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Development of Genotypes for Foliar Disease Resistance in Mungbean [*Vigna radiata* (L.) Wilczek] through Recombination and Mutation Breeding

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ABSTRACT

Background: Despite the best efforts for improving the greengram varieties, the yield potential of this crop remains low owing to both biotic and abiotic factors. Yield losses caused due to various diseases were estimated to be 24-67 per cent in anthracnose, 80-100 per cent in mungbean yellow mosaic virus and 40 per cent due to powdery mildew So, the use of resistant cultivars is the most effective and eco-friendly method of managing the disease.

Methods: A series of field experiments spanned over seven years (2014-2021) were conducted at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka. In the first set of experiments, the material comprised of eight varieties of greengram which were treated with 20kR gamma radiation. $M_1$was planted in *Kharif* 2014 and each variety was bulk harvested. M_2 was planted in summer 2014 and individual plant selection was carried out. Mutant families were advanced to the further generation in *Kharif* 2015 and *Rabi* 2015. The M_2 mutants were scored for their reaction to anthracnose, cercospora leaf spot and powdery mildew diseases with respective susceptible and resistant checks. In another set of experiments aimed at developing MYMV tolerant genotypes, an investigation was carried out with 168 F_2 individuals derived from crossing *Vigna trilobata* and DGGV2. One row infector line of DGGV-2 was raised after every three test entries to study the reaction to MYMV. Three rows of susceptible check was planted all around the border to ensure enough inoculum load. Of the 168 F_2 individuals, two lines showed resistance to MYMV. They were stabilized through a series of selfing generations.

Result: Evaluation of advanced breeding lines for their reaction to various foliar diseases revealed that the advanced breeding lines DGG-203, DGG-206, DGG-208, DGG-21, DGG-80, DGG-273, DGG-274, DGG-275, DGG-262, DGG-263, DGG-264, DGG-82, DGG-200, TARM-1 (C), DGG-215-5, DGG-302, DGG-152, IPM-3-2, DGG-20, DGG-73, IPM-14-10, DGG-121, DGG-250, DGG-109, DGG-96 had seed yields ranging from 9.8 g to 12.4 g and moderately resistant to both anthracnose and CLS. The breeding lines DGG-203, DGG-206, DGG-21, DGG-273, IPM-3-2 (Check), DGG-96 have recorded combined resistance to anthracnose, cercospora leaf spot and powdery mildew diseases.

Key words: Foliar disease resistance, Mungbean, Mutation breeding, Recombination.

INTRODUCTION

Greengram is a self-pollinated crop exhibiting less variability hence the genetic gain is limited. Mutagenesis in association with recombination breeding offers a viable option to improve adapted variety by crossing with donors of seed yield components there by releasing variability hidden in the conserved gene blocks (Kajjidoni *et al*., 2008). Therefore, an attempt has been made to enhance genetic variability by combining both recombination and induced mutation through gamma rays irradiation. For this purpose, the F_1 seeds involving diverse parental combinations were irradiated with gamma rays at BARC, Mumbai and sown to grow the F_1M_1 generation and subsequently the superior mutant lines with high GCV and genetic advances were selected from F_2M_2 onwards. Higher mutagenic effectiveness was observed with 20 kR (Majhi *et al*., 2020).

Diseases are the major problem for greengram cultivation which has a devastating effect on both quantity and quality of the product. Greengram suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also abiotic stresses. Among these, foliar diseases such as powdery mildew, anthracnose, *Cercospora* leafspot and mung bean Yellow Mosaic Virus (MYMV) are more prevalent.

Powdery mildew is caused by a fungal pathogen *Erysiphae polygoni*. It is one of the widespread diseases of green gram. More severe symptoms of this diseases are usually observed on 35 to 40 days old crop (during flowering and pod stage) causing upto 40 per cent (Khajudparn *et al*.,

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2007) yield losses. White powdery patches appear on leaves and other green parts which later become dull coloured. In severe infections, foliage becomes yellow causing premature defoliation. The fungus is ectophytic, spreading on the surface of the leaf, sending haustoria into the epidermal cells.

