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Original Research Paper

Screening for resistance to downy mildew disease [*Pseudoperonospora cubensis* (Berk. and Curt.) Rostov.] in Cucumber (*Cucumis sativus* L.)

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ABSTRACT

Downy mildew, a foliar disease caused by the oomycete *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov, is one of the most destructive disease of cucumber (*Cucumis sativus* L.). Moderately resistant cultivars are available, but yield losses are high without the use of fungicides, therefore, higher levels of resistance are required to reduce the use of fungicides. Therefore, in the present study, 12 advance breeding lines along with susceptible check of cucumber were screened against downy mildew disease under natural field condition and artificial inoculation through seedling assay technique. The results confirmed that three lines namely IIHR-177-1-1-S7, IIHR-82-1-S6 and IIHR-81-1-S6 were found to be resistant with <10 per cent disease index (PDI) and significantly outperformed against check var. Swarna Agethi for yield and quality traits. These high yielding resistant lines can be utilized as one of the parents for the development of downy mildew resistant hybrids/ varieties.

Keywords: Artificial screening technique, downy mildew, per cent disease index

INTRODUCTION

Cucumber (*Cucumis sativus* L., 2n=14) is one of the economically and commercially important widely cultivated cucurbitaceous crop. The annual global production of cucumber is 87.8 million tons in an area of 2.23 million hectare. Globally, China stands first in the cucumber production with yearly production of 70.3 million tons, which covers 80% of global production (FAOSTAT, 2021). It is very popular among the consumers for both fresh and processed consumption. However, in cucumber cultivation, there are many biotic and a biotic stresses that are causing major yield loss to the producers. Among the biotic stress, foliar fungal diseases like downy and powdery mildew causes major setback among the farmers and researchers.

Downy mildew, caused by the oomycete *Pseudoperonospora cubensis*, is by far the most devastating disease of cucumber and causing yield losses up to 70-100% in India (EI-Nagdy & Abdel-Hafez, 1990) and significant reduction in yield and quality of cucumber production (Colucci & Holmes, 2010). The pathogen gen-erally thrives in warm humid regions. The current control relies mainly on multiple fungicide applications that exert selection pressure on the fungus, increasing the risk of the development of fungicide resistance in the pathogen population (Holmes et al., 2006). Moreover, frequent use of fungicides can be harmful to the environment and detrimental to natural enemies (Kibria et al., 2010; Komarek et al., 2010). The spraying fungicides increase the pesticide residual toxicity and also increase the production cost of the farmers and consumers. Therefore, the best method is the development of resistant cultivars for controlling downy mildew in cucumber.

The main sources of resistance to downy mildew have been identified in different accessions mainly from the US, consisted of elite cultivars and breeding lines that had resistance from Indian genotype (PI 197087). Dhillon et al. (1999) reported nine downy mildew resistant cultigens of Asian and European origin. Call et al. (2012) identified six cultigens resistant to downy mildew disease. Cucumber wild species namely SM 12735, *Cucumis sativus* var *sativus*, *Cucumis metuliferus* L. and *Cucumis hardiwickii*-14 & 15 showed a high level of resistance, and six accessions namely IIHR-27, IIHR-35, IIHR-64, IIHR-82 , IIHR-303 & IIHR-433, exhibited moderate resistance

to downy mildew disease (Pitchaimuthu et al., 2012; Bommesh et al., 2017).

Accurate, fast, economic and repeatable screening methodology is crucial in breeding programmes to develop resistant varieties (Galvan, 2010). Hence, identification of new sources of resistant sources could provide farmers with economic and environmentally sound management strategies for downy mildew control. Therefore, the present study was carried out to screen the advance breeding lines with susceptible check under natural and artificial inoculation to identify the resistance sources for downy mildew disease in cucumber.

MATERIAL AND METHODS

Plant material

The experimental material consisted of 12 advance breeding lines *viz.*, IIHR-76-1-S5, IIHR-81-1-S6, IIHR-82-1-S6, IIHR-101-1-4-S5, IIHR-123 -1-2-S5, IIHR-127-1-2-S7, IIHR-177-1-1-S7, IIHR-292-2-1-S6, IIHR-293-1-2-S7, IIHR-294-1-4-S6, IIHR-296-1-2-1, IIHR-297-1-1, one susceptible check (Swarna Ageti) and two commercial checks (Pusa Bharka and Pusa Uday). The study was conducted in two stages, natural epiphytotic field screening technique and artificial screening technique.

