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Effectiveness and Efficiency of Gamma Rays, Ethylmethane Sulphonate and their Combinations in *Vigna mungo* (L.) Hepper

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ABSTRACT

A systematic study to determine the effectiveness and efficiency of gamma rays, ethylmethane sulphonate (EMS) and their combinations (gamma rays + EMS) were carried out in two urdbean (*Vigna mungo* (L.) Hepper) varieties namely, T-9 and Pant U-30. Based on effectiveness, the order of mutagens was EMS > gamma rays + EMS > gamma rays in both the varieties. Three criteria viz., pollen sterility, seedling injury and meiotic abnormalities were taken into consideration to determine the efficiency of the mutagens. The efficiency of the mutagens varies with the variety type and the mutagen doses applied depending upon the criteria chosen. Overall, the combination treatments were more efficient followed by EMS and gamma rays. Assessing mutagenic effectiveness and efficiency are beneficial to screen mutagen doses which are potent to increase genetic diversity among self-pollinated crops like urdbean for successful plant breeding strategies.

Keywords: biological damage, dose, effectiveness, mutagen
Abbreviations: CRBD, complete randomized block design; **EMS**, ethylmethane sulphonate; **kR**, kiloroentgen; **mM**, millimolar

INTRODUCTION

Urdbean (*Vigna mungo* (L.) Hepper), also known as mash or blackgram, is an important pulse crop occupying about 14% of the total area under pulse crops. In Indian context, urdbean ranks fourth in area and production after chickpea (*Cicer arietinum*), pigeonpea (*Cajanus cajan*) and mungbean (*Vigna radiata*) (Yadav 1991). Presence of various types of anthropogenic pressures and increase in human popuation the *per capita* pulses consumption over the years has come down from 61 g/day in 1951 to 30 g/day in 2008 (Reddy 2009). A solution to the problem of declining per capita availability has, therefore, to come from a rapid improvement in indigenous production levels. Since urdbean is a self-pollinated crop, mutation breeding could be rewarding for broadening the genetic base of important traits like yield. Kumar *et al.* (2011) advocates that mutation breeding can constitute a valuable tool in widening the genetic base through creation of some useful mutants, henceforth, mutation breeding finds a prominent place in the augmentation and recreation of genetic variability. Mutation breeding has played a significant role in the development of many crop varieties (Canci *et al.* 2004). In recent years, alot of work has been undertaken on induced mutagenesis through different types of mutagens (Kharakwal and Shu 2009; Moussa and Jaleel 2011; Kozgar *et al.* 2011; Wani *et al.* 2011). Physical and chemical mutagenic agents cause genes to mutate at rates above the spontaneous base line, thus producing a range of novel traits and broadening of the genetic diversity of plants (Lagoda 2007). Before the start of any sound breeding programme, knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover high frequency of desirable mutations (Smith 1972; Kumar and Mani 1997; Wani *et al.* 2011). Selecting a mutagen and its optimum dose for a genotype in any plant species is an important step in mutation breeding programme. In the backdrop, the present investigation was undertaken with the aim to evaluate the relative effectiveness and efficiency of gamma rays, EMS and their combinations on two urdbean varieties in M_2 generation.

MATERIALS AND METHODS

Uniform and healthy seeds of two urdbean (*Vigna mungo* (L.) Hepper) varieties (T-9 and Pant U-30), procured from G. B. Pant University of Agriculture and Technology, Pantnagar (Uttaranchal, India), were selected for gamma rays (a physical mutagen), ethylmethane sulphonate, EMS (a chemical mutagen) and their combination treatments. Dry seeds of each variety, with moisture content 12%, were irradiated with 100, 200, 300 and 400 Gy (10, 20, 30 and 40 kR, respectively) doses of gamma rays from a ^{60}CO source at the National Botanical Research Institute (Lucknow, India), wherein, 1 Gy is equal to 1.7 sec time duration in gamma chamber. For chemical treatments, seeds each variety were presoaked for 9 h in distilled water and treated with 0.1, 0.2, 0.3 and 0.4% of EMS, an alkylating agent, manufactured by Sissco Research Laboratories Pvt. Ltd. (Mumbai, India) for 6 h with intermittent shaking at room temperature of 25 ± 2°C. The solution of EMS was prepared in the phosphate buffer of pH 7. For combination treatments, dry seeds of each variety were first irradiated with gamma rays at 200 and 300 Gy doses and then treated with 0.2 and 0.3% EMS. Following combination treatments were given. 200 Gy+0.2% EMS, 300 Gy+0.2% EMS, 200 Gy+0.3% EMS and 300 Gy+0.3% EMS.

To facilitate uniform absorption of EMS (alone and/or in combination), large quantities of solution of mutagens, approximately three times the volume of seeds (Konzak *et al*. 1965), were used. Following these treatments, the seeds were thoroughly washed in running tap water to remove the residue mutagens from the seed surface.