Anthracnose is a severe concern for greengram grown in hot, humid climates. It results in both qualitative and quantitative losses (Sharma *et al*., 1971). *Colletotrichum lindemuthianum* is the cause. The disease can affect any section of the plant's aerial components and at any stage of development. On leaves and pods, the circular, black, sunken dots with a dark center and bright red-orange edges will appear (Mogali and Hegde, 2020). The damaged parts wither away in severe infections. Soon after seed germination, seedlings get blighted due to infection. Anthracnose-related yield losses have been estimated to be between 24-67 per cent (Deeksha and Tripathi, 2002).

Cercospora leaf spot of greengram is wide spread in India. *Cercospora cenescens* attack wide range of pulses including greengram which causes 23 to 27 per cent yield loses. Symptoms appear mainly in the above ground parts of the plant. Irregular small light brown spots measuring 2.50 to 5.00 mm, usually with grey centers and red margin are produced. These spots coalesce and cover large areas and sometime entire leaf area is engulfed resulting in death.

Mungbean plants infected with YMD generally show yellowing or chlorosis of leaves followed by necrosis, shortening of internodes and severe stunting of plants with no yield or few flowers and deformed pods produced with small, immature and shriveled seeds. It can cause yield loss of about 75-100 per cent, depending on disease incidence, virus strains, mungbean genotypes and interaction between these factors.

The management of these diseases using chemicals is a costly affair and not environmentally safe. Hence deploying genetically resistant cultivars would be cost-effective, practically feasible, eco- and farmer-friendly and a viable alternative. To address these issues breeding efforts were done to develop genotypes with resistance to various foliar diseases.

The PDI of the diseases was further subjected to arc sine transformation and was then analysed using R software version 4.2.1 to know whether the breeding lines differed significantly for their reaction to these diseases.

MATERIALS AND METHODS

Experimental details

A series of field experiments spanned over seven years (2014-2021) were conducted at F block, AICRP on MULLaRP, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India, geopositioned at 15°26′ North latitude, 75°07′ East longitude and at an altitude of 750 m above the Mean Sea Level and located in the North Western transitional agro-climatic zone of Karnataka.

In the first set of experiments, the material comprised of six varieties of greenram *viz*., LGG-460, VGG rul, Sonamung, IPM-2-3, IPM 2-17 and Vamban-2 which were crossed in different combinations and the resulting F_1s were treated with 20kR gamma radiation. $F_1M_1$was planted in *Kharif* 2014 and each variety was bulk harvested. F_1M_2 was planted in summer 2014 and individual plant selection was carried out. Mutant families were advanced to the further generation in *Kharif* 2015 and *Rabi* 2015. The F_2M_2 mutants were scored for their reaction to anthracnose, cercospora leaf spot and powdery mildew diseases with respective susceptible and resistant checks. The seeds selected from F_2M_2 population were sown as SPS (single plant selection) *Kharif* 2016 to observe for their characters and reaction to various foliar diseases. Based on the field observations, a few F_3M_3 progenies were selected and forwarded to F_4M_4 generation. Further the families conferring resistance to anthracnose, cercospora leaf spot and powdery mildew diseases were selected and stabilized through a series of selfing generations.

In another set of experiments aimed at developing MYMV tolerant genotypes, an investigation was carried out with 195 F_2 individuals derived from crossing *vigna trilobata* and DGGV 2. Each entry was sown in single row of three meter length with the spacing of 30 × 10 cm in two replications. One row infector line of DGGV-2 was raised after every three test entries to study the reaction to MYMV. Three rows of susceptible check was planted all around the border to ensure enough inoculum load. All the recommended agronomic practices were followed. No insecticidal spray was given in order to allow the whitefly population to spread the disease. Disease incidence was recorded periodically. Of the 195 F_2 individuals, four lines DGG125, DGG 96, DGG 218 and DGG 263 Showed resistance to MYMV, DGG 302, DGG 201, DGG 126 and DGG 128 recorded moderate resistance to MYMV (Table 2). They were stabilized through a series of selfing generations till *summer* 2021.

