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Identification and validation of novel source of resistance to downy mildew in cucumber (*Cucumis sativus*)

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ABSTRACT

The experiment was conducted during rainy (*kharif*) season of 2021–2023 under natural epiphytotic conditions at three locations, viz. ICAR-Indian Agricultural Research Institute, New Delhi, ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh and ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka to identify novel resistance source for downy mildew in cucumber (*Cucumis sativus* L.). One hundred fifty-six cucumber genotypes including checks were screened. Consequently, a subset of 5 cucumber genotypes showing resistant/moderately resistant disease reaction at more than one location were selected for validation through multi-location, multi-year testing of their disease response under replicated trial followed by artificial screening. Accessions IC527400 and IC572024 showed field resistance with an average PDI ranging from 11.68–40.39 and 4.08–58.10, respectively at different locations as compared to 37.6–92.9 PDI in susceptible check Pusa Uday. The disease reaction in these genotypes under artificial screening was in accordance with disease reaction under natural conditions. The overall data suggested that IC527400 and IC572024 collected from West Bengal and Lakshadweep, respectively were quite promising as they recorded resistant to moderately resistant reaction at all the three locations and performed better than two of the resistant checks PI 197085 and PI 197086. Even under artificial screening these lines were free from disease symptoms even after 25 days of inoculation. This showed that these lines may have the potential of multi strain/race/pathotype resistance which may be utilized for development of resistant cultivars.

Keywords: Artificial screening, Cucumber, Downy mildew, Field screening, Resistant source

Cucumber (*Cucumis sativus* L.) is one of the most common salad vegetables in India, grown in an area of 1.13 lakh hectares with an annual production of 1.64 million tonnes (NHB 2021–22). The global cucumber production was estimated as 91.8 million tonnes in 2022 (FAOSTAT 2023) with China, Russia, Turkey and Iran as the leading producers. Cucumber is native to the Indian gene centre (De Candolle 1885, Bisht *et al.* 2004, Sebastian *et al.* 2010) and holds enormous diversity throughout the country. The production of cucurbits is affected by more than 200 diseases caused by fungi, bacteria, viruses, and mycoplasma like organism across the world. Downy mildew (DM) caused by *Pseudoperonospora cubensis* (Berkeley and MA Curtis) Rostovzev, is the most common foliage disease of cucumber and other cucurbit crops worldwide. It is known to be one of the most devastating and widespread diseases in cucumber causing up to 100% yield loss (Lebeda and Cohen 2011, Call 2012, Savory *et al.* 2011). When conditions are favourable, the disease can defoliate a cucumber field in a matter of days, limiting the flexibility of fungicide spray. The spores of *Pseudoperonospora cubensis* spread quickly through wind, splashing rain, and/or irrigation water. The typical symptoms of downy mildew infection are angular lesions that are limited by the leaf veins and soon turn chlorotic and necrotic. The infected leaves limit the photosynthetic capabilities of the plant leading to retarded plant growth and yield. A temperature range of 5–30°C along with sufficient moisture provides congenial atmosphere for disease appearance (Thomas 1977). Though this disease can be managed through use of fungicides and cultural practices, the use of pesticides is of major concern for health and environment. Among integrated pest management practices, the use of resistant cultivars is clearly the most cost-effective and environment friendly method of disease control (Dey *et al.* 2023). Fortunately, cucumber lines with high resistance

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to the new DM strain have been identified (Call *et al.* 2012). Though global source of resistance to downy mildew in cucumber is primarily coming from Indian gene pool, the germplasm conserved in National Genebank, New Delhi has not yet thoroughly screened for DM resistance and no resistant cultivar is available so far in India. Over the past 60 years, many downy mildew resistant cucumber cultivars have been developed globally, but there has always been a lack of such cultivar that offers high level of resistance to different races/pathotypes of *Pseudoperonospora cubensis* that occur in different geographical regions. Keeping these in view the present study was conducted with the objective to identify novel source of resistance to downy mildew from Indian gene pool.

