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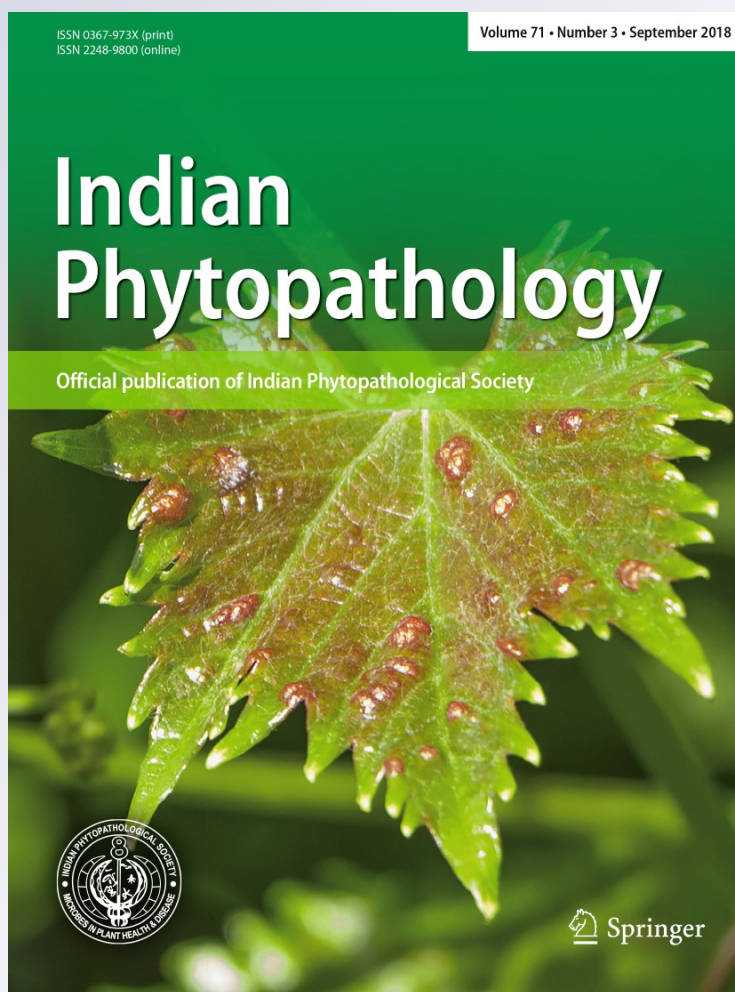
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## RESEARCH ARTICLE



# Identification and confirmation of downy mildew (*Pseudoperonospora cubensis* Berk. & Curt.) resistance sources in cucumber (*Cucumis sativus* L.)

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## Abstract

An experiment was designed to identify the resistance source for downy mildew disease in different genotypes of cucumber. Forty-one cucumber genotypes were screened under natural condition and also under artificial epiphytic condition using detached leaf assay method. Genotype IIHR-438 and *Cucumis metuliferus* L. showed field resistance with an average PDI of 17.66 and 17.46; AUDPC of 772.24 and 764.48, respectively compared to 73.12 PDI and AUDPC of 3096.64 in highly susceptible genotype of IIHR-389. The disease reaction in selected genotypes of cucumber confirmed by artificial screening was in accordance with disease reaction under natural conditions. Resistant genotype IIHR-438 (14.3 PDI) and *C. metuliferus* L. (12.8 PDI) had least average PDI as compared to susceptible check Swarna Agethi (58.00 PDI) under artificial condition. Statistical analysis of disease severity data over a period for all the forty-one genotypes using non-linear growth model revealed that 99% variability in disease progression. Screening of genotypes under field conditions, sporulation of pathogen, progress of disease, detached leaf assay and non-linear statistical analysis implied that none of genotypes were found to be immune to downy mildew. Wherein the genotype, IIHR-438 and wild cucumber (*C. metuliferus* L.) were found resistant to downy mildew disease. Hence, it can be utilized in breeding program to develop resistant cultivar in cucumber against *Pseudoperonospora cubensis* under tropical conditions of India.

**Keywords** AUDPC · Cucumber · Detached leaf assay · Downy mildew · Haemocytometer · Sporulation

## Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important cucurbitaceous vegetable crops grown extensively in tropical and sub-tropical parts of the world. The global cucumber production amounts to 62 million tonnes in 1.97 million hectares (FAO 2016). It has been an important food source for more than 5000 years, used as both culinary and

non-culinary purposes. Fruits are commonly eaten fresh as salads, pickled, cooked and used in cosmetic products, including lotions, perfumes and soaps (Seshadri 1986).

Downy mildew is one of the most devastating and widespread diseases of cultivated cucurbits worldwide (Call 2012; Lebeda and Cohen 2011). The disease is caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov, which has a host range of more than 60 species belonging to 20 genera in the Cucurbitaceae family (Lebeda and Cohen 2011; Lebeda 1992). Six pathotypes of *P. cubensis* have been reported based on their compatibility with specific host genera (Cohen et al. 2003; Thomas et al. 1987). Rainfall, dew formation or irrigation provides congenial atmosphere for disease appearance (Thomas 1977). Temperature range between 5 and 30 °C is favourable with sufficient moisture for disease progress.

Many cucumber cultivars resistant to downy mildew have been developed (Wehner and Shetty 1997) over the past 50 years. The most resistant cultigens were from US origin and were primarily elite cultivars and breeding lines with

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resistance derived from an Indian genotype (PI 197087). Dhillon et al. (1999) tested 217 cultigens in northern India for downy mildew resistance and found nine resistant cultigens of Asian and European origin. Recently, Call (2012) screened 1300 cultigens of cucumber for downy mildew resistance for 4 years in Poland that led to the identification of six cultigens (PI 330628, PI 197088, PI 197086, PI 197085, Ames 2353 and Ames 2354) showing high levels of downy mildew resistance.

The resistance sources were identified, but they become susceptible to downy mildew pathogen over a period of time. Though, the disease can be controlled with fungicides (Urban and Lebeda 2006), genetic resistance provides more economically sound and environmentally safe approach.

Identification of source of resistance is the pre-requisite for any disease resistance breeding program. Hence, the objective for this study was to evaluate the germplasm collected from National Bureau of Plant Genetic Resources (NBPGR) New Delhi, India to identify new resistant genotypes for downy mildew pathogen of cucumber.

