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Identification of new resistant sources against downy mildew disease from a selected set of cucumber germplasm and its wild relatives

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Abstract

This study was undertaken to identify new sources of resistance against downy mildew of cucumber caused by *Pseudoperonospora cubensis* [(Berk. and Cart.) Rostow]. Pot experiments were conducted with 16 accessions each of *Cucumis sativus* and *C. hardwickii* during the years 2017 and 2018 using 12 isolates of *P. cubensis* representing different agroclimatic zones of India. The accession, IC331627 from Dehradun, Uttarakhand showed plant disease index (PDI) ranging from 5.55-20.35 per cent with an average PDI of 11.56 and 11.87 per cent during the years, 2017 and 2018, respectively. Hence, IC331627 was identified to be resistant against the five isolates of *P. cubesis* (*Pc*12, *Pc*17, *Pc*19, *Pc*21 and *Pc*24) and moderately resistant against one isolate (*Pc*22). This resistant accession *C. hardwickii* (IC331627) could be utilized to develop mapping population to map genomic regions conferring the resistance to downy mildew in cucumber.

Key words: Resistant sources; *P. cubensis*; cucumber; wild relatives

Introduction

Cucumber is mainly grown for its edible tender fruits, preferred as salad ingredient, pickles, dessert fruit and also cooked as vegetable preparation. It is a very good source of vitamins such as A, C and folic acid and minerals including calcium, potassium and magnesium. The total area under cultivation and production of cucumber in India is 82.04 thousand ha and 1259.94 thousand MT respectively (Anonymous, 2018). Downy mildew, a foliar disease caused by the oomycete fungus *P. cubensis,* is one of the most destructive pathogens of cucurbits (Palti and Cohen, 1980). This disease was first described in Cuba in 1868 (Berkeley and Curtis, 1868). The symptoms of the disease in the susceptible genotypes appear mostly on foliage as small, water soaked lesions on the underside of the leaves which later become angular and turn chlorotic as they are restricted by the small leaf veins. Whereas, the resistant genotypes show hypersensitive response (HR) with small necrotic spots (Call et al. 2012).

Since several fungicides, which have earlier provided control, are reported to be ineffective because of evolution of new strains of *P. cubensis*and resistant varieties substantially reduce or eliminate fungicide requirements (Wan et al. 2010). The development of resistant cultivars is generally considered to be the best approach to control downy mildew (Olczak et al. 2011). So, identification of new sources of resistance to downy mildew is of paramount importance for agriculture and environment. Therefore, this study was undertaken with the objective to find out sources of resistance through artificial screening (pot culture) of cucumber germplasm.

Materials and methods

In order to identify the sources of resistance in cucumber against downy mildew, the pot experiments were conducted during *kharif* seasons of 2017 and 2018 at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi following

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standard package of practices using completely randomized block design (CRBD). A total of 32 accessions of cucumber including 16 germplasm accessions/ varieties of *C. sativum* and 16 wild accessions of *C. hardwickii* were obtained from the Gene Bank of ICAR-NBPGR, New Delhi and the scientists working with the cucumber (Table 1) and were screened artificially against 12 isolates of *P. cubensis.*

formalin (1%) for 15 days. After 20 days of sowing, the infected leaves preserved at ultralow temperature collected from six locations were taken out of deep freeze and allowed to thaw. For artificial inoculation, the sporangial suspension was prepared in distilled water from fresh infected cucumber leaves. After giving chilling treatment to the sporangial suspension for overnight, the four-week-old cucumber plants were inoculated on the abaxial surface of the first fully

Table 1. Collection of cucumber germplasm and its wild relative from different sources

S.No.	Species	Germplasm/ Variety	Source
1	*Cucumis sativus*	IC410654, IC410658 & IC410682	Tripura
		IC527391, IC527400, IC527402, IC527418 & IC527420	West Bengal
		IC538145, IC538147 & IC538155 and Kalyanpur Green	Uttar Pradesh
		IC595920	Kerala
		Pahari Harit (Local landrace)	Uttarakhand
		PusaBarkha&PusaUday	New Delhi
2	*C hardwickii*	IC331459	Chattisgarh
		IC331465	Madhya Pradesh
		IC331620	Himachal Pradesh
		IC331609, IC331627, IC331628 & IC331631	Uttarakhand
		IC248263 & IC277066	Goa
		IC277000, IC277029, IC277030, IC277035, IC277048 & IC277054	Maharashtra
		IC253909	-

A total of 50 samples of cucumber plants showing typical symptoms of downy mildew caused by *P. cubensis* were collected during 2013 and 2014 from different regions of India. Out of the total 50 diseased samples, six samples (isolates) each in the form of infected leaf samples from Uttar Pradesh and Delhi and three samples (isolates) each from Karnataka, Madhya Pradesh, Punjab and West Bengal were preserved at –80°C for further studies (Table 2). After molecular characterization, 12 isolates representing different geographical regions (states) were selected for screening purpose.