MATERIALS AND METHODS

Preliminary screening: The experiment was conducted during rainy (*kharif*) season of 2021–2023 under natural epiphytotic conditions at three locations, viz. ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi; ICAR-Indian Institute of Vegetable Research (ICAR-IIVR), Varanasi, Uttar Pradesh and ICAR-Indian Institute of Horticultural Research, Bengaluru (ICAR-IIHR), Karnataka. Preliminary screening of 156 genotypes of cucumber (Table 1), including released varieties, landraces, previously identified resistant germplasm (Ranjan *et al.* 2015), 3 susceptible checks and 3 resistant checks were conducted for downy mildew incidence during *kharif* season of 2021 under Collaborative Research Platform on Agro-Biodiversity at ICAR-IARI, ICAR-IIVR and ICAR-IIHR, Bengaluru. Consequently, a subset of 5 cucumber genotypes showing resistant/moderately resistant disease reaction at more than one location were selected for validation through multi-location multi-year testing of their disease response under replicated trial (Table 1).

Screening under natural epiphytotic conditions: A total of five accessions collected from different parts of India (Table 1) based on their previous response during preliminary screening were validated at three locations, viz. ICAR-IARI, New Delhi; ICAR-IIVR, Varanasi and ICAR-IIHR, Bengaluru in replicated trial during late *kharif* season of 2022 and 2023. The three Indian origin PI lines PI 197085, PI 197086 and PI 197088 which are being utilized globally as a source of resistance to downy mildew in cucumber were used as resistant check. The crop was raised as per standard package of practices except no fungicide was applied to build up enough disease inoculum. The disease score was recorded after 30 days of sowing (DAS) at 15 days interval up to 75 days. The experiment was laid under randomized block design (RBD) with three replications.

Table 1 Details of selected genotypes used for multi-year multi-location testing

Genotype	Material Type	Collected from
IC527400	Germplasm	West Bengal
IC527413	Germplasm	West Bengal
IC527431	Germplasm	West Bengal
IC 572024	Germplasm	Lakshadweep
IC538158	Germplasm	Uttar Pradesh
Pusa Long Green	Susceptible check	Released variety
Pusa Uday	Susceptible check	Released variety
Pahari Harit	Susceptible check	Local variety
PI 197085 (IC395877)	Resistant check	Assam
PI 197086 (EC1041437)	Resistant check	Assam
PI 197088 (IC622750)	Resistant check	Assam

Screening under artificial epiphytotic conditions: The genotypes which performed best under natural field conditions were also screened through artificial inoculations along with susceptible and resistant checks. Cucumber leaves were gathered from the field in the morning and stored in plastic bags with ice in a cooler to collect downy mildew inoculum. Five severely affected leaves were soaked in distilled water in the laboratory and gently scraped with a glass rod to extract the sporangia (Fig. 1). The spore suspension was then filtered through four layers of cheesecloth to remove dirt and debris. To maintain even dispersion of spores in the solution, Tween 20 (0.06 g/L) was added immediately. Using a hand sprayer, 20–25 days old seedlings were inoculated with a sporangium suspension containing 10,000/ml sporangia. Prior to inoculation, small pinholes were created on the adaxial leaf surface using small needles. Disease symptoms started appearing after 3–4 days, and the infected plants were kept in high humidity (100%) at a temperature of 20°C for 48 h, then kept at 20–25°C. Disease score was recorded for resistance and susceptibility on the eighth day till 25 days post inoculation.

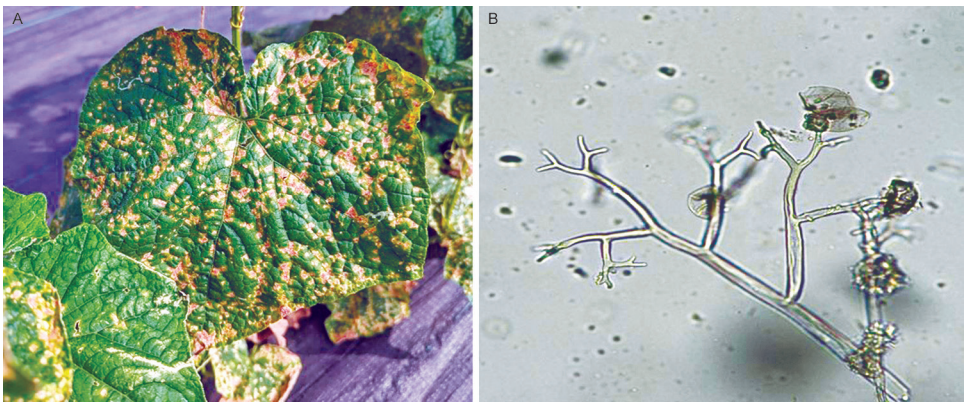

Fig. 1 (a) Symptoms of downy mildew on infected cucumber leaf (b) Sporangiophore of *Pseudpernospora cubensis*.

