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Studies on mutagen sensitivity, effectiveness and efficiency in urdbean [*Vigna mungo* (L.) Hepper]

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Abstract

Seeds of urdbean [(*Vigna mungo* (L.) Hepper] cultivars PDU-1 and T-9 were mutagenised with gamma-rays and EMS to determine their mutagen sensitivity, LD_{50}, mutagenic effectiveness and efficiency. The increasing doses of gamma-rays and EMS decreased germination, plant survival and pollen fertility. LD_{50} values revealed that PDU-1 was more radio-and chemo-resistant than T-9. LD_{50} values of gamma-rays and EMS can be helpful in experimental mutagenesis. The mutation frequency of gamma-rays was less in PDU-1 than in T-9. There was a parallelism between radio-sensitivity and chemo-mutability of urdbean genotypes. The average effectiveness and efficiency (pooled overdoses and genotypes) of EMS was 2.0-2.5 and 1.5-2.0 times higher than the gamma-rays, respectively. The lower doses of mutagens were more effective and efficient than the higher doses. Though effectiveness and efficiency computed by different methods varied in quantum, their relative proportion were comparable. A single method of studying mutagenic effectiveness and efficiency should be appropriate.

Key words: Urdbean, mutagen sensitivity, LD_{50}, mutagenesis, mutagenic effectiveness, mutagenic efficiency

Introduction

Response to seed treatment to various mutagens provide valuable information for mutation breeding as it facilitates the planning of experiments designed to get higher mutation frequency. This information should be available with the mutation breeder in the beginning of experimentation. There is no report on LD_{50} in urdbean. The mutagenic effectiveness and efficiency of mutagens and their doses are pre-requisite for induction and utilization of mutations. Effectiveness means the rate of mutations as related to dose, while efficiency usually refers to the mutation rate in relation to damage [1]. Although many studies on mutagenic effectiveness have been undertaken in mung bean, limited studies are available in urdbean [2-3]. However, there are a few reports available on the efficiency of gamma-rays and EMS in urdbean. The present study, therefore, intends to provide relative sensitivity of the urdbean genotypes, LD_{50} of the commonly used mutagens such as gamma-rays and EMS and their effectiveness and efficiency using urdbean as a genetic system.

Materials and methods

Two commercial varieties of urdbean [*Vigna mungo* (L.) Hepper] *viz.* PDU-1 and T-9 were selected for the present study. Samples of five hundred dry, healthy and uniform size seeds were treated with 100, 200, 300 and 400 Gy gamma rays in ^{60}Co gamma cell at a dose rate of 10 Gy/minute. For chemical treatments, seed samples were pre-soaked in the distilled water for 16 hours at room temperature (25±1°C), followed by treatment for five hours with 0.1, 0.2, 0.3 and 0.4% of freshly prepared aqueous solution of EMS, whose pH was adjusted at 8.5 using 0.2M solution of sodium tetra borate (Borax). Dry and pre-soaked seeds were used as control with gamma rays and EMS treatment, respectively.

The seedling height reduction (I) in different M_1 treatments was studied [4]. The extent of reduction in pollen fertility (S) due to mutagenic treatment was determined by staining the pollen grains with 1% acetocarmine solution. For this purpose, plants were selected randomly from each treatment and finally 5 flower buds from each plant were used for microscopic analysis. The pollen grains which failed to stain or showed abnormal shape and improper filling were scored as sterile. The plant survival percentage (L) was computed as the percentage of plants surviving till maturity, out of total number of plants recorded after germination. Mutagenic effectiveness and efficiency were calculated according to the formulae suggested by Konzak *et al.* [5]. LD_{50} was determined for the biological parameters of the treatment using the regression equation of Y (damage) on X (dose) as $Y = a + bX$.

Results and discussion

Germination, plant survival and pollen fertility decreased progressively with increasing doses of gamma-rays

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and EMS in both the varieties. It was indicative from the significant correlation co-efficient of biological parameters such as germination and plant survival with gamma-ray treatments of variety T-9. However, in PDU-1 only germination showed significant correlation coefficient. The EMS showed significant correlation co-efficient for germination and plant survival in T-9, while non-significant in PDU-1.

