

## Identification 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 cucumber genotypes and its crosses developed from the same genotypes in half diallel mating design (excluding reciprocal crosses). Fifteen parents and 105 crosses of cucumber along with a check (Swarna Agethi) were screened under the natural condition with manual inoculation (spore suspension). The disease rating was recorded a month after planting and at a weekly interval was observed for seven weeks until plant growth ended. Ratings were done on 0 to 9 scales. Genotypes JB/11-028-595504 and SKY/AC-270-613481 showed resistance with an average per cent disease index (PDI) of 10.42 and 14.75, respectively, compared to genotype JB/11-197-613470 (72.58 PDI) which is highly susceptible. Among the crosses, SKY/AC-316-613484 × JB/11-028-595504 and SKY/AC-251-613477 × SKY/AC-270-613481 expressed resistance with an average PDI of 8.94 and 10.23, respectively, as compared to susceptible check swarna agethi (68.92 PDI).

*Keywords* : Screening, Downy mildew, Cucumber, Genotypes and Resistance

CUCUMBER (*Cucumis sativus* L.) is one of the most popular economically important vegetable crops of the family Cucurbitaceae. It is an ideal summer vegetable crop mainly grown for its edible tender fruits preferred as salad ingredients, pickles, dessert fruit and also as cooked vegetable. The fruit of cucumber has got cooling effect prevents constipation and remedy for jaundice (Vanitha and Vasudevan, 2019). Cucumber (*Cucumis sativus* L.) having chromosome number 2n=14 (Abdul, 2019), has got a 367 Mb genome size with 7 linkage groups (Renet *et al.*, 2009). The family consists of 98 genera and 975 species with worldwide distribution (Lebeda and Cohen, 2011). Cucumber was one of the oldest vegetables under cultivation by a man with historical records of 5,000 years (Wehner and Guner, 2004).

Commercially cultivated cucumber varieties are susceptible to several pathogens of which downy mildew is a major disease. It is widely distributed in all continents of the Northern and Southern hemispheres (Call, 2012; Lebeda and Cohen, 2011). The disease

incidence was observed on cucumber as early as 19<sup>th</sup> century, but until mid-1980s it was not found to be the cause of serious disease incidence on an economically significant scale (Colucci *et al.*, 2006). The disease downy mildew is caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. It has a host range of more than 60 vegetable species belongs to the 20 genera in the cucurbitaceae family (Lebeda, 1992; Lebeda and Cohen, 2011). Cohen (1981) reported that, nine cucurbitaceous vegetables are affected by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. under natural conditions and the highest host species belongs to the genus *Cucumis* (Lebeda and Urban, 2007). Six pathotypes of *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov., have been reported worldwide based on their compatibility with a specific host range of genus Cucurbitaceae (Cohen *et al.*, 2003). Epidemics of downy mildew on the genus *Cucumis* have been observed in over 70 countries worldwide with an estimated yield loss of 60 to 70 per cent (Cohen, 1981).

Many cucumber cultivars resistant to downy mildew have been developed (Wehner and Guner, 2004) over the past 50 years. The most resistant cultigens were of US origin and were primarily elite cultivars and breeding lines with 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 in 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 became susceptible to downy mildew pathogen over some time. Though the disease can be controlled with fungicides (Urban and Lebeda 2006), genetic resistance provides a more economically sound and environmentally safe approach.

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

#### MATERIAL AND METHODS

The experimental material consisted of fifteen parents, their 105 hybrids and one check variety (Swarna Agethi- Released by ICAR-RCER, Patna, Bihar). The seeds were sown on raised bed in open field condition and subjected to disease inoculations by artificial spraying.

#### Screening Protocol followed for Challenge Inoculation of Downy Mildew Disease

Downy mildew inoculum was collected from cucumber plants maintained in the Department of Horticulture, UAS, Bangalore and Department of Plant Pathology, College of Horticulture, UHS, Bagalkot, Bengaluru campus. Fresh downy mildew infected

leaves were collected and clipped on to newly emerged cucumber plants at 2 to 3 leaves stage during the evening (after 3 PM) hours. Small strips of downy mildew infected leaves were clipped onto both sides of the leaves of cucumber seedling using a stapler (Swamy *et al.*, 1980).

