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Gamma rays induced urdbbean [*Vigna mungo* (L.) Hepper] mutants with YMV resistance, good batter quality and bold seeded type

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ABSTRACT

The seeds of urdbean varieties MDU 1 and VBN (Bg) 4 were treated with 5 doses (100, 200 300, 400 and 500 Gy) of Gamma rays to identify YMV resistant and determinate plant type. The individual plant and plant-row selections were made in M_2, M_3 and M_4 generation of MDU 1 and VBN (Bg) 4. Mutant population was screened for YMV resistance, pods protruding up above the canopy, early maturity, and purple pigmentation on plant. The investigation revealed that ACM-16-30, ACM-16-29, ACM-6-17 and ACM-16-14 mutant lines were found to be as ideal mutant lines for further improvement programme. The mutants line had YMV resistance, purple pigmentation on leaf, hypocotyls, stem and pod along with pods protruding up above the canopy, good batter quality and bold seeded type.

Key words: Urdbean, Gamma rays, Mutant, Radiation, Resistance.

INTRODUCTION

Urd bean [*Vigna mungo* (L.) Hepper] is one of the major pulse crops of the tropics and sub tropics. It is the third major pulse crop cultivated in the Indian sub-continent. Yellow mosaic disease (YMD) is the major constraint to the productivity of grain legumes across the Indian subcontinent (Varma *et al.*, 1992; Varma and Malathi 2003). YMV affects the majority of legume crops including mungbean (*Vigna radiata*), blackgram (*V. mungo*), pigeon pea (*Cajanus cajan*), soybean (*Gycine max*), mothbean (*Vigna aconitifolia*) and common bean (*Phaseolus vulgaris*) causing loss of about $300 million. The disease in southern Asia is caused by four distinct begomoviruses collectively known as the yellow mosaic viruses (YMVs); Mungbean yellow mosaic virus (MYMV), Mungbean yellow mosaic India virus (MYMIV), Dolichos yellow mosaic virus (DoYMV) and Horsegram yellow mosaic virus (HgYMV) (Qazi *et al.* 2007). Of these MYMIV and MYMV are most important as these infect large number of legumes in India. MYMIV is more predominant in northern, central and eastern regions of India (Usharani et al. 2004) and MYMV in southern region (Karthikeyan *et al*. 2004; Girish and Usha 2005; Haq *et al*. 2011) to which Tamil Nadu state belongs.

Anthocyanin, a group of reddish or purple flavonoids and have been recognized as health-promoting functional food ingredients due to their antioxidant activity (Nam *et al*., 2006;), anticancer (Kamei *et al*., 1995), hypoglycemic (Tsuda *et al*., 2003), and anti-inflammatory effects (Tsuda *et al*., 2002), and these functions provide synergic effects with various nutrients in vivo. The pigmentation in leaf due to breakdown of sugars in the presence of bright light as the level of phosphate in the leaf is reduced.

Stem growth habit is an important morphological as well as agronomical trait that has been under investigation for decades in grain legumes. It has been documented that stem growth habit has great effects on plant height, node production, leaf morphology, root architecture, maturity, water-use efficiency, and yield. The trait stem growth habit has been identified to be controlled by a few major genes in all the model legumes such as Medicago, Soybean, Peas and Field beans. Recent research findings on Arabidopsis, soybean, peas and Medicago through mutation complementation, transgenic approaches and expression analysis have indicated that the gene Terminal Flowering 1 (TFL 1) is responsible for determining flowering habit in these plants (Zhixi Tian *et al*., 2010). The wild type TFL 1 allele leads to axillary flowering habit thereby resulting in indeterminate vegetative growth, while the mutant allele tfl 1 results in terminal flowering thereby promoting determinate habit.

The timing of flowering is also an agronomically important trait in all legume species, as it decides the balance between vegetative and flowering phases. Like that of the growth habit, in legumes flowering time is also determined by a single gene Flowering Locus T (FT). Functional mutants

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in this gene, leading to isolation of alleles with different flowering time have been reported in legumes like Medicago, soybean, peas and beans in many studies ((Foucher *et al*., 2003 and Liu *et al*., 2010). As studies in legumes on key genes like TFL 1 and FT had clearly indicated that it is possible to exercise control over seemingly complex traits such as growth habit and flowering time through functional mutations.