A common belief is that doses close to LD_{50} for biological parameters such as germination or survival should be taken into consideration, while planning the mutation breeding experiments. In general, an overdose is likely to kill too many treated individuals while an under dose would produce too few mutations. LD_{50} values of gamma rays for germination and plant survival were 510.5 and 440.4 Gy respectively, while higher values of 586.5 and 539.3 Gy were recorded in T-9 under field conditions (Table 1). In case of EMS, LD_{50} value for germination and plant survival in PDU-1 were 0.81 and 0.62% respectively, while slightly higher doses (0.82 and 0.75%) were observed in T-9. These LD_{50} values can be taken into consideration while planning the experimental mutagenesis in urdbean. The results also revealed that PDU-1 was more resistant to gamma rays and EMS than T-9 as revealed by LD_{50} values. The differences of mutagen sensitivity were conspicuous in gamma rays treatments than EMS. Besides, there was a parallelism in the relative radio- and chemo-sensitivity of the urdbean genotypes under study.

Table 1. LD_{50} of gamma rays and EMS for germination and plant survival under field condition in M_1 generation

Mutagen	Biological parameter	LD_{50}	
		PDU-1	T-9
Gamma rays (Grey)	Germination	510.5	586.5
	Plant survival	444.0	539.3
EMS (%)	Germination	00.81	00.82
	Plant survival	00.62	00.75

Effectiveness (Mp/dose or Ms/dose) was the highest (2.13 and 0.32 respectively) at 100 Gy dose in PDU-1, which decreased and was the lowest (0.72 and 0.08) at 400 Gy gamma rays (Table 2). Likewise, the effectiveness (Mp/dose or Ms/dose) was the highest (4.58 and 0.59% respectively) at 0.1% EMS, which

Table 2. Mutagenic effectiveness and efficiency of gamma-rays and EMS in urdbean varieties PDU-1 and T-9

Mutagen treatment	% of mutations on the basis of		Biological damage observed in the glass-house and expressed as			Effectiveness		Efficiency					
	M_1 plant progenies (Mp)	M_2 plants (Ms)	% seedling height reduction (I)	% plant survival reduction (L)	% pollen sterility reduction (S)	Mp/ dose	Ms/ dose	Mp/ I	Mp/ L	Mp/ S	Ms/ I	Ms/ L	Ms/ S
Gamma rays (Gy)						Variety PDU-1							
100	21.25	3.22	2.1	20.5	3.5	2.13	0.32	10.12	1.04	6.07	1.53	0.16	0.92
200	25.96	3.45	8.4	27.7	10.5	1.30	0.17	3.09	0.94	2.47	0.41	0.12	0.33
300	24.95	2.89	10.9	35.4	24.8	0.83	0.10	2.29	0.70	1.01	0.27	0.08	0.12
400	28.70	3.29	11.0	41.2	30.2	0.72	0.08	2.61	0.70	0.95	0.30	0.08	0.11
Mean	25.22	3.21	8.1	31.2	17.3	1.25	0.17	4.53	0.85	2.63	0.63	0.11	0.37
EMS(%)													
0.1	22.23	2.86	1.8	21.0	3.8	4.58	0.59	12.35	1.06	5.85	1.59	0.14	0.75
0.2	26.30	3.92	6.5	26.8	9.3	2.71	0.40	4.05	0.98	2.83	0.60	0.15	0.42
0.3	31.84	5.02	8.1	26.0	22.3	2.19	0.35	3.95	1.22	1.43	0.62	0.19	0.23
0.4	38.58	5.97	8.4	31.6	30.6	1.99	0.31	4.59	1.22	1.26	0.71	0.19	0.20
Mean	29.74	4.44	6.2	26.4	16.5	2.87	0.41	6.24	1.12	2.84	0.88	0.17	0.40
Gamma rays (Gy)						Variety T-9							
100	25.35	3.43	2.6	35.2	4.3	2.54	0.34	9.75	0.72	5.90	1.32	0.10	0.80
200	33.99	4.82	8.2	42.8	12.8	1.70	0.24	4.15	0.79	2.66	0.59	0.11	0.38
300	46.01	6.63	18.3	47.5	18.8	1.53	0.22	2.51	0.97	2.45	0.36	0.14	0.35
400	53.83	6.76	19.5	53.3	26.9	1.35	0.17	2.76	1.01	2.00	0.35	0.13	0.25
Mean	39.80	5.41	12.2	44.7	15.7	1.78	0.24	4.79	0.87	3.25	0.66	0.12	0.46
EMS(%)													
0.1	43.40	4.56	1.2	25.3	6.5	8.95	0.94	36.17	1.72	6.68	3.80	0.18	0.70
0.2	47.15	7.86	4.5	32.5	12.0	4.86	0.81	10.48	1.45	3.93	1.75	0.24	0.66
0.3	53.96	8.53	14.5	36.5	18.5	3.71	0.59	3.72	1.48	2.92	0.59	0.23	0.46
0.4	51.39	9.40	18.2	42.5	22.8	2.65	0.48	2.82	1.21	2.25	0.52	0.22	0.41
Mean	48.98	7.59	9.6	34.2	15.0	5.04	0.71	13.30	1.47	3.95	1.67	0.22	0.56

