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Development of Yellow Mosaic Virus Resistance in Mung Bean through EMS Mutagenesis

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Authors' contributions

This work was carried out in collaboration between all authors. Author AR was the principal scientist of the study and provided the raw data as well as edit the final draft. Author MMH analyzed the data and wrote the first draft of the manuscript. Author SI supervised the field trial and collected data. Author LR provided intellectual guidance and overall supervision of the project. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Green gram is a variety of mungbean (*Vigna radiata*) and one of the most crucial food legumes consumed as a protein source. One of the most destructive diseases of the legume is Yellow mosaic virus. It is caused by bipartite begomoviruses namely mungbean yellow mosaic virus (MYMV). One of the ways to strengthen resistance against the virus is mutation breeding. Several programs of induced mutations have been conducted in the subcontinent successfully. Ethyl methanesulfonate (EMS) been used as a chemical mutagen in the TILLING technique for mutation breeding programs for a while now since it induces point mutation within the genome. In this experiment the seeds of BARI Mung-6 were treated with six different concentrations (0.05%, 0.1%, 0.15%, 0.20%, 0.3% and 0.4%) EMS. Seeds were grown and advanced in the greenhouse till M3 generation. Individual lines were chosen as per standard disease grade 0-3. The M4 generation was grown in the research field and 13 lines were chosen as per level of disease resistance and 100 seed weight. Three lines were identified as high yielding resistant material from those thirteen tested lines.

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1. INTRODUCTION

Pulses are an important protein source for the majority of Bangladesh. Bangladesh has faced a deficit in supply of pulses in comparison to demand for many years [1]. Green gram is a variety of mungbean (*Vigna radiata*) and one of the most consumed food legumes [2]. Mungbean is a diploid ($2n = 2x = 22$) pulse crop, cultivated predominantly in South East Asia mainly for its protein-rich edible seeds and sprouts [3]. It is an excellent source of easily digestible high-quality protein for both humans and domestic animals. The seeds provide an inexpensive source of nutritionally rich vegetable protein to the poor population. They are preferred due to their easy digestibility and absence of flatulence producing factors [3]. Yao et al. in [4]; discussed the anti-diabetic properties of extracts from mung bean sprouts and seed coat in their study of type 2 diabetic mice. Selection of varieties with high levels of polyphenol content during sprouting was recommended as a very good option for addressing diabetic problems. Being leguminous, this crop maintains soil fertility by fixing the atmospheric nitrogen [5,6].

The crop is susceptible to diseases caused by fungi, bacteria and viruses. The yellow mosaic virus is one of the most devastating diseases of mung bean. It is caused by bipartite begomoviruses namely Mung bean Yellow Mosaic Virus (MYMV), Mung bean Yellow Mosaic India Virus (MYMIV) and Horse gram Yellow Mosaic Virus (HgYMV) in different growing areas of the world. Various leaf and stem pathogens, such as powdery mildew and bacterial blight, are frequently seen, especially in growing crops.

Interspecific crosses within the genus *Vigna* have been attempted by various research groups. Barriers while crossing mung bean with other species range from failure of pollen tube to penetration into the style or stigma [7] to embryo abortion after fertilization and poor pod or seed set. Bharathi et al. in 1998, while crossing *V. radiata* as the female parent with four other species obtained highest crossability with *V. umbellata* (29.63%) followed by *V. trilobata* (8.48%) and *V. aconitifolia* (7.69%) [8]. Though dramatic results have been obtained in cereal crops by conventional breeding, the success in improving the crop yield has remained largely elusive in case of pulse crops in general and mung bean in particular [9]. Modern biotechnological approaches include insertion of novel gene/s at cellular level through various methods, such as, protoplast fusion, genetic transformation, etc. *In vitro* regeneration and appropriate genetic transformation methodology are the pre-requisites for plant genetic engineering. As most of the legumes have been reported as recalcitrant towards in vitro regeneration [10] it has proven cumbersome to improve mung bean through advanced biotechnological methodologies. Moreover, biotechnology is an expensive area of research and genetically modified crops have to go through strict regulations prior to release as a commercial variety. Several programs of induced mutations in mung bean have been conducted, especially in India, Pakistan, and Bangladesh [11]. EMS has been in used as a chemical mutagen for mutation breeding programs since it induces point mutation within the genome easily [12]. EMS was used as the mutagenic agent for improving disease resistance and yield capacity of a popular mungbean variety of Bangladesh namely BARI Mung 6.