Artificial screening of cucumber against downy mildew was undertaken as pot experiments laid out in CRBD with two replications at ICAR-NBPGR, New Delhi during the years 2017 and 2018.

Five seeds of each accession after sterilization with sodium hypochlorite (2 %) for 2 minutes and rinsing thrice in sterilized water were sown in plastic pots (20 cm diameter) filled with sterilized soil with

(a) (b)

Fig. 1. Pot experiment showing Resistant (a-IC331627) and susceptible (b-IC538145) reactions against isolate *Pc*17

expanded leaf @ 20-30 µl droplets (1 x 10^5 sporangia/ ml). High humidity (100 %) and 20° C temperature was maintained for 48 hrs to ensure infection. After 48 hrs, the plants were kept at ambient temperature (15-24° C). The observations were recorded at 10 days interval till crop maturity and PDI (%) was calculated using formula given by Pitchaimuthu et al. (2007).

Table 2. *Pseudoperonospora cubensis* isolates collected from different regions of India

S.No.	State	District	Isolate
1	Uttar Pradesh	Varanasi	***Pc*1**, *Pc*2, *Pc*3, *Pc*4, *Pc*5 &***Pc6***
2	Delhi	Central Delhi	*Pc7*, *Pc*8, *Pc*9,***Pc*15**, *Pc16*&***Pc17***
3	Karnataka	Raichur	*Pc*10, ***Pc*11**&***Pc*12**
4	Punjab	Ludhiana	***Pc*13**, *Pc*14 &***Pc*21**
5	West Bengal	Cooch Bihar	*Pc*18, ***Pc*19**&***Pc*20**
6	Madhya Pradesh	Jabalpur	***Pc*22**, *Pc*23&***Pc*24**

*Isolates written in bold font were used for artificial screening for disease resistance

$$PDI = \frac{\text{Sum of numerical values}}{\text{No. of leaves graded x maximum ratings}} \times 100$$

After calculating the PDI, the germplasm accessions were categorized into five groups namely (1) resistant (0-12), (2) moderately resistant (12.10-25.0), (3) moderately susceptible (25.10-50.0), (4) susceptible (50.10-75.0) and (5) highly susceptible (>75.0) and the data were analyzed using ANOVA.

Results and discussion

In order to provide resistant donors against downy mildew for cucumber improvement programmes towards resistance breeding, 32 accessions of cucumber were screened against 12 isolates of *P. cubensis.* The disease index of downy mildew during both the years 2017 and 2018 revealed that only two accessions i.e. IC331627 from Dehradun, Uttarakhand and IC277029 from Raigad, Maharashtra were found resistant and moderately resistant, respectively. The resistant accession, IC331627 showed PDI ranging from 5.55-20.35 % with mean PDI of 11.56 % and 11.87 % during the years 2017 and 2018, respectively. Similarly, the moderately resistant accession, IC277029 showed PDI ranging from 12.95-33.30 % during both the years with an average PDI 24.20 % and 23.90 % during both the years 2017 and 2018, respectively. Based on PDI, the accession, IC331627 was found resistant against five isolates (*Pc*12, *Pc*17, *Pc*19, *Pc*21 and *Pc*24) of *P. cubesis* and moderate resistant to one isolate (*Pc*22) during both the years 2017 and 2018. Whereas, another accession, IC277029 was found moderately resistant against three isolates (*Pc*6, *Pc*11 and *Pc*19) of *P. cubesis* and moderately susceptible to three isolates (*Pc*12, *Pc*17 and *Pc*20) during both the years.

The data presented in Supplementary Table S1 indicated that during the year 2017, out of 32 accessions, five accessions *viz*., IC538155, Kalyanpur Green, Pahari Harit, PusaBarkha, PusaUday were found moderately susceptible showing PDI of 25.10-50.0 %. Twenty-five accessions *viz*., IC410654, IC410658, IC410682, IC527391, IC527400, IC527402 etc. found susceptible showing PDI of 51.10-75.0 %. The accession, IC331627 showed lowest mean PDI (11.56%) followed by the accession, IC277029 (24.20%). Whereas, the accession, IC277000 showed highest mean PDI (63.52%) followed by the accession, IC538145 (62.75%).

