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Mining the source of resistance for downy mildew and gummy stem blight in bottle gourd (*Lagenaria siceraria*) accessions

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ABSTRACT

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] is an important vegetable crop widely cultivated during summer and *kharif* season throughout the country. Seventeen advance lines of bottle gourd were artificially screened for downy mildew. Among them, only VRBG-12 showed resistance with lowest PDI value of 6.5%. Three lines (VRBG-26, VRBG-47 and VRBG-17) were moderately resistant (15.7-21.7%), four (VRBG-11, VRBG-20, VRBG-49 and VRBG-56) moderately susceptible (30.4-40.2%), five (VRBG-52, VRBG-5, VRBG-10, VRBG-66 and VRBG-53) susceptible (59.3-69.2%) and the remaining four lines (VRBG-33, VRBG-48, DVBG-01 and VRBG-61) were highly susceptible (79.2-90.75%). Fifty one accessions were screened against gummy stem blight and none were found immune/resistant under field condition. Out of 51 lines, least severity (28.8%) was observed in VRBG-556, 13 were moderately susceptible with the PDI ranging between 28.80 to 49.44, 32 were found to be susceptible (50.4-74.4%) and 6 highly susceptible (75.2-87.2%). These potential genotypes can be used further in breeding programs of bottle gourd against downy mildew and gummy stem blight.

Key words: Bottle gourd, Downy mildew, Gummy stem blight, PDI, Resistance, Screening

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] is one of the most popular Cucurbitaceous vegetable crop grown in India and shows wide adaptability and variability for different traits due to its Indian sub-continent origin. It is mainly grown in summer and rainy season; however, few local land races are well adapted to winter season (Ram *et al*. 2007). Now-a-day, it is becoming popular for several health benefits. The fresh juice of the fruit has medicinal value due to its laxative, digestive and anti-constipation property and used to cure various ailments like flatulence, diabetes mellitus, hypertension, liver diseases as well as acts as a diuretic (Ghule *et al.* 2007). The seeds are rich in essential amino acids and oil.Yield of the crop is affected by several biotic constraints. Among fungal diseases, downy mildew caused by *Pseudoperonospora cubensis* and gummy stem blight caused by *Didymella bryoniae* are becoming the most serious diseases of bottle gourd causing severe yield losses and deterioration of fruit quality (Harika *et al*. 2012).

Downy mildew which is caused by *P. cubensis* starts with the development of chlorotic lesions on the adaxial leaf surface. Generally, lesions have angular appearance and restricted by the leaf vein. As infection progresses, the chlorotic lesions expand and may become necrotic which gets further accelerated in hot and dry weather. As the disease advances, entire leaves may die within few days, as lesions expand and coalesce. *P. cubensis* is an obligate parasite and with the rare exception of oospore production, can survive and reproduce only on living host tissue. *P. cubensis* reproduces both sexually and asexually (Cohen and Rubin 2012), and sporangia of *P. cubensis* may be dispersed for long distances rapidly. Due to the presence of both mating systems, prolonged survival ability round the year in harsh conditions, long distance dispersal of sporangia by the wind and ability to overwinter on the wild and cultivated hosts, the evolutionary potential of the pathogen is high (McDonald and Linde 2002) and hence would appear to have a high potential for rapidly overcoming host resistance and fungicide resistance (Lebeda *et al.* 2013).

Gummy stem blight disease caused by *Didymella bryoniae,* is an air-borne, seed-borne, and soil-borne pathogen. Being a facultative necrotrophic fungus, it persists on crop residue even at extreme temperatures. The disease causes crown blight, extensive defoliation and fruit rot, which can result in severe losses in the field. Initially, fungus infects on the stem parts which develop brown or

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black stripes and become girdled and withered in advance stages leading to exudation of gummy secretion that aids favourable condition for growth and secondary infection of the fungus. Foliar lesions begin as small circular spots with dark tan edges and coalesce during severe infection the plant dies.

