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Original Research Paper

Screening for resistance to gummy stem blight, powdery mildew and cucumber green mottle mosaic virus in bottle gourd [*Lagenaria siceraria* (Mol.) Standl.]

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ABSTRACT

Investigations were carried out to identify the source of resistance in 67 bottle gourd genotypes for gummy stem blight, powdery mildew and cucumber green mottle mosaic virus (CGMMV) diseases, under natural field epiphytotic conditions. The genotypes BG-95 (105.13), BG-114-1 (131.04), BG-114-3 (208.81) and BG-77-6-1 (221.80) were resistant for gummy stem blight with low AUDPC values, while, BG-125-5 (232.22), BG-6-3 found (250.00), BG-125-4 (307.78), BG-8-1 (308.89), BG-125-2 (311.11) and BG-124-2 (423.33) resistant with low AUDPC values for powdery mildew. Further, the two genotypes such as IIHR-19 and BG-131 showed field level resistance against CGMMV. The selected genotypes based on field evaluation were subjected for artificial screening under glass house conditions. The genotypes, recorded consistent resistant reactions were BG-114-3, BG-77-6-1 and BG-95 for gummy stem blight disease and BG-6-3, BG-8-1, BG-125-4 and BG-125-2 for powdery mildew. The stable and durable source of resistance identified for gummy stem blight and powdery mildew in bottle gourd genotypes will hasten the process of developing resistance varieties in bottle gourd.

Keywords : Bottle gourd, CGMMV, gummy stem blight, powdery mildew

INTRODUCTION

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] is widely cultivated cucurbitaceous vegetable throughout India and also in various parts of Asia. Its economical part is fruit which is harvested in young stage and it is available in the market throughout the year. Fruits are rich source of minerals and vitamins and also have medicinal value (Thakur *et al.,* 2015). In India, production of bottle gourd during 2021-22 was 3742.71 (000 MT) with an area of 1.58 mha. Major bottle gourd producing states in India are Bihar, Uttar Pradesh, Madhya Pradesh, Haryana and Chhattisgarh (Anona, 2022). Powdery mildew, gummy stem blight, anthracnose and cucumber green mottle mosaic virus (CGMV) are important diseases limiting bottle gourd production in India (Vasudeva and Nariani, 1952; Ullasa and Amin, 1986; Nayak *et al.,* 2017; Kousik *et al.,* 2018; Dombrovsky *et al.,* 2017).

Gummy stem blight is a devastating disease on cucurbits worldwide. In India, this disease is reported on muskmelon (Sudisha *et al.,* 2004), watermelon (Sohi and Om-Prakash, 1972), pickling cucumbers (Garampalli *et al.,* 2016) and ridge gourd (Bhat *et al.,* 2010). Cucurbit gummy stem blight is reported to be caused by *Didymella bryoniae* (*Stagonosporopsis cucurbitacearum*), *Stagonosporopsis caricae* and *Stagonosporopsis citrulli* (Stewart *et al.,* 2015). *Stagonosporopsis caricae* and *S. citrulli* were associated with gummy stem blight epidemics in gherkin cucumber (*Cucumis sativus*) in Karnataka and *Didymella bryoniae* on *Sechium edule* and *Citrullus lanatus* (Sohi and Om-Prakash, 1972). Two pathogenic fungi *Golovinomyces cichoracearum (Erysiphe cichoracearum*) and *Podosphaera xanthii (Sphaerotheca fuliginea)* are reported to cause powdery mildew disease (Cohen *et al.,* 2004). In India, *Podosphaera xanthii* is reported to cause powdery mildew disease on bottle gourd (Nayak *et al.,* 2017).

Few attempts were made by Indian researcher to identify resistance sources against gummy stem blight and powdery mildew, however, no stable resistance sources could be identified (Maheshwari *et al.,* 2012; Bhardwaj *et al.,* 2018). Hitherto, no attempts have been made for identification of resistance against CGMV in bottle gourd in India. In this context, there is a need to identify stable sources for resistance against these diseases for the development of bottle

gourd varieties/hybrids with good horticultural and resistance traits.

