2.20 ± 0.60	55.00 ± 8.66	3.13 ± 0.31	78.33 ± 4.41
GyCl-15	1.53 ± 0.31	38.33 ± 4.41	2.60 ± 0.20	65.00 ± 2.89	3.27 ± 0.31	81.67 ± 4.41
C-1	1.73 ± 0.23	43.33 ± 3.33	2.40 ± 0.20	60.00 ± 2.89	3.40 ± 0.20	85.00 ± 2.89
GYCL-10	1.80 ± 0.20	45.00 ± 2.89	2.53 ± 0.12	63.33 ± 1.67	3.47 ± 0.12	86.67 ± 1.67
Pusa Uday	1.80 ± 0.20	45.00 ± 2.89	2.73 ± 0.31	68.33 ± 4.41	3.87 ± 0.12	96.67 ± 1.67
Pusa Parthenocarpic Cucumber 6	2.27 ± 0.23	56.67 ± 3.33	3.13 ± 0.12	78.33 ± 1.67	3.93 ± 0.12	98.33 ± 1.67
DC-769	2.20 ± 0.53	55.00 ± 7.64	3.53 ± 0.31	88.33 ± 4.41	3.73 ± 0.31	93.33 ± 4.41
Sl-749	2.67 ± 0.12	66.67 ± 1.67	3.33 ± 0.31	83.33 ± 4.41	3.67 ± 0.12	91.67 ± 1.67
DPaC-41	2.53 ± 0.31	63.33 ± 4.41	3.47 ± 0.12	86.67 ± 1.67	3.67 ± 0.12	91.67 ± 1.67
Sl-746	2.87 ± 0.12	71.67 ± 1.67	3.13 ± 0.12	78.33 ± 2.04	3.73 ± 0.12	93.33 ± 1.67
DPaC-21	2.8 ± 0.20	70.00 ± 2.89	3.33 ± 0.12	83.33 ± 1.67	3.93 ± 0.12	98.33 ± 1.67
DPaC-59	2.93 ± 0.12	73.33 ± 1.67	3.53 ± 0.23	88.33 ± 3.33	3.93 ± 0.12	98.33 ± 1.67
DC-773	3.67 ± 0.31	91.67 ± 4.41	3.87 ± 0.12	96.67 ± 1.67	3.93 ± 0.12	98.33 ± 1.67

Supplementary Table S3. Whitefly screening of cucumber genotypes for resistance against ToLCNDV during 2023

Sl No.	Genotype	Whitefly-mediated inoculation							
		7 dpi		14 dpi		21 dpi		28 dpi	
		Average scoring		Average scoring	Vulnerability Index	Average scoring	Vulnerability Index	Average scoring	Vulnerability Index
1	DC-70	0.13 ± 0.07	3.33 ± 1.67	0.40 ± 0.12	10.00 ± 2.89	0.67 ± 0.24	16.67 ± 6.01	1.60 ± 0.23	40.00 ± 5.77
2	DC-61	0.13 ± 0.07	3.33 ± 1.67	0.47 ± 0.07	11.67 ± 1.67	0.93 ± 0.07	23.33 ± 1.67	1.33 ± 0.18	33.33 ± 4.41
3	DC-91	0.07 ± 0.07	1.67 ± 1.67	0.33 ± 0.07	8.33 ± 1.67	1.13 ± 0.18	28.33 ± 4.41	1.40 ± 0.20	35.00 ± 5.00
4	WBC-26	0.33 ± 0.07	8.33 ± 1.67	1.00 ± 0.12	25.00 ± 2.89	1.93 ± 0.18	48.33 ± 4.41	2.60 ± 0.12	65.00 ± 2.89
5	Sel-3 (*C. melo* var *agretis*)	0.73 ± 0.07	18.33 ± 1.67	1.47 ± 0.07	36.67 ± 1.67	1.87 ± 0.07	46.67 ± 1.67	2.47 ± 0.07	61.67 ± 1.67
6	WBC-17	0.87 ± 0.07	21.67 ± 3.33	1.53 ± 0.07	38.33 ± 1.67	1.67 ± 0.18	41.67 ± 4.41	2.67 ± 0.07	66.67 ± 1.67
7	Pusa Barkha	1.47 ± 0.18	36.67 ± 4.41	2.20 ± 0.20	55.00 ± 5.00	2.73 ± 0.18	68.33 ± 4.41	2.80 ± 0.12	70.00 ± 2.89
8	H-16 (*C. hardwickii*)	0.93 ± 0.07	23.33 ± 1.67	1.87 ± 0.07	46.67 ± 1.67	2.73 ± 0.13	68.33 ± 3.33	3.67 ± 0.13	91.67 ± 3.33
9	DC-45	1.13 ± 0.13	28.33 ± 3.33	2.00 ± 0.12	50.00 ± 2.89	2.53 ± 0.07	63.33 ± 1.67	3.87 ± 0.13	96.67 ± 3.33
10	Pusa Uday	1.07 ± 0.18	26.67 ± 4.41	1.80 ± 0.20	45.00 ± 5.00	3.33 ± 0.07	83.33 ± 1.67	3.67 ± 0.24	91.67 ± 6.01
11	GyCl-10	1.87 ± 0.13	46.67 ± 3.33	2.73 ± 0.18	68.33 ± 4.41	3.73 ± 0.07	93.33 ± 1.67	3.87 ± 0.07	96.67 ± 1.67
12	GyCl-15	2.53 ± 0.18	63.33 ± 4.41	2.87 ± 0.13	71.67 ± 3.33	2.93 ± 0.07	73.33 ± 1.67	3.93 ± 0.07	98.33 ± 1.67
13	WBC 5	1.13 ± 0.13	51.67 ± 3.33	1.75 ± 0.13	71.67 ± 3.33	3.67 ± 0.07	91.67 ± 1.67	3.87 ± 0.07	96.67 ± 1.67
14	Sl-749	2.13 ± 0.18	53.33 ± 4.41	3.27 ± 0.29	81.67 ± 7.26	3.60 ± 0.12	90.00 ± 2.89	3.93 ± 0.07	98.33 ± 1.67
15	DC-773	2.33 ± 0.07	56.67 ± 4.41	3.40 ± 0.12	76.67 ± 3.33	3.60 ± 0.12	96.67 ± 1.67	3.93 ± 0.07	98.33 ± 1.67
16	DPaC-21	2.33 ± 0.07	58.33 ± 1.67	3.67 ± 0.18	85.00 ± 2.89	3.73 ± 0.13	90.00 ± 2.89	3.87 ± 0.07	98.33 ± 1.67
17	DC-769	2.27 ± 0.18	58.33 ± 1.67	3.07 ± 0.13	91.67 ± 4.41	3.87 ± 0.07	93.33 ± 3.33	3.93 ± 0.07	96.67 ± 1.67