Screening for foliar diseases

Through the seasons, *kharif* 2019 to 2021, the test genotypes and the checks were screened for fungal diseases *i.e*., anthracnose, cercospora powdery mildew and MYMV. One set of experiment was conducted with all the recommended cultivation and disease management practices. Another set of experiment was laid out during the same season in the vicinity of the first experimental plot under unprotected conditions. Resistant and susceptible checks for all the foliar diseases were used in the experiments. For disease reaction, percentage of leaf area covered by the disease was scored manually. The incidence of disease on the leaves of mungbean was scored by using standard scoring procedure given by Mayee and Datar (1986). The percentage was then converted to the disease score and the per cent disease index was calculated using the formula given by Wheeler (1969). Further the per cent yield reduction of each breeding line due to these diseases was calculated using the yield data from both protected and unprotected conditions.

Per cent disease index (PDI) =

$$\frac{\text{Sum of numerical ratings}}{\text{Number of leaves observed} \times \text{Maximum rating (9)}} \times 100$$

During *summer* 2021, the recombinants derived from crossing DGGV2 and *Vigna trilobata* were screened for their reaction to MYMV In this season also two experiments (protected and unprotected conditions) were laid using augmented design. DGGV-2 was used as a susceptible check whereas IPM-2-14 was used as resistant check. The per cent disease incidence was calculated by using the formulae given by Bashir *et al*. (2006) at 45 DAS, 60 DAS and physiological maturity. Based on the percent disease incidence, the genotypes were categorized into different groups.

Per cent disease incidence =

$$\frac{\text{Total number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

The PDI of the diseases was further subjected to arc sine transformation and was then analysed using R software version 4.2.1 to know whether the breeding lines differed significantly for their reaction to these diseases.

RESULTS AND DISCUSSION

Kharif 2019 was highly favorable season for incidence of foliar diseases as there was heavy rainfall (338.94 mm) in the initial stage of growth, with a temperature of 21.62°C, relative humidity of 85.8 per cent and wind speed of 5.85 m/s. In the month of August-2021, a total of 307.42 mm rainfall was received as against the normal rainfall of 109.5 mm. This disrupted the crop during its grand-growth period. In the initial days of development, the incidence of anthracnose disease was very low. During 45-65 days after sowing the incidence of anthracnose drastically increased, varying from 60-90 per cent. Hence, the per plant yield was very low as there was a heavy incidence of foliar disease.

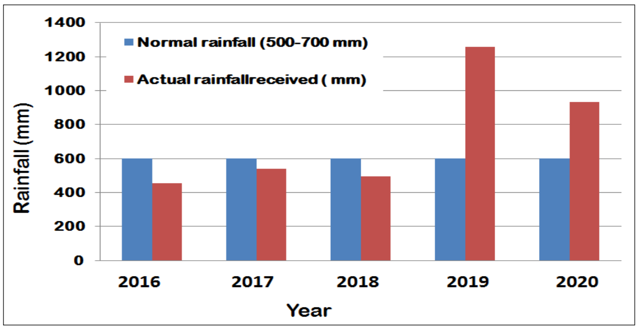
Incidence of Powdery mildew (PDI 80.0), *cercospora* leaf spots (PDI 77.4) was more during 2016-2018 due to less receipt of rainfall of 435, 539, 496 mm during 2016, 2017 and 2018 respectively when compared to normal rainfall of 500-700 mm.

During 2019-2020 anthracnose disease was very severe and recorded maximum PDI of 85.22 as the rainfall received was almost double (1259 and 931 mm during 2019 and 2020 respectively) when compared to normal rainfall. MYMV incidence was more during *Kharif* 2020 in mungbean (70 to 80%) due exposure of crop to long dry spell during seedling stage. Moderate temperature with higher humidity and frequent rains increases the disease attack. Greengram is more sensitive to erratic rainfall with uneven distribution.Further rains during flowering and pod filling stage affect the greengram yield.

The results were similar to the observations made by Kulkarni and Benagi (2012) who concluded that, the late sown crop suffered more because of coincidence of the favourable period like moderate temperature coupled with higher humidity and frequent rains with stage of the crop. Model based seasonal analysis showed that the greengram is more sensitive to change in rainfall than temperature (Sagar and Patil, 2020). Changes in erratic rainfall patterns projected under future climates are going to affect the performance and productivity of greengram and urdbean crops, especially under rainfed condition.