With this background, field experiments were conducted with an objective to identify gummy stem blight, powdery mildew and CGMMV resistance sources in bottle gourd breeding material.

MATERIALS AND METHODS

The experiments were conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru, India during of 2017-2018 and 2018-19. The experimental site was located at an altitude of 890 m above mean sea level with coordinates of 13° 7'N, 77° 29'E. The site receives an average annual rainfall of 757 mm. In both the years the plots were located at same sites to ensure the build of soil inoculums. Based on PDI calculated, genotypes were categorized as: 0 = immune; 1-10% = resistant; 11-25% = moderately resistant; 26-50% = moderately susceptible; 51-75%=susceptible; and >75%= highly susceptible as described by Bhardwaj *et al.* (2018).

Plant material

Bottle gourd genotypes comprising of 67 inbred lines were evaluated during *kharif* season for gummy stem blight, *rabi* season for powdery mildew and CGMMV during summer of 2017-18 and 2018-19. The field screening experiment was laid out in randomized block design with two replications. Eighteen plants of each entry under study were transplanted on raised beds with polythene mulching at a spacing of 1.60 m × 0.9 m. Standard package of practice was followed to raise the crop under open field condition.

Disease assessment and identification

Observations on disease incidence and severity were recorded at weekly interval from the date of first initiation of diseases. Diseases were identified based on microscopic examination of symptomatic leaf and plant samples (n=50) at periodic intervals and morphological characterization.

Powdery mildew (PM) severity was assessed based on assessment key of 0-10 scale, where 0=no visible symptoms, 1=very sparse mycelia growth on leaves with very few to no visible conidia (0 to 3%), 2=4 to 6% of leaf area covered with PM and sparse development of conidia, 3=7 to 12% leaf area covered with abundant conidia, 4=13 to 25%, 5 = 26 to 50%, 6= 51 to 75%, 7=76 to 87% leaf area covered with abundant conidia, 8= 88 to 94%, 9= 95 to 97% and 10= 98 to 100% leaf area covered with abundant conidia and leaf dying or dead (Kousik *et al.,* 2008 & 2018). For artificial inoculation, spores from powdery mildew infected bottle gourd leaves were dusted on plants at two leaves stage. Dusting was made twice at one day interval. Disease rating was done on 0-10 scale (as described above) starting from one week after inoculation. Total of nine observations were made at 3 days interval.

Gummy stem blight scoring was done on 5 leaves and stem each in 5 plants using 0-9 rating scale as per Gusmini *et al*. (2005), where, 0=no symptoms, 1=yellowing on leaves (suspect of disease only), 2=moderate symptoms (<20% necrosis) on leaves only, 3=slight symptoms (21–45% necrosis) on leaves only, 4=severe symptoms (>45% necrosis) on leaves only, 5=some leaves dead, no symptoms on stem, 6=moderate symptoms (<20% necrosis) on leaves, with necrosis also on petioles and stem (<3 mm long), 7=slight symptoms (21–45% necrosis) on leaves, necrosis also on petioles and stem (3-5 mm long), 8=severe symptoms (45% necrosis) on leaves, necrosis also on petioles and stem (>5 mm long) and 9=plant dead.

In vitro screening for resistance against gummy stem blight

A highly virulent culture of *Staganosporopsis cucurbitacearum* from previous study of Mahapatra *et al*. (2020) was used. This culture was initially isolated from watermelon and had high virulence and aggressiveness on bottle gourd. The isolate was maintained by periodic culture on quarter strength potato dextrose agar media.