Another method was followed for better inoculation, fresh downy mildew infected leaves were collected and spore suspension was prepared in water for inoculation and diluted to 6 to 12 spores per microscopic field at 10X. The spore suspension was sprayed on the leaves of cucumber plants using a hand sprayer. The inoculated plants were covered with polythene sheets to increase the humidity near the plants.

A second inoculation was also done a week after the first inoculation. After four to five days, disease development started on the cucumber leaves and the disease rating started a month after planting and at weekly intervals, it was observed for seven weeks until plant growth ended. Ratings were done on 0 to 9 scales as described by Jenkins and Wehner (1983) presented in Table 1.

#### Statistical Analysis

Fifteen parents and 105 hybrids along with a check (Swarna Agathi) were subjected to statistical analysis in which per cent disease index (PDI) was calculated by the formula mentioned below.

#### Disease incidence (%)

The disease incidence was calculated by recording the number of plants affected by the disease particularly, downy mildew from each parent and their hybrids. The scoring of downy mildew disease incidence was started a month after planting and at weekly intervals for seven weeks until plant growth ended to study disease reaction of parents and hybrids. The data was recorded on a 0 to 9 scale for downy mildew disease incidence as suggested by Jenkins and Wehner (1983).

TABLE 1  
Scale to score downy mildew disease incidence

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

Disease scored data of all the plants in each genotype has been converted to PDI using the following formula:

Per cent disease index (PDI) was calculated with the help of the following formula.

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

Based on PDI the disease reaction of genotype was classified into four groups (Table 2) according to the following scale suggested by Reddy (2002).

Table 2

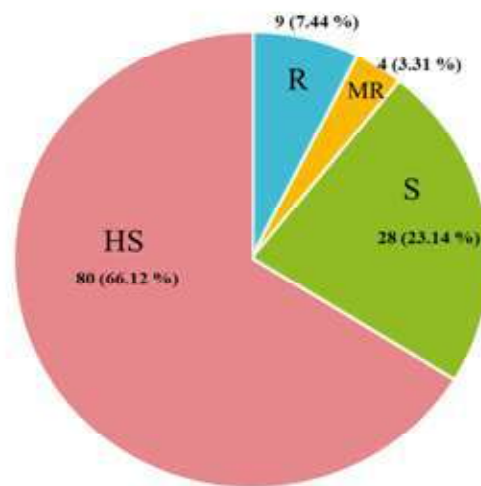
The disease reaction scale used to the grouping of genotypes

| Diseasereaction           | PDI range  |
|---------------------------|------------|
| Resistant (R)             | 0 to 20 %  |
| Moderately resistant (MR) | 21 to 40 % |
| Susceptible (S)           | 41 to 60 % |
| Highly susceptible (HS)   | >60 %      |

RESULTS AND DISCUSSION

Natural epiphytic screening of 120 cucumber genotypes (15 parents and 105 crosses) along with

susceptible check Swarna Agethi was carried out during the *rabi* season of 2019 at Horticultural Research Station experimental fields of Department of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru. All the parents and their hybrids along with a check were screened under field condition



Legend

- R : Resistant (0-20 % PDI) : 9
- MR: Moderately resistant (21-40 % PDI) : 4
- S : Susceptible (41-60 % PDI) : 28
- HS : Highly susceptible (>60 % PDI) : 80

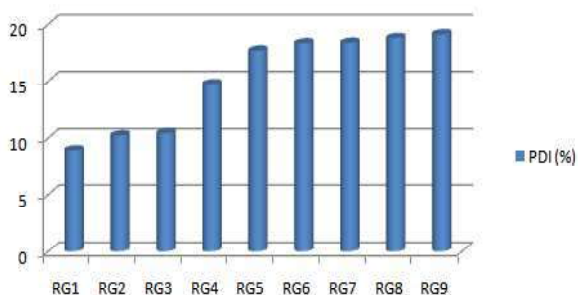
Fig. 1: Grouping of cucumber genotypes based on PDI of downy mildew disease under field condition

with artificial inoculation and scored data of disease has been converted to PDI. The PDI score of each genotype was employed to precisely identify the downy mildew resistant source from the cucumber germplasm screened. Among them, none of the genotypes was observed to be immune to downy mildew pathogen.