Fungicides have been targeted to control both downy mildew (Lebda and Cohen 2012) and stem bleeding (Afroz *et al.* 2012) but results are discouraging. Moreover, the excess uses of fungicides are not only detrimental to the environment but are a key factor of developing fungicide resistance of the pathogen. Hence, host plant resistance is considered as the best alternative for controlling the disease and can be achieved by identification of resistance source against the pathogen (Jadhav and Sharma 1983). Resistant sources may be present in the land races, indigenous cultivars, semi-wild relatives, folk cultivars, and allied species of the crop. Keeping this at the backdrop, the present study was undertaken to evaluate and identify the effective source (s) of resistance to *D. bryoniae* and *P. cubensis* by screening the available bottle gourd germplasm collections of ICAR-IIVR.

MATERIALS AND METHODS

Both, artificial screening of downy mildew and field screening of gummy stem blight were conducted during 2015, at the ICAR- Indian Institute of Vegetable Research, Varanasi, India (25.3176° N, 82.9739° E). All bottle gourd germplasm used in screening were available at ICAR-IIVR, Varanasi which were collected from different regions of the India.

For downy mildew, artificial screening was done in 17 genotypes of bottle gourd, viz. DVBG-1, VRBG-5, VRBG-10, VRBG-11, VRBG-12, VRBG-17, VRBG-20, VRBG-26, VRBG-33, VRBG-47, VRBG-48, VRBG-49, VRBG-52, VRBG-53, VRBG-56,VRBG-61 and VRBG-66. The plants of all genotypes were raised in poly bags inside the green house. Three plants per germplasm were maintained as per assay and repeated two times.

For gummy stem blight, a total of 51 germplasm/ cultivars were screened under natural field conditions. Experiment was laid down under randomized complete-block design with three replications and standard cultural practices were followed for crop production. Each genotype in each replication had 10 plants at a row to row spacing of 1.8 m and plant to plant spacing of 0.8 m.

Leaves infected with downy mildew were collected in the morning, soaked in distilled water and rubbed gently to extricate sporangia. The sporangial suspension was filtered through two layers of cheesecloth and the concentration was determined with the use of a haemocytometer (Improved Neubauer, Rohem Counting Chamber, India). The final concentration of suspension was adjusted to 1.1×10^3 sporangia/ml. At the two-true leaf stage, the plants were artificially inoculated by spraying sporangial suspension prepared in sterile water with an atomizer. The adaxial and abaxial surfaces of the leaves were sprayed with the inoculum suspensions until run-off. Later, inoculated plants were placed in the dark growth chamber at 25°C with high relative humidity (100% RH) for 21 hr to maximize sporangial sporulation. Subsequently, they were transferred to the greenhouse under ambient conditions of 26-28˚C and 94-98% relative humidity. After 7 days, the disease reactions on the host plants were recorded. A set of the host plants without fungal inoculation were used as a control. Three leaves from each plant were scored for disease using 0 to 4 rating scale (0 = No symptoms, 1= 1-10%, 2= 11-25%, 3= 26-50%, 4= 51% and above) of Thind *et al*. (1991) with some modifications.

The Per cent Disease Index (PDI) was calculated based on the observation using the formula

$$\text{PDI} = \frac{\text{Sum of all numerical values (scores)}}{\text{(Number of leaves counted} \times \text{maximum disease rating)}} \times 100 \quad (1)$$

Fifty one bottle gourd accessions were screened for the resistance against the gummy stem blight. Five plants of each germplasm were tagged randomly and data were recorded after 60 days of sowing and placed under different categories on the basis of disease intensity.

Scoring of disease was done on 5 leaves each in 5 plants using an index of necrotic tissue by the following 0-5 rating scale (0: no infection visible; 1: >0% and ≤20% leaf area necrotic; 2: >20% and ≤40% leaf area necrotic; 3: >40% and ≤60% leaf area necrotic; 4: >60% and ≤80% leaf area necrotic and 5: >80% leaf area necrotic) as per McGrath *et al*. (1993). Per cent Disease Index (PDI) was calculated based on field scoring data by using the formula given above.Based on the PDI calculated, germplasm for both the diseases studied were categorized as: 0 = Immune; 1-10% = Resistant; 11-25% = moderately resistant; 26-50% = moderately susceptible; 51-75% = susceptible; and >75% = highly susceptible described by Pandey *et al*. (2005) with some modifications.

