Legume Research, 38 (1) 2015: 30-36
Print ISSN:0250-5371 / Online ISSN:0976-0571

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.legumeresearch.in

Comparative mutagenic effectiveness and efficiency of physical and chemical mutagen and induced variability in ricebean (*Vigna umbellata* Thunb, Ohwi and Ohashi)

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Received: 14-06-2013 Accepted: 27-12-2013 DOI:10.5958/0976-0571.2015.00005.3

ABSTRACT

Healthy and pure seeds of two varieties of ricebean *viz.*, BRS-1 and Totru Local were treated with gamma rays (30, 40 and 50 kR) and ethyl methane sulphonate (EMS) (0.50, 0.60 and 0.70%). A steady reduction in germination and subsequent survival of the treated population and seedling height reduction was observed with the increasing dose/concentration of mutagens in both the cultivars regardless of the mutagens used. EMS was almost four times more effective and two times more efficient than gamma-rays and both mutagenic effectiveness and efficiency decreased with an increasing doses/ concentrations of mutagens. The coefficient of variation for all the quantitative characters were of higher magnitude compared to control in both the cultivars. The lower doses (30kR in gamma rays and 0.50% in EMS) were found to be the most important doses for inducing desirable variability in ricebean and two traits *i.e.* pods per cluster and seeds per pod showed high heritability coupled with high genetic advance in both BRS-1 and Totru Local indicating that breeding for these traits can be achieved by phenotypic selection.

Key words: Effectiveness, Efficiency, Ethyl methane sulphonate (EMS), Gamma- rays, Mutation, *Vigna umbelleta.*

INTRODUCTION

Mutagenesis is an important technique for induction of variability. This applies both to overall variability as well as variability for specific traits and second where a simply inherited defects needs to be rectified in an otherwise agronomically superior cultivar (Toker *et al.*, 2007). Extensive studies on mutagenesis have been undertaken in cereal crops (Konzak *et al.*, 1965), but its utilization for the improvement of legumes is limited (Haq *et al.*, 2003). The usefulness of a mutagen in crop improvement depends on its effectiveness and efficiency. Efficient mutagenesis being production of maximum desirable changes accompanied with minimum undesirable changes, while mutagen effectiveness is a measure of frequency of mutations induced by unit dose of mutagen. A highly effective mutagen may not necessarily show high efficiency and *vice versa*. The higher efficiency of a mutagen indicates relatively less biological damage (seedling injury, seedling height reduction, sterility *etc*.) in relation to mutations induced (Kharkwal, 1998). Hence, previous knowledge of effectiveness and efficiency of the mutagens to be used is indispensable to identify the appropriate doses or concentrations of useful mutagens and to get high frequency of desirable mutations for mutation breeding program.

Variability is the pre-requisite for selection and varietal development in crop plants and mutation induction has become a proven way of creating variation within a crop species (Novak and Brunner, 1992). It offers the possibility of inducing desired attributes that either can not be found in nature or have been lost during evolution. Shu (2009) reported the mutational enhancement of genetic diversity in 17 plant species. The mutants obtained can become an important genetic resource for breeding, gene discovery and functional analysis of various genes.

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Of the huge list of nutritionally rich crop species which have remained under-utilized from the view point of their commercial exploitation as agricultural crops, ricebean (*Vigna umbellata* Thunb, Ohwi and Ohashi) a diploid (2n=22)self-pollinated crop is one of the most fascinating and nutritionally rich pulses. It has been considered to be one of the best nutritionally balanced pulses in the world and has even been included in the school children's nutritional programmes in Philippines (NAS, 1979). The crop despite of high nutritional status has remained neglected for its improvement by breeding interventions either by hybridization or otherwise, especially in India. Mutation breeding can be a powerful tool for inducing variability in ricebean, where exploitable and useful genetic variation is meager. Also at present, very less conclusive information on relative effectiveness and efficiency of any physical and chemical mutagens is available for ricebean. The present investigation was therefore undertaken to induce variability in two ricebean genotypes by gamma radiation (a physical mutagen) and EMS (a chemical mutagen) and to identify the most effective and efficient dose of the two mutagens.

