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Identification of Sources of Multiple Disease Resistance in Advanced Breeding Lines of Bottle Gourd (*Lagenaria siceraria* (Molina) Standle)

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ABSTRACT

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Bottle gourd [*Lagenaria siceraria* (Molina) Standle] is one of the most widely grown cucurbit in India valued for its various medicinal and nutritional properties. The crop is plagued by a multitude of diseases which drastically affect the yield and quality of produce. Under South Indian conditions, Gummy Stem Blight (GSB) caused by *Didymella bryoniae* is creating havoc wiping out entire populations during recent times. Till now there are no reported sources of resistance to GSB. The present study is aimed at screening advanced breeding lines of bottle gourd for GSB along with powdery mildew, CGMMV and phyllody. We found that three lines BG 95-3, 95-4 and 95-6 were showing moderate to high resistance to GSB. Among these 95-3 and 95-4 were also showing high resistance to powdery mildew with the absence of CGMMV and phyllody infection. Twelve lines were found to have low to medium resistance to GSB however with excellent yield attributes. The lines identified can be successfully utilized to breed high yielding multi-disease resistant bottle gourd varieties.

Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standle; 2n = 22] commonly known as 'white-flowered gourd' or 'calabash gourd' belongs to the family Cucurbitaceae. One of the most ancient crops cultivated by man in the tropics, bottle gourd is grown as a rainy season and as well as summer season vegetable in India. Although known as the poor man's vegetable, it is attaining fast popularity among the health-conscious urban

elite due to its numerous medicinal properties reported (Barot *et al.*, 2005). Its juice is reported to have cooling, calming, diuretic and anti-bilious properties. It is low in saturated fat and cholesterol but high in water, dietary fibre, Vitamin C, Zinc, Riboflavin, thiamine, iron, magnesium and manganese. 100g of tender fruit contains 96g water, 0.2g protein, 0.1g fat, 2.5 g carbohydrate, 0.6 g fibre, 0.5 g minerals, 12 kilocalories of energy, 20 mg calcium, 10 mg phosphorus, 0.7 mg iron, 0.3 mg thiamine, 0.01 mg

riboflavin and 0.2 mg niacin. In addition glycosides such as Cucurbitacins B, D, G and H are present (Roopan *et al.*, 2016).

Bottle gourd is plagued by several diseases which drastically affect yield. Gummy stem blight (GSB) is one of the newly emerging and deadly disease which was reported from most of the cucurbits grown all over the world (Rennberger and Keinath, 2018); among which watermelon and cucumber (dos Santos *et al.*, 2009; Keinath 2014) are reported to be the most susceptible. The disease is caused by three genetically distinct species of *Stagonosporopsis* (Stewart *et al.*, 2015). Among the three, two *S. citrulli* and *S. cucurbitacearum* (syn. *Didymella bryoniae*), are host specific to cucurbits.

The symptoms include leaf spot, stem canker, vine wilt and black fruit rot. During the rainy season, lesions can become water-soaked and spread leading to severe defoliation. Gummy exudates may ooze from cracks and severe infection leads to plant death (Brewer *et al.*, 2015). Since inoculum is present in the soil, chemical control is proven difficult under open field conditions although cultural practices like crop rotation have been found effective in reducing the inoculum load. The presence of GSB in bottle gourd has been reported from different parts of India (Katoch *et al.*, 2017; Bhardwaj *et al.*, 2018). Here, we report GSB occurrence in bottle gourd for the first time from Bengaluru, India; resulting in wiping out of entire populations.

Powdery mildew caused by *Podosphaera fuliginea* (also known as *Podosphaera xanthii*) is another important disease of bottle gourd (Nayak and Babu, 2017) where yield losses up to 50-60% have been reported. The symptoms include the presence of tiny, white spots on the above-ground parts of plant especially the leaves, which became powdery as they enlarge. The infection results in

chlorosis and death of younger leaves while the older leaves become brown and shrivelled. Disease development accelerates with cool temperatures and low light intensity, a condition observed in the field as the vines mature. Although chemical control is commonly practised (Keinath, 2015) the enhanced costs, as well as pesticide residues, leave a huge question mark against the practical utility. Moreover, resistance to fungicides is quite common for *P. xanthii* populations (Lebeda *et al.*, 2010; McGrath, 2017; Keinath *et al.*, 2018). For effective disease management, the cultivation of resistant cultivars provides a safe and healthy option for both humans and the environment. Hence, breeding for resistance to these deadly diseases is the need of the hour and must be taken in an urgent manner along with yield improvement.

