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Evaluation and Isolation of Mungbean Yellow Mosai Virus Resistant Mutants in M_4 and M_5 Mutant Generations of Blackgram (*Vigna mungo*)

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ABSTRACT

Background: The pulse known as "Blackgram" is a significant component of the Indian diet since it provides vegetable protein and balances the diet centered on cereals. Mungbean Yellow Mosaic Virus (MYMV) outbreaks on a regular basis completely destroy crops, causing farmers to suffer enormous losses. Mutation breeding can be an effective strategy for developing crops with desirable traits, such as high yield and resistance to diseases like the Mungbean Yellow Mosaic Virus (MYMV) in blackgram.

Methods: This experiment was laid out to study the variability pattern of mutant population in M_4 and M_5 generation of blackgram variety CO 6 treated with the Gamma rays at 200 Gy, 300 Gy and 400Gy and isolation of viable mutants for Mungbean Yellow Mosaic virus resistance. Significant variability was observed among the different treatments in M_4 generation and twenty MYMV resistant mutants ((PKT-BG-200Gy-P07; PKT-BG-200Gy-P24; PKT-BG-200Gy-P29; PKT-BG-200Gy-P35; PKT-BG-200Gy-P36; PKT-BG-200Gy-P45; PKT-BG-300Gy-P13; PKT-BG-300Gy-P16; PKT-BG-300Gy-P28; PKT-BG-300Gy-P33; PKT-BG-300Gy-P41; PKT-BG-300Gy-P4; PKT-BG-300Gy-P43; PKT-BG-300Gy-P76; PKT-BG-300Gy-P81; PKT-BG-300Gy-P88; PKT-BG-300Gy-P93; PKT-BG-400Gy-P15; PKT-BG-400Gy-P21; PKT-BG-400Gy-P23) were selected and evaluated in a randomized block design with three replications under natural conditions. Scoring was done by the modified scale of All India Coordinated Research Project on MuLLaRP.

Result: Incidence ranged from scale 0.01 (PKT-BG-300Gy-P41and PKT-BG-300Gy-P42) to 5.30 (PKT-BG-300Gy-P28). The mutants PKT-BG-300Gy-P41 and PKT-BG-300Gy-P42 recorded with maximum yield of 1500 kg/ha and 1445kg/ha were found to be observed as high yielding and MYMV resistant lines for summer season in the Cauvery delta region.

Key words: Blackgram, Mutation breeding, MYMV, Resistant and high yielding lines.

INTRODUCTION

Blackgram, also known as urad dal or black lentil, is indeed a vital crop in the Indian diet, offering essential protein and nutrients. Its high protein content, combined with its ability to complement a cereal-based diet, makes it a valuable dietary component. This crop is resilient to adverse weather conditions, making it a reliable and stable agricultural crop. Blackgram is also a vital part of Indian cuisine, enriching diets centered on cereals by adding vegetable protein. It boasts an impressive protein content of approximately 26%, nearly three times that of cereals and a rich source of essential vitamins and minerals. Furthermore, it is fed to milch cows in particular as nutrient-rich fodder. Globally India is the largest producer of black gram, accounting for more than 70% of production followed by Myanmar and Pakistan.

Mutation breeding is employed in a number of national and state universities/research centres across India, including Tamil Nadu Agricultural University (TNAU), Indian Agricultural Research Institute (IARI), Bhabha Atomic Research Institute (BARC) and National Botanical Research Institute. A broad range of mutants impacting different properties were produced by induced mutagenesis in oilseeds and grain legumes utilizing X ray, Beta, Gamma, Fast neutrons, and Ethyl methane sulfonate. Gamma-ray and EMS-induced mutations are widely used for development of early mutants with higher pod counts, seed counts per pod, weights per 100 seeds, and protein content. MYMV resistant mutants and desirable plant types were isolated using gamma rays. Gamma rays, a form of ionizing radiation, have been used in plant breeding to induce mutations, which can potentially lead to improved crop varieties.