MATERIALS AND METHODS

Plant material and mutagen dose : The material for the study comprised of two contrasting ricebean genotypes *viz.*, BRS 1, having black grains (like urdbean), high yield and widely grown but late maturing, and Totru Local having creamish grains, low yield, early maturing and locally grown. Four hundred dry and healthy seeds with moisture content of 10-12% were used for each treatment. Three doses each of physical and chemical mutagens were administered, 30, 40 and 50 kR gamma rays; 0.50, 0.60 and 0.70% ethyl methane sulphonate (EMS).

Mutagenic treatment: Gamma rays were secured from Gamma Cell-200 having 2500 Curie ^{60}Co source installed in the Division of Genetics, at National Physical Laboratory, Indian Agricultural Research Institute, New Delhi. Untreated dry seeds were used as a control. The treatments of EMS were given in 0.1M phosphate buffer adjusted to pH 7.0. The seeds were pre soaked in distilled water for 6 hours and then immersed in freshly prepared mutagen solution for 6 hours (with periodic shaking), followed by post treatment washing in gentle flow of tap water to remove the traces of chemical from the seed surface and then air dried on blotting paper at room temperature. Untreated dry seeds were used as a control (checks).

Experimental design and analysis: Sowing of M_1 generation was done immediately after treatment with the mutagen during *kharif* 2005 and single plants were harvested individually and planted as M_2 family rows during the next crop season. In M_1 generation the data on reduction in germination and subsequent survival (lethality) and seedling height reduction (injury) was recorded as per Sharma (1990) to estimate the damage caused by the mutagens.

Half of the M_2 seeds of each plant were used for raising M_2 generation during *kharif* 2006 and half were kept for raising M_2 generation during *kharif* 2007 along with M_3 to avoid environmental effects. All M_2 family plots consisted of single 5 m long rows with spacing of 45 X 20 cm and 30 X 15 cm for BRS-1 and Totru Local, respectively. An augmented design was used to generate M_3 seeds. Different kinds of chlorophyll mutants were scored at different stages of growth by using modified classification of Lamprecht (1960) and Kharkwal (1998) and mutation frequency was worked out as per cent of mutated M_2 families/plants as per the method suggested by Konzak *et al*., (1965) and Kharkwal (1998). The next year, remaining M_2 seeds were sown along with M_3 seeds. M_2 generation was raised in single plant completely randomized design and M_3 generation was raised in RCBD with 2 replications.

The number of families studied in M_2 generation under gamma rays were 32, 31 and 29 in BRS-1 whereas these were 22, 32 and 15 in Totru Local under 30, 40 and 50 kR doses respectively, and 18, 26 and 12 in BRS-1 while 21, 12 and 10 in Totru Local under 0.50, 0.60 and 0.70% EMS dose, respectively. Data in M_2 generation were recorded on a single plant basis dose-variety wise. Observations were recorded on days to flowering, days to maturity, pod clusters/ plant, pods/cluster, seeds/pod, pod length (cm), seed yield/ plant (g) and 100-seed weight (g).

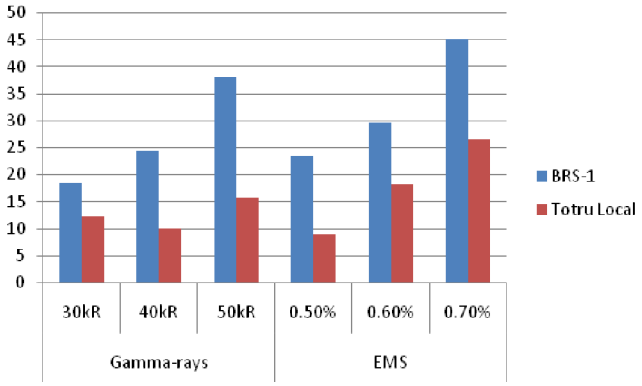
Estimates of genetic parameters were computed according to Sharma (1998).