2. METHODOLOGY

The research was carried out at the greenhouse (Gulshan Avenue, Dhaka) and in the field of Central Research Station (Mawna, Gazipur) of the Advanced Seed Research and Biotech Center, ACI Limited. One popular mungbean variety called BARI Mung 6 (green gram) which is susceptible to yellow mosaic virus was used as the parent germplasm. The duration of the whole experiment was 1 year and 6 months (From March 2015 to September 2016). Soil was prepared for mungbean with urea, TSP, MP and cow dung according to the concentration mentioned in Table-1. The seeds of BARI Mung-6 were soaked overnight in distilled water supplemented with six different concentrations (0.05%, 0.1%, 0.15%, 0.20%, 0.3% and 0.4%) of Ethyl methanesulfonate (EMS).

Table 1. Fertilizers required for soil preparation

Name of the item	Concentration (kg\ha)
Urea	40
TSP	80
MP	30
Cow dung	4-5

Bio-Ferti (micronutrient source for improving plant health), Exel Gold (micronutrient source), ACI Mix (pesticide), and Volume Flexi (pesticide for pod borer) were also used. For each treatment 100 seeds were used with 3 replications. Seeds grown in the green house were sown in individual pots and advanced till M3 generation. Disease affected plants were graded in 5 categories as per Table 2.

Seeds from all the EMS treated plants, i.e. M_0 generation, were maintained as a separate line. Later on, lines were chosen as per disease grades 0-3. In case of M_4 generation the plants were tested in experimental field plots along with susceptible lines as control. The size of each plot was 5m x 3m with plant to plant distance of 5 cm and row to row distance of 30 cm. This is the standard spacing recommended by the Bangladesh Agricultural Research Institute for Mung bean. In each plot 1000 seeds were sown. The whole field experiment was done in 3 replications. In terms of M_4 generation, data was recorded for disease grade, pod length, number of seeds per pod and weight of 100 seeds per line. Statistical analyses were done using the software IBM SPSS Statistics 25 for windows.

3. RESULTS AND DISCUSSION

Mutagenic agents are used to bring change in genotype that may be used as breeding lines [13]. Modifications by EMS basically introduce mismatch and change in nitrogenous base of DNA [14]. Most of the EMS treatment induces changes from C/G to T/A [15]. As EMS introduces random point mutation throughout the genome of a plant, it is used to study the function of a particular gene and to understand specific role of an amino acid in proteins along with mutation breeding programs. In general, in seed-propagated plants, germinating seeds are used so that the mutagenic agent is absorbed by the embryo and reach the meristematic region of the embryo [16].

Since disease tolerance capacity and yield per plant are the desired characters of this experiment, good seeds were collected from the healthy plants of M_0 generation showing acceptable phenotypic characteristics (Figs. 1 and 3). Characteristics changed as concentration of EMS treatment increased. Initially rouging was done to reduce population depending on mortality rate, stunted growth, delayed flowering and vine like plants. Mortality rate was found to be the highest at 0.4% concentration as expected, while one third of the plants showed stunted growth or delayed flowering at 0.3% concentration. Afterwards, plants were screened based on disease scale till M_3 generation and in case of M_4 generation plants were selected based on the weight of 100 seeds along with disease resistance (Figs. 2 and 4). Two lines selected as per disease grade till M3 generation, failed to be resistant in the M4 generation field trial, when exposed to ambient conditions of high humidity and temperature. Finally a total of 55 lines were screened out from M_3 generation where none scored greater than 2 in disease scale used in this study. From M_4 generation 13 promising lines were found to be MYMV tolerant\resistant as well as high yielding (Fig. 4).

Managing the Mungbean Yellow Mosaic disease is much cumbersome as it has a wide range of host plants including weeds [17]. Attempts were taken to improve various quantitative traits of mungbean, *viz*., fertile branches per plant, pods per plant and seed yield per plant [18]. Though they did not report the mentioned characteristics

Table 2. Disease scale for Mungbean Yellow Mosaic Virus Disease [19]

Score	Symptoms	Disease reaction
0	No visible symptoms on leaves or very minute yellow specks (1-2) on leaves	Highly resistant
1	Small yellow specks with restricted spread covering up to 5% leaf area	Resistant
2	Yellow molting covering up to 15% leaf area	Moderately resistant
3	Yellow molting and discoloration of up to 30% leaf area	Moderately susceptible
4	Pronounced yellow molting and discoloration of leaves and pods, reduction in leaf size and stunting of plants covering up to 75% foliage	Susceptible
5	Severe yellow discoloration of leaves covering 75% of foliage, stunting of plants and reduction in pod size	Highly Susceptible

Fig. 1. Mungbean plants in pod of M_3generation (a) Control plant showing MYMV disease symptom (b) mutant plant showing no disease symptom

