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Screening for pod shattering in mutant population of mungbean (*Vigna radiata* (L.) Wilczek)

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Abstract: Mungbean, (*Vigna radiata* (L.) Wilczek) occupies a unique position in Indian agriculture and has been grown under various agro-ecological conditions. It is cultivated in 1.61mha with production of 3.38MT and productivity of 474kg/ha in India. Mungbean pods are thin and brittle when dry, so shattering is a major problem. The loss of seeds by pod dehiscence is one of the major reasons for low yield in mungbean; thus, reducing the frequency of pod dehiscence is an important objective in mungbean breeding. Induced mutations, have offered a single and short alternative to conventional breeding including isolation, screening, selection and testing generation after generation. In this study, variability was induced by gamma rays and Ethyl methane sulphonate (EMS) in two greengram genotypes *viz.,* CO (Gg) 7 and NM 65. Screening for pod shattering was carried out in M_2 and M_3 populations of greengram. The scoring for shattering was recorded at physiological maturity of the pod. The shattering percentage ranged from 14.56 (400 Gy) to 93.45 per cent (20 mM). A total of 100 shattering tolerant mutants were selected from field based on visual observation. These mutants were again scored under laboratory condition as per IITA method. A total of 12 mutants of CO (Gg) 7 and 10 mutants of NM 65 which were tolerant to pod shattering were identified in M_2 generation and forwarded to M_3 generation. These mutants were scored for pod shattering under laboratory condition and nine mutants *viz.,* M26, M44, M46, M58, M70, M71, M84, M92 and M98 were found to be tolerant in M_3 generation. This study on identification and screening of the mutants tolerant to pod shattering with high yielding potential will help to increase the production of the pods to a greater extent.

Keywords: Induced mutation, Mungbean, Pod shattering, Scoring, Elite mutants

INTRODUCTION

Legumes generally loose different alleles for high productivity, seed quality, pests and disease resistance during the processes of adaptation to environmental stress. A large number of legume species hitherto unexploited possess great potential for contributing to not only protein rich food for humans, but also excellent quality forage for animals. Among such novel legumes, mungbean (*Vigna radiata* (L.) Wilczek), belonging to family Fabaceae is quite notable (Wani *et al.,* 2012). Mungbean is a cheap source of dietary protein for the poor, with high levels of folate and iron compared with many other legumes.

In Pulses, pod shattering is a major concern for the breeder. Pod shattering, when crops reach maturity in hot and dry condition could lead to serious seed yield losses (Adeyeye *et al.,* 2014). Shattering resistance is one of the primary traits that crops have acquired in the process of domestication (Fuller 2007). Seed loss is generally divided into two periods, shattering before and during harvesting (Chandler *et al*., 2005). Seed losses of 34-99% are often associated with pod shattering in susceptible varieties and delayed harvesting after maturity (Tiwari and Bhatnagar, 1991). This problem of mechanical damage is likely to be much affected by other plant attributes such as pod angles, pod length and width (Thompson and Hughes, 1986). So that breeding should be concentrate on development of high yielding varieties with pod shattering resistance. Hence screening the genotypes for pod shattering resistance is the initial process of crop breeding programme. The loss of seeds by pod dehiscence is one of the major reasons for low yield in mungbean; thus, reducing the frequency of pod dehiscence is an important objective in mungbean breeding. Most of the mungbean genotypes are prone to shattering. The indeterminate flowering habit of this crop leads to a spread of flowering and pod maturity on a single plant over the entire reproductive phase. Consequently, pods which develop at the earliest flower may shatter prior to 100% pod maturity. Mutation breeding is a proven supplement and an effective substitute of conventional breeding so as to confer specific improvement in a variety without significantly affecting its acceptable phenotype (Sanjay Gandhi *et al*., 2014). Although selection for economically useful spontaneous mutants still takes place with some level of success (Wilde *et*