Materials and Methods

Plant materials

Germplasm comprising of 115 lines of bottle gourd were planted in the open field during Rabi (2016-17) along with a susceptible check for GSB; 'Desi local' in an Augmented Block Design with ten plants per genotype. The three completely susceptible lines identified in the field during previous season screening viz; BG118-3, BG118-4 and BG 119-2 were also included as checks. The experimental field comprised an area of 50m² in the vegetable research field of ICAR-IIHR farm at Hessaraghatta.

The presence of *Didymella* in the field was established by culture studies by the pathologist and for effective spread of inoculum 'Desi local'; the susceptible line was planted all around the field also. Standard agronomic practices recommended for the crop was followed without any chemical spraying.

Scoring of disease incidence in the field

Based on the symptoms on the leaf, stem cracking, gum oozing and wilting of plants; a visual score of 1-5 was given for GSB incidence. All the plants were scored and data was taken on a weekly basis. Based on the severity of symptoms, powdery mildew incidence in the field was given a score of 1-10, with ten given for complete drying. A genotype was scored as completely susceptible when more than half of the population showed wilting for either or both of the diseases.

Phenotyping for other traits

The genotypes were scored for germination percentage and vigour before field planting. In the field observations taken include date of emergence of the female flower, the number of fruits, yield/genotype and fruit shape. In addition the presence or absence of phyllody and CGMMV (Cucumber Green Mottle Mosaic Virus) was visually scored.

Calculation of AUDPC scores

The area under the disease progress curve (AUDPC) is used to assess disease intensity over time, or across years, locations, or management tactics. The trapezoidal method is most commonly used method for estimating the AUDPC based on different time variable (hours, days, weeks, months, or years) help to calculate the average disease intensity between each pair of adjacent time points (Madden *et al.*, 2007).

The AUDPC scores for GSB as well as powdery mildew were calculated by taking the average score for biweekly interval based on the formula

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

using R software. In the formula, n= the total number of observations, y_i =crop health data at the i^{th} time period, and t = time at i^{th} observation. The first term in the equation gives the height of the rectangle (the midpoint between y_i and y_{i+1}) whereas the second term is the width of the rectangle. The combined resistance for both the diseases for individual lines showing resistance was also worked upon by modification of the formula.

Analysis of phenotypic data

For individual genotypes including the check varieties, the average yield and the average number of fruits per plant were calculated in Excel based on field data. This data along with combined disease score was used to predict the yield performance. A regression analysis was conducted using the software SYSTAT ver.13 by keeping average yield as the dependent variable and the disease scores (intensities) of GSB and powdery mildew as the independent variables.

Results and Discussion

Screening for Gummy Stem Blight (GSB)

The disease progress was quite rapid under hot humid conditions with recurrent rainfall. 30 days after transplanting we observed small brown lesions (score of 1-2) on most of the lines. However, some plants remained asymptomatic at this stage (score 0). By 60 DAT, the brown lesions coalesced into big brown patches and leaf and vine drying was observed. At this stage, most of the susceptible plants and checks (12% of the total population) were given a score of 3-4. By 85 DAT; stem cracking, oozing of gummy exudates and complete wilting was noticed in 23% of the total population corresponding to a score of 5. A score 5 was given when complete wilting was noticed in more than 50% plants in a particular line. Almost 90%

disease incidence (score of 4-5) with a few plants showing complete wilting was noticed in about 57 lines (49% of the total population). All the plants in all the replicates of check lines BG118-3, BG118-4 and BG 119-2 showed complete wilting at this stage. After 120 days, the disease wiped out 80% of the germplasm with a disease incidence of 90-100%. Complete drying of plants was noticed 46% population corresponding to 53 lines.

The AUDPC (Madden *et al.*, 20007) scores were worked out based on the monthly average of GSB score calculated in percentage. An AUDPC score of 250 and below were classified as having moderate resistance to GSB while a score of 250-300 was classified as low to moderate resistance. Only 7 lines (<1% of the population) showed moderate to high resistance with a disease incidence of 40-60% (GSB score of 2-3) by the end of the cropping season. These lines are BG 95-3, BG 95-4 and BG 95-6 and the AUDPC scores were 210, 210 and 240 respectively. A total of 12 germplasm lines were found to show low to moderate resistance with AUDPC score ranging 270-300. These lines are BG 124-3, BG-68, BG 114-3, BG 77-6, BG 101, BG 102, BG 116-4, BG 117-3, BG 121-5, BG 124-4, BG 126 and IIHR 22349. The complete devastation of susceptible lines in the field is given in Fig-1. The details are given in Table-1 along with AUDPC score.