Disease scoring: Regular monitoring for the date of disease onset and subsequent symptom development of downy mildew was performed. Once the initial downy mildew symptom was observed on the plant grown in open field, the lesion colour changed from light yellow/ dark yellow to light brown to dark brown/necrotic with the progress of the disease. The nature of the spread of the disease was observed through visual observation from the initiation of the disease at 15 days interval till 75 DAS. Genotypes were screened on 0–9 scale (Jenkins and Wehner 1983) based on the percentage of symptomatic leaf area (0, 0%; 1, 1–5%; 2, 6–10%; 3, 11–20%; 4, 21–30%; 5, 31–50%; 6, 51–65%; 7, 66–80%; 8, 81–99%, and 9, 100%). The percent disease index (PDI) was calculated by the following formula given by Wheeler (1969):

$$PDI = \frac{N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4 + N_5 \times 5 + N_6 \times 6 + N_7 \times 7 + N_8 \times 8 + N_9 \times 9}{\text{Total number of observed leaves} \times \text{Maximum grade}} \times 100$$

Where N_1 to N_9 represents total number of leaves falling under 1–9 scales, respectively.

Based on PDI the disease reaction of genotype was classified into four groups namely resistant (0–20%); moderately resistant (21–40%); susceptible (41–60%) and highly susceptible (>60%) based on the average PDI (Reddy 2002).

Statistical analysis: The differences among genotypes for PDI value was analyzed through mixed model analysis of ANOVA using SPSS16.0 software and Tukey test was used to compare the means.

RESULTS AND DISCUSSION

Preliminary screening: The preliminary screening data suggested (data not presented) that most of the genotypes showed susceptibility to the DM at one or more locations. Only five accessions namely IC527400, IC527413, IC527431, IC572024 and IC538158 were recorded to show resistant or moderately resistant reaction at more than one location. The resistance of accession to a local isolate of pathogen does not necessarily mean that it will be resistant to isolates that prevail in other locations because of the presence of different pathotypes at different location and their interaction with environmental conditions causing differential reaction. Interactions between pathogen, host and environment are complex and not easily determined. Hence, these five genotypes along with resistant and susceptible check need to be validated at different locations.

Screening under natural epiphytotic conditions: All the five genotypes along with checks were screened under natural epiphytotic condition and typical symptom of disease first appeared at 30 DAS in most of the genotypes with varying degree of infection. The disease incidence increased gradually with number of days to sowing at all the locations. Initially the symptom appeared as angular lesion turning chlorotic which ultimately turned necrotic (Fig. 1). Eventually the entire leaf became necrotic and plant die. The susceptible checks showed highly chlorotic and necrotic symptoms while resistant checks, IC527400 and IC572024 had small chlorotic spots and sparse sporulation. Cespedes-Sanchez *et al.* (2015) reported that the symptoms vary depending on relative susceptibility of the cultigens. The most resistant cultigens displayed a hypersensitive response (HR) with small necrotic or chlorotic flecks and sparse sporulation, whereas the most susceptible cultigens or lines were highly necrotic and chlorotic, as demonstrated by the current study, which is similar to the observation made by Call *et al.* (2012) in cucumber. Barnes and Epps (1954) first described hypersensitive type resistance in cucumber genotype PI 197087. Furthermore, different genotypes react to disease differently at different stages of plant development. Older plants, even those classified as resistant, exhibited more disease symptoms, whereas some genotypes maintained their resistance even at late developmental stages. This could be because of their rapid, unpredictable growth, which enables them to outgrow the disease (Vanden and Wehner 2016). Analysis of variance using the mixed model analysis for PDI showed significant location, genotype, genotype × location, and year × genotype × location interaction (Table 2).