RESULTS AND DISCUSSION

Damage in M_1 generation: The initial damage caused by the mutagenic treatments was scored by recording the reduction in germination and subsequent survival of the

treated plants and seedling height reduction (injury) in M_1 generation. Both gamma rays and EMS proved to be hazardous for germination at higher dose (in gamma rays the germination percentage was minimum under 50 kR while under EMS it was minimum under 0.70%) in both BRS-1 and Totru Local (Table 1). This reduction in germination may be attributed to the damage to the enzyme system encompassing repair mechanism or due to the production of toxic substances in the treated cells. Jain and Khandelwal (2009) have reported similar reduction in germination with increasing doses of mutagens. Like germination, mutagenic treatments also reduced plant survival drastically with increasing dose of both the mutagens. Lethal hits or relatively more chromatid or chromosomal breaks due to gamma rays and physical toxicity leading to lethality at higher doses of chemicals, have in general, been held responsible for drop in plant survival. Similar trend of reduction in survival with increasing dose has been reported by Karthika and Lakshmi, (2007) in soybean and Mahla *et al.*, (2010) in clusterbean. Reduction in the germination and plant survival was higher under EMS treatments than gamma rays in both the varieties. Some plants were killed after germination also but killing was not drastic and percentage of germination and plant survival at maturity showed linear and positive relationship. Also, both the mutagens were more effective for BRS-1 while, they were more efficient for Totru Local (Fig 2). Which indicates that biological damage is more in Totru Local as compared to BRS-1. Such difference in the effects of mutagens on different material might be due to the seed metabolism and onset of DNA synthesis. Kundi *et al.*,(1997) reported differential sensitivity within crop and even genotype. The sensitivity depends upon its genetic architecture and the mutagens employed (Blixit, 1970) besides, the amount of DNA, its replication time in the initial stages and degree of heterochromatin.

FIG 1: Seedling height reduction after mutagenic treatment in two ricebean genotypes

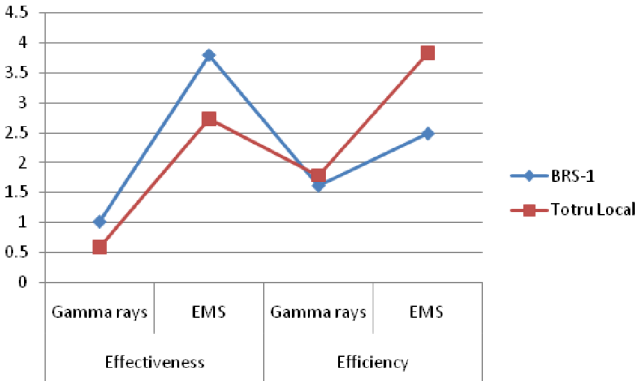


Mutation frequency, effectiveness and efficiency in M_2 generation: The chlorophyll deficient mutants are the most frequently observed and easily identified factorial mutations in M_2 generation. The frequency of these mutants reflects the effectiveness of the mutagens and are used as tests for evaluation of genetic action of mutagenic factors. Mutagenic effectiveness and efficiency were calculated to find out the

TABLE 1: Effect of mutagens on germination and plant survival in M_1 generation

Treatments		Germination %		Survival %		Reduction in survival
		Actual	Relative	Actual	Relative	over control (%)
BRS-1						
Gamma rays	Control	93.34	100.00	91.97	100.00	—
	30 kR	62.28	66.72	61.34	66.70	33.30
	40 kR	45.56	48.81	39.86	43.34	56.66
	50 kR	34.87	37.36	29.98	32.60	67.40
EMS	Control	91.44	100.00	89.94	100.00	—
	0.50%	56.56	61.85	54.67	60.78	39.22
	0.60%	38.97	42.62	37.73	41.95	58.05
	0.70%	21.56	23.58	16.97	18.87	81.13
Totru Local						
Gamma rays	Control	84.78	100.00	81.98	100.00	—
	30 kR	67.73	79.89	65.61	80.03	19.97
	40 kR	54.92	64.78	51.56	62.89	37.11
	50 kR	44.00	51.90	39.87	48.63	51.37
EMS	Control	82.37	100.00	79.92	100.00	—
	0.50%	61.23	74.34	60.51	75.71	24.29
	0.60%	41.24	50.07	39.97	50.01	49.99
	0.70%	16.98	20.61	10.66	13.33	86.67