Induced mutations have been found useful in creating useful variability for yield traits, plant type and resistance to various stresses. Commercial crops like cereals (rice, barley, maize, wheat) and legumes (bean, green pea) (Petra and Vladmir 2012) were improved mostly. Radiation, including γ-rays, X- rays, fast neutrons, thermal neutrons etc. has been widely applied to induce mutations and made great progress in plant breeding (Souframanien *et al*., 2016).More than 3,000 mutant crop varieties have been produced over the past 60 years (http://www-mvd.iaea.org). Most of those varieties were developed using ionizing radiation mainly γ-rays (64%) followed by X-rays (22 %). Among different ionizing radiations, γ-rays have been commonly used, and numerous mutants have been produced in black gram (Souframanien and Pandey 2006). In this research we employed gamma rays to induce mutations in the morphogenesis and flowering related traits in the indeterminate blackgram varieties of MDU 1 and VBN (Bg) 4 and also to screen for YMV resistance.

Batter is a liquid mixture of one or more flours made with ground grains or soaked grains that are ground. The word batter comes from the old French word batter which means to beat, as many batters require vigorous beating or whisking in their preparation. Though processing plays a major role in battering quality, genotypes also have its own role in contributing quality characters. Moreover, the battering quality of blackgram is found to be deteriorating within 2-3 months after the harvest. This is major problem faced by the farmers, traders and consumers (Veni *et al*., 2016). Bio chemically, the soft spongy texture observed in the leavened in the steamed idli made out of blackgram is due to the presence of two components, namely surface active protein (globulin) and an arabinogalactan (polysaccharide) in blackgram. The mucilaginous principle of blackgram is a complex carbohydrate containing galactose and arabinose together with a small amount of rhamnose and galacturonic acid (Umadevi *et al*., 2008).

MATERIALS AND METHODS

The seeds of urd bean varieties MDU 1 and VBN (Bg) 4 were treated with 100, 200, 300, 400, 500 Gy gamma rays. The seeds packed in butter paper covers, were placed into the gamma cell and exposed to gamma irradiation. The cobalt-60 gamma chamber at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore was utilized in the present study. Raising of M_1 to M_4 was conducted from 2014 to 2016 at Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai.

About 500 seeds in each dose was sown to raise M_1 generation during *rabi* 2014 using RBD with spacing of 30 x 10 cm. Each row was 3 m long. At maturity, pod from each survived M_1 plant was harvested on single plant basis and forwarded to M_2 generation.

A total of more than fifteen thousand plants irrespective of the doses in both the varieties were raised in the M_2 generation. Selection of desirable characteristics was made and the selected plants were tagged. Each tagged plant was separately harvested and forwarded to M_3 generation. The biometrical traits such as plant height, base length, number of clusters per plant, number of pods/plant, number of seeds/pod, hundred seed weight and single plant yield were recorded for 150 plants per treatment. Desirable mutants were harvested individually and forwarded to raise M_3 generation in plant progeny row during *rabi* 2015.

Selection in M_3 generation was conducted by selecting individual rows of homozygous line with desirable characters. Plants producing high yield and having synchronized maturity in a row were identified and made as bulk. Thirty families, fifteen each in MDU 1 and VBN (Bg)4 were selected and forwarded to M_4 generation.

M_4 generation was raised in the plot size of 3x4 m in three various environments *viz.*, National Pulses Research Centre, Vamban, Agricultural Research Station, Vaigaidam and Agricultural College and Research Institute, Madurai using RBD with two replications during summer 2016 for stability analysis. YMV disease screening was carried out in all the environments. In the M_4 generation all thirty mutants' families were subjected to observation of various quantitative characters such as yield (days to fifty percent flowering, plant height, base length, number of clusters per plant, number of pods per plant, number of seeds per pod, hundred seed weight, peduncle length and single plant yield), and YMV screening, Various biochemical traits (phenol, total sugar), molecular marker characterization, batter quality characters, Protein content (Lowry *et al*., 1951), Arabinose (Bial, M. 1902), Globulin and Albumin (Landry and

Table 1: Details of molecular markers linked to YMV resistance used for validation study.

Marker Name	Marker type	Crop	Primer sequences (5'- 3')	Annealing temperature (°C)	Expected product size (bp)	Reference
$ISSR811_{1357}$	ISSR	Black gram	GAGAGAGAGAGAGAGAC	50-55	1357	Souframanien and Gopalakrishna, 2006
CEDG180	SSR	Black gram	F:GGTATGGAGCAAAACAATC R:GTGCGTGAAGTTGTCTTATC	55	136/163	Gupta *et al*., 2013

Moureaux, 1970), microbial studies in idli batter during before and after fermentation (Harrigan and Margaret McCance, 1970, Stiles and Holzapfel, 1997) were carried out as per the protocols. DNA from YMV resistant mutants and parents were used for PCR amplification using ISSR and SSR primers (Table 1) to validate the makers linked to YMV resistance reported previously (Souframanien and Goplalakrishna 2006; Gupta *et al.*, 2013).