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al., 2012), the purposeful induction of a specifically desired mutation at a specific time and place, and in a selected genotype for a selected purpose is a much more attractive option. Induced mutation is a suitable source of producing variation through mutation breeding pro-cedure (Domingo *et al*., 2007). Mutated genes have therefore; become valuable material to plant breeders and molecular biologists for understanding not only the function but also in shuffling and isolating the genes between varieties (Souframanian *et al.,* 2002). Mutation breeding offers scope for achieving in many instances what cannot be accomplished through backcross breeding and selection (Lavanya *et al.,* 2011). Induced mutation using physical and chemical mutagens is one way to create genetic variation resulting in new varieties with better characteristics. The practical role of induced mutation in the improvement of crop plants can best be assessed on the basis of quantitatively inherited characters. It combines quite a few advantages in plant improvement by up- grading an explicit character without altering the original genetic makeup of the cultivar. In that sense, it provides a speedy method to improve the crop varieties, without resorting to hybridization and back crossing. The identification of resistant sources for pod shattering is one of the most important aspects in the management of pod shattering. Hence the present investigation was carried out with Mutant population of greengram for screening of elite mutants tolerant to pod shattering.

MATERIALS AND METHODS

Two greengram genotypes viz., Co (Gg) 7 and NM 65 obtained from the Department of Pulses, Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The genotypes were subjected to gamma irradiation at the doses of 400, 500 and 600 Gray and Ethyl Methane Sulphonate treatments of 10, 20 and 30 milli Molar. Gamma irradiation was done using cobalt 60 sources in the Gamma chamber, installed at Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The chemical mutagen, ethyl methane sulphonate ($CH_3SO_2OC_2H_5$) with molecular weight 124.16, from the sigma chemical company, USA was used for treating the seeds. The treated seeds were sown with a spacing of 30 x 10 cm in a randomized block design. The trial was conducted in the research farm of Agricultural College and Research Institute, Madurai during Kharif season 2013. The weather in the site is usually warm and dry with the mean annual rainfall of 851 mm and a maximum and minimum temperature of 35.5 ^oC and 23.5^oC, respectively. The M_2 generation was raised as individual M_1 plant basis. The M_3 generation was raised under Randomized Block Design. The treated and control populations of M_2 and M_3 generation were carefully screened for pod shattering resistance.

Screening for pod shattering resistance: Pod shattering resistance was evaluated both in laboratory and field conditions and found out that laboratory method is not influenced by the environment and hence can only be used as a tool for identification of pod shattering resistance genotypes Agarwal *et al*. (2000). The pod shattering resistance was recorded at physiological maturity of the pod. The screening was done under laboratory condition by following the methodology adopted by IITA (Dashell and Bello, 1988). The results were recorded as percentage of pod shattering.

IITA method of calculating pod shattering under lab conditions:

A sample of 25 pods were collected and kept in oven at 40°C for 7 days.

On the 7th day, the number of shattered pods were counted and expressed in percentage as below,

Pod shattering percentage (%) = Number of pods shattered / Total number of pods x 100

The genotypes were classified into different categories based on their reaction to pod shattering. The scoring rate was followed according to method adopted by IITA.

Category	Resistant reaction
No pod shattering	Shattering resistant
<25% pod shattering	Shattering tolerant
25-50% pod shattering	Moderately shattering
51-75% pod shattering	Highly shattering
>75% pod shattering	Very highly shattering

RESULTS AND DISCUSSION

Induced mutations are also useful when it is desired to improve easily identifiable characters (Roychowdhury and Tah, 2013). Mutant plants displayed a range of reduction in shattering (5 to 15%) depending upon the combination of mutations used. This variation is being utilized for variety development.

Visual screening is the most effective and efficient method for identifying mutant phenotypes. In the present study, hundred mutants were selected based on field observations to shattering. Similar method of screening was reported by Yamada, *et al.* (2009) and Khan *et al.* (2013) in Soyabean. Under laboratory condition, the lowest pod shattering percentage was recorded by the mutants, M77 (30 mM) of CO (Gg) 7 and M58 (400 Gy) of NM 65. Highest shattering percentage was recorded by the mutants M1 (300 Gy) of CO (Gg) 7 and M95 (20 mM) of NM 65. Similar findings were given by Mohammad (2010) and Khan *et al.* (2013) in Soyabean. CO (Gg) 7 was under very highly shattering type (76.56 %) and NM 65 showed highly shattering percentage of 67 % in M_2 generation.

Among the 100 mutants, 22 mutants were identified as tolerant types, 42 mutants observed to be medium shattering, 29 mutants showed highly shattering and 7 mutants falls under very highly shattering categories. These findings are similar to genotypic studies in Soyabean given by Gadde (2006), Khan *et al.* (2013). None of the mutants showed resistance to pod shatter-

Table 1. Screening for pod shattering in M_2 mutant lines of greengram.