During the year 2018, the data presented in Supplementary Table S2 indicated that out of 32 accessions, four accessions *viz*., Kalyanpur Green, Pahari Harit, PusaBarkha, PusaUday were found moderate susceptible showing PDI of 25.10-50.0 %. Twenty-six accessions *viz*., IC410654, IC410658 etc. were found susceptible showing PDI of 51.10-75.0 %. The accession, IC331627 (Fig. 1a) showed lowest mean PDI (11.87%) followed by the accession, IC277029 (23.90%). Whereas the accession, IC595920 showed highest mean PDI i.e. 63.05 % followed by the accession, IC538145 having mean PDI (62.59%). The disease development was found very slow in case of resistant accessions while it was found reverse in case of susceptible accessions (Fig. 1b).

Lisitsin and Pluzhnikova (1990) identified promising sources of resistance by screening cucumber varieties against *P. cubensis* and a bacterial disease. The most useful varieties with resistance to both pathogens, were Levina F_1, Kamon F_1, 6501, 6502, Belcanto F_1 and PER Industria BF_1p510c. Dhillon et al. (1999) screened genotypes of cucumber for downy mildew resistance in North India under natural field conditions and found that resistant genotypes

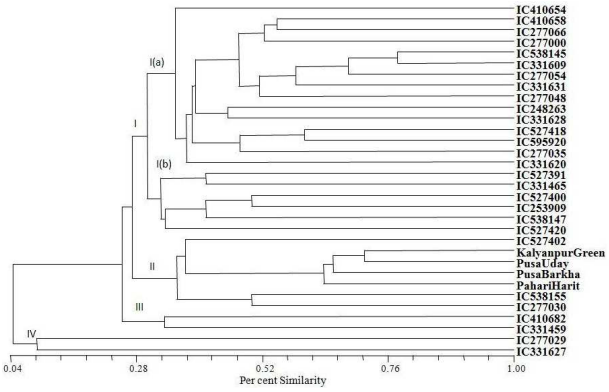

Fig. 2. Dendrogram showing similarity among/between germplasm accessions based on reactions caused by different isolates of *P. cubensis*

were of Japanese origin. Petrov et al. (2000) described that in resistant plants, the resistance expressed as small, chlorotic, water soaked lesions with no sporulation of fungus. Pitchaimuthu et al. (2012) also evaluated different species of cucumber including wild cucumber (*C. hardiwickii*), *C. sativus* var. *sativus* and SM 12735 against powdery and downy mildew diseases under natural conditions and reported that some accessions, *C hardiwickii*-14 and 15, *C. sativus* var. *sativus* and SM 12735 exhibited high level of resistance against both the diseases with PDI ranging between 0-25%.

Moreover, based on the average PDI, clustering revealed that only one accession each observed as resistant (IC331627) and moderately resistant (IC277029) fell into the same cluster i.e., IV. Whereas, rest of the accessions were grouped into three clusters i.e., cluster I, cluster II and cluster III which showed susceptible reactions based on average PDI of infection recorded against 12 isolates (Fig. 2). However, the response of individual accession varied with different isolates independently.

The result of pot experiments carried out during the years 2017 and 2018 with a view to find out new sources of resistance against a very destructive fungal disease, downy mildew (*P. cubensis*) revealed that none of the accessions of *C. sativus* was resistant, whereas, only 2 accessions of *C. hardiwickii* showed resistance. During both the years, an accession of *C. hardwickii* from Dehradun, Uttarakhand (IC331627) was found resistant against all the tested isolates of *P. cubensis*, whereas, another accession of *C. hardwickii* from Raigad, Maharashtra (IC277029) was found moderate resistant.

The identified accessions of crop wild relatives of cucumber can be utilized to develop mapping population to map genomic regions conferring the resistance to downy mildew in cucumber. Wang et al. (2016) reported QTLs for resistance to downy mildew: *dm1.1*, *dm5.1*, *dm5.2*, *dm5.3* and *dm6.1*. Several QTLs were identified on chromosomes 1, 3, 4 and 5 (Li et al. 2018). It was also established that resistance in some genotypes is also governed by three recessive resistance genes, *dm-1, dm-2* and *dm-3*. These reported genes/QTLs can be validated in these newly identified accessions. Further, fine mapping would help in cloning of genes that would help in understanding the molecular mechanism of resistance. The identified genes/QTLs can be introgressed in the susceptible but otherwise agronomically superior cucumber varieties through marker-assisted selection. Moreover, the deployment of these identified resistant sources will also be helpful in minimizing the excessive use of pesticides thereby reducing the environmental pollution as well as the cost of cultivation. The downy mildew resistant cultivars would provide more yield and profit to the farmers thereby generating livelihood.