Virulence was maintained by periodic inoculation and reisolatiion from bottle gourd plants. Since, the isolate was poorly sporulating on different media assayed, mycelial plugs were used as inoculum in this evaluation. Briefly, 8 mm plugs were taken from margin of five days old actively growing culture on potato dextrose agar with a cork borer. Mycelium plugs were inoculated on to stem of five weeks old seedlings with 2 to 4 true leaves. Mycelial plugs were fastened with cello tape. Inoculated plants were kept in plant growth chamber with 12 hour light cycle set at temperature 28°C and relative humidity above 85%. Starting from five days after inoculation, up to 15 days, disease severity scoring was made by adopting

0-5 scale modified from Zhang *et al.* (1997), where 1=no damage, 2=expanding lesion on stem without girdling, 3=lesions with extensive girdling of stem, 4=withered stem, 5=dead seedling with stem shredding Area under disease progress curve was worked out as per equation (Wilcoxson *et al.,* 1975)

Per cent disease index (PDI) was calculated based on field scoring data by using the formula.

$$PDI = \frac{Sum\ of\ all\ disease\ rating}{Total\ no.of\ observations\ X\ Maximum\ disease\ grade}\ X\ 100$$

$$A = \sum_{i-1}^{K} \frac{1}{2(Si + Si - 1)} \times d$$

where, Si=disease severity at the end of week i, k = the number of successive evaluation of disease and d= interval between two evaluations.

In the present experiment, the 67 genotypes of bottle gourd were screened against CGMMV disease resistance under natural field conditions and with artificial screening under glasshouse conditions. Mechanical sap inoculation was carried out with Cucumber green mottle mosaic virus (G: Tobamovirus, F: Virgaviridae) infected leaf samples collected from ICAR-IIHR experimental plots. Viral sap was extracted with 0.05 M potassium phosphate buffer by grinding CGMMV infected leaves with an antioxidant 5 µl (β-mercapto ethanol). Viral sap was inoculated on 10-12 days old test seedlings of bottle gourd genotypes by smearing on the emerging leaves.

After 10 minutes, it was washed with distilled water. Inoculated plants were kept for observations under glass house conditions with insect proof net (20 mesh size) to prevent damage from possible glass house insects to the seedlings.

After 12-15 days of inoculation, symptomatic leaves were observed under Transmission Electron Microscopy (TEM) and captured the rigid rod virus particles indicating the presence of *Tobamovirus* particles. Further, symptomatic leaves were tested and confirmed with CGMMV specific immuno strips (Agdia^{R}). Test seedlings were assessed based on 0-5 scale (0: no symptoms, 1: initiation of greenish lesions and vein greening, 2: enlarged lesions and thickened vein greening, 3: blisters and mottling, 4: leaf distortion and 5: veinal distortion and loss of chlorophyll). Same assessment scale was used for field assessment of CGMMV severity.

RESULTS AND DISCUSSION

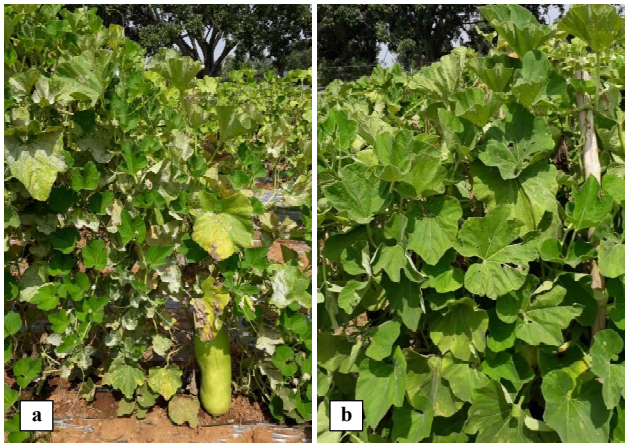
A total of 67 bottle gourd genotypes were subjected to field evaluation to identify source of resistance against powdery mildew, gummy stem blight and *CGMMV*. Among them, the genotypes; the genotypes BG-95 (105.13), BG-114-1 (131.04) BG-114-3 (208.81), and BG-77-6-1 (221.80) were found promising for gummy stem blight resistance with low AUDPC values and PDI (Table 1 and Fig. 1), whereas, BG-125-5 (232.22), BG-6-3 (250.00), BG-125-4 (307.78), BG-8-1 (308.89), BG-125-2 (311.11), and BG-124-2 (423.33) were found promising for powdery mildew with low disease index and AUDPC values (Table 2 and Fig. 2). The genotypes IIHR19 and BG131 were recorded field resistant to CGMMV (Table 3).