RESULTS AND DISCUSSION

As genetic variability is essential for any crop improvement programme, induced mutations provide an important source for variability. Selecting the appropriate mutagen and the treatment methods are desirable to not only induce a higher mutation rate in a target trait, but also to have less of an effect on the remaining genetic background. In the present study, 5 different doses of gamma rays were used to induce genetic variability in two different varieties. Viable and desired mutants were observed in 300 and 400 Gy of gamma rays. Low and intermediate doses inducing viable mutants than higher doses were previously reported for γ-rays in black gram (Deepalakshmi and Ananda Kumar 2004; Surendar *et al*. 2014). The M_2 generation of MDU 1 and VBN (Bg) 4 treated with gamma rays were raised at Agricultural College and Research Institute Madurai. All the plants harvested in M_1 generation were sown in plant to progeny fashion along with parent variety repeated after every 10 rows. Several desirable plants were selected in M_2 generation. A severe incidence of YMV occurred during this season. The M_2 mutants with high yielding synchronized maturity showing resistance to YMV were selected and forwarded to M_3 generation.

The M_3 generation sown in *rabi* 2015 was severely infected by YMV disease. The infection started at seedling stage and its severity enhanced with the increasing age of mutant. YMV resistance with terminal pod plant were identified. Thirty mutant families were selected in M_3 generation and forwarded to next generation. Out of thirty, fifteen mutant families were from MDU 1 and fifteen mutant families were from VBN (Bg) 4. In M_4 generation, four stable blackgram mutants *viz* ACM-16-14, ACM16-17, ACM16-29, ACM16-30 were identified with special character among three locations. The description of this blackgram mutants are given below

ACM 16-14 mutant line is bold seeded type (Fig. 1) which is derived from MDU 1 irradiated with 400 Gy of gamma rays. ACM-16-14 mutant line found to be early flowering (31.50 days), short (32.99 cm), more number of clusters per plant (18.20), pods per plant (51.59) and seeds per pod (8.00). Hundred seed weight (6.08) and single plant yield (14.82) was high in ACM-16-14 mutant compared to parent MDU 1 (Table 1). Biochemical traits like phenols, total sugars and MYMV disease score under field condition were 1.36 g, 28.15g and 5 respectively Table 2. Based on the YMV screening, ACM-16-14 line rated as 4.1 to 5.0, which come under the reaction of moderately susceptible (Table 4). With respect to batter quality, lactobacillus activity was high compared to control of MDU1 in serial dilution before and after fermentation of idli batter (Table 7 and Table 8). ACM-16-14 mutant line had high globulin content of 10.61% and and total protein content of 24.06 compared to MDU 1 (Table 9).

Fig 1: Black gram mutant ACM-16-14 with bold seeded.

Table 2: Parentage of blackgram mutants.

Mutant lines	Variety and Dose	Mutagen
ACM-16-14	MDU 1-400 Gy	Gamma rays
ACM-16-17	MDU 1-500 Gy	Gamma rays
ACM-16-29	MDU 1-300 Gy	Gamma rays
ACM-16-30	MDU1-300Gy	Gamma rays

The ACM-16-17 mutant line was obtained from MDU 1 irradiated with 500 Gy of gamma rays. This mutant line showed terminal pod (Fig. 2), narrow leaf with YMV resistance while control MDU 1 had bushy type, broad leaf with susceptible to YMV. ACM-16-17 mutant line found to more base length (5.73), more number of clusters (23.30), number of branches (3), number of pods per plant (58.60) and high peduncle length (11.71 cm). It had high amount of phenol (2.43g) and low amount of sugar (15.26g) content. Based on the YMV screening, ACM-16-17 mutant lines rated as 1.0 to 2.0, which come under the resistance reaction.

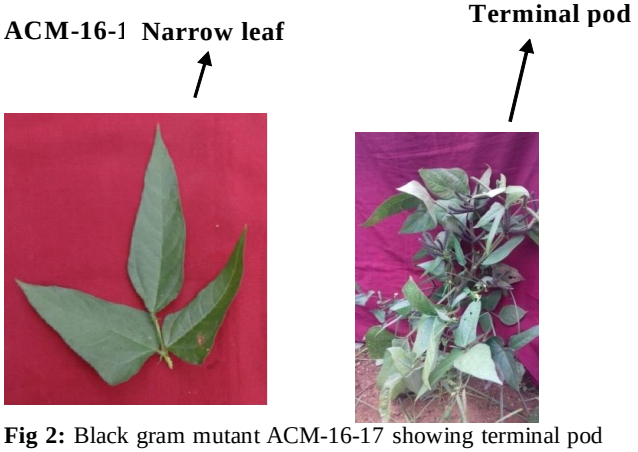

Fig 2: Black gram mutant ACM-16-17 showing terminal pod bearing.