Natural epiphytotic field screening technique

Twelve advance breeding lines, susceptible check and two commercial checks, were screened on raised beds (8 m x 1.2 m) at spacing of 60 cm plant to plant and 2-meter row to row, during *rabi* 2015. Field screening was performed in randomized block design with three replications. Each lines/check has maintained five plants per replication. The standard agronomic package of practices was followed to get good growth and maintenance. However, there was no fungicide sprayed till the end of the crop growth.

Artificial screening technique

The seeds of advance breeding lines and susceptible check (Swarna Ageti) were sown in polythene cover for artificial inoculation at twenty-five days after germination. Since, this pathogen is an obligate parasite and cannot be cultured on artificial media, hence, sporangia were harvested directly from the naturally infected leaves. Diseased leaves at initial stage of infection were collected and brought in the laboratory. The care was taken while selecting the

Table 1 : Scale to score downy mildew disease incidence

Numerical Scale	Leaf area affected (%)	Reaction
0	0	No disease
1	0-3	Few small leaf lesions
2	3-6	Few lesions on few leaves with no stem lesions
3	6-12	Few lesions on few leaves or with superficial stem lesions
4	12-25	Few well-formed leaf lesions or superficial stem lesions
5	25-50	Few well-formed leaf lesions or enlarging stem lesions
6	50-75	Many large leaf lesions or deep stem lesions with abundant sporulation or plant more than 50 per cent defoliated
7	75-87	Many large coalescing leaf or stem lesions, over 75 per cent of plant area affected or defoliated
8	87-99	Plants largely defoliated, leaves or stem with abundant sporulation lesions
9	100	Plants dead

Disease scored data of all the plants were used to calculate the per cent disease index (PDI) by using the following formula:

$$PDI = \frac{\text{Sum of numerical values}}{\text{Total number of leaves examined} \times \text{maximum rating}} \times 100$$

leaves that pathogen must be in its reproducing phase and ready for secondary inoculums with sufficient sporangiophores and sporangia. Infected leaves were gently washed in sterilized distilled water so that sporangia could come out in water.

Three advance breeding lines namely IIHR-177-1-1-S7, IIHR-82-1-S6 and IIHR-81-1-S6 were selected based on natural field screening per cent disease index data and each line/check has maintained five plants per replication. All seedlings were inoculated with strain of *P. cubensis* at fully opened second true leaf. Both sides of each leaf of the seedlings were sprayed uniformly using a hand-pumped sprayer. The inoculums concentration used was 8×10^4 sporangia/mL. After inoculation, plants were kept in the dark at 100% relative humidity (RH) for 24 h, followed by 7 to 10 days at 80/100% RH by day/night at a temperature of 20 to 23°C. Disease scoring was carried out on the basis of per cent leaf area infected by the downy mildew lesions and 0-9 rating scale was adopted for disease ratings.

Disease assessment

The disease incidence was calculated by recording the number of plants affected by downy mildew from each line/check. The scoring of downy mildew disease incidence was started a month after planting and at weekly intervals for six weeks until plant growth ended. The data were recorded on a 0 to 9 scale for downy mildew disease incidence as suggested by Jenkins & Wehner (1983) (Table 1).

The disease reaction of genotype was classified into four groups based on their PDI data i.e. 0-20 (resistant), 21-40% (moderately resistant), 41-60% (susceptible) and >60% (highly susceptible) (Reddy, 2002).

Statistical analysis

Twelve advance breeding lines and three commercial checks were subjected to statistical analysis for per cent disease index (PDI).

RESULTS AND DISCUSSION

Screening under natural field condition

Among 12 advance breeding lines and 3 check varieties screened, line IIHR-177-1-1-S7 recorded 9.0 per cent disease index (PDI) followed by IIHR-81-1-S6 (<10%) and IIHR-82-1-S6 (10%) and showed resistance reaction to downy mildew. Five lines *viz.*, IIHR 127-1-2-S7 (22.99 PDI), IIHR-292-2-1-S6

Table 2 : Disease reaction of cucumber advance breeding lines and checks against downy mildew incidence under open field condition