## Materials and methods

### Screening under natural epiphytotic conditions

The experiments were conducted at the experimental farm of Division of Vegetable Crops, ICAR-IIHR, Hessarghatta, Bengaluru, Karnataka, India, during *rabi* season of 2016. The experimental materials consisted of 41 genotypes obtained from the National Bureau of Plant Genetic Resources, New Delhi, which includes indigenous collections (36), exotic collections (3) and one wild *Cucumis* species. They were evaluated for resistance to downy mildew and Swarna Agethi was maintained as susceptible check.

The disease scoring was started a month after planting at weekly interval for 7 weeks until the end of the crop growth. Variance analysis was performed based on a randomized block design with one factor (the genotype) and three replicates for 41 genotypes. PDI (per cent disease index) of screening data were arc sine transformed for statistical analysis in Statistical Analysis System by developing a programming code (SAS, package available at ICAR-IIHR, Bengaluru).

### Disease assessment

Disease incidence in field was recorded based on evaluation of the intensity of the disease symptoms on five leaves each at the top, the median and at the base of the plant using the 0–9 scale. The scale was based on percentage of symptomatic leaf area (0: 0%, 1: 1–3%, 2: 3–6%, 3: 6–12%, 4: 12–25%, 5: 25–50%, 6: 50–75%, 7: 75–87%, 8: 87–99% and

9: 100%) as described by Jenkins and Wehner (1983). Using symptomatic leaf area data, the PDI was calculated using the given formula and the genotypes were categorized into four groups namely resistant (0–20%), moderately resistant (21–40%), susceptible (41–60%) and highly susceptible (> 60%) (Reddy 2002).

$$\text{PDI} = \frac{\text{Sum of numerical values}}{\text{Number of leaves graded} \times \text{Maximum ratings}} \times 100.$$

### Artificial screening using detached leaf assay

Two resistant genotypes and eight highly susceptible genotypes were selected based on natural screening to confirm the resistance reaction using detached leaf method assay by the method of artificial inoculation. Leaves were collected from plants maintained under greenhouse conditions at the flowering stage, before the incitation of natural infection. Heavily infected leaves were soaked in distilled water and rubbed gently with a glass rod to dislodge the sporangia. The spore suspension was filtered through four layers of cheese-cloth to remove dirt and debris and the final concentration was made to 1000 sporangia/ml by using haemocytometer. Tween 20 (0.06 g/l) was added to the inoculum suspension to keep the sporangia well dispersed in the solution. The abaxial surface of leaf was kept up and each leaf inoculated with 8–10 droplets of the solution (6 ml each). The Petri dishes with the inoculated leaves were immediately placed in a growth chamber at dark at 20 °C for 12 h and then kept in light for 10 days. Disease progress was assessed after the inoculation by scoring the severity of the symptoms on 8th day onwards using the scale as described by Cohen et al. (2000).

- 0: No lesions;
- 0.1: Circular minute lesions, 1–2 mm in diameter, usually chlorotic with a water-soaked appearance, occupying 1–5% of the inoculated area, no visible sporulation;
- 0.5: As above, but lesions 2–3 mm in diameter occupying 5–10% of the inoculated area, negligible sporulation;
- 1: Chlorotic, 3–5 mm water-soaked lesions occupying 10–20% of the inoculated area, weak sporulation;
- 2: Chlorotic, 6–10 mm lesions of circular or irregular shape, occupying 50% of the inoculated area, moderate sporulation;
- 3: As above, lesions chlorotic turning partially necrotic, occupying 50–75% of the inoculated area, heavy sporulation on the chlorotic parts of the lesions;
- 4: Lesions coalesced, mostly necrotic, occupying > 75% of the inoculated area, sporulation moderate due to necrosis;



### Apparent infection rate (r)

The apparent rate of disease development ( $r$ ) is a measure of the speed at which an epidemic develops. Disease incidence data recorded at weekly interval for 7 weeks was used to calculate the apparent infection rate by using the formula suggested by Van der Plank (1968)

$$r = 2.3/t_2 - t_1 \{ \log(X_2(1 - X_1)/X_1(1 - X_2)) \}$$

where  $r$  is the apparent infection rate in non-logarithmic phase,  $X_1$  is the disease index at time  $t_1$ ,  $X_2$  is the disease index at subsequent week time  $t_2$ .

### Enumeration of sporulation

The freshly infected leaves were collected from field and the sporangia were dislodged from infected portions. The sporangia were enumerated using haemocytometer for 41 genotypes with 3 replications in a completely randomized desi. One cm<sup>2</sup> of infected leaf section was taken and made into a volume to 1 ml with distilled water. Tween 20 was added to the suspension to keep the sporangia well dispersed. The sporulation count was taken thrice for all genotypes at 15-day interval and the average was calculated (sporangia/cm<sup>2</sup>/ml) for each genotype (Criswell et al. 2008).

### Non-linear regression analysis by logistic model

Non-linear growth models which describe the growth behaviour over time are used in many biological fields (Venugopalan and Vijay 2015). The utility of model is that, it helps us to gain insight into the underlying mechanism of the system and also in identification of efficient resistant source. The formula for logistic model is given below:

$$Y_t = \frac{C}{(1 + b\bar{e}^{at})} + e, b = \frac{C}{Y_0} - 1$$

where  $Y_t$  the percentage of disease incidence during the time  $t$ ;  $a$ ,  $b$ ,  $C$  are the parameters,  $e$  the error term.  $a$  Intrinsic growth rate.  $b$  Incremental relative rate of relative growth rate of the disease.  $Y_{(0)}$  corresponds to age of theoretical zero size, which also represents time when the growth curve crosses the  $t$ -axis;  $C$  Carrying capacity for each model.

In order to fit these non-linear growth model for the disease incidence data, Levenberg–Marquardt technique (Ratkowsky 1990) was utilized and programming codes were developed using statistical analysis system (SAS) package available at IIHR, Bengaluru. PROC NLIN subroutine was utilized to construct SAS codes (SAS-Cucumber-DM). Global convergence of the parameter estimates was ensured by trying different sets of initial values.