Table 3: Mean performance of urdbean mutants for ten quantitative traits.

Genotypes	DFF	PHT(cm)	B.L(cm)	N.C.P	N.P.P	PD.L(cm)	N.S.P	N.P.B	H.S.W(g)	S.P.Y(g)
ACM-16-14	31.50	32.99	6.59	18.20	51.59	5.84	8.00	2.20	6.08	14.82
ACM-16-17	34.25	49.20	5.73	23.30	58.60	11.71	7.9	3.00	5.29	12.92
ACM-16-29	31.50	35.06	6.40	16.30	45.90	6.28	7.00	2.63	5.31	13.96
ACM-16-30	34.50	22.45	4.24	8.50	25.50	9.86	6.00	4.27	4.80	7.01
VBN(Bg) 4	38.45	39.71	5.32	12.15	35.35	9.25	7.19	1.23	4.87	12.26
MDU 1	34.55	34.84	4.21	13.81	40.25	8.35	7.19	2.21	5.38	13.19
Mean	34.13	35.71	5.42	15.38	42.87	8.55	7.21	2.59	5.29	12.36
SE	0.40	0.73	0.13	0.53	1.52	0.29	0.09	0.11	0.07	0.47

DFF-Days to fifty percent flowering, PHT-Plant height, B.L-Base length, N.C.P-Number of cluster per plant, N.P.P- Number of pods per plant, PD.L- Peduncle length, N.S.P-Number of seed per pod, N.P.B- Number of primary branches, H.S.W- Hundred seed weigth, S.P.Y- Single plant yield.

This mutant line had high globulin (11.02%) and protein content (25.10%).

The ACM-16-29 mutant line was derived from MDU 1 irradiated with 300 Gy of gamma rays. This mutant line identified as an ideal plant type compared to control of MDU 1 for various quatitative characters such as days to fifty per cent flowering, plant height, base length, number of clusters per plant, number of pods per plant, peduncle length, number of seeds per pod, number of branches per plant, hundred seed weight and single plant yield (Table 3). ACM-16-29 mutant line found to be early flowering (31.50 days), high base length (6.40 cm), more number of clusters per plant (16.30), branches per plant (2.63) and pods per plant (45.90), compared to MDU 1 (Table 3). ACM-16-29 had high phenol content (2.52) and low total sugar (17.15) with YMV disease score of 2. (Table 4). Based on the YMV screening, ACM-16-29 showed the rating of 1.0 to 2.0, which come under the reaction of resistance (Table 5). In the case of molecular level, the marker $ISSR811_{1357}$ and CEDG 180 linked to YMV resistance did not get amplified (Table 5.) This may be due variation at the primer binding sites or the mutant may carry newer *R* gene. Mutagenesis enables the identification of wild *R* genes or the creation of novel *R* genes. Induced mutagenesis offers scope to identify newer *R* genes, especially when there is no reliable source of resistance found in the nature that makes it impossible to introduce to susceptible cultivars by hybridization (Petra and Vladimir 2012). Induced mutagenesis by radiation mutagens have advantages over other insertional methods, since mutagens introduce random changes throughout genome and can generate variety of mutations within a single plant.

Table 4: Screening of blackgram mutants against yellow mosaic virus during *rabi*, 2015.

Mutant lines	Phenols (mg/g)	Total sugars (mg/g)	MYMV disease score
ACM-16-14	1.36	28.15	5
ACM-16-17	2.43	15.26	1
ACM-16-29	2.52	17.15	2
ACM-16-30	2.89	15.46	1
MDU 1	1.52	31.26	7
VBN (Bg) 4	2.13	14.56	1

With respect to batter quality, lactobacillus activity was high compared to control of MDU1 in serial dilution before and after fermentation of idli batter (Table 7 and Table 8). ACM-16-29 mutant line had high globulin content (11.23%) and low albumin content of (9.10%) compared to MDU 1 (Table 9).The ACM-16-29 mutant line showed purple pigmentation on leaf and stem (Fig. 3). According to the preliminary observation, it was speculated to be back mutation that was from recessive green plant to dominant purple. This character is controlled by a single gene. Hypocotyl, leaf, stem and pod colors can be used as genetic markers in genetic study and cultivar identification.

Swain (1965) reported that the color of seed coat and hypocotyl depended on the concentration of anthocyanin and chlorophyll. Ishikura *et al.* (1981) found 2 kinds of anthocyanin, A-I (delphinidin-3-glucoside) and A-IV (cyanidin-3- glucoside), in blackgram (*Vigna mungo*) with purple-red hypocotyl. According to the study of Pandey *et al.* (1989), *Vigna* had 4 kinds of anthocyanin, A-I, AII, AIII and A-IV. Anthocyanins in seed coat and hypocotyls are independent from each other. The report of Pandey *et al.* (1989) also stated that mungbean seedlings of purple-red had 3 kinds of anthocyanin namely A-I, A-II and A-III whereas green seedlings were free of anthocyanin.