FIG 2: Pooled mutagenic effectiveness and efficiency of gamma-radiation and EMS in BRS-1 and Totru Local



potential mutagen out of gamma rays and EMS (Table 2). In gamma rays, mutagenic efficiency (family basis) ranged from 0.63 to 2.89 in BRS-1 and 1.28 to 2.21 in Totru Local. Whereas for EMS mutagenic efficiency was in the range of 1.81 to 3.79 in BRS-1 and 1.88 to 6.88 in Totru Local. In both the cultivars, 30 kR dose in case of gamma rays and 0.50% dose in EMS were the most efficient doses. EMS at 0.50% was found to be more efficient dose for induction of mutations than gamma rays in both the varieties. Similarly, the effectiveness decreased with the increase in dose or concentration. Similar trend of decreasing effectiveness and efficiency with increasing dose of gamma rays and EMS has been reported by Waghmare and Mehra (2001) in *Lathyrus sativus* and Gnanamurthy *et al*.,(2011) in *Vigna mungo*. Greater effectiveness and efficiency in lower treatments of chemical mutagens has also been reported earlier by Bhosle and Kothekar (2010). According to Konzak *et al*., (1965) the reason for greater efficiency at lower doses/treatments is due to the fact that with the increase in mutagenic treatments the rate of biological damage like injury, lethality and sterility increases at a faster rate than the mutations. In other words lower doses/concentrations cause relatively less damage thereby enabling the organism to express the induced mutations successfully.

EMS was almost four times more effective than gamma rays whereas its efficiency was two times higher than that of gamma rays. Also, With the increasing doses of EMS/ gamma -rays, seedling height (in the present study taken as biological criteria for mutation effect) in M_1 generation decreased (Fig.I). The reduction in biological criteria may be attributed to a decrease in the auxin level (Gordon and Webber, 1955), inhibition of auxin synthesis (Skoog, 1935), chromosomal aberrations (Sparrow, 1961) or due to decline of assimilation mechanism (Quastler and Baer, 1950). Thilagavathi and Mullainathan (2009) in blackgram and Velu *et al*., (2008) in cluster bean also reported the greater efficiency and effectiveness of EMS than gamma rays.

Induced variability: The analysis of variance for 1460 entries (taken over samples) i.e. 160 controls + 1300 mutant populations in M_2 generation showed significant differences among the entries for all the seven traits. The variance due to entries was further partitioned into controls, mutants and control *vs* mutants. Significant differences among controls, mutants and control *vs* mutants were found for all the traits studied. Partitioning of control into between parents and

TABLE 2: Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens

Treatments		% Seedling	Mutation rate		Mutagenic effectiveness		Mutagenic efficiency	
		height reduction	Family basis	Plant basis	Family basis	Plant basis	Family basis	Plant basis
BRS-1								
Gamma rays	30kR	18.41	53.13	4.45	1.77	0.15	2.89	0.24
	40kR	24.50	32.26	3.01	0.81	0.08	1.32	0.12
	50kR	38.23	24.14	2.49	0.48	0.05	0.63	0.07
EMS	0.50%	23.47	88.89	7.88	5.11	0.45	3.79	0.34
	0.60%	29.68	53.85	4.58	2.65	0.23	1.81	0.15
	0.70%	45.19	83.33	8.13	3.59	0.35	1.84	0.18
Totru Local								
Gamma rays	30kR	12.34	27.27	1.70	0.91	0.06	2.21	0.14
	40kR	10.03	18.75	1.17	0.47	0.03	1.87	0.12
	50kR	15.67	20.00	1.26	0.40	0.03	1.28	0.08
EMS	0.50%	9.00	61.90	3.69	3.56	0.21	6.88	0.41
	0.60%	18.30	50.00	3.05	2.46	0.15	2.73	0.17
	0.70%	26.58	50.00	3.07	2.16	0.13	1.88	0.12

within parents showed no differences within each parental population for all the traits, while the two populations as different units differed significantly for all the traits. Homogeneity of individual parental populations indicated that these were most suitable for treatment with mutagens to induce variability for breeding purpose.