The Bhabha Atomic Research Centre (BARC) has been a pioneering institute for genetic improvement of blackgram

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through induced mutations for the past thirty years. Through extensive mutation investigations of the EC-168200 genotype, numerous mutants with unique morphological characteristics have been isolated. A significant breakthrough obtained from the mutation breeding was identification of TAU-5, an early maturing and yellow mosaic virus (YMV) mutants in All-India Pulse Improvement Project, Kanpur (Pawar *et al*., 2000). Elite cultivars such as T-9, TPU-4 and LBG-17 were successfully crossed with TAU-5. The introduction of the blackgram variety 'Trombay' has played a crucial role in increasing its productivity in several states of the country. TAU I, the most popular blackgram variety cultivated in Maharashtra, Karnataka and Andhra Pradesh for its large seed size (Pawar, 2000). Some black gram mutation varieties popularly grown in Tamil Nadu are ADT 3, Co 6, TU 17-9, VBN3, T-9 and Pant U-30.

However, the Mung Bean Yellow Mosaic Virus (MYMV) poses a significant threat to blackgram crops. MYMV is a major concern for farmers because it can cause severe yield losses, which in turn impacts their livelihoods. The virus is known for its capacity to spread rapidly, exacerbating the damage and making it challenging for farmers to manage. The black gram productivity and production are negatively impacted by the genus Begomo virus, which causes the Mungbean Yellow Mosaic Virus (MYMV) disease. The species *V. radiata, V. mungo, V. aconitifolia, V. unguiculata, Cajanus cajan, Glycine max* and *Phaseolus vulgaris* are among the important YMV hosts (Karthikeyan *et al*., 2014). As alternatives, it has also been established that *V. hainiana* and *V. trilobata* are natural hosts of other leguminous organisms. Two genes regulate the expression of resistance to MYMD and the chi-square goodness-of-fit test on an F_2 population demonstrated repressive gene activity in blackgram (Subramaniyan *et al*., 2021). It implies that there are several pathogenicity-varying strains of the causing virus, which might be the reason for the complex genetics of MYMD resistance. The main reasons for low productivity in black gram are due to the disease caused by pathogen which affects the production. Among them, the Mungbean Yellow Mosaic Virus (MYMV) disease is the most devastative disease causing yield loss upto hundred percentage and the disease is spread through the vector whitefly (*Bemisia tabaci*). The Yellow Mosaic Virus also popularly called as "Yellow plague of *Kharif* pulses". Although there are many strategies available for managing YMV disease such as vector management, chemical control and modifying cultural practices which are not more effective for controlling this disease. Therefore, there is a urgent need to develop a alternate technology to control the spread of this disease. Development of MYMV resistant varieties is a better method of management technology to break the chain of disease spread. With this background, the present research was under taken with a view to identify Mungbean Yellow mosaic virus resistance mutants for higher yield.

MATERIALS AND METHODS

Blackgram variety CO 6 was treated with the mutagen Gamma rays at 200 Gy, 300 Gy and 400Gy and raised M_1 and M_2 generation at Department of Pulses, Tamil Nadu Agricultural University, Coimbatore during *Rabi* season 2019 and summer season 2020 and the M_3 generation was raised along with susceptible check CO 6 at MYMV hot spot centre- National Pulses Research Centre, Vamban during summer irrigated condition of the year 2020 and M_4 generation was advanced at Agricultural Research Station, Pattukkottai along with infector rows of susceptible check variety CO 6 and yield check variety ADT 5 during rabi season 2020 and 75 single plants were selected based MYMV resistance. From these 75 single plants, based on MYMV disease incidence, twenty high yielding plant/ genotypes were selected for further screening for MYMV resistance based on the biometrical observations.