ACM-16-30 is a determinate mutant type (Fig. 4a) which was derived from MDU 1 irradiated with 300 Gy of gamma rays. ACM-16-30 mutant line was found to be short

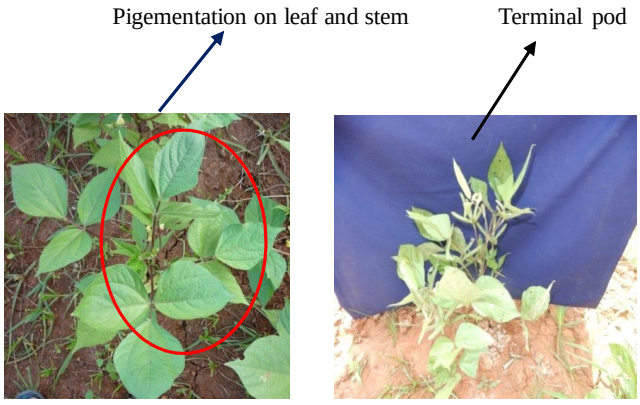

Fig 3: ACM-16-29:

Table 5: Grouping of blackgram mutants screened against YMV during *rabi*, 2015.

Rating	Reaction	Genotypes
1.0 to 2.0	Resistant (R)	ACM-16-29, ACM-16-30, ACM-16-17
2.1 to 4.0	Moderately Resistant (MR)	-
4.1 to 5.0	Moderately Susceptible (MS)	ACM-16-14
5.1 to 7.0	Susceptible (S)	-
7.1 to 9.0	Highly susceptible (HS)	-

Fig 4a: Black gram mutant ACM-16-30 exhibiting determinate growth habit.

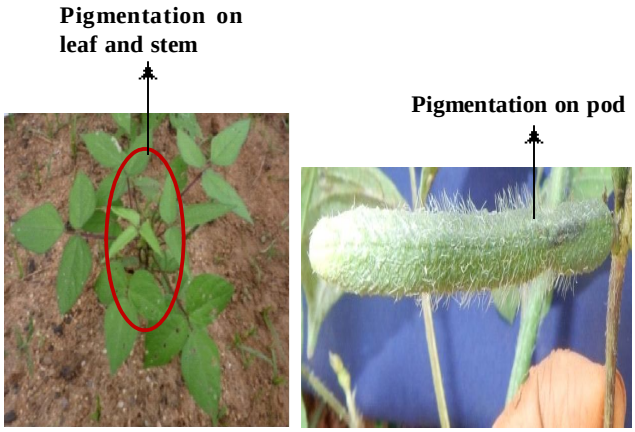

Fig 4b: ACM-16-30

Table 6: Amplification profile of molecular markers linked to YMV resistance on black gram mutant lines.

Genotypes	Reaction to YMV	$ISSR811_{1357}$	CEDG 180
ACM-16-29	Resistant	-	-
ACM-16-30	Resistant	-	+
MDU 1	Susceptible	-	-
VBN (Bg)4	Resistant	+	+

(22.45 cm), high base length (4.24 cm) and peduncle length (9.86 cm) compared to MDU 1 (Table 3). It also had purple pigmentation on leaf, hypocotyls, stem and pod (Fig. 4b). The importance of terminal flowering when coupled with optimized vegetative growth are synchronized maturity, ease the way for mechanical harvesting or single manual harvesting can be done as the crop uniformity is very high, and its more responsiveness to fertilizer uptake, ultimately resulting in increase yield.

Several legumes, such as common bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* (L.) Merr.) and pigeonpea (*Cajanus cajan* (L.) Huth) can show either DT (Determinate growth) or IDT (Indeterminate) growth habit. One single recessive gene causes the common bean plants to grow determinately (Talmann Neto and Alberini, 1989), and in soybean and pigeonpea the character is controlled by oligogenically (Palmer and Kilen, 1987). Investigation leading to successful isolation of determinate types with terminal flowering habit through mutation of TFL 1 gene from indeterminate varieties has already reported in the leguminaceae crops like pea (Foucher *et al*., 2003) and soybean (Liu *et al*., 2010).

Though some germplasm accession in the gene pool having terminal flowering habit are present, they do not possess optimized vegetative growth resulting in weak plant types resulting in reduced yield as compared to the indeterminate type. The mutant line ACM-16-30 possessed pods protruding up above the canopy and synchronized maturity.