In the M_2 generation for estimation of induced variability through mutagenesis, the mean values, PCV, GCV, heritability and genetic advance were calculated for yield and yield components (Table 3). An increase in genetic variability was observed as there was shift in mean values due to the occurrence of extreme types, both on the positive and negative side of the control.

In genotype BRS-1, 30kR proved to be most suitable dose in gamma rays where maximum traits showed moderate to very high PCV, GCV, h^2_{bs} and GA. EMS at 0.50 and 0.60% showed similar trend. In these doses all the selection parameters were recorded to be high to very high for the traits pods per cluster, seeds per pod and pod length indicating the relative importance of these traits for selection.

In Totru Local, 30 kR and 0.50% alongwith 0.70% EMS were the most suitable doses for inducing maximum variability in the characters, thereby showing high to very high values of selection parameters. The important traits which could be most effective for making selection in Totru Local were clusters of pods per plant, pods per cluster and seeds per pod.

On the joint survey of gamma rays doses over BRS-1 and Totru Local, 30 kR and of chemical mutagen 0.50% EMS were the most suitable doses for producing mutagenized

TABLE 3: Estimates of mean values , shift in mean, coefficient of variation, heritability (h^2_{bs}) and genetic advance (GA) for different traits in M_2 generations of BRS-1 and Totru Local.