In M_5 generation, 20 mutants (PKT-BG-200Gy-P07; PKT-BG-200Gy-P24; PKT-BG-200Gy-P29; PKT-BG-200Gy-P35; PKT-BG-200Gy-P36; PKT-BG-200Gy-P45; PKT-BG-300Gy-P13; PKT-BG-300Gy-P16; PKT-BG-300Gy-P28; PKT-BG-300Gy-P33; PKT-BG-300Gy-P41; PKT-BG-300Gy-P42 ; PKT-BG-300Gy-P43; PKT-BG-300Gy-P76; PKT-BG-300Gy-P81; PKT-BG-300Gy-P88; PKT-BG-300Gy-P93 ; PKT-BG-400Gy-P15 ; PKT-BG-400Gy-P21; PKT-BG-400Gy-P23) were isolated and raised in randomized block design with three replications along with checks ADT5 and CO 6 in summer irrigated condition during 2021 at Agricultural College and Research Institute, Eachangkkottai. Regular agronomic practices were followed to maintain healthier population. Regular monitoring was conducted for whitefly surveillance. The following biometric observations were recorded on ten plants for plant height, days to 50% flowering, number of pods/ plant, days to maturity, seed yield and MYMV resistance.

Screening for MYMV Screening for MYMV resistance was performed by following infector row method in the field with three rows of the mutants and one row of susceptible check CO6 in natural epidemic conditions. Disease infestation was estimated according to the formula of Devi *et al*. (2016). The severity of disease was recorded by modified (0-9 scale; Table 1) of All India co-ordinated Research Project on MULLARP (Alice and Nadarajan 2007) and categorization was done based on (Gantait and Kantidas, 2009).

Per cent of disease incidence (PI) =

$$\frac{\text{No. of infected plants in the plot}}{\text{Total number of plants in the plot}} \times 100$$

The severity of disease was recorded by modified (0-9 scale) of All India co-ordinated Research Project on MULLARP (Alice and Nadarajan, 2007) and categorization was done based on (Gantait and Kantidas, 2009; Table 2). The estimates of mean, variance and standard error were analysed as per the methods suggested by Panse and

Sukhatme (1985). The estimates of variability parameters were worked out according to the method suggested by Johnson *et al*. (1955). Phenotypic and genotypic coefficient of variations was calculated based on the method by Burton (1952). Heritability in the broad sense by Lush (1940) and Genetic advance (GA) by Johnson *et al*. (1955) was estimated.

RESULTS AND DISCUSSION

The combined effect of gamma ray-induced mutations and advancements in agronomic practices has led to significant increases in grain yield per hectare over the past 50 years (Borlaug, 1983). The wide range of variation observed for all the quantitative traits suggests that there is sufficient variability in these characters to exploit. Our study demonstrated that the application of physical mutagens gamma rays, led to significant improvements in various growth traits of black gram plants in the M_4 generation. Among the different dose of gamma rays, a gradual increase of mean values was observed up to 300Gy when compared to control in M_4 generation. Beyond the optimal dose of 300Gy mutagen showed decreasing of mean values of quantitative traits (Table 3). Variability analysis showed an increase all the traits. The analysis of variance (ANOVA) revealed the significance degree among the treatment and control. The wide range of variation was recorded at 300Gy and Control variety compared to other two treatments. Those ranges are *viz*., plant height (38.6 and 36.4 cm), day to 50 percent flowering (37.2 and 40.1 days), Number of pods (44.1 and 37.2), days to maturity (72.5 and 75.35 days) and yield per ha (1325 and 1056 kg). The similar kind of wide range was observed more in the treatment 300Gy in earlier generation of M_2 and M_3 in the similar population. These findings suggest that the mutagenic treatments induced genetic variability in the black gram genome, which in turn contributed to the observed improvements in growth and yield characteristics as similar reported in chickpea in M_2 generation of chickpea (Wani and Anis, 2001). In the study by Odeigah *et al*. (1998), the researchers investigated the effects of ethyl methane sulfonate (EMS) and gamma rays on various quantitative traits in cowpea during the M2 generation. Their findings indicated that the treatments led to significant improvements in several traits compared to the control. Specifically, plant height, the number of peduncles per plant, the number of pods per plant, the weight of 1000 seeds and the number of seeds per pod all showed increased values due to the influence of EMS and gamma rays. This suggests that these mutagenic treatments had a positive impact on the growth and yield characteristics of cowpea, highlighting their potential for enhancing crop performance.