The area under the disease progress curve (AUDPC) has been proven as a reliable method for quantitative disease in crop cultivars thereby avoiding the need for repeated measurements between two data points (Jeger and Viljanen-Rollinson, 2001). This method has been widely utilized in resistance studies against fungal pathogens like *Didymella bryoniae* causing GSB in melons (Dalcin *et al.*, 2017; Dalcin *et al.*, 2018; Rennberger and Keinath, 2018).

Previously reported study (Bhardwaj *et al.*, 2018) failed to identify any immune or resistant lines which we also found true in the present study. However the moderate to high resistance shown by the advanced breeding lines can be utilized for further development of a resistant cultivar.

Screening for powdery mildew

Powdery mildew is an important foliar disease affecting cucurbit crops grown all over the world (McGrath, 2017). The disease symptoms appeared late when compared to GSB incidence and reached maximum severity after 5-6 harvest completed. The disease progress was also slow compared to GSB. Since a majority of plants succumbed to GSB by this stage, only lines showing some resistance to GSB could be scored effectively for powdery mildew. Among the lines screened, 7 lines were found to be highly resistant to powdery mildew with AUDPC scores <100. However, 4 of these lines were found to be susceptible to GSB. These lines are BG 8-1, BG 05, BG 53 and BG 108-1. We found the lines BG 95-3 and BG 95-4 which showed a moderate to high resistance reaction to GSB to be highly resistant to powdery mildew as well. In addition, high resistance was also shown by BG-68, with low to moderate resistance to GSB. Many lines including BG 5-6, BG 6-3 and the check line BG 119-2 showed a very low powdery mildew incidence (score 1-2), however, were completely wilted due to GSB before the AUDPC scores could be calculated. Similarly, AUDPC scores were used for the identification of powdery mildew resistant rootstock lines in bottle gourd by Kousik *et al.*, 2018. Here also the resistance was expressed as none to few lesions and sparse mycelia on the leaves and exposed plant surfaces. The combined resistance for both the diseases is presented in Fig-2.

Table.1 The AUDPC scores calculated for gummy stem blight (GSB) along with average fruit weight (Av_frt_wt) in kilo grams and total number of fruits harvested (Total_frts) for bottle gourd advanced breeding lines. The three checks are suffixed by symbol ©