Taggar *et al*. (2014) quantified antioxidative compounds *viz*., phenols, dihydroxy phenols, tannin and flavonols content from nine blackgram genotypes over a period of two years for potential whitefly resistance. The observed antioxidative compounds showed negative correlation with whitefly population suggesting that enhanced levels of these biochemical may contribute to bio protection of blackgram plants against *B. tabaci* infestation. Moreover, it was noted that total phenol contents, increased significantly

Table 7: Microbial Activity in Idli Batter: Before fermentation of batter.

Mutant lines	Population of Lactobacillus spp.(Cfu/g of fresh weight of dough)			
	10^3	10^4	10^5	10^6
MDU 1	$58x10^3$(4.76)	$25x10^4$ (5.39)	$68x10^5$(6.83)	$50x10^6$(6.69)
VBN (Bg) 4	$50x10^3$(3.69)	$45x10^4$ (5.65)	$55x10^5$(6.74)	$65x10^6$ (7.81)
ACM-16-14	$51x10^3$(4.71)	$10.2x10^5$ (7.01)	$12.5x10^6$(8.09)	$10.9x10^7$(9.04)
ACM-16- 29	$81x10^3$(4.91)	$80x10^4$ (5.90)	$96x10^5$(6.98)	$90x10^6$ (7.95)
ACM-16- 30	$10.5x10^4$(5.02)	$70x10^4$ (5.84)	$81x10^5$(6.91)	$55x10^6$ (7.74)

Table 8: Microbial Activity in Idli Batter: After Fermentation of batter.

Mutants lines	Population of Lactobacillus spp.(Cfu/g of fresh weight of dough)			
	10^6	10^7	10^8	10^9
MDU 1	$70x10^6$(7.84)	$60x10^7$(8.79)	$75x10^8$(9.87)	$72x10^9$(10.86)
VBN (Bg) 4	$10.5x10^7$(8.02)	$87x10^7$(8.94)	$80x10^8$(9.90)	$70x10^9$(9.84)
ACM-16-14	$12.5x10^7$(8.09)	$11.2x10^8$(9.05)	$10.9x10^9$(10.04)	$10.5x10^{10}$(11.02)
ACM-16-29	$10.5x10^7$(8.02)	$10.1x10^8$(9.00)	$15x10^9$(10.18)	$14x10^{10}$(11.15)
ACM-16-30	$15.9x10^7$(8.20)	$11.6x10^8$(9.06)	$12.8x10^9$(10.11)	$11x10^{10}$(11.04)

(Figures in the parenthesis indicates log values)

Table 9: Mean performance of four bio chemical traits for batter quality character in blackgram mutants.

Mutant lines	Arabinose (%)	Globulin (%)	Albumin (%)	Total protein(%)
ACM-16-14	10.81	10.61	10.81	24.06
ACM-16-17	9.07	11.02	9.07	25.10
ACM-16-29	9.74	11.23	9.10	20.85
ACM-16-30	10.90	13.46	9.90	24.51
MDU 1	11.62	8.57	11.62	20.62
VBN (Bg) 4	12.50	13.64	12.10	18.07
Mean	10.77	11.42	10.43	22.20
SE	0.37	0.60	0.37	0.84

upon infestation of whitefly, particularly in resistant blackgram genotypes as compared to susceptible ones.

ACM-16-30 mutant line had high amount of phenol (2.89 g) and low amount of sugar (15.46 g) compared to control of MDU 1 (Table 4). MYMV disease scoring under field condition was 1 (Table 2). Based on the YMV screening, ACM-16-30 line rated as 1.0 to 2.0, which comes under the reaction of resistance (Table 4 and Fig.5). In the case of molecular level, the marker $ISSR811_{1357}$ and CEDG 180 linked to YMV resistance but $ISSR811_{1357}$ did not amplified whereas CEDG 180 was amplified at 136 bp (Table 6). With respect to batter quality, lactobacillus activity was high compared to control MDU1 in serial dilution before and after fermentation of idli batter (Table 7 and Table 8). ACM-16-30 mutant line had high globulin content of 13.46% and total protein content of 24.51% compared to MDU-1(Table 9).