The treated seeds were directly sown in the field along with untreated controls. Three replications of 100 seeds per treatment were sown in a complete randomized block design (CRBD) to raise M_1 generation in 2007 *kharif* (summer or monsoon) season. The M_1 plants were harvested separately and the seeds sown the next *kharif* season (2008) in plant progeny rows to raise M_2 generation. Distance between the seeds in a row and between the rows

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Short Communication

Table 1 Effectiveness and efficiency of gamma rays, EMS and their combinations in M_2 generation of urdbean var. T-9.

Treatment	I	S	Me	Mp	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
Gamma rays								
100 Gy	10.41	6.72	4.53	2.00	0.020	0.19	0.30	0.44
200 Gy	13.42	13.08	5.76	10.00	0.050	0.74	0.76	1.74
300 Gy	30.34	18.35	7.85	12.00	0.040	0.39	0.65	1.53
400 Gy	36.96	24.56	9.66	14.00	0.035	0.38	0.57	1.45
EMS								
0.1%	8.69	6.36	2.28	4.00	6.66	0.46	0.63	1.75
0.2%	13.07	13.03	5.45	16.00	13.33	1.22	1.23	2.93
0.3%	21.47	17.91	6.84	16.00	8.89	0.74	0.89	2.34
0.4%	34.06	24.32	8.73	20.00	8.33	0.59	0.82	2.29
Gamma rays + EMS								
200 Gy+0.2%	11.59	13.49	4.72	14.00	0.058	1.21	1.04	2.97
300 Gy+0.2%	16.91	19.54	6.80	20.00	0.055	1.18	1.02	2.94
200 Gy+0.3%	33.47	22.78	8.59	20.00	0.055	0.60	0.88	2.33
300 Gy+0.3%	41.75	28.60	10.45	22.00	0.041	0.53	0.77	2.10

I: % Seedling injury; S: % Pollen sterility; Me: % Meiotic abnormalities; Mp: % Mutated plant progenies

Table 2 Effectiveness and efficiency of gamma rays, EMS and their combinations in M_2 generation of urdbean var. Pant U-30.

Treatment	I	S	Me	Mp	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
Gamma rays								
100 Gy	9.85	7.41	4.63	4.00	0.040	0.41	0.54	0.86
200 Gy	13.04	14.48	6.29	12.00	0.060	0.92	0.83	1.91
300 Gy	25.91	21.61	8.51	16.00	0.053	0.62	0.74	1.88
400 Gy	40.58	27.78	10.82	20.00	0.050	0.49	0.72	1.85
EMS								
0.1%	7.25	8.20	2.73	6.00	10.00	0.83	0.73	2.20
0.2%	11.88	13.58	6.08	18.00	15.00	1.51	1.32	2.96
0.3%	16.81	20.77	7.20	20.00	11.11	1.19	0.96	2.78
0.4%	27.54	26.90	9.38	20.00	8.33	0.73	0.74	2.13
Gamma rays + EMS								
200 Gy+0.2%	10.20	13.37	5.00	14.00	0.058	1.37	1.05	2.80
300 Gy+0.2%	13.91	19.81	7.45	22.00	0.061	1.58	1.11	2.95
200 Gy+0.3%	32.46	26.78	9.02	22.00	0.061	0.68	0.82	2.44
300 Gy+0.3%	41.16	28.75	12.54	28.00	0.051	0.68	0.97	2.23

I: % Seedling injury; S: % Pollen sterility; Me: % Meiotic abnormalities; Mp: % Mutated plant progenies

was kept at 30 and 60 cm, respectively. Frequency of chlorophyll mutants scored was calculated as percentage of M_2 segregating progenies as well as that of mutant seedlings in M_2 generation and classified according to the methodologies adopted by Gustafsson (1940) and proceeded with to evaluate the mutagenic effectiveness and efficiency of the mutagens used as per suggested by Konzak *et al.* (1965).

RESULTS AND DISCUSSION

Data on effectiveness and efficiency for gamma rays, EMS and their combination treatemnets in two varieties of urdbean are presented in **Table 1** and **Table 2**, respectively. It was found that effectiveness and efficiency were generally higher at the moderate doses of gamma rays and EMS; on the other hand, in case of the combination treatments, 200 Gy+0.2% EMS and 300 Gy+0.2% EMS treatments were most effective and efficient. The estimates of effectiveness ranged from 0.020 to 0.050 in the var. 'T-9' and 0.040 to 0.060 in var. 'Pant U-30' of gamma rays treatments, whereas the effectiveness of EMS treatments ranged from 6.66 to 13.33 and 8.33 to 15.00 in the varieties 'T-9' and 'Pant U-30', respectively. Mutagenic effectiveness decreased at the highest concentration of all the three mutagenic doses in both the varieties. Based on effectiveness in both the varieties, the order of mutagenic effectiveness was EMS > gamma rays + EMS > gamma rays. The decline in the mutagenic effectiveness recorded at higher doses shows that the increase in mutation rate was not proportional to the increase in the doses of various mutagens. This type of trend was also reported in *Lathyrus sativus* by Waghmare and Mehra (2001).