The average PDI of genotypes ranged from 8.94 to 74.28. Among 121 genotypes (including check) screened, the lowest average PDI was expressed by genotypes JB/11-028-595504 (10.42 %) and SKY/AC-270-613481 (14.72 %) among the parents and SKY/AC-316-613484 × JB/11-028-595504 (8.94 %), SKY/AC-251-613477 × SKY/AC-270-613481 (10.23 %), SKY/AC-270-613481 × JB/11-205-595510 (17.60 %), SKY/AC-251-613477 × JB/11-205-595510 (18.35 %), KP/SC-1494-613474 × JJK/10-601-595518 (18.39 %), SKY/AC-270-613481 × JB/11-217-595512 (18.80 %) and KP/SC-1494-613474 × JS/06-01-541367 (19.17 %) among the crosses showed resistance reaction (Fig. 2). Four crosses *viz.*, SKY/AC-251-613477 × JB/11-197-613470 (25.42 %), SKY/AC-319-613485 × Tripura local (35.54 %), SKY/AC-251-

613477 × JB/11-091-613462 (35.61 %) and SKY/AC-270-613481 × JB/11-197-613470 (38.18 %) showed moderate resistance to downy mildew disease with PDI in the range of 21 to 40 per cent (Fig. 3). Twenty-eight genotypes recorded susceptibility with PDI in the range of 41 to 60 per cent and 80 genotypes with > 60 PDI were highly susceptible to downy mildew disease (Table3 and Fig. 1).

**PDI (%)**

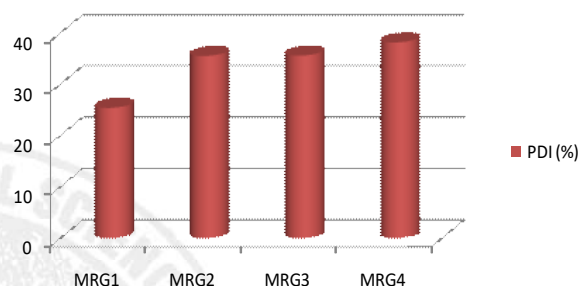


**Legend**

- RG<sub>1</sub> : SKY/AC-316-613484 × JB/11-028-595504
- RG<sub>2</sub> : SKY/AC-251-613477 × SKY/AC-270-613481
- RG<sub>3</sub> : JB/11-028-595504
- RG<sub>4</sub> : SKY/AC-270-613481
- RG<sub>5</sub> : SKY/AC-270-613481 × JB/11-205-595510
- RG<sub>6</sub> : SKY/AC-251-613477 × JB/11-205-595510
- RG<sub>7</sub> : KP/SC-1494-613474 × JJK/10-601-595518
- RG<sub>8</sub> : SKY/AC-270-613481 × JB/11-217-595512
- RG<sub>9</sub> : KP/SC-1494-613474 × JS/06-01-541367

Fig. 2 : Resistance group of cucumber genotypes based on PDI of downy mildew disease

**PDI (%)**



**Legend**

- MRG<sub>1</sub> : SKY/AC-251-613477 × JB/11-197-613470
- MRG<sub>2</sub> : SKY/AC-319-613485 × Tripura local
- MRG<sub>3</sub> : SKY/AC-251-613477 × JB/11-091-613462
- MRG<sub>4</sub> : SKY/AC-270-613481 × JB/11-197-613470

Fig. 3 : Moderately resistance group of cucumber genotypes based on PDI of downy mildew disease