Mutants	Treatments	Shattering %	Grade
M1		83.54	Very highly shattering
M2		75.08	Highly shattering
M3	CO (Gg) 7	80.67	Very highly shattering
M4		65.89	Highly shattering
M5	300 Gy	23.12	Tolerant
M6		76.34	Very highly shattering
M7		70.88	Highly shattering
M8		43.28	Moderately shattering
M9		20.19	Tolerant
M10		50.90	Moderately shattering
M11		34.00	Moderately shattering
M12		53.25	Highly shattering
M13		46.77	Moderately shattering
M14		49.08	Moderately shattering
M15		27.89	Moderately shattering
M16		30.84	Moderately shattering
M17		56.33	Highly shattering
M18		23.99	Tolerant
M19		32.85	Moderately shattering
M20	CO (Gg) 7	25.62	Moderately shattering
M21	400 Gy	26.78	Moderately shattering
M22		39.00	Moderately shattering
M23		25.52	Moderately shattering
M24		35.67	Moderately shattering
M25		42.32	Moderately shattering
M26		21.90	Tolerant
M27		40.75	Moderately shattering
M28		56.78	Highly shattering
M29		34.56	Moderately shattering
M30		30.00	Moderately shattering
M31		78.65	Very highly shattering
M32		73.21	Highly shattering
M33	CO (Gg) 7	65.77	Highly shattering
M34		62.09	Highly shattering
M35	500 Gy	60.54	Highly shattering
M36		24.50	Tolerant
M37		66.67	Highly shattering
M38		22.52	Tolerant
M39		77.00	Very highly shattering
M40		69.43	Highly shattering
M41		50.97	Moderately shattering
M42		23.00	Tolerant
M43		54.68	Highly shattering
M44		19.00	Tolerant
M45		70.32	Highly shattering
M46		22.98	Tolerant
M47		24.45	Tolerant
M48		45.00	Moderately shattering
M49	NM 65	43.66	Moderately shattering
M50	300 Gy	42.81	Moderately shattering
M51		31.99	Moderately shattering
M52		30.00	Moderately shattering
M53		32.88	Moderately shattering
M54		15.00	Tolerant
M55		17.87	Tolerant
M56		32.10	Moderately shattering
M57	NM 65	46.76	Moderately shattering
M58		14.56	Tolerant
M59	400 Gy	53.89	Highly shattering
M60		67.87	Highly shattering
M61	NM 65	65.43	Highly shattering
M62		78.90	Very highly shattering
M63	500 Gy	53.22	Highly shattering
M64		58.76	Highly shattering
M65		68.90	Highly shattering
M66		25.00	Tolerant
M67		65.00	Highly shattering
M68	CO (Gg) 7	43.98	Moderately shattering
M69		54.90	Highly shattering
M70	10 mM	22.87	Tolerant
M71		21.94	Tolerant
M72	CO (Gg) 7	30.12	Moderately shattering
M73		35.44	Moderately shattering
M74	20 mM	56.34	Highly shattering
M75		32.14	Moderately shattering
M76		34.76	Moderately shattering
M77		15.45	Tolerant
M78		70.99	Highly shattering
M79		67.80	Highly shattering
M80		45.00	Moderately shattering
M81		34.99	Moderately shattering
M82		45.87	Moderately shattering
M83		43.21	Moderately shattering
M84		15.45	Tolerant
M85		65.45	Highly shattering
M86		78.90	Highly shattering
M87		46.75	Moderately shattering
M88		54.33	Highly shattering
M89		23.45	Tolerant
M90		35.90	Moderately shattering
M91		24.00	Tolerant
M92		20.98	Tolerant
M93		45.43	Moderately shattering
M94		65.80	Highly shattering
M95		93.45	Very highly shattering
M96		48.70	Moderately shattering
M97		35.78	Moderately shattering
M98		16.99	Tolerant
M99		26.55	Moderately shattering
M100		32.00	Moderately shattering
Co (Gg) 7		76.56	Very highly shattering
NM 65		67.00	Highly shattering

Mean	Range
45.08	14.56 - 93.45

< 25 % shattering- Tolerant, 25-50 % - Moderately shattering, 51-75 %- Highly shattering, > 75 % - Very highly shattering.

Table 2. M_3 mutants scoring for tolerance to pod shattering.