The data presented in Table 3 showed that overall disease severity was higher at New Delhi location and lowest at Varanasi. At ICAR-IARI, New Delhi, the average PDI ranged from 30.41–92.90 and 38.98–89.20 during 2022 and 2023, respectively. The overall disease incidence and AUDPC was lowest at Varanasi centre for all the genotypes and average PDI ranged from 4.08–38.91 and 5.25–45.34 during 2022 and 2023, respectively. At Bengaluru, the average PDI ranged from 13.79–47.22 and 29–70.83 during 2022 and 2023, respectively. Variations in temperature, humidity, precipitation, and wind-driven inoculum movement have been documented to impact downy mildew infection severity (Cohen 1977).

At New Delhi location, the susceptible checks along with two of the resistant checks PI 197085 and PI 197086

Table 2 ANOVA using mixed model analysis of multi-location and multi-year trial

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Year	252.32	1	252.32	0.54	0.538
Location	31411.97	2	15705.98	18.79	0.003
Genotype	12614.07	10	1261.40	2.45	0.042
Replication	47.40	2	23.70	3.93	0.052
Year × Location	927.69	2	463.84	3.28	0.058
Year × Genotype	845.51	10	84.55	0.59	0.797
Genotype× Location	10261.54	20	513.07	3.63	0.003
Year × Genotype × Location	2825.79	20	141.29	11.22	0.000

showed susceptible or highly susceptible disease reaction with more than >40% PDI (Table 3). Only one resistant check (PI 197088) showed moderately resistant reaction during both the year. Among five accessions screened for DM resistance only two accessions IC527400 and IC572024 showed moderately resistant reaction in year 2022, however, in year 2023 only IC527400 was found moderately resistant to this disease. IC527400 showed lowest PDI (30.41) during 2022 and 40.27% during 2023 which was significantly lower than all the three resistant checks and this genotype has also lowest AUDPC among the five accessions during both the years (Fig. 2). At Varanasi location, all the five accessions showed resistant (IC527400, IC527413, IC527431, IC572024) to moderately resistant (IC538158) disease reaction including the susceptible checks except Pusa Long Green (Table 3). All the resistant checks were showing resistant and moderately resistant disease reaction. PDI was lowest for two accessions which did not differ significantly i.e. IC572024 (4.08) and IC527431 (5.25), the PDI was significantly lower than the three known resistant sources in year 2022. Similar observations were recorded in case of AUDPC for both the accessions (Fig. 2). At Bengaluru, all the five accessions showed resistant to moderately resistant reaction except IC538158 while susceptible checks showed higher PDI (Table 3). The three resistant checks PI 197085, PI 197086 and PI 197088 showed resistant/moderately resistant reaction in both the years. The average PDI was lowest for IC527431 (13.79) in 2022 while IC572024 (29.00) and IC527431 (29.83) recorded lowest average PDI during 2023. AUDPC value was found lowest for IC527431 (529.58) in 2022 and for IC572024 (1021.56) in 2023 (Fig. 2). The PDI and AUDPC of both the accessions were significantly lower than all the three resistant sources. IC527400 recorded moderately resistant reaction. The progress of disease was found to be exponential after the onset of the disease at every location reaching maximum at the later stage of the growth (Fig. 3).

The data suggested that genotypes exhibited consistent response at different locations (Fig. 2) as disease expression is highly dependent on environmental condition specially temperature and humidity. The genotype showing resistant reaction at Varanasi is either moderately resistant or even susceptible at other locations (Table 3). Call and Wehner (2010) noted a change in rank of resistant and moderate resistant cultigens from screening against DM and after a change in the pathogen population. Cultigens highly resistant in 1988 and 1989 were only moderately resistant in studies conducted from 2005 to 2009. Those cultigens identified as highly resistant in the most recent studies were only moderately resistant in 1988 and 1999 (Call and Wehner 2010). At New Delhi condition, IC572024 was showing susceptible reaction and even the resistant checks PI 197085 and PI 197086 were showing high average PDI during 2023. Highly variable nature of this pathogen has been reported (Lebeda and Urban 2004) and multiple pathotypes and races have been identified (Lebeda and Widrlechner 2003) and even in some cases within a geographical region more than one pathotype has been identified (Lebeda and Urban 2004). This is why out of 10 germplasm which showed resistance during 2011–12 (Ranjan *et al.* 2015) only few are showing resistance till now. The environmental condition and races 10-years back might be different from present day. However, it is important to note that the line IC527400 and IC572024 collected from West Bengal and Lakshadweep respectively are showing consistent resistance response. These lines have resistance, equally good and sometimes better than the global source of resistance PI 197085, PI 197086 and