### Measures of model adequacy

As a measure of goodness of fit, the value of coefficient of determination ( $R^2$ ) (Kvalzeth 1985) was calculated as

$$R^2 = 1 - \left[ \frac{(\sum(Y_t - \hat{Y}_t)^2)}{(\sum Y_t - \bar{Y})^2} \right]$$

where  $Y_t$  represents the percent disease incidence during the period  $t$ .  $\hat{Y}_t$  predicted percent disease incidence during the period  $t$ .  $\bar{Y}$  mean observed percent disease incidence during the period  $t$ .

### Residual analysis

Before concluding on the statistical adequacy of the selected model, residual analysis was also carried out using the one sample run-test, for testing the randomness assumption and the normality assumption of residuals were tested using Shapiro–Wilk test (Siegel and Castellan 1988).

### Area under disease progress curve (AUDPC)

AUDPC is another criteria that represents the speed of progression of pathogen in plant tissue and used to differentiate between resistant and susceptible genotypes. Disease incidence data recorded at a weekly interval was used to calculate the area under disease curve progression (AUDPC) as a measure of quantitative disease resistance involving repeated disease assessments. The AUDPC was computed based on the disease scores using the following formula (Jeger and Rollinson 2001):

$$Ak = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

where  $y_i$  is the proportion of disease on the  $i$ th observation,  $t_i$  is the time (days) of observation expressed as days after sowing (DAS) and  $N$  is the total number of disease severity readings (PDI) taken throughout the experimental period.

For data sets, weekly growth of downy mildew was evaluated by computing the values of the derivative for different values of  $t$ , in Logistic model. Furthermore, it can be seen that time ( $t$ ) for which the downy mildew severity rate of growth was maximum, is given by

$$t = \text{Ln} \frac{b}{a}$$

$a$  Intrinsic growth rate.  $b$  Incremental relative rate of relative growth rate of the disease.

## Results and discussion

### Screening under natural epiphytotic conditions

Natural epiphytic screening of 41 cucumber genotypes along with susceptible check Swarna Agethi was carried out during the *Rabi* season 2016 at the experimental fields of ICAR-IIHR, Bengaluru. Among them none of the genotype was observed to be immune to downy mildew pathogen. Genotype IIHR-438 and wild cucumber (*C. metuliferus* L.) showed resistance with average PDI of 17.66 and 17.46, respectively. Ten genotypes recorded susceptibility with PDI ranged from 41 to 60 and rest of the genotypes with > 60 PDI were highly susceptible to downy mildew disease (Table 1) wherein IIHR-433 was found to be moderately resistant (35.12 PDI). Highest average PDI was observed in genotype IIHR-389 (73.12), which was higher than that of the susceptible check Swarna Agethi (67.06 PDI).

The response of resistant genotypes in cucumber is characterized by the recognition (compatibility/incompatibility) of a host plant by its oomycetes pathogen (Fig. 1). Petrov et al. (2000) described resistant plants as having only small, chlorotic, water-soaked lesions and most susceptible cultigens showed yellowing, chlorosis and high sporulation ratings. Our findings were similar to those reported from Poland and North Carolina from 2005 to 2009 found that only twenty of 1300 tested cucumber cultigens were highly resistant to downy mildew and none was immune (Call 2012). Bhutia et al. (2005) screened 114 cucumber genotypes and result revealed that ten were resistant, 18 were moderately resistant, 37 moderately susceptible and 49 genotypes with susceptible reaction. Criswell et al. (2008), Reshmi (2006) and Wan et al. (2010) also reported the resistant sources for downy mildew pathogen in cucumber.

### Enumeration of sporulation

The diseased leaves from cucumber genotypes were collected and spores were counted using haemocytometer. The resistant genotype IIHR-438 and wild cucumber (*C. metuliferus* L.) had 3.1 and 3.3 average number of spores per cm<sup>2</sup>, respectively whereas Swarna Agethi (Susceptible check) had 3.80 spores per (Table 1).

The disease intensity was correlated with sporulation, resistant genotype recorded less sporulation due to lower rate of disease intensity and Swarna Agethi recorded more disease intensity with high sporulation. There was decrease in spore number with disease progressions in all the genotypes. As the downy mildew disease progresses, entire leaves may die within few weeks following the initial infection, as

lesions expand and coalesce (Thomas 1996), and sporulation is much more intense on chlorotic lesions than in necrotic lesions. Thus the advances in disease severity results in the decrease of sporulation. Some physical and biotic factors like temperature, humidity and pathogenicity limits sporulation level in plants. At lower temperatures, sporulation occurs later but lasts longer in cucumber (Cohen 1977; Cohen and Eyal 1977; Neufeld and Ojiambo 2012). Lebeda and Prasil (1994) screened 155 cucumber cultivars based on intensity of sporulation for determining resistance or susceptibility to downy mildew.

### Apparent infection rate (r)

Apparent infection rate (r) of genotypes at weekly intervals showed a wide variation. There was increase in the infection rate of downy mildew growth observed per day in all the genotypes and thus the chance of causing epidemics was more in almost all the genotypes of cucumber. The highest average 'r' value was observed in the susceptible genotypes of IIHR-389 and IIHR-393 is 1.23. *C. metuliferus* (0.74) and IIHR-438 (0.79) showed the least 'r' value as compared to susceptible check Swarna Agethi (1.20). The maximum rate of speed was observed in the sixth and seventh week after inoculation of pathogen in many genotypes (Table 2).

In the result the apparent infection rate value varied and at times they did not remain consistent for given genotype. The apparent infection rate was maximum in most of susceptible genotype and it increases with growth of the plant (Fig. 2). The 'r' value of IIHR-389 and IIHR-393 was higher than Swarna Agethi, due to high incidence of average PDI of these two genotypes than Swarna Agethi. This information will be useful to decide the resistance level in genotypes with age of plant. The severity of disease may be attributed to susceptibility of the genotypes and also the congenial environmental factors which might contribute for the favour of disease development. A similar observation was made by Wilcoxson et al. (1975) and Patil (1997).

### Confirmation by artificial screening

The cucumber genotypes were categorized based on the resistance reaction screened under field condition. Ten genotypes showing resistance and high susceptibility were selected for the detached leaf assay by artificial inoculation method to confirm the resistance. Average PDI of *C. metuliferus* and IIHR-438 was 12.8 and 14.3, respectively which was much lesser than that of other genotypes and susceptible check (58 PDI) (Table 3).