decreased with increasing doses of EMS and was the lowest at 0.4% (1.99 and 0.31), indicating the proportionate increase in the mutation rate (Mp or Ms) was much lower than the proportionate increase in dose of the mutagens. The comparison of biological damage expressed in terms of per cent seedling height reduction (I), per cent plant survival reduction (L) and per cent pollen sterility reduction (S) induced by different doses of mutagens in both the genotypes revealed that 100, 200, 300 and 400 Gy gamma ray treatments were comparable with 0.1, 0.2, 0.3 and 0.4% EMS, respectively (Table 2). At biologically comparable doses of both the mutagens, EMS was 2.0-3.5 times more effective than the gamma rays. These results were supported by the average mutagenic effectiveness pooled over both the genotypes Birhman *et al.* [6] reported that in *Vigna radiata* effectiveness was the highest at 300 Gy gamma rays. Jebaraj and Marrapan [7] also reported higher mutagenic effectiveness at lower doses of gamma rays and EMS in mungbean.

The mutagen efficiency for both the mutagens and their doses was calculated using two mutation rates (Mp and Ms) and three parameters of biological damage observed in the glass-house (I = % seedling height reduction, L = % plant survival reduction and S = % pollen sterility reduction). The efficiency computed by all six methods was higher at the lowest doses of the mutagens in both the varieties, which decreased further with the increasing doses of the mutagens, indicating that proportionate increase in the mutation rate was much less than the proportionate increase in the biological damage. At the higher doses of mutagens, there was no further decrease in the mutagen efficiency and the values appeared to have become static. At these doses, the proportionate increase in the mutation rate was parallel to the increase in the biological damage. At biologically comparable doses of both the mutagens in both the genotypes, EMS doses were 1.0-3.7 times more efficient than the doses of gamma-rays. Similar results were obtained when the doses were pooled over genotypes.

EMS and its doses were found to be a more effective and efficient mutagen than the gamma rays. Among doses, lower ones of EMS and gamma rays were more effective and efficient than the higher doses. Efficient mutagens and their treatments are essential for the economic use of the mutagen as a tool for the induction of useful mutations and their direct and indirect utilization. Although both mutagenic effectiveness and efficiency generally decreased with the increasing doses of the mutagens (with a few minor exceptions), nonetheless, the higher mutation rates were obtained with the higher doses of gamma rays and EMS in both the genotypes. It would be thus seen that higher mutagenic effectiveness and efficiency did not reflect the *per se* mutation frequency and these cannot be used as an index for the maximization of mutation rates.

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