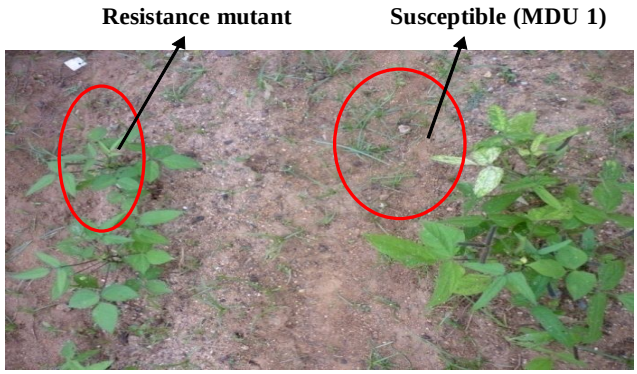

Fig 5: YMV

CONCLUSION

The use of gamma rays in two blackgram varieties, MDU 1 and VBN (Bg)4 resulted in many kind of viable mutation in M_2 generation. Plants selected in M_2 and M_3 generation carried the characters of pods protruding up above the canopy, YMV resistant, bold seeded type, purple pigmentation on plant and good batter quality. The ACM-16-30 and ACM-16-29 mutant lines was found to be resistant to YMV, pods protruding up above the canopy, purple pigmentation on plant and good batter quality. The ACM-16-14 mutant line possessed bold seeded type and moderately susceptible to YMV. The ACM-16-17 mutant line had YMV resistance with terminal pod. The mutants identified in the present study will evaluated for their use directly as variety or better utilized for crop improvement programme through hybridization programme.

REFERENCES

Bial, M. (1902). Die Diagnose der pentosurie. Deut med. Wochschr., **28:** 253-254.

Deepalakshmi, A. J and Ananda Kumar C.R. (2004). Creation of genetic variability for different polygenic traits in black gram [*Vigna mungo* (L.) Hepper] through induced mutagenesis. *Legume Research* **27**: 188-192.

Foucher, F., Morin, J., Courtiada, J., Cadioux, S., Ellis, N., Banfield M.J. and Rameau. C. (2003). Determinate and late flowering are two terrminal flower/Centroradialis homologs that control two distinct phases of flowering initiation and development in pea. *Plant Cell*, **15**: 2742-2754.

Girish, K.R., and Usha, R. (2005). Molecular characterization of two soybean-infecting begomoviruses from India and evidence for recombination among legume-infecting begomoviruses from South-East Asia. *Virus Res.* **108**:167–176.

Gupta, S., Gupta, D.S., Anjum, T.K., Pratap, A., Kumar, J. (2013). Inheritance and molecular tagging of MYMIV resistance gene in black gram (*Vigna mungo* L. Hepper). *Euphytica*, **193**: 27-37.

Haq, Q.M.I., Rouhibakhsh, A., Ali, A and Malathi, V.G. (2011). Infectivity analysis of a blackgram isolate of Mungbean yellow mosaic virus and genetic assortment with MYMIV in selective hosts. *Virus Genes*. **42**:429–439.

Harrigan WF. and ME. McCance. (1970). Laboratory methods in food and dairy microbiology in fermenting grain mashes used for food preparations. Bull. Central Food Technol. Res. Inst. Mysore (India). *Academic Press Inc.*, **2:** 224. -288.

Ishikura, N.M., Iwata and Miyazaki, S. (1981). Flavonoids in some Vigna plants in Leguminosae. *Bot. Mag*. **94**:197-205.

Kamei, H., Kojima, T., Hasegawa, M., Koide, T., Umeda, T., Yukawa, T. and Terabe, K. (1995). Suppression of tumor cell growth by anthocyanins in vitro. *Cancer Invest* **13**:590-594.

Karthikeyan, A.S., Vanitharani, R., Balaji, V., Anuradha, S., Thillai Chidambaram, P., Shivaprasad, P.V., Parameswari, C., Balamani, V. and Samiyappan Veluthambi, V.K. (2004). Analysis of an isolate of Mungbean yellow mosaic virus (MYMV) with a highly variable DNA component. *Arch Virol*. **149**:1643–1652.

Liu, B., Watanabe, S., Uchiyama, T., Kong, F., Kanazawa, A., Xia, Z., Nagamatsu, A., Arai, M., Yamada, T., Kitamura, K., Masuta, C., Harada, K. And Abe, J. (2010). The soybean stem growth habit gene Dt1 is an ortholog of Arabidopsis terminal flower1. *Plant Physiol.,* **153**: 198-210.

Landry, J., and T. Moureaux. 1970. Heterogenicity of the glutelins of the grain core. Selective extraction and composition in amino acids of the three isolated fractions. Bull. *Soc. Chem. Biol***. 52**:1021–1037.

Lowry, O.H, N.J. Roseburgh, A.L farr, and R.J Randall, (1951). *J.of Biological Chemistry***.,** 193, p-265.

Nam, S.H., Choi. S.P., Kang, M.Y., Koh, H.J., Kozukue, N. and Friedman, M. (2006). Antioxidative activities of bran from twenty one pigmented rice cultivars. *Food Chemistry*, **94**:613-620.

Pandey, R.N., Pawar, S.E., Chintalwar, G.J. and Bhatia, C.R. (1989). Seed coat and hypocotyls pigments in greengram and backgram. *Proc. Indian Acad. Sci. (Plant Sci.)* **99**: 301-306.