Phenotypic and genotypic coefficient of variation of quantitative traits (PCV and GCV) are parameters for selecting genotypes possessing higher yield and growth traits depends largely on the existence and exploitation of genetic variability of the fullest extent. The estimates of range, phenotypic and genotypic coefficient of variability was presented in Table 3. The phenotypic and genotypic coefficient of variation expressed in terms of per cent was comparatively high at 200 Gy of gamma rays than control *viz*., plant height (35.98; 37.85), day to 50 per cent flowering (24.50;24.59), Number of pods (85.89;85.98), days to maturity (140.21;141.05) and yield per ha (9.81;9.87). The PCV and GCV values were significant at P-0.05 and P-0.01

Table 1: The severity of disease was recorded by modified (0-9 scale) of All India Co-ordinated Research Project on MULLARP (Alice and Nadarajan, 2007).

Disease severity per cent	Rating	Reaction
0.1-5	1.0-2.0	Resistant (R)
5.1-15	2.1-4	Moderately Resistant (MR)
15.1-30	4.1-5	Moderately susceptible (MS)
30.1-75	5.1-7	Susceptible (S)
75.1-100	7.1-9	Highly Susceptible (S)

Table 2: Modified All India Co-ordinated Research Project on MULLARP (Alice and Nadarajan, 2007).

Scale	Description
0	No visible symptoms on leaves.
1	Very minute yellow specks on leaves.
2	Small yellow specks with restricted spread covering 0.1-5.0% leaf area of plant.
3	Yellow mottling of leaves covering. 1-10.0% leaf area of plant.
4	Yellow mottling of leaves covering 10.1-15.0% leaf area of plant.
5	Yellow mottling and discoloration of 15.1-30.0% leaf area of plant.
6	Yellow discoloration of 30.1-50.0% leaf area of plant.
7	Pronounced yellow mottling and discoloration of leaves and pods, reduction in leaf size and stunting of plants covering 50.1-75.0% foliage of plant.
8	Severe yellow discoloration of leaves covering 75.1-90.0% foliage, stunting of plants and reduction of pod size.
9	Severe discoloration of leaves covering above 90.1% of foliage of plants, stunting of plants and no pod formation.

level, which positively correlated with their mean values of quantitative traits. The highest genetic coefficient of variation (GCV) was observed for the number of pods per plant, indicating that simple selection for yield may be more advantageous compared to focusing on individual yield components. Previous research by Kumar (2008) also reported high GCV for traits such as the number of pods per plant, number of seeds per pod, and 100-grain weight in peas. These findings are consistent with the current study and suggest that phenotypic selection for these traits could be highly effective in improving yield.

Heritability (h^2), genetic advance (GA %) as percent of mean of quantitative traits. The wide range of variability was exhibited by heritability and genetic advance as per cent of mean. The heritability and GA as percentages of mean were high almost all dose of gamma ray treatment (Table 3). However, 300Gy of gamma rays revealed highest values of heritability with genetic advance as per cent of mean for plant height (79.2; 39.46), day to 50 per cent flowering (60.1; 54.86), Number of pods (78.2; 95.20), days to maturity (44.2; 68.23) and yield per ha (79.1; 91.66).

The study revealed high heritability with high genetic advance as percent of mean in all the quantitative traits at 300Gy of gamma rays in M_4 generation. When a trait shows high heritability coupled with high genetic advance, it suggests that the trait is largely influenced by additive genetic effects. In other words, the effects of individual genes on the trait are cumulative and not masked by interactions with other genes (non-additive gene action). This additive nature makes the trait more amenable to selection because the genetic progress achieved through selective breeding is likely to be more predictable and effective (Unche *et al*., 2008). Kalpande *et al*. (2008) recorded high heritability for plant height, seed cotton yield /plant, number of bills/plant, number of sympodia/plant and average boll weight in F_3 generation of cotton. It indicated that predominance of additive gene action.