Authors' contribution

Conceptualization of research (JA, DG); Designing of the experiments (JA, RN, ABG); Contribution of experimental materials (JA, SA); Execution of field/ lab experiments and data collection (DG, JA, ANI); Analysis of data and interpretation (JA, DG, ABG, GSJ); Preparation of manuscript (JA, DG, SA, BT).

Declaration

The authors declare no conflict of interest.

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Supplementary Table S1. Disease index of downy mildew caused by different isolates of *P. cubensis* during 2017

S.No.	Accession	Disease Index (per cent)												
		*Pc*1	*Pc*6	*Pc*11	*Pc*12	*Pc*13	*Pc*15	*Pc*17	*Pc*19	*Pc*20	*Pc*21	*Pc*22	*Pc*24	S.D.
1	IC410654	68.45 (75.42)	57.35 (61.99)	46.25 (48.37)	64.75 (71.72)	57.35 (61.99)	31.15 (31.72)	44.40 (45.67)	51.80 (45.96)	49.95 (52.97)	53.65 (57.02)	75.85 (86.50)	49.95 (52.97)	±10.94
2	IC410658	48.10 (50.23)	70.30 (80.32)	70.30 (80.32)	48.10 (50.23)	66.60 (73.92)	51.80 (45.96)	57.35 (61.99)	53.65 (57.02)	53.65 (57.02)	53.65 (57.02)	55.50 (59.10)	59.20 (63.67)	±7.25
3	IC410682	64.75 (71.72)	40.70 (42.52)	77.70 (89.88)	62.90 (68.03)	49.95 (52.97)	44.40 (45.67)	42.55 (44.20)	81.40 (96.31)	83.25 (99.15)	57.35 (61.99)	51.80 (45.96)	75.85 (86.50)	±15.56
4	IC527391	44.40 (45.67)	72.15 (82.57)	35.15 (35.93)	46.25 (48.37)	49.95 (52.97)	57.35 (61.99)	51.80 (45.96)	62.90 (68.03)	42.55 (44.20)	70.30 (80.32)	68.45 (75.42)	66.60 (73.92)	±11.84
5	IC527400	40.70 (42.52)	62.90 (68.03)	48.10 (50.23)	64.75 (71.72)	64.75 (71.72)	74.0 (83.31)	48.10 (50.23)	61.05 (67.50)	77.70 (89.88)	70.30 (80.32)	61.05 (67.50)	51.80 (45.96)	±9.39
6	IC527402	42.55 (44.20)	37.0 (37.90)	68.45 (75.42)	57.35 (61.99)	83.25 (99.15)	79.55 (93.63)	64.75 (71.72)	64.75 (71.72)	47.75 (50.0)	57.35 (61.99)	42.55 (44.20)	64.75 (71.72)	±13.67
7	IC527418	64.75 (71.72)	75.85 (86.50)	62.90 (68.03)	53.65 (57.02)	48.10 (50.23)	40.70 (42.52)	72.15 (82.57)	59.20 (63.67)	57.35 (61.99)	53.65 (57.02)	44.40 (45.67)	75.85 (86.50)	±11.60
8	IC527420	49.95 (52.97)	48.10 (50.23)	44.40 (45.67)	48.10 (50.23)	75.85 (86.50)	86.95 (105.55	68.45 (75.42)	75.85 (86.50)	70.30 (80.32)	46.25 (48.37)	61.05 (67.50)	55.50 (59.10)	±13.83
9	IC538145	55.50 (59.10)	66.60 (73.92)	64.75 (71.72)	57.35 (61.99)	66.60 (73.92)	57.35 (61.99)	70.30 (80.32)	62.90 (68.03)	59.20 (63.67)	55.50 (59.10)	70.30 (80.32)	66.60 (73.92)	±5.06