Twenty four genotypes *viz.*, SKY/AC-265-613480, JJK/10-601-595518, JB/11-197-613470, SKY/AC-251-613477 × SKY/AC-319-613485, SKY/AC-251-613477 × JB/11-217-595512, SKY/AC-265-613480 × JJK/10-601-595518, SKY/AC-265-613480 × JB/11-217-595512, SKY/AC-265-613480 × Tripura local, SKY/AC-270-613481 × KP/SC-1494-613474, SKY/AC-270-613481 × Tripura local, SKY/AC-319-613485 × JB/11-028-595504, KP/SC-1494-613474 × JB/11-028-595504, JJK/10-601-595518 × JB/11-197-613470, JJK/10-601-595518 × JB/11-205-595510, JS/06-01-541367 × JB/11-091-613462, JS/06-01-541367 × JB/11-197-613470, JS/06-01-541367 × JB/11-205-595510, JB/11-028-595504 × JB/11-217-595512, JB/11-028-595504 × Tripura local, JB/11-091-613462 × JB/11-197-613470, JB/11-091-613462 × JB/11-217-595512, JB/11-205-595510 × Tripura local, JB/11-217-595512 × Tripura local and Swarna Agethircorded a PDI of 100 per cent at final observation. Hence all these were highly susceptible to downy mildew. Parent JB/11-197-

TABLE 3  
Grouping of cucumber genotypes based on PDI of downy mildew disease under field condition

| Disease reaction group | Genotypes                             |                                       | No. of genotypes/group                |                                       |
|------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| R                      | Parents                               | SKY/AC-270-613481                     | JB/11-028-595504                      | 9                                     |
|                        | Crosses                               | SKY/AC-251-613477 × SKY/AC-270-613481 | SKY/AC-316-613484 × JB/11-028-595504  |                                       |
|                        |                                       | SKY/AC-251-613477 × JB/11-205-595510  | KP/SC-1494-613474 × JJK/10-601-595518 |                                       |
|                        |                                       | SKY/AC-270-613481 × JB/11-205-595510  | KP/SC-1494-613474 × JS/06-01-541367   |                                       |
|                        |                                       | SKY/AC-270-613481 × JB/11-217-595512  |                                       |                                       |
| MR                     | Crosses                               | SKY/AC-251-613477 × JB/11-091-613462  | SKY/AC-270-613481 × JB/11-197-613470  | 4                                     |
|                        |                                       | SKY/AC-251-613477 × JB/11-197-613470  | SKY/AC-319-613485 × Tripura local     |                                       |
| S                      | Parents                               | KP/SC-1494-613474                     | JB/11-217-595512                      | 28                                    |
|                        | Crosses                               | SKY/AC-247-613476 × SKY/AC-316-613484 | SKY/AC-316-613484 × JJK/10-601-595518 |                                       |
|                        |                                       | SKY/AC-247-613476 × SKY/AC-319-613485 | SKY/AC-316-613484 × JB/11-205-595510  |                                       |
|                        |                                       | SKY/AC-247-613476 × JB/11-197-613470  | SKY/AC-316-613484 × JB/11-217-595512  |                                       |
|                        |                                       | SKY/AC-247-613476 × JB/11-217-595512  | SKY/AC-319-613485 × KP/SC-1494-613474 |                                       |
|                        |                                       | SKY/AC-251-613477 × SKY/AC-265-613480 | SKY/AC-319-613485 × JS/06-01-541367   |                                       |
|                        |                                       | SKY/AC-251-613477 × SKY/AC-316-613484 | SKY/AC-319-613485 × JB/11-205-595510  |                                       |
|                        |                                       | SKY/AC-251-613477 × JS/06-01-541367   | KP/SC-1494-613474 × JB/11-091-613462  |                                       |
|                        |                                       | SKY/AC-251-613477 × Tripura local     | KP/SC-1494-613474 × Tripura local     |                                       |
|                        |                                       | SKY/AC-265-613480 × SKY/AC-316-613484 | JJK/10-601-595518 × JS/06-01-541367   |                                       |
|                        |                                       | SKY/AC-265-613480 × JB/11-028-595504  | JS/06-01-541367 × JB/11-028-595504    |                                       |
|                        |                                       | SKY/AC-265-613480 × JB/11-091-613462  | JS/06-01-541367 × Tripura local       |                                       |
|                        |                                       | SKY/AC-270-613481 × JB/11-028-595504  | JB/11-028-595504 × JB/11-091-613462   |                                       |
|                        |                                       | SKY/AC-316-613484 × SKY/AC-319-613485 | JB/11-197-613470 × JB/11-217-595512   |                                       |
|                        |                                       | HS                                    | Parents                               |                                       |
| SKY/AC-251-613477      | JB/11-091-613462                      |                                       |                                       |                                       |
| SKY/AC-265-613480      | JB/11-197-613470                      |                                       |                                       |                                       |
| SKY/AC-316-613484      | JB/11-205-595510                      |                                       |                                       |                                       |
| SKY/AC-319-613485      | Tripura local                         |                                       |                                       |                                       |
| JJK/10-601-595518      |                                       |                                       |                                       |                                       |
| Crosses                | SKY/AC-247-613476 × SKY/AC-251-613477 |                                       |                                       | SKY/AC-265-613480 × SKY/AC-270-613481 |
|                        | SKY/AC-247-613476 × SKY/AC-265-613480 |                                       | SKY/AC-265-613480 × SKY/AC-319-613485 |                                       |
|                        | SKY/AC-247-613476 × SKY/AC-270-613481 |                                       | SKY/AC-265-613480 × KP/SC-1494-613474 |                                       |
|                        | SKY/AC-247-613476 × KP/SC-1494-613474 |                                       | SKY/AC-265-613480 × JJK/10-601-595518 |                                       |
|                        | SKY/AC-247-613476 × JJK/10-601-595518 |                                       | SKY/AC-265-613480 × JS/06-01-541367   |                                       |
|                        | SKY/AC-247-613476 × JS/06-01-541367   |                                       | SKY/AC-265-613480 × JB/11-197-613470  |                                       |
|                        | SKY/AC-247-613476 × JB/11-028-595504  |                                       | SKY/AC-265-613480 × JB/11-205-595510  |                                       |
|                        | SKY/AC-247-613476 × JB/11-091-613462  |                                       | SKY/AC-265-613480 × JB/11-217-595512  |                                       |
|                        | SKY/AC-247-613476 × JB/11-205-595510  |                                       | SKY/AC-265-613480 × Tripura local     |                                       |
|                        | SKY/AC-247-613476 × Tripura local     |                                       | SKY/AC-270-613481 × SKY/AC-316-613484 |                                       |
|                        | SKY/AC-251-613477 × SKY/AC-319-613485 |                                       | SKY/AC-270-613481 × SKY/AC-319-613485 |                                       |
|                        | SKY/AC-251-613477 × KP/SC-1494-613474 |                                       | SKY/AC-270-613481 × KP/SC-1494-613474 |                                       |
|                        | SKY/AC-251-613477 × JJK/10-601-595518 |                                       | SKY/AC-270-613481 × JJK/10-601-595518 |                                       |