Traits	BRS-1						Totru Local					
	30kR	40kR	50kR	0.50%	0.60%	0.70%	30kR	40kR	50kR	0.50%	0.60%	0.70%
Days to Flowering												
	Control (77.24)						**Control (68.95)**					
Mean ± SE	76.98^n	77.48	76.70^n	77.73^p	76.79 ^{n}	76.86^n	69.43 ^{p}	69.69 ^{p}	70.28 ^{p}	68.07 ^{n}	69.48 ^{p}	69.03 ^{p}
	±0.14	±0.18	±0.15	± 0.28	±0.18	±0.28	±0.18	±0.09	±0.06	±0.21	±0.08	±0.19
PCV	5.93	13.19	13.19	20.99	9.90	10.88	10.88	4.04	-	13.29	-	4.34
GCV	5.13	12.86	12.86	20.77	9.46	10.47	10.38	2.43	-	12.87	-	2.88
h^2_{bs}(%)	74.85	95.01	95.05	97.88	91.20	92.60	91.08	36.09	-	93.82	-	44.19
GA	9.14	25.82	25.83	42.33	18.60	20.75	20.41	3.00	-	25.69	-	3.95
Days to Maturity												
	Control (130.16)						**Control (110.13)**					
Mean ± SE	130.71^p	131.07^p	133.23^p	130.48^p	129.56^n	131.05^p	110.54^p	109.16^n	111.70^p	108.59^n	112.32^p	114.88^p
	±0.08	±0.09	±0.07	±0.10	±0.08	±0.09	±0.07	±0.06	±0.11	±0.07	±0.09	±0.12
PCV	6.88	4.90	3.36	6.24	5.68	3.08	4.63	3.33	2.90	4.57	2.01	2.93
GCV	6.75	4.72	3.11	6.10	5.52	2.79	4.36	2.92	2.47	4.28	1.32	2.51
h^2_{bs}(%)	96.38	92.94	85.72	95.59	94.42	82.17	88.80	77.06	73.02	87.66	43.12	73.72
GA	13.65	9.38	5.94	12.29	11.04	5.22	8.47	5.29	4.36	8.26	1.79	4.44
Clusters of pod/ plant												
	Control (13.18)						**Control (7.23)**					
Mean ± SE	12.66 ^{n}	13.95 ^{p}	12.32 ^{n}	12.81 ^{n}	12.80 ^{n}	12.38 ^{n}	6.89 ^{n}	7.52 ^{p}	7.52 ^{p}	6.99 ^{n}	6.76 ^{n}	6.89 ^{n}
	±0.03	±0.03	±0.03	±0.05	±0.08	±0.08	±0.04	±0.04	±0.04	±0.05	±0.08	±0.07
PCV	11.45	14.44	9.76	15.29	52.51	18.98	46.49	34.19	16.35	26.62	45.56	48.76
GCV	8.59	12.99	6.73	13.69	51.98	17.33	42.86	32.12	11.34	23.38	43.48	46.24
h^2_{bs}(%)	56.35	80.86	47.63	80.06	97.98	83.35	84.97	88.21	48.11	77.15	91.09	89.90
GA	13.29	24.06	9.57	25.23	105.98	32.60	81.38	62.14	16.20	42.30	85.49	90.31
Pods/cluster												
	Control (4.91)						**Control (3.92)**					
Mean ± SE	4.63 ^{n}	6.02 ^{p}	5.02 ^{p}	4.80 ^{n}	4.60 ^{n}	5.02 ^{p}	3.29 ^{n}	4.61 ^{p}	4.01 ^{p}	4.32 ^{p}	3.22 ^{n}	3.88 ^{n}
	±0.03	±0.04	±0.03	±0.05	±0.04	±0.06	±0.04	±0.04	±0.05	±0.04	±0.06	±0.05
PCV	49.60	35.05	17.26	45.33	70.54	30.15	67.72	22.12	43.11	46.08	64.69	70.81
GCV	45.47	32.3	5.34	41.34	67.86	24.49	61.67	11.9	38.07	42.31	59.19	66.96
h2bs (%)	84.03	84.94	9.58	83.15	92.54	66.00	82.94	28.97	78	84.30	83.71	89.41
GA	85.86	61.33	3.41	77.64	134.48	40.99	115.7	13.2	69.27	80.03	111.56	130.42
Seeds/pod												
	Control (5.41)						**Control (4.18)**					
Mean ± SE	5.38	6.55^p	5.99^p	5.78 ^{p}	5.69 ^{p}	5.21 ^{n}	4.04n	4.89 ^{P}	4.78 ^{p}	4.74 ^{p}	3.92 ^{n}	4.06 ^{n}
	±0.02	±0.03	±0.02	±0.04	±0.03	±0.04	±0.03	±0.05	±0.03	±0.03	±0.03	±0.05
PCV	29.12	13.09	10.68	20.87	25.84	27.53	39.07	65	35.11	25.26	25.89	32.93
GCV	26.37	7.94	1.73	17.61	23.04	24.16	35	63.75	32.21	21.95	19.07	27.91
h2bs (%)	81.98	36.79	2.63	71.24	79.49	77.00	80.29	96.2	84.15	75.50	54.22	71.83
GA	49.18	9.92	0.58	30.63	42.31	43.67	64.61	128.81	60.86	39.29	28.92	48.72

p = Significant positive shift in mean n = Significant negative shift in mean

populations with high magnitudes of four different parameters of variability. The two characters *viz.,* pods per cluster and seeds per pod suitable for making selection in BRS-1 were also suitable in Totru Local.

Increase in the variability parameters in mutagenized populations of ricebean have also been reported by Lokesha *et al.,* (1991) and Lokesha and Veeresh (1993). Mahla *et al.,* (2010) in clusterbean and Singh *et al.,* (2007) in lentil also reported induction of variability through gamma rays and EMS. An increase in genetic variability might be due to the occurrence of extreme types, both on the positive and negative side of the control. This is due to the fact that the effect of mutagens on the quantitative traits has been interpreted as that owing to detrimental mutations which are supposed to occur more frequently than favorable ones. Brock (1965) reported that in the species which had previously been selected to breeding, random mutations resulted in an increase in variance and a shift in the mean away from the direction of previous selection. He further suggested that the increased genetic variation permitted effective selection response in each direction even in characters approaching the limits within the species.

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