10	IC538147	53.30 (57.02)	53.65 (57.02)	48.10 (50.23)	55.50 (59.10)	48.10 (50.23)	53.65 (57.02)	46.25 (48.37)	49.95 (52.97)	51.80 (45.96)	85.10 (102.20)	62.90 (68.03)	49.95 (52.97)	±10.47
11	IC538155	35.15 (35.93)	46.25 (48.37)	42.55 (44.20)	49.95 (52.97)	61.05 (67.50)	40.70 (42.52)	62.90 (68.03)	42.55 (44.20)	44.40 (45.67)	79.55 (93.63)	59.20 (63.67)	61.05 (67.50)	±11.55
12	IC595920	70.30 (80.32)	62.90 (68.03)	62.90 (68.03)	72.15 (82.57)	48.10 (50.23)	42.55 (44.20)	57.35 (61.99)	55.50 (59.10)	59.20 (63.67)	61.05 (67.50)	48.10 (50.23)	79.55 (93.63)	±10.24
13	IC248263	79.55 (93.63)	42.55 (44.20)	70.30 (80.32)	79.55 (93.63)	49.95 (52.97)	44.40 (45.67)	49.95 (52.97)	59.20 (63.67)	46.25 (48.37)	59.20 (63.67)	74.0 (83.31)	68.45 (75.42)	±12.34
14	IC253909	33.30 (33.68)	48.10 (50.23)	51.80 (45.96)	38.85 (39.53)	70.30 (80.32)	53.65 (57.02)	38.85 (39.53)	40.70 (42.52)	72.15 (82.57)	81.40 (96.31)	59.20 (63.67)	55.50 (59.10)	±13.58
15	IC277000	40.70 (42.52)	42.55 (44.20)	53.65 (57.02)	75.85 (86.50)	85.10 (102.20)	72.15 (82.57)	79.55 (93.63)	66.60 (73.92)	70.30 (80.32)	57.35 (61.99)	66.60 (73.92)	51.80 (45.96)	±12.37
16	IC277029	18.50 (18.57)	22.20 (22.41)	24.05 (24.40)	27.75 (28.12)	22.20 (22.41)	25.90 (26.28)	33.30 (33.68)	18.50 (18.57)	27.75 (28.12)	14.80 (14.87)	24.05 (24.40)	31.45 (32.05)	±5.13
17	IC277030	51.80 (45.96)	46.25 (48.37)	48.10 (50.23)	83.25 (99.15)	70.30 (80.32)	37.0 (37.90)	81.40 (96.31)	42.55 (44.20)	44.40 (45.67)	51.80 (45.96)	44.40 (45.67)	51.80 (45.96)	±15.30
18	IC277035	61.05 (67.50)	51.80 (45.96)	48.10 (50.23)	37.0 (37.90)	44.40 (45.67)	48.10 (50.23)	57.35 (61.99)	83.25 (99.15)	66.60 (73.92)	59.20 (63.67)	85.10 (102.20)	79.55 (93.63)	±15.74
19	IC277048	53.65 (57.02)	46.25 (48.37)	66.60 (73.92)	42.55 (44.20)	68.45 (75.42)	51.80 (45.96)	68.45 (75.42)	68.45 (75.42)	62.90 (68.03)	68.45 (75.42)	55.50 (59.10)	64.75 (71.72)	±9.24
20	IC277054	62.90 (68.03)	61.05 (67.50)	57.35 (61.99)	42.55 (44.20)	53.65 (57.02)	70.30 (80.32)	49.95 (52.97)	53.65 (57.02)	55.50 (59.10)	72.15 (82.57)	46.25 (48.37)	62.90 (68.03)	±8.82
21	IC277066	48.10 (50.23)	57.35 (61.99)	57.35 (61.99)	55.50 (59.10)	51.80 (45.96)	44.40 (45.67)	79.55 (93.63)	64.75 (71.72)	64.75 (71.72)	75.85 (86.50)	51.80 (45.96)	55.50 (59.10)	±10.05