RESULTS AND DISCUSSION

Artificial screening of downy mildew

The study clearly revealed that out of seventeen advanced lines of bottle gourd artificially screened under controlled conditions against downy mildew, only one line VRBG-12 showed resistant reaction with lowest PDI value of 6.5 (Table 1). Three lines, viz. VRBG-26, VRBG-47 and VRBG-17 were moderately resistant exhibiting PDI value ranging from 15.7 to 21.1%. Four lines (VRBG-11, VRBG-20, VRBG-49 and VRBG-56) were moderately susceptible and five lines (VRBG-52, VRBG-5, VRBG-10, VRBG-66 and VRBG-53) were susceptible. Similar finding were reported by Harika *et al.* (2012) who screened twenty five genotype of bottle gourd and found three (Kaveri, Gutkha and Sarika) showing resistance against downy mildew. Initially, symptoms appeared as small water soaked lesions with sporulation on the underside of

Table 1 Disease resistance reaction against downy mildew (*P. cubensis*) in bottle gourd genotypes under artificial screening.

Disease resistance reaction	Germplasm accessions (PDI %)
Resistant	VRBG-12 (6.5)
Moderately resistant	VRBG-17 (21.7), VRBG-26 (15.7), VRBG-47 (19.2)
Moderately susceptible	VRBG-11 (33.7), VRBG-20 (35.4), VRBG-49 (40.2), VRBG-56 (30.4)
Susceptible	VRBG-5 (59.5), VRBG-10 (69.2), VRBG-52 (61.7), VRBG-53 (59.3), VRBG-66 (65.71)
Highly susceptible	DVBG-1 (90.75), VRBG-33 (81.7), VRBG-48 (85.8), VRBG-61 (79.2)

leaves. Lesions were angular, being bound by leaf veins, and turning chlorotic to varying degrees. Chlorotic lesions ultimately turned necrotic. Eventually the entire leaf will become necrotic and die. Symptoms varied depending on relative susceptibility of the cultigens whereas lesions were more circular and not restricted by the leaf veins (Cespedes-Sanchez *et al.* 2015). Similar to the observation by Call *et al.* (2012) in cucumber, present study also showed that the most susceptible cultigens or lines were highly chlorotic and necrotic, while the most resistant cultigens exhibited a hypersensitive response (HR) with small necrotic or chlorotic flecks and sparse sporulation. The HR type resistance was first described by Barnes and Epps (1954) in cucumber genotype PI197087, from a single resistance gene *dm-1*. However, cultigens responded differently to disease across plant developmental stages. In general, older plants had more disease symptoms, even those classified as resistant while some cultigens held their resistance even at late developmental stages which may be due to their rapid, indeterminate growth, that allows them to outgrow the disease (Vanden Langenberg and Wehner 2016). Anatomical and cytological factors also contribute to the level of host resistance and Habdas *et al*. (1996) found that the epidermis in all highly resistant cucumber accessions was covered with a thick cuticle that also partly covered the stomata. Efforts are directed towards sequencing of the cucumber genome which will provide the opportunity to acquire, in a short time frame, reliable and accurate information on resistance genes to downy mildew for the *cucurbits*, their location, construction, and function (Olczak-Woltman *et al*. 2011). The sequenced genome enables identification of the gene sequences, which, in turn, will enable the elucidation of the resistance mechanism and the metabolic pathways activated by these genes. This will make possible the determination of the conditions in which the genes are activated and of the metabolic pathways that are involved in the expression of resistance.

Field screening of gummy stem blight

On bottle gourd, symptoms such as necrosis on stem with exudation of gummy fluid and irregular brown lesions towards the margin of the leaves were seen. The lesions later extended towards the centre of the leaf and were surrounded by yellow halo. Gummy exudation on the stem eventually caused drying of the entire plants. Blackpycnidia were observed on the infected dried stem portion. Similar symptoms were described by Keinath (2013) on the cucurbitaceous crops. Occurrence of this disease was severe during *kharif*-2015 at the ICAR-IIVR farm with an average disease severity of 57%. Incidence was found to be maximum during the post rainy season coupled with high humidity (84.89%) and minimum temperature (30.31ºC) (Table 2). This finding was in corroboration with the findings of Van Steekelenburg (1985) that infection caused by *D. bryoniae* seemed to depend on relative humidity, with more infection occurring at 95% than at 50%.