Scores for severity of disease on artificially inoculated leaf were positively associated with disease severity in plants

**Table 1** Screening of cucumber germplasm for downy mildew resistance under field condition

Sl. no.	Accessions	(IHR number) <sup>a</sup>	PDI at weekly interval							PDI (%)	Number of spores/cm <sup>2</sup>				Disease reaction
			I	II	III	VI	V	VI	VII		I	II	III	Average spores/cm <sup>2</sup>	
1	Faridabad Local	82	38.19 (38.2)	42.92 (40.9)	63.19 (52.6)	71.53 (57.7)	90.28 (71.8)	93.75 (75.5)	100.00 (89.4)	68.55 (57.6)	5.6	4	3.6	4.4	HS
2	Orissa local	177	36.81 (37.3)	44.31 (41.7)	57.64 (49.4)	61.81 (51.8)	68.06 (55.6)	90.28 (71.8)	100.00 (89.4)	62.70 (54.04)	4.2	2.6	4.8	3.9	HS
3	EC-581059	385	27.08 (31.3)	39.58 (39.0)	55.56 (48.2)	63.19 (52.6)	77.78 (61.9)	86.11 (68.1)	84.72 (67.0)	62.00 (51.9)	6.6	3.8	2.6	4.3	HS
4	EC-587057	387	38.19 (38.2)	46.33 (42.9)	52.78 (46.6)	57.64 (49.4)	72.92 (58.6)	86.81 (68.7)	94.44 (76.3)	62.30 (53.2)	4.4	4.4	5.2	4.7	HS
5	EC-587056	388	9.03 (17.5)	18.75 (25.6)	36.11 (36.9)	62.50 (52.2)	59.72 (50.6)	78.47 (62.3)	88.19 (69.9)	50.40 (45.2)	5.4	3.2	4.2	4.3	S
6	IC-753493	389	38.19 (38.2)	45.14 (42.2)	61.11 (51.4)	72.92 (58.6)	95.14 (77.2)	99.31 (85.2)	100.00 (89.4)	73.12 (58.7)	5.4	1.8	1.6	2.9	HS
7	IC-613458	392	23.61 (29.1)	43.75 (41.4)	59.03 (50.2)	68.06 (55.6)	95.83 (78.2)	97.22 (80.4)	97.22 (80.4)	69.25 (56.30)	7.4	5.2	5.4	6.0	HS
8	IC-613475	393	33.33 (35.2)	39.58 (39.0)	62.50 (52.2)	75.00 (60.0)	99.31 (85.2)	100.00 (89.4)	100.00 (89.4)	72.82 (58.55)	4.8	5	3.8	4.5	HS
9	IC-613583	394	30.56 (33.5)	34.72 (36.1)	55.56 (48.2)	58.33 (49.8)	81.94 (64.8)	87.50 (69.3)	98.61 (83.2)	63.89 (53.04)	5.8	3.2	3.8	4.3	HS
10	IC-613447	395	24.31 (29.5)	34.72 (36.1)	50.00 (45.0)	68.75 (56.0)	86.81 (68.7)	92.36 (73.9)	93.75 (75.5)	64.38 (53.34)	3.8	4.8	2.8	3.8	HS
11	IC-613460	396	32.64 (34.8)	40.28 (39.4)	56.25 (48.6)	69.44 (56.4)	82.64 (65.4)	91.67 (73.2)	96.53 (79.2)	67.06 (54.96)	3.8	1.8	2	2.5	HS
12	IC-613484	397	25.69 (30.4)	34.72 (36.1)	47.22 (43.4)	63.19 (52.6)	83.33 (65.9)	93.06 (74.7)	95.83 (78.2)	63.29 (52.69)	6.6	3.4	1.2	3.7	HS
13	IC-613472	398	32.64 (34.8)	45.83 (42.6)	65.28 (53.9)	65.28 (53.9)	84.03 (66.4)	85.42 (67.5)	95.83 (78.2)	67.76 (55.38)	5.2	2.4	3.4	3.7	HS
14	IC-613463	399	12.50 (20.7)	13.89 (21.9)	36.81 (37.3)	36.81 (37.3)	47.22 (43.4)	71.53 (57.7)	75.69 (60.4)	42.06 (40.42)	5.6	6.6	3.8	5.3	S
15	IC-613481	400	29.17 (32.7)	38.89 (38.6)	53.47 (47.0)	67.36 (55.1)	84.72 (67.0)	93.06 (74.7)	96.53 (79.2)	66.17 (54.41)	3.8	5.4	4.2	4.5	HS
16	IC-613473	401	17.36 (24.6)	23.61 (29.1)	40.97 (39.8)	56.25 (48.6)	79.86 (63.3)	85.42 (67.5)	96.53 (79.2)	57.14 (49.09)	4.4	4.6	3.2	4.1	S
17	IC-613462	403	36.81 (37.3)	39.58 (39.0)	51.39 (45.8)	59.03 (50.2)	70.83 (57.3)	80.56 (63.8)	93.75 (75.5)	61.71 (51.75)	7.6	11	4.6	7.7	HS
18	IC-613466	404	27.08 (31.3)	35.42 (36.5)	50.00 (45.0)	51.39 (45.8)	61.11 (51.4)	81.94 (64.8)	88.89 (70.5)	56.55 (48.74)	4.8	3.4	3.2	3.8	S
19	IC-613479	405	24.31 (29.5)	34.72 (36.1)	50.00 (45.0)	63.19 (52.6)	84.72 (67.0)	88.89 (70.5)	91.67 (73.2)	62.50 (52.22)	6.8	5.8	2.2	4.9	HS
20	IC-613482	406	27.08 (31.3)	30.56 (33.5)	46.53 (43.0)	56.25 (48.6)	59.72 (50.6)	77.08 (61.4)	93.06 (74.7)	55.75 (48.28)	6.8	4.2	3.4	4.8	S
21	IC-613457	407	23.61 (29.1)	40.97 (39.8)	52.08 (46.2)	67.36 (55.1)	83.33 (65.9)	88.19 (69.9)	100.00 (89.4)	65.08 (53.75)	6.4	3.6	2.6	4.2	HS
22	IC-613476	408	21.53 (27.6)	29.86 (33.1)	50.69 (45.4)	68.06 (55.6)	79.86 (63.3)	91.67 (73.2)	97.22 (80.4)	62.70 (52.34)	7	5.6	1.8	4.8	HS
23	IC-613461	410	28.47 (32.2)	38.19 (38.2)	52.78 (46.6)	67.36 (55.1)	83.33 (65.9)	86.11 (68.1)	92.36 (73.9)	64.09 (53.16)	9.2	5.4	1.6	5.4	HS
24	IC-613485	413	26.39 (30.9)	29.17 (32.7)	44.44 (41.8)	59.03 (50.2)	69.44 (56.4)	86.11 (68.1)	93.75 (75.5)	58.33 (49.78)	6.2	8.4	2.6	5.7	S
25	IC-613469	414	29.86 (33.1)	36.81 (37.3)	57.64 (49.4)	61.11 (51.4)	69.44 (56.4)	86.81 (68.7)	98.61 (83.2)	62.90 (52.45)	9.8	13.8	1.2	8.3	HS
26	IC-612082	415	15.28 (23.0)	32.64 (34.8)	46.53 (43.0)	58.33 (49.8)	63.19 (52.6)	75.00 (60.0)	81.25 (64.3)	53.17 (46.80)	5	3.8	1.6	3.5	S
27	IC-613471	417	35.42 (36.5)	43.75 (41.4)	54.86 (47.8)	65.28 (53.9)	77.08 (61.4)	88.19 (69.9)	94.44 (76.3)	65.58 (54.05)	7.2	5.2	3.6	5.3	HS
28	IC-613468	418	26.39 (30.9)	44.44 (41.8)	60.42 (51.0)	67.36 (55.1)	73.61 (59.1)	81.25 (64.3)	84.03 (66.4)	62.50 (52.22)	7.8	6.6	2.6	5.7	HS
29	IC-613474	419	25.69 (30.4)	38.89 (38.6)	50.69 (45.4)	69.44 (56.4)	87.50 (69.3)	97.22 (80.4)	98.61 (83.2)	66.87 (54.83)	3.6	2.6	1.4	2.5	HS