Among the 20 mutants evaluated in M_5 generation, the mutants BG-200Gy-P07 and BG-200Gy-P24 recorded for early 50% flowering (38 and 42 days). The mutant BG-200Gy-P07 was found to be observed as dwarf with plant height of 38.5 cm, however there was no significant difference observed in the remaining lines. Mutant lines

Table 3: Analysis of variances, Standard error, CD, CV, PCV, GCV, Heritability and Genetic advance observed in the homozygosity of M_4 generation.

Characters	GM	Genotype MMS	Error MMS	SEd	CD	CV	GCV	PCV	Heritability	Genetic advance
M_4 Generation 200Gy treatment										
Plant height (cm)	38.6	20.78	0.60	0.63	1.33	9.39	35.98	37.85	60.4	70.47
Days to 50% flowering	39.3	8.48	0.02	0.11	0.25	2.25	24.50	24.59	39.3	50.28
No of pods/plant	42.5	159.88	0.90	0.77	1.63	3.81	85.89	85.98	79.8	82.76
Days to maturity	74.5	549.81	0.20	0.36	0.77	4.93	140.21	141.05	78.2	95.20
Seed yield (kg/ha)	1295	176.70	4.96	1.81	3.81	2.92	9.81	9.87	60.2	18.56
M_4 Generation 300Gy treatment										
Plant height (cm)	39.2	255.76	4.99	1.82	3.81	3.12	9.43	9.87	79.2	39.46
Days to 50% flowering	37.2	68.28	2.15	1.19	2.50	2.51	7.54	7.90	60.1	54.86
No of pods/plant	44.1	77.00	1.06	0.84	1.75	2.03	22.93	23.12	78.2	95.20
Days to maturity	72.5	0.002	0.005	0.02	0.04	3.04	4.23	4.51	44.2	68.23
Seed yield (kg/ha)	1325	122.02	5.09	1.84	3.84	2.91	5.23	5.84	79.1	91.66
M_4 Generation 400Gy treatment										
Plant height (cm)	38.4	356.94	3.10	1.43	3.01	2.90	19.30	19.45	60.2	18.56
Days to 50% flowering	39.2	42.54	0.02	0.12	0.24	2.86	71.33	71.37	65.2	14.83
No of pods/plant	45.1	539.10	0.07	0.21	0.45	3.21	140.96	141.05	60.2	46.83
Days to maturity	72.4	15.05	0.00	0.05	0.10	2.21	79.65	80.07	55.2	8.70
Seed yield (kg/ha)	1276	73.89	26.53	4.21	8.79	3.87	97.96	98.03	59.1	9.63
Control (Co6)										
Plant height (cm)	36.4	233.76	4.59	1.32	2.91	2.12	19.23	19.87	63.4	28.56
Days to 50% flowering	40.1	58.28	3.05	1.76	2.50	2.59	6.21	6.75	63.4	17.63
No of pods/plant	37.2	65.42	1.06	0.74	1.06	2.26	22.04	23.12	65.7	33.83
Days to maturity	75.35	0.001	0.005	0.03	0.04	2.04	4.01	4.51	51.5	9.70
Seed yield (kg/ha)	1056	103.02	3.09	1.23	2.29	2.10	6.78	6/91	53.2	10.63

Table 4: Mean performance for yield and yield related traits and MYMV disease score in blackgram.