Table 1 : Bottle gourd genotypes field reaction against gummy stem blight

PDI (%)	Reaction	Genotype
0	Immune	-
1-10	Resistant	BG-95, BG-114-3 and BG-114-1
11-25	Moderately resistant	BG-77-6-1(221.80), BGAIC-6, BG-91, BG-108-2, BG-124-4, BG-124-2
26-50	Moderately susceptible	BG-23-5-6, BG-23-5-10, BG-136, BG-78, BG-135, BG-98, BG-98-3, BG-143, BG-67, BG-123-3-1, BG-62-1-1, BG-131, BG-114-46, BG-75-2, BG-108-1
51-75	Susceptible	BG-99, BG-47, BG-79, BG-18, BG-24, BG-108-2-3, BG-8-1, BG-139, BG-6, BG-125-5, BG-6-3, BG-141, BG-138, BG-118-3, BG-115-2, BGAIC-11, BG-125-4, BG-120-5, BG-125-2, BG-81, BG-104, BG-4, IIHR-22349, BG-61-10, BG-105, BG-23, BG-115-5b, BG-118-3-3, BG-62-1, BG-49, BG-49-5, BG-119-2, BG-122-5, BG-123-3, BG-44, BG-11, BG-39-112-1, BG-93, P.Lauki-8
>75	Highly susceptible	BG-64, BG-75, BG-108, IIHR-19

a) BG-64 (susceptible) b) BG-77-6-1(resistant)

Fig. 1 : Gummy stem blight disease reaction on bottle gourd genotypes



a) BG-11(susceptible) b) BG-124-2 (resistant)

Fig. 2 : Powdery mildew disease reaction on bottle gourd genotypes

Table 2 : Bottle gourd genotypes field reaction against powdery mildew disease

PDI (%)	Reaction	Genotype
0	Immune	-
1-10	Resistant	BG-6-3, BG-8-1, BG-125-4, BG-125-2, BG-124-2, BG-125-5
11-25	Moderately resistant	BG-64, BG-118-3, BG-114-3, BG-114-1, BG-115-2, BGAIC-11, BGAIC-6, BG-120-5, BG-81, BG-104, BG-4, IIHR-22349, BG-61-10, P.Lauki-8
26-50	Moderately susceptible	BG-105, BG-18, BG-23, BG-98, BG-6, BG-91, BG-115-5b, BG-118-3-3, BG-62-1, BG-49, BG-49-5, IIHR-19, BG-119-2, BG-122-5, BG-123-3, BG-44, BG-24,
51-75	Susceptible	BG-11, BG-39-112-1, BG-78, BG-67, BG-93, BG-95, BG-77-6-1, BG-108-2, BG-124-4, BG-23-5-6, BG-23-5-10, BG-136, BG-135, BG-98-3, BG-143, BG-123-3-1, BG-62-1-1, BG-131, BG-114-46, BG-75-2, BG-108-1, BG-99, BG-47, BG-79, BG-108-2-3, BG-139, BG-141, BG-138, BG-75, BG-108
>75	Highly susceptible	-

Table 3 : Reaction of bottle gourd genotypes against CGMMV under natural field conditions

Score	Reaction	Genotype
0	Immune	-
1	Resistant	IIHR-19, BG-131
2	Moderately resistant	BG-95, BG-62-1-1, BG-75-2, BG-124-4, BG-108-1, BG-67
3	Moderately	BG-108-2-3, BG-108-2, BG-98, BG-114 -46, BG-8-1, BG-114-1
4	Susceptible	BG-108, BG-78, BG-114-3, BG-24, BG-136, BG-143BG-118-3, BG-115-2, BGAIC-11, BG-125-4, BG-120-5, BG-125-2, BG-81, BG-104, BG-4, IIHR-22349, BG-61-10, BG-105, BG-23, BG-115-5b, BG-118-3-3, BG-62-1, BG-49, BG-49-5, BG-119-2, BG-122-5, BG-123-3, BG-44, BG-11, BG-39-112-1, BG-93, P. Lauki-8BG-64, BG-75, BG-77-6-1, BG-124-2, BG-18, BG-99, BG-139, BG-123-3-1, BG-141, BG-23-5-6, BG-47, BG-79
5	Highly susceptible	BG-138, BG-6, BG-125-5, BG-91, BG-98-3, BG-6-3, BGAIC-6, BG-135, BG-23-5-10