**Table 1** (continued)

Sl. no.	Accessions	(IIHR number) <sup>a</sup>	PDI at weekly interval							PDI (%)			Number of spores/cm <sup>2</sup>			Disease reaction
			I	II	III	VI	V	VI	VII	I	II	III	Average spores/cm <sup>2</sup>			
30	IC-613478	420	33.33 (35.2)	38.89 (38.6)	60.42 (51.0)	65.28 (53.9)	81.25 (64.3)	86.81 (68.7)	93.75 (75.5)	65.67 (54.11)	5.2	5.8	3.6	4.9	HS	
31	IC-420510	422	15.28 (23.0)	25.69 (30.4)	45.14 (42.2)	50.00 (45.0)	69.44 (56.4)	79.86 (63.3)	81.94 (64.8)	52.48 (46.40)	6.6	4.2	3.4	4.7	S	
32	IC-420636	424	27.08 (31.3)	43.06 (41.0)	44.44 (41.8)	54.86 (47.8)	50.69 (45.4)	81.25 (64.3)	97.22 (80.4)	56.94 (48.97)	6.4	3.8	3.6	4.6	S	
33	IC-416835	425	33.33 (35.2)	37.50 (37.7)	52.78 (46.6)	59.72 (50.6)	72.92 (58.6)	84.03 (66.4)	93.06 (74.7)	61.90 (51.87)	5.8	8.6	3.8	6.1	HS	
34	IC-412889	426	31.94 (34.4)	38.19 (38.2)	52.08 (46.2)	61.81 (51.8)	75.69 (60.4)	92.36 (73.9)	98.61 (83.2)	64.38 (53.34)	4.6	2.8	1.2	2.9	HS	
35	IC-416870	427	33.33 (35.2)	31.94 (34.4)	59.03 (50.2)	63.19 (52.6)	82.64 (65.4)	97.22 (80.4)	98.61 (83.2)	66.57 (54.65)	5.4	6.4	3.6	5.1	HS	
36	IC-447284	430	21.53 (27.6)	28.47 (32.2)	36.81 (37.3)	43.75 (41.4)	51.39 (45.8)	77.08 (61.4)	88.89 (70.5)	49.70 (44.81)	6.6	7.2	1.8	5.2	S	
37	IC-435783	431	33.33 (35.2)	37.50 (37.7)	52.08 (46.2)	69.44 (56.4)	91.67 (73.2)	93.06 (74.7)	96.53 (79.2)	67.66 (55.32)	7.4	4.4	4.2	5.3	HS	
38	IC-447388	433	4.17 (11.8)	7.64 (16.0)	22.92 (28.6)	29.86 (33.1)	34.72 (36.1)	68.75 (56.0)	77.78 (61.9)	35.12 (36.33)	3.8	2.4	3.8	3.3	MR	
39	IC-613488	438	2.08 (8.3)	5.56 (13.6)	19.44 (26.2)	19.44 (26.2)	22.92 (28.6)	26.39 (30.9)	27.78 (31.8)	17.66 (24.84)	2.8	4.2	2.4	3.1	R	
40	<i>C. metuliferus</i>	–	0.69 (4.8)	2.08 (8.3)	15.28 (23.0)	15.97 (23.5)	17.36 (24.6)	36.11 (36.9)	34.72 (36.1)	17.46 (24.69)	3.8	2.4	3.8	3.3	R	
41	Swarna Agethi	–	38.19 (38.1)	41.67 (40.1)	57.64 (49.3)	59.72 (50.5)	77.78 (61.8)	94.44 (76.3)	100.00 (89.3)	67.06 (54.95)	6.2	3.4	1.8	3.8	HS	
	SE±m		0.276	0.276	0.436	0.547	1.208	1.442	1.516	0.524	0.093	0.090	0.045			
	C.D.		0.777	0.778	1.231	1.541	3.405	4.067	4.275	1.476	0.261	0.180	0.126			

S susceptible, HS highly susceptible, R resistant, MR moderately resistant, EC exotic collection, IC indigenous collection

<sup>a</sup>IIHR number-accessions number given by ICAR-Indian Institute of Horticultural Research, Bengaluru. Transformed value in parenthesis



**Fig. 1** Downy mildew infected plants in susceptible checks and resistant genotype screened under natural conditions



grown under field conditions approximately 1 month after transplanting (Fig. 3). Resistant genotypes found resistance reaction under both field and artificial condition. These findings are in line with Cohen et al. (2000) and Lebeda and Urban (2007).