Table 3 Average PDI (%) of cucumber genotypes at three locations in the years 2022 and 2023

Genotype	ICAR-IARI, New Delhi		ICAR-IIVR, Varanasi		ICAR-IIHR, Bengaluru		Mean[$]
	2022	2023	2022	2023	2022	2023	
IC527400	30.41	40.27	11.68	16.95	40.39	36.32	29.33[a, b]
IC527413	51.09	51.27	9.18	9.18	40.56	40.56	33.64[b]
IC527431	89.20	89.20	5.25	5.25	13.79	29.83	38.75[c]
IC 572024	40.91	58.10	4.08	19.20	21.11	29.00	28.73[a]
IC538158	76.48	60.88	26.75	26.75	45.00	57.41	48.87[d]
Pusa Long Green	92.90	55.02	38.91	45.34	47.22	63.33	57.12[e]
Pusa Uday	82.13	57.10	37.63	38.09	47.22	70.83	55.50[e]
Pahari Harit	59.51	56.79	19.97	17.90	47.22	65.56	44.49[d]
PI 197085	62.44	58.33	19.29	27.71	25.28	32.78	37.63[c]
PI197086	50.07	49.05	18.60	20.91	41.11	40.78	36.75[c]
PI 197088	40.97	38.98	7.77	13.59	35.85	37.58	29.12[a]
CD (p=0.05)	5.33	7.13	2.60	3.47	8.22	4.17	-
CV%	5.09	23.14	4.14	3.65	22.05	5.35	-

PDI for resistant (0–20%); Moderately resistant (21–40%); Susceptible (41–60%) and Highly susceptible (>60%).

[$]Tukey test was conducted using the mixed model of ANOVA and the different letter represent the significant difference between the means.

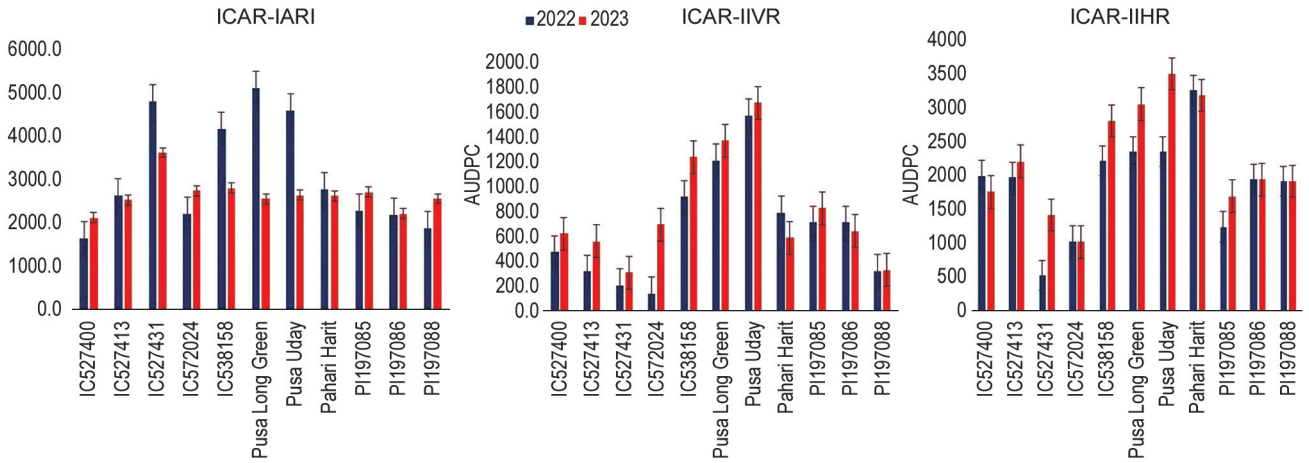

Fig. 2 AUDPC of different cucumber genotypes at three locations in year 2022 and 2023.