Anthracnose (*Colletotrichum lindemuthianum*) is a major problem of greengram cultivated in hot and humid areas. In northern Karnataka losses in yield and disease incidence of anthracnose have been estimated to be in the range of 24 to 67 per cent (Deeksha and Tripathi 2002a) and 18.20 to 86.57 per cent respectively (Laxman 2006). Therefore current study was aimed at development of anthracnose tolerant genotypes. *Kharif* -2019 season was highly favourable for anthracnose disease development as there was incidence of heavy rainfall (857.2 mm) in the initial stage of growth, with temperature of 21°C, relative humidity of 94-95 per cent and wind speed of 5-6 km/hr. In the month of august-2020, a total of 451 mm rainfall was received as against the normal rainfall of 70-100 mm (Graph 1). This disrupted the crop during its grand-growth period. In the initial days of development the incidence was very low. During 45-65 days after sowing the incidence of anthracnose drastically increased, varying from 60-90 per cent.

Evaluation of hundred breeding lines developed by various breeding methods for anthracnose disease on a scale of 0 to 9 (Mayee and Datar 1986) revealed that the breeding lines DGG-203, DGG-206, DGG-208, DGG-21, DGG-80, Sonamung, DGG-273, DGG-274, DGG-275, DGG-262, DGG-263, DGG-264, DGG-82, DGG-200, TARM-1 (C), DGG-215-5, DGG-302, DGG-152, IPM-3-2, DGG-20, DGG-73, IPM-14-10, DGG-121, DGG-250, DGG-109, DGG-96 recorded moderately resistant reaction with PDI of 25, 16.6, 13.33and 16.66 per cent respectively. Resistant reaction was recorded by IPM-2-14 with PDI 12.8 per cent. Agronomicaly superior ocal check DGGV-2 showed susceptible reaction



Graph 1: Variation in normal rainfall and actual rainfall which favoured development of foliar diseases of mungbean in Northern transitional tract of Karnataka.

with PDI of 62 per cent and DGG-7 showed moderately susceptible reaction with PDI of 40 per cent .These breeding lines can be utilized in breeding programes aimed at developing anthracnose resistant greengram varieties, which can augment the yielding potential under varied climatic conditions.

Reaction of advanced breeding lines to Anthracnose disease during *kharif*-2021

In the present study, a set of forty breeding lines derived from F_7 generation were screened for Anthracnose disease with susceptible and resistant checks during *kharif*-2021. The *kharif* season was highly favourable as there was receipt of heavy rainfall (338.94 mm) in the initial stage of growth, with a temperature of 21.62°C, relative humidity of 85.8 percent and wind speed of 5.85 m/s. In the month of August-2021, a total of 307.42 mm rainfall was received as against the normal rainfall of 109.5 mm. This disrupted the crop during its grand-growth period. In the initial days of development, the incidence was very low. During 45-65 days after sowing the incidence of anthracnose drastically increased, varying from 60-90 percent. Hence, the per plant yield was very low as there was a heavy incidence of foliar disease. Among ninety breeding lines, twenty-two breeding lines have recorded moderately resistant reactions to anthracnose with good performance and yield. The rest of the genotypes showed susceptibility to a highly susceptible reaction. However, the resistance has to be confirmed over seasons before including in the hybridization program. Close results were observed by Marak *et al*. (2018).

Correlation between Anthracnose disease and yield parameters

Anthracnose disease correlation study with yield contributing traits provides insight into reduced yield in anthracnose susceptible lines, *vis a vis* high yield in anthracnose resistant lines. All yield-attributing variables, such as number of branches per plant, number of pods per plant and number of seeds per pod, were found to be negatively correlated with per cent disease incidence in a correlation study. With the exception of percent disease infection, all yield indices exhibited a positive correlation with seed yield per plant. We may deduce that when disease incidence is high, the seed yield per plant will be reduced. Similar results were revealed in the study of Rekha *et al*. (2020); Nair *et al*. (2019).