### Disease progression and determination of AUDPC

The non-linear logistic model showed significant variation among the genotypes screened. Figure 4 shows the difference among genotypes for the disease intensity and disease progression. Genotype IIHR-438 had least disease progression until the end of crop growth (17.66 PDI) compared to susceptible check which reached to 100% at seventh week. The AUDPC indicates the progress of the disease in a given crop growth period (Table 4). Both the resistant genotypes IIHR-438 and *C. metuliferus* L. showed less AUDPC value of 772.24 and 764.49 respectively, compared with other genotypes under screened for resistance. The highest AUDPC value was recorded in genotype IIHR-393 (3098.85) with average PDI of 72.82 and susceptible check value was 2797 with average PDI of 67.06.

The disease progression curve shows the wide variation in the average per cent disease index in different genotypes screened under field conditions. Differences in field resistance were characterized by a delay in the onset of infection and a slower rate of disease progression under strong infection pressure (Lebeda 1999). AUDPC indicates the resistance reaction in genotype over the time period (Fig. 5). AUDPC value was almost same in all the genotypes except in four genotypes where it was less. Thus the resistance source for downy mildew disease was limited in the cucumber genotypes. As the pathogen was obligate and the congenial environmental factor contributes for the development of disease. Pathogen progression is generally slower in the resistant genotype which usually delays and

limits the pathogen colonization and the expansion of disease symptoms (Mhada et al. 2015). Low temperatures can delay symptom development and colonization in the leaf tissues, whereas in higher temperatures lesion formation and chlorosis will be faster which may inhibit growth of pathogen (Cohen 1977). Neykov and Dobrev (1987) described that if the leaf area is covered with small, necrotic lesions on less than 25% of leaves, it is categorized as resistant. The findings of the present study are supported by Bjoern and Kampmann (2000) and Cohen et al. (2000).

### Non-linear statistical model (logistic model)

The results of logistic model for downy mildew resistance are presented in Table 4. Parameter estimate of fitted models, measures of goodness of fit of logistic model ( $R^2$  and MSE) along with tested measures of model adequacy and AUDPC are also represented (Nagarajan and Muralidharan 1995). Result indicates that the severity of downy mildew infection during plant growth can be explained with a logistic model. The accuracy of 99.9% and 94.60% in cucumber genotype IIHR-417 and IIHR-438 with error mean square of 1.08 and 8.19 respectively were recorded. In run test stat value, assumptions about residuals showed that error is distributed well within the critical region for all 41 genotypes.

In the non-linear model the intrinsic growth rate 'a' was in the range of 0.17 (IIHR-177) to 1.40 (IIHR-438) and carrying capacity 'C' (Maximum disease growth rate) was minimum in resistant genotype IIHR-438 whereas susceptible check had comparatively high C value of 188.76. The 't' value in resistant genotype was 19.44% in fourth week, whereas in susceptible variety it was 57.64% in the third week itself.

A non-parametric logistic growth statistic method requires few assumptions about the data to draw valid conclusion with considerable better chance of detecting

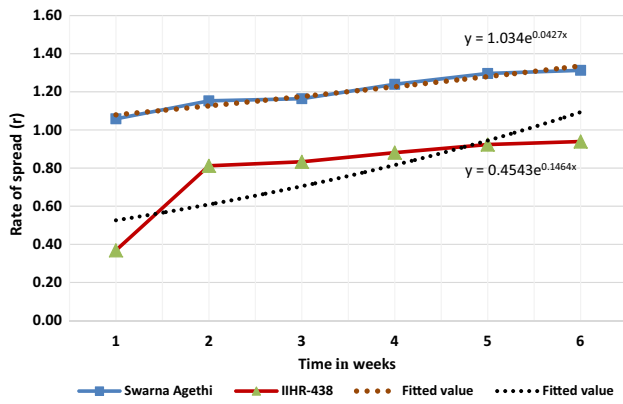
**Table 2** Apparent infection rate (r) per unit per day of with progression of downy mildew disease in cucumber

Sl. no.	Genotypes (IIHR)	1–2 weeks	2–3 weeks	3–4 weeks	4–5 weeks	5–6 weeks	6–7 weeks	Average 'r'
1	82	1.07	1.18	1.21	1.28	1.29	1.31	1.22
2	177	1.07	1.15	1.17	1.20	1.28	1.31	1.20
3	385	1.04	1.14	1.18	1.24	1.27	1.26	1.19
4	387	1.09	1.13	1.15	1.22	1.27	1.29	1.19
5	388	0.81	1.01	1.17	1.16	1.24	1.27	1.11
6	389	1.08	1.17	1.22	1.30	1.31	1.31	1.23
7	392	1.07	1.16	1.20	1.30	1.30	1.30	1.22
8	393	1.04	1.17	1.23	1.31	1.31	1.31	1.23
9	394	1.00	1.14	1.16	1.25	1.27	1.31	1.19
10	395	1.00	1.11	1.20	1.27	1.29	1.29	1.19
11	396	1.05	1.14	1.21	1.26	1.29	1.30	1.21
12	397	1.00	1.09	1.18	1.26	1.29	1.30	1.19
13	398	1.08	1.19	1.19	1.26	1.27	1.30	1.21
14	399	0.73	1.01	1.02	1.09	1.21	1.23	1.05
15	400	1.04	1.13	1.20	1.26	1.29	1.30	1.20
16	401	0.89	1.05	1.14	1.25	1.27	1.30	1.15
17	403	1.04	1.12	1.16	1.21	1.25	1.29	1.18
18	404	1.01	1.11	1.12	1.17	1.25	1.28	1.16
19	405	1.00	1.11	1.18	1.26	1.28	1.29	1.19
20	406	0.97	1.09	1.14	1.16	1.24	1.29	1.15
21	407	1.05	1.12	1.20	1.26	1.28	1.31	1.20
22	408	0.96	1.11	1.20	1.25	1.29	1.30	1.18
23	410	1.03	1.13	1.20	1.26	1.27	1.29	1.19
24	413	0.95	1.07	1.16	1.21	1.27	1.29	1.16
25	414	1.02	1.15	1.17	1.21	1.27	1.31	1.19
26	415	0.98	1.09	1.15	1.18	1.23	1.25	1.15
27	417	1.07	1.14	1.19	1.24	1.27	1.29	1.20
28	418	1.07	1.16	1.20	1.22	1.25	1.26	1.20
29	419	1.04	1.11	1.21	1.27	1.30	1.31	1.21
30	420	1.04	1.16	1.19	1.25	1.27	1.29	1.20
31	422	0.91	1.08	1.11	1.21	1.25	1.25	1.13
32	424	1.07	1.08	1.14	1.11	1.25	1.30	1.16
33	425	1.03	1.13	1.16	1.22	1.26	1.29	1.18
34	426	1.03	1.12	1.17	1.23	1.29	1.31	1.19
35	427	0.98	1.16	1.18	1.26	1.30	1.31	1.20
36	430	0.94	1.02	1.07	1.12	1.24	1.28	1.11
37	431	1.03	1.12	1.21	1.29	1.29	1.30	1.21
38	433	0.52	0.87	0.96	1.00	1.20	1.24	0.96
39	438	0.37	0.81	0.83	0.88	0.92	0.94	0.79
40	<i>C. metuliferus</i>	0.21	0.67	0.77	0.80	1.01	1.00	0.74
41	Swarna Agethi (susceptible check)	1.06	1.15	1.16	1.24	1.29	1.31	1.20