Mutagenic efficiency gives an idea of the proportion of mutations in relation to biological damage caused in M_1 generation. Mutagenic efficiency, calculated on the basis of seedling injury (Mp/I); pollen sterility (Mp/S) and meiotic abnormalities (Mp/Me), of 200 Gy dose among gamma ray treatments and 0.2% concentration among EMS treatments were most efficient. Among combination treatments, 200 Gy+0.2% EMS treatment was most efficient in the var. 'T-9', whereas in the var. 'Pant U-30', 300 Gy+0.2% EMS treatment was found to be most efficient. On the basis of seedling injury, pollen sterility and meiotic abnormalities, the efficiency of mutagens in a descending order was: gamma rays + EMS > EMS > gamma rays (**Table 3**).

Mutagenic effectiveness relates mutagen dose to the mutational events, while mutagenic efficiency indicates the extent of genetic damage recorded in M_2 generation in relation to the biological damage caused in M_1 (Konzak *et al.* 1965). Mutagenic effectiveness and efficiency were estimated on the basis of the frequency of progenies segregating for chlorophyll mutations. EMS alone proved more effective than the combined gamma rays + EMS treatments while combined treatments proved more effective than gamma rays. Different concentrations of EMS concentrations (1.0 to 5%) has been regarded as superior to the doses (10 to 50 kR) of gamma rays in inducing useful mutations in *Oryza sativa* (rice) (Mikaelsen *et al.* 1971; Kaul and Bhan 1977), *Lens culinaris* (lentil) (Solanki and Sharma 1994), mungbean (Singh 2007), chickpea (Shah *et al.* 2008), urdbean (Thilagavathi and Mullainathan 2009), *Vigna unguiculata* (cowpea) (Girija and Dhanavel 2009) and *Glycine max* (soybean) (Khan and Tyagi 2010). The decline in the mutagenic effectiveness recorded at higher doses shows that the increase in mutation rate was not proportional to the increase in the doses of mutagens.

The mutagenic efficiency calculated in relation to seedling injury (Mp/I), pollen sterility (Mp/S) and meiotic ab-

Table 3 Mutational rate of the mutagens in relation to biological damage in two varieties of urdbean.

Treatment	Var. T-9			Var. Pant U-30		
	MRI	MRS	MRMe	MRI	MRS	MRMe
Gamma rays	0.42	0.57	1.29	0.61	0.71	1.62
EMS	0.75	0.89	2.33	1.06	0.94	2.52
Gamma rays + EMS	0.88	0.93	2.58	1.08	0.99	2.60

MRI: Mutation rate based on seedling injury (seedling height reduction).
MRS: Mutation rate based on pollen sterility.
MRMe: Mutation rate based on meiotic abnormalities.

normalities (Mp/Me) showed that the combination treatments were more efficient followed by EMS and gamma rays. However, Zeerak (1992), on the basis of his results on brinjal, rated the combined treatments of gamma rays and EMS to be less efficient than EMS and gamma rays. In the present study, higher frequency of chlorophyll mutations induced by gamma rays + EMS combined treatments appears to be the reason for the higher efficiency. Like effectiveness, the efficiency was also higher at moderate doses. The greater efficiency at lower/moderate doses of mutagen is because the biological damage generally increased with the enhancement in the dose at a higher rate than the mutations yielded in M_2 at the same dose (Konzak *et al.* 1965).

The mutagenic efficiency varied depending upon the criterion chosen for its estimation. Based on Mp/Me, the efficiency was generally higher as compared to Mp/S and Mp/I. This may be due to the fact that induced meiotic abnormalities were less in separate or combined mutagen treatments than the amount of pollen sterility and seedling injury. The efficiency of mutagenic agents not only depends on the biological system but also on the degree to which physiological damage, chromosomal aberrations and sterility is induced in addition to mutations. The higher effectiveness and efficiency at lower and intermediate doses have been reported in *Vigna mungo* (Deepalakshmi and Anandakumar 2003) at 20 mM (millimolar) EMS and 200 Gy) gamma rays, in *Vigna radiata* (Goyal *et al.* 2009) at 0.2% EMS, 0.02% SA and in *Glycine max* by (Khan and Tyagi 2010) at o.1% EMS and 15 kR gamma rays. Similar type of findings has also been reported by Dixit and Dubey (1986) in lentil (*Lens culinaris*). Higher efficiency at lower and intermediate doses of mutagens may be to the biological damage which gets increased with the dose at a rate greater than the frequency of the mutations (Konzak *et al.* 1965).

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