Mutants	Treatments	Shattering %	Grade
M5	CO (Gg) 7	45.67	Moderately shattering
M9	300 Gy	64.44	Highly shattering
M18	CO (Gg) 7	39.80	Moderately shattering
M26	400 Gy	12.56	Tolerant
M36	CO (Gg) 7	70.90	Highly shattering
M38	500 Gy	57.89	Highly shattering
M42		25.13	Moderately shattering
M44		20.90	Tolerant
M46	NM 65	13.56	Tolerant
M47	300 Gy	33.78	Moderately shattering
M54		27.89	Moderately shattering
M55		26.67	Moderately shattering
M58	NM 65	25.00	Tolerant
M66	400 Gy	23.25	Moderately shattering
M70	CO (Gg) 7 10 mM	10.90	Tolerant
M71	CO (Gg) 7 20 mM	16.78	Tolerant
M77	CO (Gg) 7 30 mM	28.90	Moderately shattering
M84	NM 65 10 mM	22.88	Tolerant
M89	NM 65	60.55	Highly shattering
M91	20 mM	27.65	Moderately shattering
M92		23.12	Tolerant
M98	NM 65 30 mM	22.56	Tolerant
V_1 Control	CO (Gg) 7	82.15	Very highly shattering
V_2 Control	NM 65	60.45	Highly shattering

ing. Gadde (2006) also found similar kind of results in Soyabean. Mutant plants displayed a range of reduction in shattering (5 to 15%) depending upon the combination of mutations used. This variation is being utilized for variety development.

Agrawal *et al.* (2003) reported that segregation of pod shattering was highly complex in F_2 generation in Soyabean and showed quantitative response in the cross of susceptible and resistant varieties and concluded that success of any conventional breeding program aimed at pod shattering resistance depends upon the desirable segregates.

Hence, 22 mutants identified as tolerant types have to be further evaluated in laboratory condition in succeeding generation.

The pod shattering tolerant types for gamma rays was found in 500 Gy for Co (gg) 7 and 300 Gy for NM 65. All other doses show medium shattering percentage with the scale of 3. In EMS, pod shattering tolerant types was found in 30 mM for both the genotypes as 7.84 and 5.74 per cent respectively. Lower doses of 10mM and 20mm are having the medium shattering percentage. The shattering percentage was higher in gamma rays than EMS in both the genotypes. None of the genotypes were immune or resistant to pod shattering in Soyabean (Gadde, 2006).

Screening of mutants for pod shattering revealed 22 mutants as tolerant types, 42 mutants as moderately shattering, 29 mutant as highly shattering and seven mutants falls under very highly shattering categories in M_2 generation. The shattering percentage was higher in gamma rays than EMS in both the genotypes.

In M_3 generation, out of 22 tolerant mutants, CO (Gg) 7 contains 12 mutants while 10 mutants belongs to NM 65. Eight mutants of CO (Gg) 7 viz., M5, M9, M18, M26, M36, M38, M42 and M44 and five mutants of NM 65 viz., M46, M47, M54, M55 and M58 were gamma irradiated population.

In EMS treatments, four mutants of CO (Gg) 7 *viz.,* M66, M70, M71 and M77 for CO (Gg) 7, while five mutants of NM 65 namely M84, M89, M91, M92 and M98 fall under tolerant categories. Twenty two mutants were scored for pod shattering under laboratory condition and nine mutants *viz.,* M26 (19.14%), M44 (20.85%), M46 (13.48%), M58 (23.17%), M70 (10.47%), M71 (16.64%), M84 (22.79%), M92 (23.11%) and M98 (22.53%) were found to be tolerant in M_3 generation. The control, CO (Gg) 7 scored under very highly shattering and NM 65 under highly shattering category. Similar results were reported by Bhara *et al.* (2013) in Soyabean. These shattering tolerant mutants can be further evaluated for yield contributing characters in succeeding generations for the selection of elite mutants for resistance to novel trait.

Conclusion

Pod shattering is one of the major constraints in greengram, which reduces the yield potential considerably. Hence, the identification of resistant sources for pod shattering is one of the most important aspects in the management of pod shattering. Mutagenesis is a well recognized potential tool to induce high genetic variability for effective selection towards improvement in yield and quality. Nine mutants of two genotypes of greengram were found to be tolerant to pod shattering. The identified mutants can be screened further and used in hybridization programme for development of resistant variety.

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