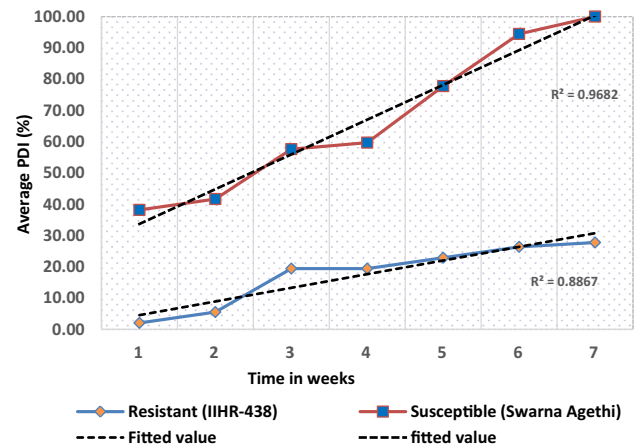
difference between genotypes to identify source of resistance (Nayak et al. 2018). The results of non-parametric model were in accordance with the PDI of genotypes in linear method.

IIHR-438 recorded high 'a' value which leads to least disease growth rate (C) and mean PDI was also minimum

for this genotype. Results can be correlated with disease progression data (shown in graph). Maximum downy mildew severity rate (t) (indicates the pathogen reaction with genotype in weeks), it will identify the week at which disease severity was high for a genotype with high accuracy. This information will give a way to select the stable resistant



**Fig. 2** Apparent infection rate (r) for downy mildew disease in susceptible and resistant cucumber genotypes. (Equations given for fitted value)



**Fig. 4** Disease progression of downy mildew in resistant and susceptible check under field condition

**Table 3** Screening of selected cucumber genotypes by artificial inoculation for downy mildew resistance using detached leaf assay

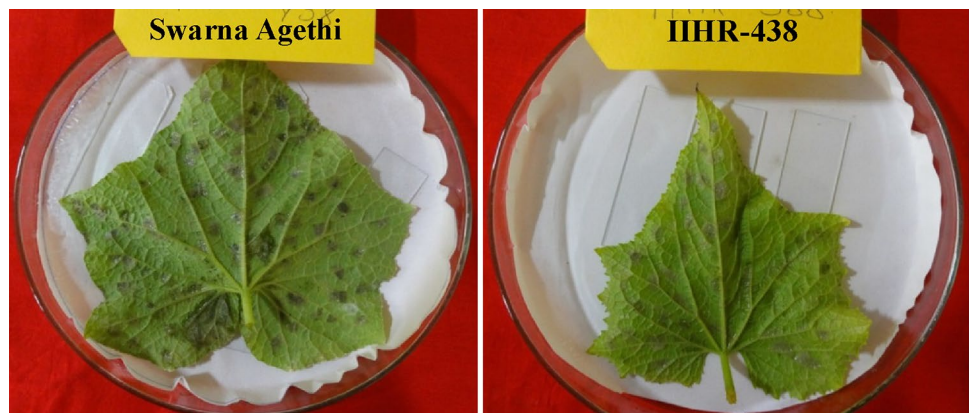
Sl. no.	Accessions number	Average PDI (%)
1	IIHR-82	38 (38.04)
2	IIHR-177	44 (41.54)
3	IIHR-388	28 (31.94)
4	IIHR-389	29 (31.94)
5	IIHR-399	46 (42.69)
6	IIHR-413	52 (46.13)
7	IIHR-433	31 (33.82)
8	IIHR-438	14.3 (22.21)
9	<i>C. metuliferus</i>	12.8 (20.95)
10	Swarna Agethi	58 (49.58)
C.D.		6.602
SE ± M		2.205

Transformed value in parenthesis

genotypes for resistance breeding programme. Similar growth models using non-linear statistic have been established development of powdery mildew in mango (Sinha and Prajneshu 2002) and in downy mildew of grape (Venugopalan and Vijay 2015).

The information gained out of this investigation based on the screening under natural epiphytic condition, artificial condition and non-linear statistical model confirmed that the genotypes IIHR-438 and wild cucumber (*C. metuliferus* L.) showed resistance with less disease progression and can be utilized in breeding programs for disease resistance. The high yielding genotype with downy mildew resistance and desired agronomic traits can be exploited to develop varieties suitable under conditions of disease epidemics.