Fig. 2. Mungbean plants of M4 generation in field (a) Control plant showing MYMV disease symptom (b) mutant plant showing no disease symptom

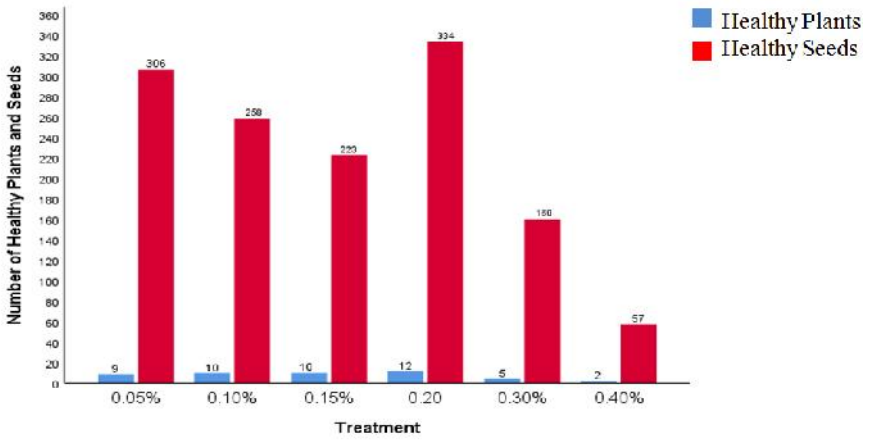

Fig. 3. Number of healthy plants and good seeds derived from M_0 generation

against disease resistance, particularly against MYMV; they suggested that further development of mung bean is possible through induced mutation using EMS and gamma radiation. Higher doses of EMS reduce various quantitative characteristics of mung bean [12]. Therefore,

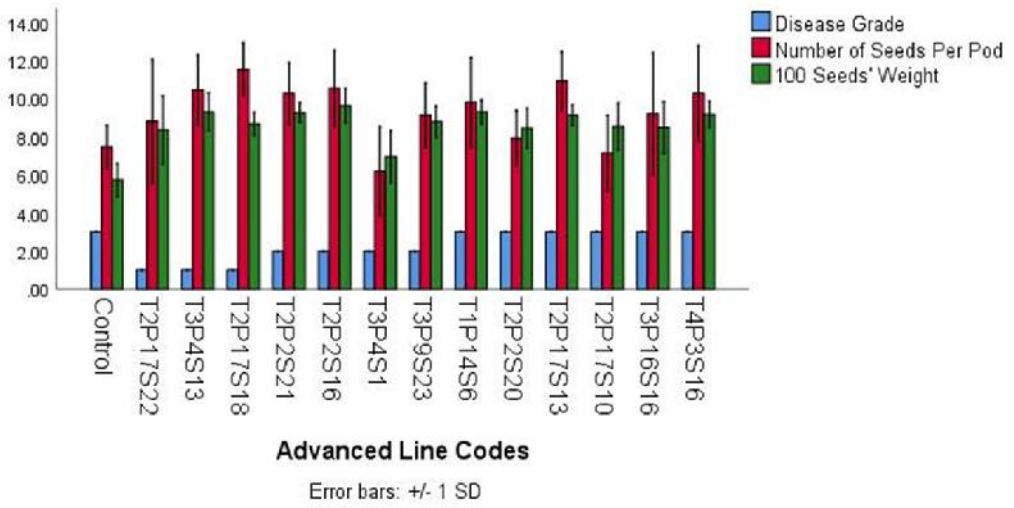

Fig. 4. Average of 100 seeds' weight (g) and number of seeds per pod along with disease grade of selected lines from M_4 generation

various quantitative characters were measured in this study to be certain the yield quality is not compromised while selecting disease tolerant lines. During the field trial of M_4 generation among the selected 13 lines from M_3 generation, higher yield than control (BARI Mung-6) was recorded for all the lines. Three lines, e.g. T2P17S22, T3P4S13 and T2P17S18 scored 1 in terms of disease scale which are also resistant lines whereas the control variety scored 3, i.e. moderately susceptible (Fig. 4). Four lines (T2P2S16, T2P2S21, T3P4S1 and T3P9S23) scored 2 (moderately resistant) and rest of the six lines, e.g. T1P14S6, T2P2S20, T2P17S10, T2P17S13, T3P16S16 and T4P3S16 scored as similar as control (Fig. 4). In terms of yield, a number of seeds per pod and weight of 100 seeds were considered. However, lines showing highest disease resistant capacity in M_4 were also as high in case of both the previously mentioned quantitative traits as the control (Fig. 4).