22	IC331459	53.65 (57.02)	49.95 (52.97)	44.40 (45.67)	66.60 (73.92)	40.70 (42.52)	49.95 (52.97)	75.85 (86.50)	68.45 (75.42)	70.30 (80.32)	48.10 (50.23)	74.0 (83.31)	46.25 (48.37)	±12.64
23	IC331465	40.70 (42.52)	74.0 (83.31)	77.70 (89.88)	46.25 (48.37)	55.50 (59.10)	51.80 (45.96)	48.10 (50.23)	38.85 (39.53)	44.40 (45.67)	48.10 (50.23)	64.75 (71.72)	70.30 (80.32)	±12.59
24	IC331609	57.35 (61.99)	53.65 (57.02)	64.75 (71.72)	53.65 (57.02)	55.50 (59.10)	77.70 (89.88)	57.35 (61.99)	70.30 (80.32)	57.35 (61.99)	61.05 (67.50)	49.95 (52.97)	74.0 (83.31)	±8.71
25	IC331620	61.05 (67.50)	49.95 (52.97)	51.80 (45.96)	70.30 (80.32)	42.55 (44.20)	61.05 (67.50)	57.35 (61.99)	83.25 (99.15)	57.35 (61.99)	77.70 (89.88)	61.05 (67.50)	48.10 (50.23)	±12.02
26	IC331627	16.65 (16.73)	5.55 (5.56)	12.95 (12.99)	11.10 (11.16)	9.25 (9.27)	16.65 (16.73)	11.10 (11.16)	9.25 (9.27)	9.25 (9.27)	9.25 (9.27)	20.35 (20.50)	7.40(7.41)	±4.02
27	IC331628	49.95 (52.97)	64.75 (71.72)	46.25 (48.37)	50.45 (53.61)	44.40 (45.67)	64.75 (71.72)	81.40 (96.31)	53.65 (57.02)	44.40 (45.67)	59.20 (63.67)	55.50 (59.10)	75.85 (86.50)	±11.87
28	IC331631	59.20 (63.67)	53.65 (57.02)	64.75 (71.72)	42.55 (44.20)	66.60 (73.92)	38.85 (39.53)	61.05 (67.50)	59.20 (63.67)	61.05 (67.50)	66.60 (73.92)	72.15 (82.57)	66.60 (73.92)	±9.96
29	Kalyanpur Green	44.40 (45.67)	49.95 (52.97)	42.55 (44.20)	51.80 (45.96)	37.0 (37.90)	46.25 (48.37)	49.95 (52.97)	49.95 (52.97)	46.25 (48.37)	49.95 (52.97)	48.10 (50.23)	48.10 (50.23)	±4.05
30	Pahari Harit	38.85 (39.53)	40.70 (42.52)	48.10 (50.23)	40.70 (42.52)	46.25 (48.37)	42.55 (44.20)	44.40 (45.67)	46.25 (48.37)	48.10 (50.23)	40.70 (42.52)	40.70 (42.52)	61.05 (67.50)	±5.71
31	PusaBarkha	49.95 (52.97)	38.85 (39.53)	46.40 (48.27)	45.95 (47.45)	83.25 (99.15)	38.85 (39.53)	49.95 (52.97)	53.65 (56.76)	46.25 (48.37)	51.80 (45.96)	48.10 (50.23)	40.70 (42.52)	±11.71
32	PusaUday	42.55 (44.20)	42.55 (44.20)	42.55 (44.20)	38.85 (39.53)	48.10 (50.23)	49.95 (52.97)	57.35 (61.99)	49.95 (52.97)	40.70 (42.52)	46.25 (48.37)	44.40 (45.67)	49.95 (52.97)	±5.08
CD at 5 per cent for I		0.0608												
CD at 5 per cent for G		0.0992												
CD at 5 per cent for I x G		0.3438												
SEm		0.0063												
CV		0.3206												