| Disease reaction group | Genotypes                             | No. of genotypes/group               |
|------------------------|---------------------------------------|--------------------------------------|
|                        | SKY/AC-251-613477 × JB/11-028-595504  | SKY/AC-270-613481 × JS/06-01-541367  |
|                        | SKY/AC-251-613477 × JB/11-217-595512  | SKY/AC-270-613481 × JB/11-091-613462 |
|                        | SKY/AC-270-613481 × Tripura local     | JJK/10-601-595518 × Tripura local    |
|                        | SKY/AC-316-613484 × KP/SC-1494-613474 | JS/06-01-541367 × JB/11-091-613462   |
|                        | SKY/AC-316-613484 × JS/06-01-541367   | JS/06-01-541367 × JB/11-197-613470   |
|                        | SKY/AC-316-613484 × JB/11-091-613462  | JS/06-01-541367 × JB/11-205-595510   |
|                        | SKY/AC-316-613484 × JB/11-197-613470  | JS/06-01-541367 × JB/11-217-595512   |
|                        | SKY/AC-316-613484 × Tripura local     | JB/11-028-595504 × JB/11-197-613470  |
|                        | SKY/AC-319-613485 × JJK/10-601-595518 | JB/11-028-595504 × JB/11-205-595510  |
|                        | SKY/AC-319-613485 × JB/11-028-595504  | JB/11-028-595504 × JB/11-217-595512  |
|                        | SKY/AC-319-613485 × JB/11-091-613462  | JB/11-028-595504 × Tripura local     |
|                        | SKY/AC-319-613485 × JB/11-197-613470  | JB/11-091-613462 × JB/11-197-613470  |
|                        | SKY/AC-319-613485 × JB/11-217-595512  | JB/11-091-613462 × JB/11-205-595510  |
|                        | KP/SC-1494-613474 × JB/11-028-595504  | JB/11-091-613462 × JB/11-217-595512  |
|                        | KP/SC-1494-613474 × JB/11-197-613470  | JB/11-091-613462 × Tripura local     |
|                        | KP/SC-1494-613474 × JB/11-205-595510  | JB/11-197-613470 × JB/11-205-595510  |
|                        | KP/SC-1494-613474 × JB/11-217-595512  | JB/11-197-613470 × Tripura local     |
|                        | JJK/10-601-595518 × JB/11-028-595504  | JB/11-205-595510 × JB/11-217-595512  |
|                        | JJK/10-601-595518 × JB/11-091-613462  | JB/11-205-595510 × Tripura local     |
|                        | JJK/10-601-595518 × JB/11-197-613470  | JB/11-217-595512 × Tripura local     |
|                        | JJK/10-601-595518 × JB/11-205-595510  | Swarna Agethi                        |
|                        | JJK/10-601-595518 × JB/11-217-595512  |                                      |