| line | AUDPC | Av_frt_wt | Total_frts | line | AUDPC | Av_frt_wt | Total_frts |
|-------------|-------|-----------|------------|----------|-------|-----------|------------|
| BG 105_1 | 480 | 1.62963 | 27 | BG_05 | 350 | 1.666667 | 9 |
| BG 106_2 | 415 | 2.192308 | 26 | BG_100 | 450 | 1.235484 | 31 |
| BG 108_1 | 415 | 1.3075 | 40 | BG_101 | 300 | 0.911538 | 26 |
| BG 108_2 | 395 | 1.359091 | 22 | BG_102 | 300 | 1.426667 | 15 |
| BG 112_1 | 380 | 1.18 | 20 | BG_103 | 340 | 1.105 | 20 |
| BG 113_6 | 415 | 1.6125 | 32 | BG_104 | 340 | 1.230769 | 13 |
| BG 114_1 | 415 | 1.433333 | 36 | BG_105 | 340 | 1.16087 | 23 |
| BG 114_3 | 280 | 1.857143 | 14 | BG_108 | 335 | 1.282143 | 28 |
| BG 114_3b | 420 | 1.542308 | 26 | BG_109 | 340 | 1.247826 | 23 |
| BG 114_4 | 395 | 1.636111 | 36 | BG_11 | 415 | 1.230435 | 46 |
| BG 114_4b | 450 | 1 | 23 | BG_110 | 340 | 1.552941 | 17 |
| BG 115_1 | 415 | 1.556 | 25 | BG_116_1 | 335 | 1.458333 | 12 |
| BG 115_2 | 430 | 1.21 | 40 | BG_116_3 | 335 | 2.1 | 10 |
| BG 115_3 | 385 | 1.471429 | 28 | BG_116_4 | 300 | 1.478571 | 14 |
| BG 115_4 | 395 | 1.25 | 6 | BG_116_5 | 340 | 1.48 | 5 |
| BG 115_4b | 395 | 1.405405 | 37 | BG_117 | 340 | 1.32 | 20 |
| BG 115_5 | 445 | 1.366667 | 21 | BG_117_2 | 340 | 1.294118 | 17 |
| BG 115_5b | 415 | 1.3 | 24 | BG_117_3 | 300 | 1.263158 | 19 |
| BG 118_3© | 450 | 1.588 | 121 | BG_117_4 | 360 | 1.408 | 20 |
| BG 118_4© | 480 | 1.6 | 36 | BG_117_5 | 340 | 1.418519 | 27 |
| BG 119_2© | 480 | 1.78 | 76 | BG_12 | 440 | 1.845 | 20 |
| BG 39_112_1 | 340 | 1.344444 | 18 | BG_120_3 | 380 | 1.2 | 2 |
| BG 77_6 | 280 | 1.994444 | 18 | BG_120_5 | 380 | 0.9 | 1 |
| BG 90_8 | 380 | 1.4725 | 40 | BG_121_2 | 335 | 1.41875 | 16 |
| BG 95_3 | 210 | 1.683333 | 18 | BG_121_3 | 335 | 1.62 | 10 |
| BG 95_4 | 210 | 1.705769 | 26 | BG_121_4 | 335 | 1.533333 | 15 |
| BG 95_6 | 240 | 1.583333 | 18 | BG_121_5 | 300 | 1.323077 | 13 |
| BG_03 | 420 | 1.35 | 6 | BG_122_5 | 340 | 1.273333 | 15 |
| BG_04 | 395 | 1.34 | 20 | BG_123_3 | 335 | 1.347368 | 19 |
| BG_124_2 | 335 | 1.68 | 5 | BG_123_5 | 335 | 1.414286 | 7 |
| BG_124_3 | 270 | 1.7 | 15 | BG_53 | 420 | 1.469444 | 36 |
| BG_124_4 | 300 | 2.014286 | 7 | BG_6_3 | 470 | 0.825 | 12 |
| BG_125_2 | 305 | 1.32 | 20 | BG_60 | 420 | 1.523077 | 26 |
| BG_125_4 | 305 | 1.185714 | 21 | BG_61 | 340 | 1.345 | 20 |
| BG_125_5 | 305 | 1.246667 | 15 | BG_62 | 470 | 1.604762 | 21 |
| BG_126 | 300 | 1.066667 | 6 | BG_63 | 415 | 1.255 | 20 |
| BG_13 | 395 | 1.443902 | 41 | BG_64 | 410 | 1.388462 | 26 |
| BG_15 | 440 | 1.504762 | 21 | BG_65 | 410 | 1.281481 | 27 |
| BG_16 | 440 | 2.042308 | 26 | BG_67 | 420 | 1.654545 | 22 |
| BG_17 | 440 | 1.092857 | 14 | BG_68 | 270 | 0.593939 | 33 |
| BG_22 | 470 | 1.44 | 15 | BG_70 | 340 | 1.325 | 24 |
| BG_23 | 440 | 2.005882 | 17 | BG_74 | 440 | 0.989474 | 19 |

| | | | | | | | |
|--------|-----|----------|----|------------|-----|----------|----|
| BG_24 | 485 | 0.962963 | 27 | BG_75_2 | 400 | 1.645 | 20 |
| BG_25 | 485 | 1.245455 | 22 | BG_79 | 355 | 1.503226 | 31 |
| BG_26 | 385 | 1.24359 | 39 | BG_8_1 | 355 | 0.852941 | 34 |
| BG_28 | 420 | 1.533333 | 21 | BG_8_3 | 315 | 1.222222 | 9 |
| BG_29 | 450 | 1.366667 | 30 | BG_92 | 415 | 1.212195 | 41 |
| BG_33 | 385 | 1.504762 | 21 | BG_93 | 385 | 1.339394 | 33 |
| BG_34 | 340 | 1.75 | 2 | BG_94_1 | 385 | 1.613725 | 51 |
| BG_36 | 385 | 1.286957 | 23 | BG_94_3 | 415 | 1.510204 | 49 |
| BG_4_6 | 330 | 1 | 6 | BG_96 | 415 | 1.242424 | 33 |
| BG_43 | 415 | 1.026087 | 23 | BG_99 | 340 | 1.441667 | 24 |
| BG_44 | 410 | 0.962 | 50 | IIHR_19 | 415 | 2.031034 | 29 |
| BG_45 | 440 | 1.223333 | 30 | IIHR_22349 | 300 | 1.208333 | 12 |
| BG_47 | 470 | 1.781818 | 11 | BG_5_6 | 440 | 2.283333 | 6 |
| BG_49 | 440 | 1.195 | 20 | BG_50 | 420 | 1.133333 | 15 |
| BG_5_4 | 340 | 1.2 | 1 | | | | |