In the present study, out of 51 germplasm screened under field conditions against the pathogen, none of the germplasm lines showed resistance under field conditions. Thirteen lines were grouped under the category of moderately susceptible with the percent disease index ranging between 28.8 to 49.44. Least severity was observed in VRBG-556 (28.8) followed by VRBG-52 (34.4) and VRBG-41 (36.0). Out of remaining germplasm lines, 32 were found to be susceptible and 6 were highly susceptible (Table 3). Similarly, germplasm of muskmelon were screened by McGrath *et al.* (1993) under artificial conditions and two accessions PI266935 and PI266934 were highly resistant to gummy stem blight. Maheshwari *et al.* (2015) screened seventeen genotypes of bottle gourd for resistance and none of them were found resistant against *Cercospora* leaf spot. In the present study, no resistant sources could be identified among the germplasm screened in bottle gourd. In earlier reports, where 49 cucumber lines evaluated for gummy blight resistance under field conditions, Homegreen 2 and PI 200818 were found resistant. Among the phenotypic character, earliness in fruit maturity was positively correlated with susceptibility to gummy stem blight (Wyszogrodzka *et al*. 1986). In *Cucumis melo*, root stocks RS 841, P 360, ES 99-13 and Elsi were found resistant to gummy stem blight with the absence of crown lesions and low leaf disease index among the 8 root stocks evaluated under artificial screening in Italy (Crino *et al*. 2007).

Table 2 Meteorological data of ICAR-IIVR farm during the field screening of bottle gourd germplasm in 2015

Month	Temperature (ºC)		Humidity (%)		Rain (mm)
	Max.	Min.	Max.	Min.	
June	40.84	30.05	70.5	42.11	9.83
July	34.15	27.32	82.54	66.96	13.81
August	34.11	27.19	83.94	65.09	5.37
September	36.22	27.13	85.96	51.43	0.43
October	34.48	22.23	85.61	46.54	0.54
November	30.31	17.7	84.89	44.29	0.42

Table 3 Disease resistance reactions against gummy stem blight in bottle gourd germplasm under field conditions

Disease resistance reaction	Germplasm accessions (PDI %)
Resistant and moderately resistant	None
Moderately susceptible	VRBG-47 (49.4), VRBG-14 (48.5), DVBG-1 (47.6), VRBG-6 (47.2), VRBG-62 (47.2), VRBG-2 (44.2), IC-594588 (42.4), VRBG-27 (41.6), VRBG-20 (40.8), PusaSantusti (40), VRBG-41 (36), VRBG-52 (34.4), VRBG-556 (28.8)
Susceptible	VRBG-48 (74.4), VRBG-46 (70.4), DVBG-2 (70.4), KL-4 (69.2), VRBG-50 (68.8), VRBG-67 (67.2), VRBG 6 (65.6), VRBG-5 (64.2), VRBG-1556 (62.4), VRBG-8 (61.6), VRBG-64 (61.6), VRBG-66 (61.1), VRBG-57 (59.2), VRBG-35 (58.4), DRW-67 (56.8), VRBG-53 (56.8), Pusa Sandesh (56.0), VRBG-61 (55.7), VRBG-33 (55.6), VRBG-104 (54.8), VRBG-63 (54.4), EC-750698 (53.6), VRBG-12 (52.0), VRBG-548 (52.0), VRBG-61-3 (51.6), VRBG-1 (51.3), VRBG-4 (51.2), VRBG-22 (51.2), VRBG-45 (51.2), VRBG-15-1 (51.2), VRBG-15-2 (50.4), VRBG-17 (50.4)
Highly susceptible	VRBG-59 (87.2), IC594549 (87.2), VRBG-10 (83.2), VRBG-3 (80), VRBG-18 (78.4), VRBG-11 (75.2)

Line VRBG-12 was identified as resistant and VRBG-26, VRBG-47 and VRBG-17 as moderately resistant to downy mildew. The resistant and moderately resistant lines can be used in further resistant breeding programme against the downy mildew of bottle gourd. In addition, highly susceptible lines were also identified and can be used as susceptible check in further research programme.

None of the 51 lines screened in the study expressed resistance to gummy stem blight. However, 13 lines as moderately susceptible, 32 as susceptible and 6 as highly susceptible were identified for use in further studies. Though several strategies have been demonstrated for the management of gummy stem blight disease on cucurbits, crop resistance is the most effective tool for its efficient and economical management. Further screening should be intensified for the identification of resistant progenies.In addition to exploitation of resistance sources in breeding programmes, these will be better utilized in grafting.

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