On correlation analysis of Per cent disease Infection (PDI) of anthracnose and yield attributing traits, it is observed that PDI value increased as we move from highly susceptible genotypes to moderately resistant genotypes. seed yield per plant showed a slight increase as we move from susceptible genotypes to resistant genotypes.

The advanced breeding lines *viz.*, DGG-203, DGG-206, DGG-208, DGG-21, DGG-80, DGG-273, DGG-274, DGG-275, DGG-262, DGG-263, DGG-264, DGG-82, DGG-200, TARM-1 (C), DGG-215-5, DGG-302, DGG-152, IPM-3-2, DGG-20, DGG-73, IPM-14-10, DGG-121, DGG-250, DGG-109, DGG-96 had seed yields ranging from 9.8 g to 12.4g, with a per cent incidence of 11-20 per cent, making these genotypes moderately resistant to both anthracnose and CLS (Table 1). Seed yields of susceptible lines DGGV-230, DGGV-303, DGGV-306 and DGGV-193 ranged from 3.04 g to 4.66 g, with PDIs ranging from 50.10 to 64.8 per cent. As a result of the preceding findings, it can be concluded that percent disease incidence is negatively correlated to yield contributing features, particularly seed yield per plant. Similar results were drawn by Kiptoo *et al*. (2020), in which the

Table 1: Advanced breeding lines conferring combined resistance to Anthracnose and Cercospora leaf spot in greengram.

Entry	ANTH	CLS
DGG-203	MR	MR
DGG-206	MR	MR
DGG-208	MR	MR
DGG-21	MR	MR
DGG-80	MR	MR
Sonamung	MR	MR
DGG-273	MR	MR
DGG-274	MR	MR
DGG-275	MR	MR
DGG-262	MR	MR
DGG-263	MR	MR
DGG-264	MR	MR
DGG-82	MR	MR
DGG-200	MR	MR
TARM-1 (C)	MR	MR
DGG-215-5	MR	MR
DGG-302	MR	MR
DGG-152	MR	MR
IPM-3-2 Mutant	MR	MR
DGG-20	MR	MR
DGG-73	MR	MR
IPM-14-10	MR	MR
DGG-121	MR	MR
DGG-250	MR	MR
DGG-109	MR	MR
DGG-96	MR	MR

Table 2: Advanced breeding lines conferring resistance to MYMV in greengram.

Entry	Reaction to MYMV
DGG-125	R
DGG-96	R
DGG218	R
DGG263	R
DGG-302	MR
DGG-201	MR
DGG-126	MR
DGG-128	MR
436714 (GPM 50)	R
DGG 237	MR

results revealed tolerant and resistant genotypes with lower incidences and severity than those of resistant controls while susceptible genotypes recorded higher incidences and severity than those of the susceptible controls.

Green gram yield enhancement has been a challenge due to the existence of pests and diseases (Mogali and Hegde (2020). During the summer season from January to March-2021 rainfall was in the range of 3.4 mm to 12.44 mm, the maximum mean temperature was in the range of 30.62°C to 37.51°C, minimum mean temperature was in the range of 16.21°C to 19.73°C, mean relative humidity of 44.9 per cent to 63.4 per cent and wind speed of 1.67 m/s to 1.93 m/s at 2 meters and 2.59 m/s to 2.94 m/s at 10 meters. These conditions were congenial for the spread of MYMV through whiteflies. In the current study, it was observed that the disease incidence was much higher in the summer than in the other seasons, ranging from 0 to 100 per cent.