Table.2 Multiple linear regression analysis using yield as the dependent variable with GSB and powdery mildew intensities as independent variables. Statistically significant relationships are highlighted

| Regression Coefficients $B = (X'X)^{-1}X'Y$ | | | | | | |
|---------------------------------------------|-------------|----------------|------------------|-----------|-------------|---------|
| Effect | Coefficient | Standard Error | Std. Coefficient | Tolerance | t | p-Value |
| CONSTANT | 4.23 | 1.63 | 0 | . | 2.59 | 0.02 |
| GSB | -0.02 | 0.02 | -0.25 | 0.75 | -0.98 | 0.35 |
| PM | 0.05 | 0.02 | 0.75 | 0.75 | 3.01 | 0.01 |
| Analysis of Variance | | | | | | |
| Source | SS | df | Mean Squares | F-Ratio | p-Value | |
| Regression | 17.4 | 2 | 8.69 | 4.7 | 0.03 | |
| Residual | 22.2 | 12 | 1.85 | | | |

Fig.1 The complete devastation of bottle gourd lines due to gummy stem blight (GSB). The susceptible line BG 118-3 is shown at different time intervals where A) stem cracking and oozing of gummy exudates at flowering stage B) Drying off the entire plant C) Field view of susceptible lines at crop maturity



Fig.2 Combined resistance to GSB and powdery mildew in bottle gourd advanced breeding lines as a function of AUDPC scores. The X-axis represent the time period whereas the Y-axis represent the percentage infection. The graph represents the progress of disease along with time in line BG 94-1 where the late onset of disease was noticed, susceptible check BG 118-3 and the resistant cultivar BG 95-3. The blue line represents the disease severity of GSB whereas the yellow line represents the disease severity of powdery mildew

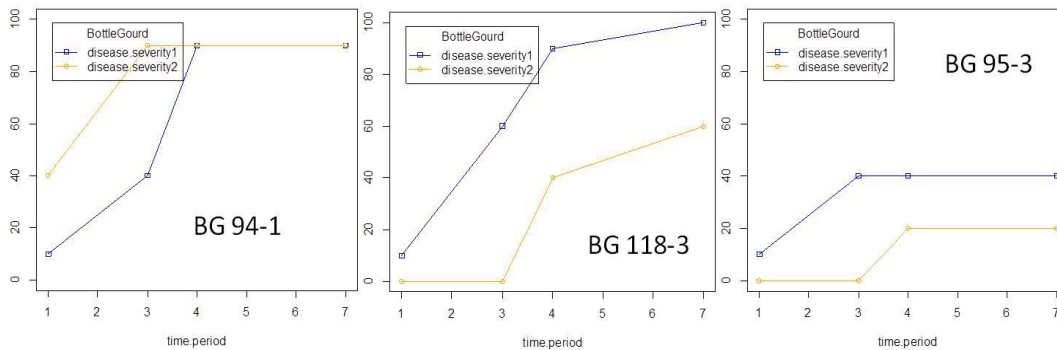
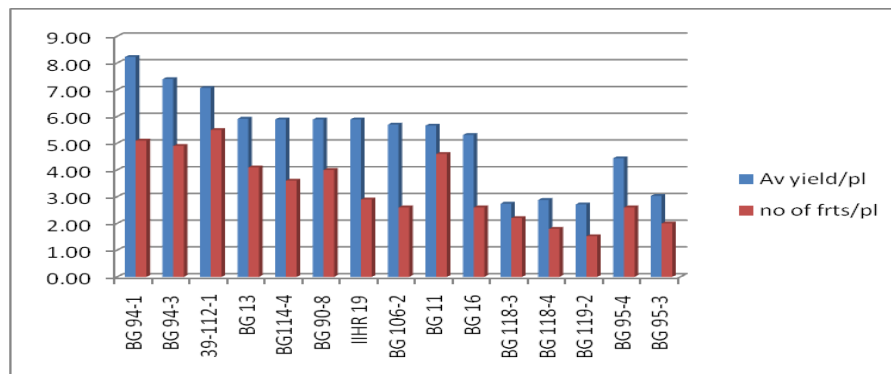