Supplementary Table S2. Disease index of downy mildew caused by different isolates of *P. cubensis* during 2018

S.No.	Accession	Disease Index (per cent)												
		*Pc*1	*Pc*6	*Pc*11	*Pc*12	*Pc*13	*Pc*15	*Pc*17	*Pc*19	*Pc*20	*Pc*21	*Pc*22	*Pc*24	S.D.	
1	IC410654	48.10 (50.23)	57.35 (61.99)	44.40 (45.67)	55.50 (59.10)	53.65 (57.02)	33.30 (33.68)	48.10 (50.23)	64.75 (71.72)	51.95 (54.68)	59.20 (63.67)	53.65 (57.02)		61.05 (67.50)	±8.40
2	IC410658	48.10 (50.23)	79.55 (93.63)	44.40 (45.67)	44.40 (45.67)	66.60 (73.92)	48.10 (50.23)	57.35 (61.99)	57.35 (61.99)	70.30 (80.32)	57.35 (61.99)	61.05 (67.50)		61.05 (67.50)	±10.77
3	IC410682	77.70 (89.88)	40.70 (42.52)	57.35 (61.99)	70.30 (80.32)	48.10 (50.23)	42.55 (44.20)	42.55 (44.20)	77.70 (89.88)	64.75 (71.72)	74.0 (83.31)	55.50 (59.10)		81.40 (96.31)	±15.21
4	IC527391	49.95 (52.97)	53.65 (57.02)	37.0 (37.90)	38.85 (39.53)	49.95 (52.97)	57.35 (61.99)	49.95 (52.97)	70.30 (80.32)	46.25 (48.37)	49.95 (52.97)	66.60 (73.92)		75.85 (86.50)	±11.90
5	IC527400	51.80 (45.96)	70.30 (80.32)	38.85 (39.53)	49.95 (52.97)	64.75 (71.72)	70.30 (80.32)	49.95 (52.97)	49.95 (52.97)	62.90 (68.03)	59.20 (63.67)	75.85 (86.50)		62.90 (68.03)	±10.90
6	IC527402	46.25 (48.37)	48.10 (50.23)	68.45 (75.42)	68.45 (75.42)	61.05 (67.50)	81.40 (96.31)	57.35 (61.99)	62.90 (68.03)	49.95 (52.97)	48.10 (50.23)	74.0 (83.31)		53.65 (57.02)	±11.39
7	IC527418	72.15 (82.57)	57.35 (61.99)	66.60 (73.92)	81.40 (96.31)	44.40 (45.67)	40.70 (42.52)	68.45 (75.42)	61.05 (67.50)	55.50 (59.10)	61.05 (67.50)	46.25 (48.37)		61.05 (67.50)	±11.89
8	IC527420	70.30 (80.32)	66.60 (73.92)	33.30 (33.68)	72.15 (82.57)	55.50 (59.10)	57.35 (61.99)	64.75 (71.72)	53.65 (57.02)	72.15 (82.57)	46.25 (48.37)	57.35 (61.99)		66.60 (73.92)	±11.62
9	IC538145	61.05 (67.50)	66.60 (73.92)	55.50 (59.10)	53.65 (57.02)	61.05 (67.50)	55.50 (59.10)	74.0 (83.31)	66.60 (73.92)	70.30 (80.32)	57.35 (61.99)	66.60 (73.92)		62.90 (68.03)	±6.40
10	IC538147	57.35 (61.99)	66.60 (73.92)	44.40 (45.67)	59.20 (63.67)	74.0 (83.31)	74.0 (83.31)	57.35 (61.99)	42.55 (44.20)	72.15 (82.57)	62.90 (68.03)	64.75 (71.72)		37.0 (37.90)	±12.43
11	IC538155	40.70 (42.52)	40.70 (42.52)	29.60 (29.88)	44.40 (45.67)	64.75 (71.72)	38.85 (39.53)	74.0 (83.31)	48.10 (50.23)	40.70 (42.52)	55.50 (59.10)	55.50 (59.10)		59.20 (63.67)	±12.68
12	IC595920	59.20 (63.67)	70.30 (80.32)	51.80 (45.96)	64.75 (71.72)	59.20 (63.67)	46.25 (48.37)	68.45 (75.42)	74.0 (83.31)	79.55 (93.63)	75.85 (86.50)	40.70 (42.52)		66.60 (73.92)	±12.03
13	IC248263	74.0 (83.31)	59.20 (63.67)	68.45 (75.42)	61.05 (67.50)	40.70 (42.52)	49.95 (52.97)	46.25 (48.37)	59.20 (63.67)	64.75 (71.72)	66.60 (73.92)	57.35 (61.99)		64.75 (71.72)	±9.64
14	IC253909	61.05 (67.50)	31.45 (32.05)	49.95 (52.97)	44.40 (45.67)	66.60 (73.92)	68.45 (75.42)	42.55 (44.20)	38.85 (39.53)	70.30 (80.32)	51.80 (45.96)	70.30 (80.32)		68.45 (75.42)	±13.88
15	IC277000	49.95 (52.97)	42.55 (44.20)	61.05 (67.50)	57.35 (61.99)	61.05 (67.50)	61.05 (67.50)	70.30 (80.32)	72.15 (82.57)	66.60 (73.92)	70.30 (80.32)	62.90 (68.03)		59.20 (63.67)	±8.59
16	IC277029	25.90 (26.28)	12.95 (12.99)	18.50 (18.57)	29.60 (29.88)	31.45 (32.05)	12.95 (12.99)	27.75 (28.12)	22.20 (22.41)	25.90 (26.28)	29.60 (29.88)	29.60 (29.88)		20.35 (20.50)	±6.48