Supplementary Table S4. *Agrobacterium*-**mediated screening of cucumber genotypes for resistance against ToLCNDV during 2023**

Sl No.	Genotype	Agrobacterium-mediated inoculation							
		7 dpi		14 dpi		21 dpi		28 dpi	
		Average scoring	Vulnerability index	Average scoring	Vulnerability index	Average scoring	Vulnerability index	Average scoring	Vulnerability index
1	DC-70	0.07 ± 0.07	1.67 ± 1.67	0.27 ± 0.07	6.67 ± 1.67	0.47 ± 0.07	11.67 ± 1.67	0.73 ± 0.07	18.33 ± 1.67
2	DC-61	0.07 ± 0.07	1.67 ± 1.67	0.27 ± 0.18	6.67 ± 4.41	0.53 ± 0.13	13.33 ± 3.33	1.33 ± 0.18	23.33 ± 1.67
3	DC-91	0.13 ± 0.07	3.33 ± 1.67	0.27 ± 0.07	6.67 ± 1.67	0.73 ± 0.18	18.33 ± 4.41	1.40 ± 0.18	28.33 ± 4.41
4	Pusa Uday	1.40 ± 0.23	35.00 ± 5.77	2.67 ± 0.07	66.67 ± 1.67	3.80 ± 0.12	95.00 ± 2.89	3.93 ± 0.07	98.33 ± 1.67
5	WBC-26	1.47 ± 0.13	36.67 ± 3.33	1.93 ± 0.07	48.33 ± 1.67	2.60 ± 0.12	65.00 ± 2.89	3.13 ± 0.07	78.33 ± 1.67
6	GyCl-10	1.47 ± 0.13	36.67 ± 3.33	1.87 ± 0.07	46.67 ± 1.67	3.27 ± 0.27	81.67 ± 6.67	3.73 ± 0.13	93.33 ± 3.33
7	WBC-17	1.53 ± 0.18	38.33 ± 4.41	2.13 ± 0.07	53.33 ± 1.67	2.67 ± 0.24	66.67 ± 6.01	3.33 ± 0.24	83.33 ± 6.01
8	Pusa Barkha	1.87 ± 0.13	46.67 ± 3.33	2.73 ± 0.13	68.33 ± 3.33	3.00 ± 0.01	75.00 ± 0.00	2.80 ± 0.12	70.00 ± 2.89
9	H-16 (*C. hardwickii*)	2.13 ± 0.07	53.33 ± 1.67	2.53 ± 0.13	63.33 ± 3.33	3.73 ± 0.07	93.33 ± 1.67	3.73 ± 0.07	93.33 ± 1.67
10	DC-45	1.93 ± 0.07	48.33 ± 1.67	2.80 ± 0.12	70.00 ± 2.89	3.53 ± 0.18	88.33 ± 4.41	3.93 ± 0.07	98.33 ± 1.67
11	Sel-3 (*C. melo* var *agretis*)	1.73 ± 0.07	43.33 ± 1.67	2.87 ± 0.07	71.67 ± 1.67	3.73 ± 0.07	93.33 ± 1.67	3.87 ± 0.07	96.67 ± 1.67
12	WBC-5	1.25 ± 0.07	56.67 ± 1.67	1.62 ± 0.07	73.33 ± 1.67	3.60 ± 0.12	90.00 ± 2.89	3.67 ± 0.13	91.67 ± 3.33
13	GyCl-15	2.93 ± 0.07	73.33 ± 1.67	2.80 ± 0.12	70.00 ± 2.89	2.93 ± 0.07	73.33 ± 1.67	3.87 ± 0.13	96.67 ± 3.33
14	DPaC-21	2.60 ± 0.12	65.00 ± 2.89	3.13 ± 0.07	78.33 ± 1.67	3.73 ± 0.18	93.33 ± 4.41	3.93 ± 0.07	98.33 ± 1.67
15	Sl-749	2.40 ± 0.12	60.00 ± 2.89	3.67 ± 0.18	91.67 ± 4.41	3.87 ± 0.07	96.67 ± 1.67	3.93 ± 0.07	98.33 ± 1.67
16	DC-769	2.73 ± 0.07	68.33 ± 1.67	3.73 ± 0.18	93.33 ± 4.41	3.93 ± 0.07	98.33 ± 1.67	3.93 ± 0.07	98.33 ± 1.67
17	DC-773	2.93 ± 0.07	73.33 ± 1.67	3.60 ± 0.12	90.00 ± 2.89	3.93 ± 0.07	98.33 ± 1.67	3.93 ± 0.07	98.33 ± 1.67