Palmer, R.G. and T.C. Kilen. 1987. Qualitative genetics and cytogenetics. In: Soybeans: Improvement, Production, and Uses (Wilcox, J.R., ed.). 2nd edn. *ASA/CSSA/SSSA, Madison***,** pp.180-197.

Petra, K and Vladimir, M. (2012). Mutagenesis in plant breeding for disease and pest resistance. In: Mutagenesis (Eds. R. Mishra). Pp. 195-220. http://dx.doi.org/10.5772/50332

Qazi, J., Ilyas, M., Mansoor, S., Briddon, R.W. (2007). Legume yellow mosaic viruses: genetically isolated begomoviruses. *Mol Plant Pathol*. **8**:343–348.

Satue-Gracia, M., Heinonen, I.M. and Frankel, E.N. (1997). Anthocyanins as antioxidants on human low-density lipoprotein and lecithinliposome system. *J Agric Food Chem*, **45**:3362-3367.

Souframanien, J and Pandey R.N. (2006). Mutational and biotechnological approaches for urdbean improvement; a review. *Indian Journal of Pulses Research* **19**: 155-164.

Souframanien, J., Gopalakrishna, T.(2006). ISSR and SCAR markers linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram [*Vigna mungo* (L.) Hepper]. *Plant Breed*., **125**: 619-622.

Souframanien.J, Kandali S Reddy, V.C. Petwal and J. Dwivedi, (2016). Comparative effectiveness and efficiency of electron beam and 60Co γ-rays in induction of mutations in black gram [Vigna mungo (L.) Hepper].

Stiles, M.E. and W.H. Holzapfel. (1997). Lactic acid bacteria of foods and their current taxonomy. *Int. J. Food Microbiol.,* **36:** 1-29.

Surendar R., C. Vanniarajan and Souframanien J. (2014) Effectiveness and efficiency of mutagens in gamma irradiated and EMS treated population of blackgram (*Vigna mungo* (L.) Hepper). *International Journal of Tropical Agriculture* **32:** 79-84.

Swain, T. (1965). Nature and properties of flavonoids; In Chemistry and Biochemistry of Plant Pigments [ed) T. W. Goodwin] Academic Press p: 225.

Taggar, G,K., Gill, R.S., Gupta A.K. and Singh. S. (2014). Induced changes in the antioxidative compounds of *V. mungo* genotypes due to infestation by *B. Tabaci* (Gennadius). *J.Environ. Bio.,* **35***:* 1037-1047.

Talmann Neto, A. and J. Alberini, (1989). Release to farmers of Carioca Arbustive Precoce 1070' (CAP-1070), a bushy bean mutant inducd by gamma rays in Brazil. *Mutat. Breed. Newsl.* **34***:12*-13.

Tsuda, T., Horio, F and Osawa, T. (2002). Cyanidin 3-O-β-glucoside suppresses nitric oxide production during a zymosan treatment in rats. *J. Nutr. Sci. Vitaminol*., **48**:305-310.

Tsuda, T., Horio, F., Uchida, K., Aoki, H. and Osawa, T. (2003). Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia. *J. Nutr*, **133**:2125-2130.

Umadevi, M., Meenakshi Ganesan, N and Muthiah, AR. (2008). Batter quality –Need for Traditional Indian Fermented Urdbean – Rice Food. *Madras Agric.J.,* **95** : 440-447.

Usharani, K.S., Surendranath, B., Haq, O.M.I, Malathi, V.G. (2004). Yellow mosaic virus infecting soybean in Northern India is distinct from the species infecting soybean in Southern and Western India. *Curr Sci*. **86**: 845–850.

Varma, A., Dhar, A.K, Mandal B. (1992). MYMV transmission and control in India. In: Green SK, Kim D, editors. Mungbean yellow mosaic disease. Taipei: *Asian Vegetable Research and Development Centre*; p. 8–27.

Varma A, Malathi VG. (2003). Emerging geminivirus problems: a serious threat to crop production. *Ann Appl Biol*. **142**:145–164.

Veni.K, E.Murugan, Mini, M.L., Vanniarajan and Radhamani, T. (2016). Genetic relationship between yield and battering quality in blackgram (*Vigna Mungo* (L.). *Legume Research*, **39** (3): 355-358.

Zhixi Tiana, Xiaobo Wangb, Rian Leec, Yinghui Lib, James E. Spechtd, Randall L. Nelsone, Phillip E. McCleanc, Lijuan Qiub, and Jianxin Maa, (2010). Artificial selection for determinate growth habit in soybean. *PNAS*. **107**(19): 8563–8568.