Name of the mutant	Plant height (cm)	Days to 50% flowering	No. of pods /plant	Days to maturity	Seed yield (kg/ha)	Disease severity percentage (%)
PKT-BG-200Gy-P07	38.5	38	43.3	73	1120	1.34
PKT-BG-200Gy-P24	41.8	42	50.3	75	960	1.52
PKT-BG-200Gy-P29	42.6	43	53.5	72	1150	0.45
PKT-BG-200Gy-P35	44.3	48	47.3	73	1335	0.14
PKT-BG-200Gy-P36	42.5	43	49.2	69	945	0.55
PKT-BG-200Gy-P45	49.2	38	45.3	73	1250	1.32
PKT-BG-300Gy-P13	46.5	43	50.3	73	1360	2.36
PKT-BG-300Gy-P16	46.2	45	49.2	76	1345	0.13
PKT-BG-300Gy-P28	44.0	45	53.7	78	1125	5.30
PKT-BG-300Gy-P33	43.2	43	45.3	65	1345	0.75
PKT-BG-300Gy-P41	43.5	45	50.2	73	1500	0.01
PKT-BG-300Gy-P42	42.6	42	62.4	66	1445	0.01
PKT-BG-300Gy-P43	46.7	43	47.3	75	1300	4.32
PKT-BG-300Gy-P76	44.4	42	46.9	74	1125	0.35
PKT-BG-300Gy-P81	43.5	45	46.8	74	1364	0.55
PKT-BG-300Gy-P88	43.2	45	47.9	73	1345	5.30
PKT-BG-300Gy-P93	45.9	43	68.5	75	1350	0.50
PKT-BG-400Gy-P15	46.5	44	32.4	75	1260	1.56
PKT-BG-400Gy-P21	42.5	43	50.5	73	986	0.05
PKT-BG-400Gy-P23	42.8	45	46.5	72	1035	1.24
Checks ADT5 (For yield)	47.3	43	45.3	72	1350	5.3
CO5 (For susceptible)	38.3	47	38.0	73	650	12.5
Mean	41.6	43.5	36.4	73.4	1245	0.44
SE	0.73	0.53	0.29	0.09	0.79	0.44
CV	2.57	0.98	3.78	2.23	3.12	0.45

BG-300Gy-P93 recorded more number of branches per plant (68.5nos.) followed by BG-300Gy-P42 (62.4nos).The mutant BG-300Gy-P33 and BG-300Gy-P42 were found to be observed as early maturing mutants (66 and 67 days). The mutants BG-300Gy-P41and BG-300Gy-P42 recorded with maximum yield of 1500 kg/ha and 1445kg/ha (Table 4). In this study 20 mutants were screened for MYMV resistance by infector row method using Co5 as susceptible check and categorization was done (Gantait and Kantidas, 2009). Bandi (2018) likely provided valuable insights into the genetic resistance of blackgram to MYMV, so aligning with the results suggests that methods and interpretations are sound. The results showed that the mutants PKT-BG-300Gy-P41, PKT-BG-300Gy-P42, PKT-BG-400Gy-P21 and PKT-BG-300Gy-P16 were resistant to MYMV with disease severity percentage between 0.01 to .0.052. Whereas the mutants PKT-BG-300Gy-P28 (5.3), PKT-BG-300Gy-P88 (5.3) and PKT-BG-300Gy-P43 (4.3) were found to be moderately resistant with disease severity percentage between 4 to 6. The overall performance of the mutants showed resistant nature even MYMV was in peak epidemic area in the particular season in the region. Murugan and Nadarajan (2012) reported that the resistance to MYMV is dominant and suggested that the presence of a single dominant allele is sufficient to confer resistance to the disease. Verma and Singh (1986) reported that the resistance to MYMV is recessive and implies that two recessive alleles are needed for the expression of resistance. The observed phenotypic expressions of MYMV disease incidence among the F_1 progeny revealed inconsistencies with the expected inheritance patterns, as reported by Vadivel *et al*. (2023).

CONCLUSION

The present investigation reveals significant variability in traits, coupled with high heritability and substantial genetic advance for key yield traits in the M_4 generation. This suggests that selection is effective for isolating resistant mutant lines at this stage. Based on further intensive replicated trials, the lines PKT-BG-300Gy-P41, PKT-BG-300Gy-P42, PKT-BG-400Gy-P21, and PKT-BG-300Gy-P16 have been identified as exhibiting complete resistance. To confirm the stability of genetic inheritance for these lines, it is recommended to conduct extensive trials in diverse farming environments. This will help ensure that a resistant variety can be developed as a precautionary measure against MYMV disease outbreaks.

Conflict of interest

All authors declare that they have no conflict of interest.

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