4. CONCLUSION

Three resistant lines of mung bean towards the most devastating disease Mung bean Yellow Mosaic Virus with high yielding capacity were developed in the current experiment (T2P17S22, T3P4S13 and T2P17S18) following induced mutation methodology. Ethyl methanesulfonate (EMS) was used as the mutagenic agent in this experiment. Such putative mutant lines will be subjected for large-scale field trial and release as a commercial variety if they outperform the original parent.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Das S, Kabir W. Pulses production in Bangladesh: Status and Drivers for Enhancement; 2016.
DOI: 10.13140/RG.2.1.3112.7283
2. Kakati P, Deka SC, Kotoki D, Saikia S. Effect of traditional methods of processing on the nutrient contents and some antinutritional factors in newly developed cultivars of green gram [*Vigna radiata* (L.) Wilezek] and black gram [*Vigna mungo* (L.) Hepper]. International Food Research Journal. 2010;17:377-384.
3. Sahoo L, Sugla T and Jaiwal PK. *In vitro* regenearion and transformation of cowpea, mung bean and adzuki bean. Applied

Genetics of Leguminosae Biotechnology. 2003;89–120.

4. Yao Y, Chen F, Wang M, Wang J and Ren G. Antidiabetic activity of mungbean extracts in diabetic KK-Ay mice. Journal of Agricultural Food Chemistry. 2008;56(19): 8869–8873.
5. Senanayake L, Knievel DP, Stevena SE. Nodulation and symbiotic nitrogen fixation of cowpea (*Vigna unguiculata* L.). Plant Soil. 1987;99:435-439.
6. Zapata F, Danso SKA, Hardarson G, Fried M. Nitrogen fixation and translocation in field-grown fababean. Agronomy Journal. 1987;79:505-509.
7. Chowdhury RK, Chowdhury JB. Intergeneric hybridization between *Vignamungo* L. Hepper and *Phaseolus calcaratus* Roxb. Indian Journal Agricultural Science. 1977;47:117–121.
8. Bharathi A, Vijay KS, Veerabadhiran P, Subba BL. Crossability barriers in mungbean (*Vigna radiata* L. Wilczek) with its wild relatives. Indian Journal of Crop Science. 2006;1(1–2):120–124.
9. Vir R, Lakhanpaul S, Malik S, Umdale S, Bhat KV. Utilization of germplasm for the genetic improvement of Mung bean [*Vigna radiata* (L.) Wilczek]: The constraints and the opportunities. In: Rajpal VR, Rao SR, Raina SN Editors. Gene Pool Diversity and Crop Improvement. Vol 1. Switzerland Springer International Publishing; 2016.
10. Bhojwani SS, Mukhopadhyay A. Some aspects of plant regeneration in tissue cultures of legumes. In: Gupta PK and Bahl JR, editors. Genetics and Crop Improvement. Meerut, Rastogy and Co.; 1998.
11. Shanmugasundaram S. Mungbean Varietal Improvement. A catalog of mung bean cultivars released around the world. Asian Vegetable Research and Development Center. 1988;20.
12. Kozgar MI, Goyal S, Khan S. EMS induced mutational variability in *Vigna radiata* and *Vigna mungo*. Research Journal of Botany. 2011;6:31-37.
13. Lee SY, Cheong JI, Kim TS. Production of doubled haploids through another culture of M1 rice plants derived from mutagenized fertilized egg cells. Plant Cell Reports. 2003;22(3):218-23.
14. Kim Y, Schumaker KS, Zhu JK. EMS mutagenesis of *Arabidopsis*. Methods Molecular Biology. 2006;323:101-3.
15. Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ, Reynolds SH, Enns LC, Burtner C, Johnson JE, Odden AR, Comai L, Henikoff S. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in *Arabidopsis*. Genetics. 2003;164(2):731-40.
16. Serrat X, Esteban R, Guibourt N, MoyssetL, Nogués S. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods. 2014;0:5.
17. Sharma SR, Varma A. Control of yellow mosaic of mung bean through insecticides and oil. Journal of Entomological Research. 1982;6:130-136.
18. Khan S, Goyal S. Improvement of mung bean varieties through induced. Mutations African Journal of Plant Science. 2009;3(8):174-180.
19. Kitsanachandee R, Somta P, Chatchawankanphanich O, Akhtar KP, Shah TM, Nair RN, et al. Detection of quantitative trait loci for mung bean yellow mosaic India virus (MYMIV) resistance in mung bean (*Vigna radiata* (L.) Wilczek) in India and Pakistan. Breeding Science. 2013;63:367–373.

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