Table 4 : *In vivo* evaluation of promising genotypes with artificial inoculation against powdery mildew

Genotype	PDI**	AUDPC*	Class
BG-6-3	11.11	266.67	R
BG-8-1	13.58	300.00	R
BG-125-4	14.81	343.27	R
BG-125-2	17.77	378.83	R
BG-124-2	37.77	811.49	MS
BG-125-5	33.33	717.64	MS
BG-11 (susceptible check)	68.56	1222.83	S

*AUDPC calculated based on nine observations at 3 days interval
**PDI is average of two replications (n=20)
R= resistant, MS= moderately susceptible, S= susceptible

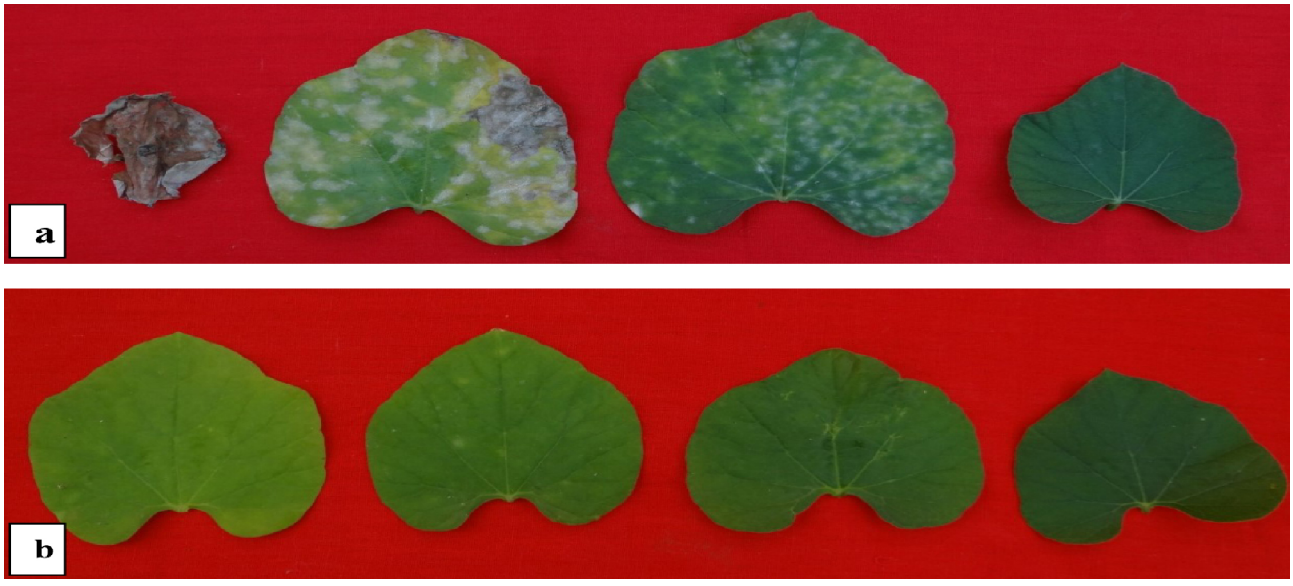


Fig. 4 : Powdery mildew disease reaction on bottle gourd genotypes *in vivo* screening with artificial inoculation, four leaves from left to right shows disease progress starting from basal leaf to young leaf a) BG-11 (susceptible), b) BG-6-3 (resistant)

Table 5 : Resistance reaction of bottle gourd genotypes against gummy stem blight under artificial screening

Genotype	Artificial inoculation	
	Per cent disease index	Resistant class
BG-114-3	9.45	R
BG-114-1	9.25	R
BG-77-6-1	10.00	R
BG-95	8.89	R
BG-98	41.67	MS
BGAIC-6	15.56	MR
Warad	54.45	S
BG-24	67.22	S
BG-18	65.56	S
Arka Bahar	48.34	MS
Pusa Naveen	71.67	S