Advanced breeding line/check	Per cent disease index (PDI)	Disease reaction
IIHR-76-1-S5	36.00 (36.86)	Moderately resistant
IIHR-81-1-S6	9.433 (17.88)	Resistant
IIHR-82-1-S6	10.00 (18.43)	Resistant
IIHR-101-1-4-S5	54.00 (47.29)	Susceptible
IIHR-123-1-2-S5	55.00 (47.87)	Susceptible
IIHR-127-1-2-S7	22.99 (28.65)	Moderately resistant
IIHR-177-1-1-S7	9.00 (17.45)	Resistant
IIHR-292-2-1-S6	26.00 (30.65)	Moderately resistant
IIHR-293-1-2-S7	36.00 (36.47)	Moderately resistant
IIHR-294-1-4-S6	45.00 (42.12)	Susceptible
IIHR-296-1-2-1	50.00 (45.00)	Susceptible
IIHR-297-1-1-1	36.00 (36.86)	Moderately resistant
Swarna Ageti (Susceptible check)	68.00 (55.55)	Highly Susceptible
Pusa Bharka (check)	34.00 (35.66)	Moderately resistant
Pusa Uday (check)	40.96 (39.79)	Susceptible
CD @ 5%	2.325	-
CD @ 1%	1.725	-
CV (%)	2.907	-

Values in parenthesis are transformed value

(26 PDI), IIHR-76-1-S5 (36 PDI), IIHR-293-1-2-S7 (36 PDI) and IIHR-297-1-1-1(36 PDI) showed moderately resistance reaction, whereas, four lines *viz.*, IIHR-294-1-4-S6, IIHR-296-1-2-1, IIHR-101-1-4-S5 and IIHR-123-1-2-S5 recorded susceptible reaction. However, the susceptible check Swarna Ageti recorded highly susceptible reaction (68.5 PDI) (Table 2). Innark et al. (2014), Ranjan et al. (2015) and Bommesh et al. (2017) also reported the resistant sources for downy mildew in cucumber.

Resistant genotypes responses in cucumber are characterized by the ability of the host plant to recognize (compatibility/incompatibility) of the pathogen of the host plant. According to Eckardt & Taler (2004), the pathogen did not penetrate into the resistant host's mesophyll cell walls due to a massive build-up of callose on the host's cell walls and inner wall surface on the mycelium. According to Lebeda (1992), *C. metauliferus* and 9 other *Cucumis* strains showed resistance against *P. cubensis*, and Lebeda (2007) proposed the use of wild strains of *Cucumis* to broaden the genetic basis of cucumber resistance breeding against downy mildew in cucumbers.

Screening under artificial inoculation

Artificial inoculation of the pathogen on cucumber revealed that disease initiated on 5th day of inoculation. Three advance breeding lines *viz.*, IIHR-177-1-1-S7, IIHR-82-1-S6 and IIHR-81-1-S6 (resistant to downy mildew disease) were selected based on the natural open field screening, were screened artificially through seedling assay technique under controlled condition during Summer, 2016. After 10 days of inoculation, symptoms were scored weekly basis and area under disease progressive curve has been worked out. Among the lines screened, IIHR-177-1-1-S7 showed an average PDI (18.33), and AUDPC (929.95) compared to Swarna Agethi (53.13 PDI) and AUDPC (2668.7). Out of 3 lines screened against *Pseudoperonospora cubensis*, IIHR-177-1-1-S7 was found resistant and IIHR-82-1-S6 & IIHR-81-1-5-7 showed moderately resistant to the disease. However, check varieties Swarna Ageti and Pusa Uday showed >60 PDI and recorded as highly susceptible to downy mildew disease (Table 3 & 4). The lines IIHR-177-1-1-S7 did not show any infection after 8 days of inoculation but disease appeared later.

Table 3 : Reaction of *Pseudoperonospora cubensis* after challenge inoculation on Cucumber

Disease reaction	Per cent disease index (%)	Advance breeding line/check
Resistant	0-20	IIHR-177-1-1-S7
Moderately resistant	21-40	IIHR-82-1-S6 and IIHR-81-1-5-7
Susceptible	41-60	Swarna Agethi, Pusa Uday and Pusa Bharka
Highly susceptible	> 60	Nil

Table 4 : Area under diseases progressive curve of selected downy mildew resistant lines

Advanced breeding line/check	34 DAS	41 DAS	48 DAS	55 DAS	62 DAS	69 DAS	76 DAS	83 DAS	90 DAS	Average	AUD PC
IIHR-177-1-1-S7	-	7.50	10.50	15.50	17.70	20.40	22.50	25.00	27.50	18.33	929.95
IIHR-101-1-4-S5	10.00	22.50	30.00	45.00	50.00	57.50	65.00	75.00	82.50	48.61	2738.75
IIHR-81-1-5-7	11.50	17.00	21.50	24.00	32.00	36.00	38.50	41.00	42.50	29.33	1350.6
IIHR-82-1-S6	-	12.50	15.00	20.00	26.00	32.50	36.50	39.50	43.00	25.00	1156.00
Swarna Agethi (susceptible check)	-	17.50	25.00	40.00	50.00	62.50	65.00	77.50	87.50	53.13	2668.75
Pusa Uday (check)	7.50	15.00	17.50	35.00	57.50	60.00	65.00	75.00	80.00	45.83	2581.25
Pusa Bharka (check)	7.50	12.50	17.50	35.00	57.50	60.00	65.00	87.50	95.00	48.61	2703.75