**Fig. 3** Infection of downy mildew in susceptible checks and resistant genotype by detached leaf assay



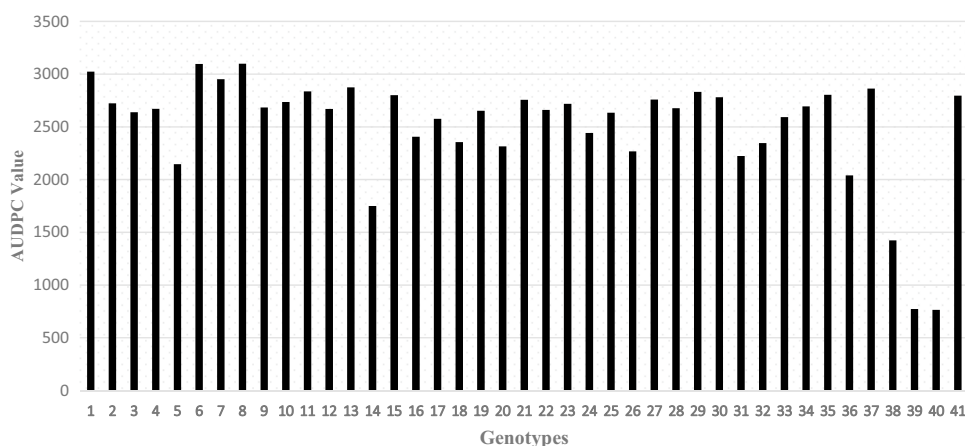
**Table 4** Nonlinear statistical analysis (logistic model) for downy mildew resistance in cucumber genotypes

Sl. no.	Genotypes	a	b	C	R <sup>2</sup> (%)	MSE	Run test (Z)	SW stat	AUDPC	t/time (weeks)
1	82	0.49	3.65	113.14	98.20	16.72	0.0432	0.960	3023.09	42.92 (2.0)
2	177	0.17	29.00	964.67	97.80	17.55	0.0049	0.884	2723.55	68.06 (5.0)
3	385	0.62	4.50	92.37	99.00	7.94	0.0107	0.963	2639.12	39.58 (2.0)
4	387	0.19	10.34	364.07	98.70	8.58	0.0086	0.966	2671.31	57.64 (4.0)
5	388	0.80	16.48	89.83	96.60	45.64	0.2286	0.950	2146.38	36.11 (3.0)
6	389	0.52	3.92	114.44	97.50	25.02	0.0815	0.887	3096.64	45.14 (2.0)
7	392	0.71	6.51	105.06	97.30	35.08	0.0081	0.994	2953.15	41.4 (2.0)
8	393	0.67	5.61	109.28	97.10	35.80	0.1466	0.892	3098.85	39.58 (2.0)
9	394	0.43	5.20	124.96	97.60	25.25	0.0238	0.939	2684.20	55.56 (3.0)
10	395	0.70	7.54	101.90	99.10	10.30	0.1263	0.919	2736.24	34.72 (2.0)
11	396	0.50	4.31	110.16	99.60	3.35	0.0352	0.987	2837.44	40.28 (2.0)
12	397	0.57	6.79	110.96	99.10	11.40	0.1092	0.857	2670.67	47.22 (3.0)
13	398	0.50	3.34	103.02	96.90	24.10	0.0320	0.992	2875.46	45.83 (2.0)
14	399	0.50	12.66	106.34	95.30	43.80	0.0326	0.894	1749.83	36.81 (3.0)
15	400	0.55	5.23	109.51	99.50	5.64	0.0565	0.867	2800.93	38.89 (2.0)
16	401	0.68	12.32	106.42	99.00	11.00	0.1077	0.901	2405.97	40.97 (3.0)
17	403	0.21	8.33	273.56	99.50	3.09	0.0039	0.908	2576.15	59.03 (4.0)
18	404	0.27	7.37	188.88	97.30	20.54	0.0103	0.890	2355.13	50.00 (3.0)
19	405	0.64	6.73	100.94	98.90	12.68	0.0881	0.957	2652.39	34.72 (2.0)
20	406	0.25	11.56	272.41	98.10	15.83	0.0240	0.916	2315.49	56.25 (4.0)
21	407	0.56	5.73	109.57	99.30	7.57	0.0374	0.937	2756.56	40.97 (2.0)
22	408	0.68	8.49	104.27	99.70	4.24	0.0331	0.913	2660.44	50.69 (3.0)
23	410	0.59	5.02	100.36	99.30	6.24	0.0505	0.786	2718.69	38.19 (2.0)
24	413	0.44	6.71	124.27	99.20	8.42	0.0461	0.970	2442.70	44.44 (3.0)
25	414	0.32	5.41	153.18	97.60	21.81	0.0344	0.889	2634.23	57.64 (3.0)
26	415	0.67	6.69	83.96	98.40	12.90	0.1286	0.813	2268.09	32.64 (2.0)
27	417	0.36	3.67	123.60	99.90	1.08	0.0102	0.937	2759.04	43.75 (2.0)
28	418	0.74	4.16	84.46	99.90	6.43	0.0498	0.890	2677.52	44.44 (2.0)
29	419	0.62	6.60	110.45	99.30	9.55	0.0836	0.893	2832.86	38.89 (2.0)
30	420	0.49	3.83	105.30	98.30	14.10	0.0136	0.969	2780.82	38.89 (2.0)
31	422	0.66	9.09	90.63	98.50	15.12	0.0264	0.973	2224.26	45.14 (3.0)
32	424	0.25	11.56	272.41	98.10	15.83	0.1253	0.841	2346.40	54.86 (4.0)
33	425	0.33	4.55	135.71	99.30	5.02	0.0099	0.971	2593.17	52.78 (3.0)
34	426	0.36	5.30	141.83	99.40	6.18	0.0259	0.860	2694.33	52.08 (3.0)
35	427	0.48	5.27	119.87	96.30	42.80	0.0794	0.896	2804.45	31.94 (2.0)
36	430	0.24	203.53	317.35	98.30	15.86	0.0147	0.969	2039.97	77.08 (6.0)
37	431	0.57	5.21	109.35	96.90	33.50	0.1507	0.834	2862.34	37.50 (2.0)
38	433	0.61	37.97	122.62	96.40	43.70	0.0581	0.945	1423.48	29.86 (4.0)
39	438	1.40	40.85	25.76	94.60	8.19	0.1044	0.950	772.24	19.44 (4.0)
40	<i>C. metuliferus</i>	0.55	15.85	46.65	88.30	34.23	0.9686	0.929	764.49	15.97 (4.0)
41	Swarna Agethi	0.27	5.56	188.76	97.70	20.81	0.0231	0.955	2797.00	57.64 (3.0)

*t* maximum downy mildew severity rate/weeks, *a* intrinsic growth rate, *b* incremental relative rate of relative growth rate of the disease, *C* carrying capacity for each model



**Fig. 5** AUDPC for different genotypes of cucumber screened under natural condition for downy mildew disease



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### Compliance with ethical standards

**Conflict of interests** The authors declare that they have no conflicts of interest.

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