Fig.3 A comparison of yield parameters, average yield/plant and the number of fruits/plant in highly resistant and susceptible lines of bottle gourd. Most of the high yielding lines although showed susceptibility to GSB were characterized by the late onset of disease. The lines showing resistance to GSB viz. BG 95-4 and 95-3 were found to be moderately yielding



Screening for CGMMV and phyllody

Bottle gourd is used as a rootstock especially for watermelon grafting is highly susceptible to CGMMV, a highly devastating pathogen (Liu *et al.*, 2017). The visual screening was done for CGMMV and the accessions BG 114-1, BG77-6, BG-114-4b, BG 113-6, BG-118-4, BG-03, BG-11, IIHR-19, BG 118-3, BG 39-112, BG-04, BG-05, BG 8-1, BG-119, BG 119-2, BG-33, BG-68, BG-70 and BG 121-5 were found highly susceptible. Among

these BG-68 was found to be highly susceptible and the fruits were highly bitter. Bottle gourd phyllody caused by ‘*Candidatus Phytoplasma asteris*’ (Tripathy *et al.*, 2017) is another emerging disease which has the potential to become a serious threat to bottle gourd due to severe yield losses, floral malformation and sterility (Ashwathappa *et al.*, 2020). The following lines were found highly susceptible to phyllody caused by phytoplasma; BG-04, BG-05, BG 119-2, BG-33, Desi local, BG 115-4, BG 114-3b, BG

108-2, BG 13, BG 79, BG 116-3, BG 117-4, BG 36, BG 118-3 and BG 43. The lines BG 04, BG 05, BG 33 and BG 119-2 were found to be highly susceptible to CGMMV and phyllody.

Yield attributes of germplasm

A wide variation in fruit size and shape was noticed including elongate, cylindrical, oblong, globose and crooked neck type. The highest yield obtained was for the line BG 94-1 (82.3kg) followed by BG 94-3 (74kg). The total number of fruits harvested in these lines was 51 and 49 respectively with average fruit weights of 1.61kg and 1.51kg. These lines also recorded a higher average yield/plant; 8.23kg/plant and 7.4kg/plant. The average fruit weight and the average yield/plant were comparable with most of the popular varieties and hybrids (Harika *et al.*, 2012; Varalakshmi *et al.*, 2018) cultivated in South India. The highest number of fruits harvested was 55 for the line BG 39-112-1; although the average fruit weight was low 1.26kg. A negative correlation between the number of fruits harvested and fruit weight was reported previously (Shubha *et al.*, 2019). Most of the advanced breeding lines used in the study were found to be better yielding when compared to other evaluation studies (Jain *et al.*, 2017 & 2018; Shubha *et al.*, 2019). Among the checks 118-3 recorded an average yield of 3.49kg/plant. A comparison of yield parameters; average yield/plant and number of fruits/plant of highly resistant and susceptible lines are given in Fig-3 while individual yield parameters are presented in Table-1.

Regression analysis

Multiple regression models have been successfully utilized in the prediction of yield losses caused by multiple diseases and climatic conditions in crops like wheat (Jevtic

et al., 2017; Ficke *et al.*, 2018; Jevtic *et al.* 2020), French bean (Kumar and Kudada, 2018), cucumber (Ding *et al.*, 2019), bitter melon (Prasanth *et al.*, 2019), potato (Yellareddygar *et al.*, 2018) to name a few. Multiple linear regression using yield as dependent variable and GSB and powdery mildew intensities as independent variables was conducted. The analysis was conducted as a yield forecasting means in the events of combined disease incidence. The disease severity of powdery mildew had a significant effect on yield (Table-2) as revealed by P value= 0.011. The overall effect of regression of independent factors on yield was also significant as revealed by the F test (P value=0.031). The effect of GSB on yield was found non-significant; possibly due to the fact that severely affected plants dried off completely without any fruit formation.

In conclusions advanced breeding lines of bottle gourd was screened for devastating diseases gummy stem blight and powdery mildew. Since GSB is soil-borne and chemical control is ineffective breeding efforts must be accelerated to develop resistant varieties. In this context the newly identified sources of resistance to GSB particularly BG 95-3 and BG 95-4 assumes greater significance. Although moderately yielding, they exhibit resistance to powdery mildew. Hence can be successfully incorporated into breeding programs to develop high yielding and multiple disease-resistant bottle gourd varieties.

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