PI 197088 both under natural epiphytotic condition as well as under artificial screening.

Accessions IC527400 and IC572024 showed good response at all the three locations, however, IC527431 has found promising at Bengaluru and Varanasi consistently for two years but susceptible at New Delhi. The identified lines IC527400 and IC572024 were reported at par or sometimes better than PI 197088. It is important to note that out of three known resistant lines only PI 197088 exhibited resistance at all the three locations showing multiple isolate/race resistance. Previous studies suggested that PI 197088 and PI 330628 exhibit multiple-isolate resistance (Wang *et al.* 2018, Chen *et al.* 2020). Multilocation resistance recorded in lines IC527400 and IC572024 (Supplementary Fig. 1) may be due to multiple isolate/race resistance which needs to be confirmed through further study.

The overall data suggested that IC527400 and IC572024 were quite promising as they recorded resistant to moderately resistant reaction at all the three locations and performed better than two of the resistant checks PI 197085 and PI 197086 (Table 2). In the same way IC527431 has performed best under Bengaluru and Varanasi condition even better than best check, thus is quite promising as resistant source for these two locations. However, the degree of heterozygosity for most of the accessions evaluated is not known. Owing to highly cross-pollinated nature of cucumber, heterozygosity for most of the accessions can be very high. Moreover, due to small sample size and possible heterozygosity, the susceptible accessions may have the chance to carry recessive alleles for resistance (Wehner and Shetty 1997).

Screening under artificial epiphytotic conditions: The lines were also screened under controlled environment for

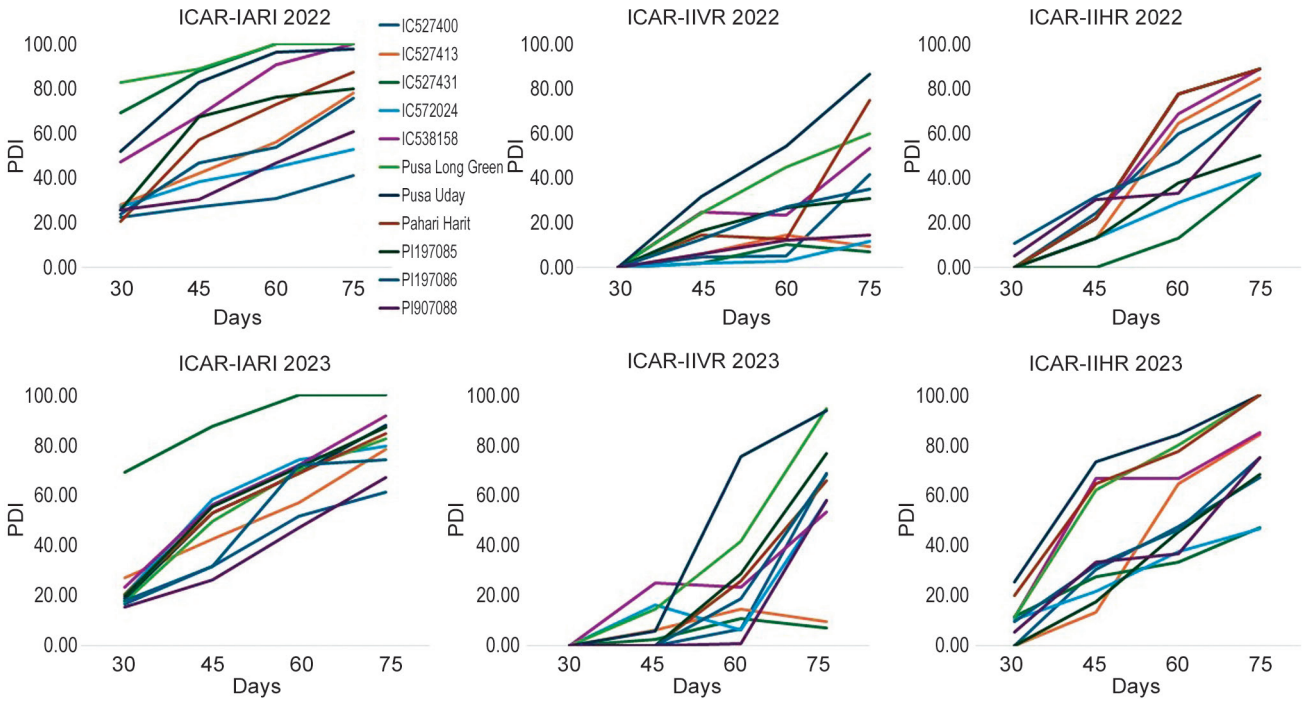

Fig. 3 Progress of disease in different genotypes at different locations during different years.