#### Legend

|  |              |                                     |                |
|--|--------------|-------------------------------------|----------------|
| R: Resistant (0-20 % PDI)              | : 9 (7.44 %) | S: Susceptible (41-60 % PDI)        | : 28 (23.14 %) |
| MR: Moderately resistant (21-40 % PDI) | : 4 (3.31 %) | HS: Highly susceptible (> 60 % PDI) | : 80 (66.12 %) |

613470 and cross JS/06-01-541367 × JB/11-197-613470 showed highly susceptible reaction with maximum average PDI of 72.58 and 74.28, respectively, which was higher than susceptible check Swarna Agathi (68.92 %) (Fig. 1).

The response of resistant genotypes in cucumber is characterized by the recognition (compatibility / incompatibility) of a host plant by its *oomycetes pathogen*. Eckardt (2004) and Taler *et al.* (2004) found that, the pathogen failed to penetrate into the mesophyll cells of the resistant host due to massive accumulation of callose along the host cell walls as well as the inner wall surface of the mycelia. Lebeda (1992) reported that, *C. metuliferus* and other nine *Cucumis* species showed resistance to *P. cubensis*

and also suggested the use of wild *Cucumis* species to broaden the genetic base for cucumber resistance breeding to downy mildew. Our findings were similar to those reported from Poland and North Carolina from 2005 to 2009 who found that only 20 of 1300 tested cucumber cultigens were highly resistant to downy mildew and none was immune (Call, 2012). Bhutia (2015) screened 114 cucumber genotypes and the results revealed that 10 were resistant, 18 were moderately resistant, 37 moderately susceptible and 49 genotypes showed susceptibility. Cohen *et al.* (2000), Lebeda and Urban (2007), Crisswell *et al.* (2008), Wan *et al.* (2010), Pitchaimuthu *et al.* (2012), Innark *et al.* (2014), Holdsworth *et al.*, (2014) and Ranjan *et al.* (2015) also reported for the downy mildew resistance sources in cucumber.

The information gained out of this investigation based on the screening under natural epiphytic condition confirmed that the genotypes JB/11-028-595504 and SKY/AC-270-613481 among the parents and SKY/AC-316-613484 × JB/11-028-595504, SKY/AC-251-613477 × SKY/AC-270-613481, SKY/AC-270-613481 × JB/11-205-595510, SKY/AC-251-613477 × JB/11-205-595510, KP/SC-1494-613474 × JJK/10-601-595518, SKY/AC-270-613481 × JB/11-217-595512 and KP/SC-1494-613474 × JS/06-01-541367 among the crosses 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.

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