During the summer of 2021, thirty-seven of the 112 breeding lines produced from the F_6 generation showed moderate resistance response, with per cent disease incidence of 0 to 64. Even though we looked at the mean percent disease incidence values of breeding lines from a certain cross as a whole, when we looked at each progeny

DGG 21 (Moderately resistant to Powdery mildew, cercospora leaf spot and Anthracnose)

DGG-96 (Resistant to cercospora leaf spot and Anthracnose)

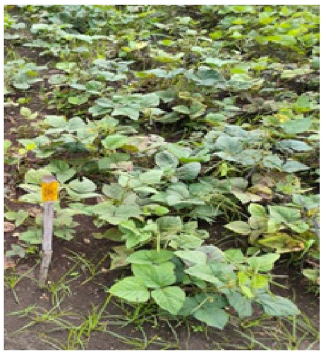
DGGV-2 (Susceptible to cercospora leaf spot and Anthracnose)

DGG 111

DGG 1

DGG 302

DGG 20



DGG 111 (Resistant to cercospora leaf spot and Anthracnose)

DGG-302 (Moderately resistant to MYMV)

DGG-215-2 (Moderately resistant to MYMV)

DGGV 2 (Susceptible to cercospora leaf spot, Anthracnose and powdery mildew)

DGG 73 (Resistant to cercospora leaf spot, Anthracnose and powdery mildew)

IPM 3-2 (Resistant to cercospora leaf spot, Anthracnose and powdery mildew)

Plate 1: Greengram advanced breeding lines resistant to different foliar diseases.

Table 3: Advanced breeding lines conferring combined resistance to anthracnose cercospora leaf spot and powdery mildew diseases in greengram.

Entry	ANTH	CLS	PM
DGG-203	MR	MR	MR
DGG-206	MR	MR	MR
DGG-21	MR	MR	MR
DGG-273	MR	MR	MR
IPM-3-2	MR	MR	MR
DGG-96	MR	MR	MR
DGG218	-	-	R
DGG-80	MR	MR	R
DGG-215-7	-	-	MR
DGG-21	MR	MR	MR
DGG182-3	-	-	MR

row separately, some of the breeding lines showed resistance to the MYMV. As a result, the identified resistance lines can be used for further reactivity confirmation as well as the use of certain progenies in breeding programs to produce Mungbean Yellow Mosaic Virus (MYMV) resistant cultivars with good performance. Similar results were observed by Kingsly *et al.* (2015); Sudha *et al*. (2013); Kabi *et al*. (2017) and Dharajiya *et al.* (2018). The ABLs DGG 302, DGG201, DGG126, DGG 128, DGG237 have recorded moderate resistance to MYMV,while DGG 125,DGG 96, DGG218, DGG263 and Accession no 436714 have recorded resistance to MYMV (Table 2).

The current investigation emphasized on development of greengram genotypes tolerant to biotic stresses like anthracnose, CLS, powdery mildew and MYMV through recombination, followed by mutation breeding and also pedigree method. The advanced breeding lines *viz*., DGG-203, DGG-206, DGG-208, DGG-21, DGG-80, DGG-273, DGG-274, DGG-275, DGG-262, DGG-263, DGG-264, DGG-82, DGG-200, TARM-1 (C), DGG-215-5, DGG-302, DGG-152, IPM-3-2, DGG-20, DGG-73, IPM-14-10, DGG-121, DGG-250, DGG-109, DGG-96 have recorded moderate resistance to both anthracnose and CLS. Further the breeding lines DGG-203, DGG-206, DGG-21, DGG-273, IPM-3-2 (check), DGG-96 have recorded combined resistance to anthracnose, cercospora leaf spot and powdery mildew diseases (Table 3). None of the genotypes are comparable to the desirable pod features and high yield potential of the released agronomically superior and popular variety DGGV-2. Hence all these traits can be introgressed and back crossed to DGGV-2 to breed high yielding genotypes conferring tolerance to the major biotic stresses prevalent in the area.

The breeding lines DGG-203, DGG-206, DGG-21, DGG-273, IPM-3-2 (check), DGG-96 have recorded combined resistance to anthracnose, cercospora leaf spot and powdery mildew diseases. (Plate 1).

CONCLUSION

The current investigation emphasized on development of greengram genotypes tolerant to biotic stresses like anthracnose, CLS, powdery mildew and MYMV through recombination, followed by mutation breeding and also pedigree method. The promising foliar disease resistant line can be stringently screened under artificial epiphytotic conditions and on confirmation of resistance can be utilized in breeding programmes aimed at developing foliar disease resistant green gram varieties, which can augment the yielding potential under varied climatic conditions.

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Conflict of interest: None.

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