Average of two replications, n=20, PDI based on modified 0-9 scale (Gusmini ýet *al.,* 2005); 0 = immune; 1-10% = resistant; 11-25% = moderately resistant; 26-50% = moderately susceptible; 51-75%=susceptible; and >75%= highly susceptible (Bhardwaj *et al.,* 2018)

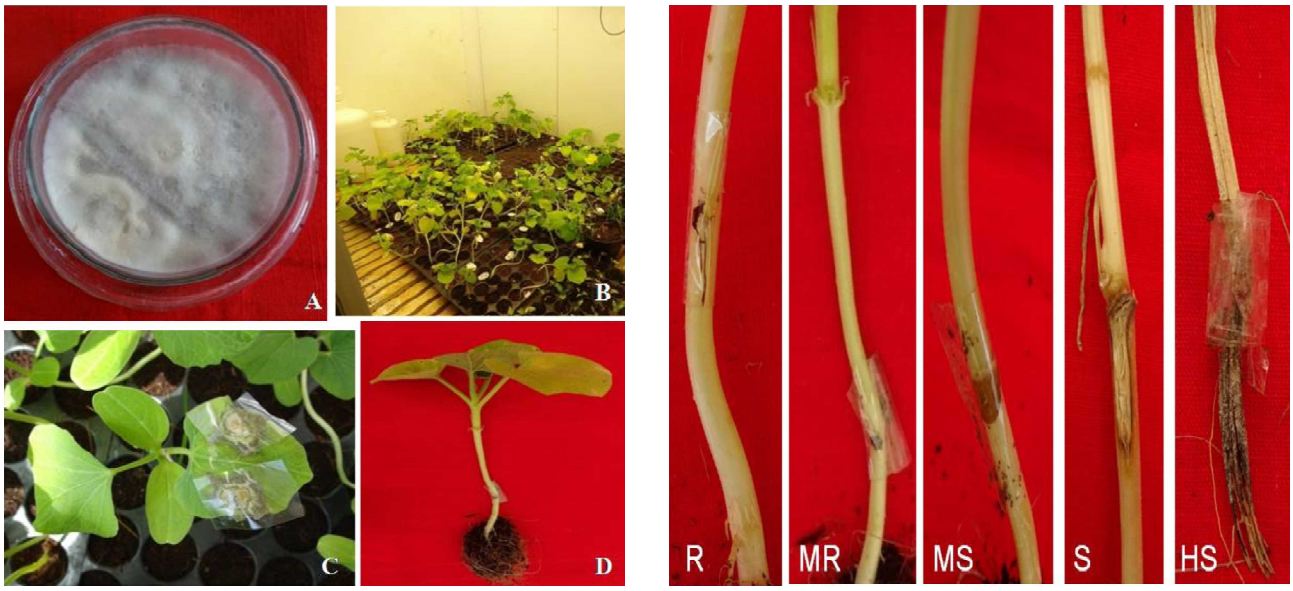


Fig. 5 : Screening method (A-D) and different disease response categories observed

Further, promising genotypes from field evaluation were subjected to *in vivo* screening for powdery mildew, gummy stem blight (3 resistant and 6 moderately resistant) and CGMMV with artificial challenge under glass house conditions. Under *in vivo* evaluation; out of six genotypes only BG-6-3 (266.67), BG-8-1 (300.0), BG-125-4 (343.27) and BG-125-2 (378.83) were found resistant for powdery mildew (Table 4 and Fig. 4). The genotypes BG-114-3, BG-77-6-1 and BG-95 were found resistant for gummy stem blight disease (Table 5 and Fig. 5), whereas, promising genotypes for CGMMV from field evaluation showed susceptible reaction under artificial screening.

CONCLUSION

The bottle gourd resistant genotypes identified could be utilized as potential source for incorporation of resistance in to bottle gourd breeding lines. Further, they can also be utilized as rootstocks for grafting in other cucurbits.

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