DAS: days after sowing

Swarna Ageti
(68.5 PDI)

IIHR-177-1-1-S7
(9 PDI)

Fig. 1b: Natural open field screening

Swarna Ageti
(55 PDI)

IIHR-177-1-1-S7
(18.33 PDI)

Fig. 1b: Artificial screening through seedling assay technique

Fig. 1: Screening of cucumber advance breeding line with susceptible check against downy mildew under natural open field condition and artificial inoculation

Scores for severity of disease on artificially inoculated leaf were positively associated with disease severity in plants grown in polythene covers approximately 10 days after inoculation The resistant line IIHR-177-1-1-S7 recorded resistance reaction under both field and artificial condition (Fig. 1). Bommesh et al. (2017) found 2 resistant accessions (1 cultivated type & 1 wild species) out of 41 accessions screened. Lebeda & Urban (2007) also reported similar findings through artificial screening for downy mildew resistance in cucumber.

Yield and quality parameters

Twelve cucumber advance lines along with three commercial check varieties namely Swarna Ageti (susceptible check), Pusa Bharka and Pusa Uday (checks for yield) were evaluated for yield and quality traits. Among them, line IIHR-177-1-1-S7 recorded

Table 5 : Evaluation of cucumber advance breeding lines with checks for yield and quality

Advanced breeding line/check	Fruit length (cm)	Fruit diameter (cm)	Fruits plant (Nos.)	Fruit weight (g)	Yield (kg/ plant)	Remarks
IIHR-76 -1-S5	21.83	6.43	10.00	471.33	2.10	Green
IIHR-81-1-S6	20.90	4.03	13.50	237.33	3.25	Light green
IIHR-82-1-S6	20.67	3.63	14.00	361.00	3.85	Green
IIHR-101-1-4-S5	18.00	5.36	5.50	228.33	2.50	Dark green
IIHR-123 -1-2-S5	21.23	4.26	12.50	284.66	2.28	Green
IIHR 127 -1-2-S7	16.70	5.06	13.50	123.33	3.00	Dark green
IIHR-177 -1-1-S7	15.57	4.43	16.50	234.75	3.89	Green
IIHR-292 -2-1-S6	17.23	3.56	14.50	134.66	3.00	Green
IIHR-293 -1-2-S7	20.60	6.50	11.50	410.66	1.56	Dark green
IIHR-294 -1-4-S6	16.17	5.23	12.50	160.33	1.25	Green
IIHR-296 -1-2-1	18.93	5.83	8.50	375.00	1.99	Green
IIHR-297 -1-1-1	16.33	4.80	19.00	203.00	3.10	Green
Swarna Ageti (susceptible check)	15.43	4.46	11.50	188.33	2.20	Dark green
Pusa Bharka (check)	13.63	5.43	14.00	294.66	3.10	Green
Pusa Uday (check)	14.2	6.00	11.00	224.66	3.50	Light green
CD @ 5%	10.25	4.52	12.50	NS	2.56	-

highest yield (3.89 kg/plant), followed by IIHR-82-1-S6 (3.85 kg/plant) against check variety Pusa Uday (3.5 kg/plant), and IIHR-81-1-S6 (3.25 kg/plant) against check variety Pusa Bharka (3.10 kg/plant) which significantly differed from the rest of the lines tested. Fruit length ranged from 13.63 cm (Pusa Bharka) to 21.83 cm (IIHR-76-1-S5), fruit diameter 3.36 cm (IIHR-82-1-S6) to 6.50 cm (IIHR-293-1-2-S7). The lines IIHR-177-1-1-S7 recorded 16.5 fruits per plant with an average fruit weight of 234.75 g. However, the maximum fruit weight was recorded 471.33 g in IIHR-76 -1-S5 (Table 5).

CONCLUSION

Based on the screening under natural epiphytic condition, line IIHR-177-1-1-S7, IIHR-81-1-S6 and IIHR-82-1-S6 were recorded highest yield, fruit quality and resistant for downy mildew, while, on artificial screening, IIHR-177-1-1-S7 showed disease resistant, whereas, IIHR-81-1-S6 and IIHR-82-1-S6 were showed susceptible reaction to downy mildew disease. However, line IIHR-177-1-1-S7 showed resistance with less disease progression and can be utilized in developing downy mildew disease resistance varieties.

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