appearance of disease after pathogen inoculation. Disease reaction appeared 8-days post inoculation in check variety and progressed gradually. After 25 days of inoculation, check variety Pusa Uday and DC 773 showed highly susceptible reaction (avg PDI> 60) while very few symptoms appeared on the leaves of IC527400, IC572024 and PI 197088 (Supplementary Fig. 2) with average PDI 20.56, 39.23 and 20.41, respectively showing resistant disease reaction which suggests IC527400 as resistant and IC572024 as moderately resistant germplasm. These germplasms performed at par or even better than the resistant check PI 197088. Artificial screening is mostly used for the confirmation of resistance at seedling stages. Protocols for artificial screening is well established in cucumber and widely used for screening of DM in large set of germplasm (Criswell 2008, Call 2010). PI 197088 was recently described as highly resistant to downy mildew in a large germplasm screening study and a multiple year re-evaluation of the most resistant and susceptible cultigens conducted at North Carolina State University (Criswell 2008, Call 2010).

Several genetic resources of cucumber including PI 197085, PI 197086, PI 197087, PI 197088, PI 330628, Chinese Long, TH118FLM and *Cucumis hystrix* have been reported to carry resistance against downy mildew (Barnes and Epps 1954, Sitterly 1972, Call 2012, Call *et al.* 2012, Pang *et al.* 2013) and different genes/QTLs (Quantitative Trait Loci) have been identified across seven chromosomes (Li *et al.* 2018). Several studies have reported inheritance of DM resistance in cucumber (Van Vliet and Meysing 1974, Fanourakis and Simon 1987, Zhang *et al.* 2013, Yoshioka *et al.* 2014, Wang *et al.* 2016). The researchers from India have also attempted to identify the resistance sources against the DM disease. Bidramali *et al.* (2023) have recently figured out how the DM is inherited from the native sources DC-70 (R) and DC-773 (S). They determined that the resistance in the DC-70 genotype is controlled by a main gene in a recessive manner based on the PDI and AUDPC. After screening 120 indigenous sources gathered by NBPGR, New Delhi, Reddy *et al.* (2022) found that SKY/AC-270-613481 and JB/11-028-595504 were resistant. Gautam *et al.* (2020) screened against the DM using 32 genotypes of wild and cultivated cucumber. The genotype IC331627 was shown to be extremely resistant to a number of isolates of DM, according to their findings. Bhutia *et al.* (2015) reported 10 resistant lines from screening of 114 genotypes of cucumbers while Ranjan *et al.* (2015) screened 40 genotypes of indigenous cucumbers, and found that IC410617, IC527419, and IC538130 were extremely resistant and IC527400, IC527431 and IC527413 were highly resistant. Pitchaimuthu *et al.* (2012) screened 42 *Cucumis* spp. accessions, including SM 12735, *Cucumis sativus* var. *sativus*, and the wild type *Cucumis hardiwickii*. They discovered that the wild species *Cucumis hardiwickii*-14 and 15, *Cucumis sativus* var. *sativus*, and SM 12735 were extremely resistant to diseases caused by powdery and downy mildew.

The major limitation of these finding is that these studies were mainly limited to single location thus their utilization is limited to that agroclimatic regions. The genotypes IC527400, IC572024 and PI 197088 showed resistance to multiple locations covering wide range of areas. Thus, these lines are promising and must need attention for their utilization in breeding for development of resistant cultivars in India. Moreover, previously reported and utilized all PI lines from India were collected from Assam. However, the germplasm identified in this study have been collected from West Bengal and Lakshadweep which draw attention for more focused exploration of these areas for search of new source of resistance to downy mildew in cucumber.

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