17	IC277030	61.05 (67.50)	35.15 (35.93)	44.40 (45.67)	72.15 (82.57)	57.35 (61.99)	35.15 (35.93)	53.65 (57.02)	37.0 (37.90)	48.10 (50.23)	62.90 (68.03)	42.55 (44.20)		62.90 (68.03)	±12.43
18	IC277035	68.45 (75.42)	64.75 (71.72)	44.40 (45.67)	48.10 (50.23)	44.40 (45.67)	48.10 (50.23)	57.35 (61.99)	64.75 (71.72)	61.05 (67.50)	68.45 (75.42)	64.75 (71.72)		53.65 (57.02)	±9.27
19	IC277048	51.80 (45.96)	44.40 (45.67)	55.50 (59.10)	53.65 (57.02)	59.20 (63.67)	70.30 (80.32)	70.45 (80.60)	44.40 (45.67)	81.40 (96.31)	53.65 (57.02)	77.70 (89.88)		68.45 (75.42)	±12.44
20	IC277054	72.15 (82.57)	72.15 (82.57)	66.80 (63.35)	37.0 (37.90)	61.05 (67.50)	62.90 (68.03)	53.65 (57.02)	66.60 (73.92)	57.35 (61.99)	68.45 (75.42)	59.20 (63.67)		72.15 (82.57)	±10.08
21	IC277066	35.15 (35.93)	66.60 (73.92)	62.90 (68.03)	51.80 (45.96)	61.05 (67.50)	44.40 (45.67)	55.50 (59.10)	59.20 (63.67)	72.15 (82.57)	61.05 (67.50)	68.45 (75.42)		64.75 (71.72)	±10.53
22	IC331459	57.35 (61.99)	42.55 (44.20)	46.25 (48.37)	62.90 (68.03)	31.45 (32.05)	46.25 (48.37)	79.55 (93.63)	68.45 (75.42)	61.05 (67.50)	40.70 (42.52)	51.80 (45.96)		46.25 (48.37)	±13.44
23	IC331465	42.55 (44.20)	68.45 (75.42)	85.10 (102.20	53.65 (57.02)	59.20 (63.67)	74.0 (83.31)	46.25 (48.37)	44.40 (45.67)	49.95 (52.97)	48.10 (50.23)	57.35 (61.99)		57.35 (61.99)	±12.92
24	IC331609	61.05 (67.50)	61.05 (67.50)	66.60 (73.92)	66.60 (73.92)	66.60 (73.92)	62.90 (68.03)	62.90 (68.03)	57.35 (61.99)	55.50 (59.10)	61.05 (67.50)	44.40 (45.67)		66.60 (73.92)	±6.41
25	IC331620	59.20 (63.67)	35.15 (35.93)	61.05 (67.50)	68.45 (75.42)	46.25 (48.37)	61.05 (67.50)	64.75 (71.72)	59.20 (63.67)	62.90 (68.03)	72.15 (82.57)	79.55 (93.63)		59.20 (63.67)	±11.43
26	IC331627	9.25(9.27)	16.65 (16.73)	11.10 (11.16)	9.25 (9.27)	12.95 (12.99)	9.25 (9.27)	5.55 (5.56)	11.10 (11.16)	18.50 (18.57)	9.25 (9.27)	20.35 (20.50)	9.25(9.27)	±4.42	
27	IC331628	66.60 (73.92)	57.35 (61.99)	55.50 (59.10)	61.05 (67.50)	42.55 (44.20)	59.20 (63.67)	62.90 (68.03)	57.35 (61.99)	68.45 (75.42)	55.50 (59.10)	68.45 (75.42)		70.30 (80.32)	±7.74
28	IC331631	55.50 (59.10)	72.15 (82.57)	59.20 (63.67)	61.05 (67.50)	64.75 (71.72)	42.55 (44.20)	48.10 (50.23)	66.60 (73.92)	55.50 (59.10)	62.90 (68.03)	66.60 (73.92)		77.70 (89.88)	±9.79
29	Kalyanpur Green	38.85 (39.53)	40.70 (42.52)	48.10 (50.23)	44.40 (45.67)	38.85 (39.53)	44.40 (45.67)	44.40 (45.67)	49.95 (52.97)	37.0 (37.90)	53.65 (57.02)	44.40 (45.67)		44.40 (45.67)	±4.85
30	Pahari Harit	48.10 (50.23)	46.25 (48.37)	40.70 (42.52)	44.40 (45.67)	44.40 (45.67)	48.10 (50.23)	44.40 (45.67)	40.70 (42.52)	53.65 (57.02)	37.0 (37.90)	53.65 (57.02)		48.10 (50.23)	±5.00
31	PusaBarkha	44.40 (45.67)	48.10 (50.23)	44.40 (45.67)	49.95 (52.97)	42.55 (44.20)	48.10 (50.23)	46.25 (48.37)	48.10 (50.23)	46.25 (48.37)	38.85 (39.53)	46.25 (48.37)		46.25 (48.37)	±2.96
32	PusaUday	37.0 (37.90)	55.50 (59.10)	44.40 (45.67)	44.40 (45.67)	46.25 (48.37)	40.70 (42.52)	37.0 (37.90)	40.70 (42.52)	48.10 (50.23)	38.85 (39.53)	33.30 (33.68)		42.55 (44.20)	±5.93
CD at 5per cent for I		0.0637													
CD at 5per cent for G		0.1040													
CD at 5per cent for I x G		0.3604													
SEm		0.0067													
CV		0.3379													