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Mutagenic Effectiveness and Efficiency of Gamma Rays, Sodium Azide and Their Synergistic Effects in Urd Bean (*Vigna mungo* L.)

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Abstract: Mutagenic effectiveness and efficiency of gamma rays sodium azide and their combination treatments were studied in the genotype of urdben variety T9. The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit mutagen dose, whereas mutagenic efficiency is measure of proportion of mutations in relation to undesirable changes like lethality, sterility and meiotic aberrations etc. Synergetic as well as antagonistic effects may occur when various physical and chemical mutagens are used in combinations. The frequency of mutagenic efficiency and effectiveness was found to be highest at lower doses.

Key words: Effectiveness · Efficiency · Gamma rays · Sodium azide · Urd bean

INTRODUCTION

Since chlorophyll mutations are most conspicuous and are easily detectable. They have been extensively used to find out sensitivity of crop plants to mutagens and to elucidate effectiveness and efficiency of mutagen [1]. Effectiveness refers to the ability of mutagen to induce desirable mutations [2] and therefore, it is a measure of mutation rate relative to dose/concentration. Efficiency, on the other hand, gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility induced by the mutagen.

As genetic variability is essential for any crop improvement programme, the creation and management of genetic variability becomes central base to crop breeding. Experimentally, induced mutations provide an important source for variability. Many physical and chemical mutagens have been used for induction of useful mutations in number of crops. The practical utility of induced mutations for the improvement of quantitatively inherited characters in urdbean is well recognized.

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, pollen sterility, ovule sterility etc.,) in relation to the mutagens induced [3,4]. Selection of effective and efficient mutagen is very essential to recover high frequency of the desirable mutations in a mutation breeding studies. Hence, previous knowledge of effectiveness and efficiency of most commonly used chemical mutagens in relation to the ionization radiations in a number of genotypes is indispensable to classify the range of dose concentrations of useful mutagens in a number of breeding programme. At present no conclusive information on relative effectiveness and efficiency of different physical and chemical mutagan is available for urdbean (*Vigna mungo* L.Hepper). The present experiment/ investigation was undertaken to assess the frequency, effectiveness and efficiency of gamma rays and sodium azide (SA) and their combinations and to study the synergistic effects of combination treatments of mutagens.

MATERIALS AND METHODS

Seeds of Urd bean (*Vigna mungo* L.Hepper), cv. T9, was used for the gamma rays treatments. Seven samples of 300 seeds stabilized at 8% moisture each were exposed to gamma- radiations 60(Co) at 5, 10, 20, 30, 40, 50 and 60kR doses (IR=2.58x10 -4C/Kg) at NBRI, Lucknow - U.P (INDIA). Seven samples comprising 300 seeds (stabilized

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at 8% moisture) were used for gamma ray treatment. Seven samples comprising 300 seeds each were presoaked for eight hours in distilled water and were treated with different sodium azide (SA) concentrations 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07% concentration for six hours in phosphate buffer (pH7.0). For combination treatments, four samples of 300 seeds each were first irradiated with gamma rays at 10,20,30 and 40 kR doses and then treated with 0.02% SA in same manner as described above .The seeds were given intermittent shaking through out the period of treatment to maintain uniform concentration. After treatments, the seeds were thoroughly washed with running tap water to remove the traces of chemical from the seeds, if any. A total 20 treatments including two controls were immediately sown at the rate of 100 seeds per plot in a randomized block design (RBD) with three replications at a spacing of 15x30 cm within and between the rows at the Research Farm of Allahabad Agriculture Institute-U.P, India during the *kharif* (15th July – 15th September) season 2005 and the M_2 generation was grown in the *rabi* (15th March- 15th June 2006). Pollen sterility in first five buds and ovule sterility from the pods at the time of maturity were recorded. The matured anthers from 20 randomly selected plants from each treatment were selected and pollen sterility was tested for each treatment by using two per cent freshly prepared acetocarmine solution and examined under microscope. Dark stained and normal size pollen grains were considered as fertile and those of irregular shape and size with light or no stain were considered as sterile.

All the surviving individual plants were harvested in each treatment in M_1 generation. M_1 plants having sufficient seeds in different treatments were grown in plants to progeny rows to rise M_2 generation. M_2 generation was screened for lethal chlorophyll mutations during the first four weeks after germination, whereas viable chlorophyll and morphological viable mutations were scored throughout the crop duration. Mutation frequency was calculated as percentage of mutated M_2 progenies for both chlorophyll and morphological mutations in each treatment. Mutagenic effectiveness and efficiency was calculated on the basis of formulae suggested by [5].

Mutagenic Effectiveness:

$$\text{Effectiveness (Physical mutagen)} = \frac{\text{Mutation rate on the basis of M1 plant progenies (Mp) or M2 population (Ms)}}{\text{Dose in Kilo roentgens (kR)}}$$

$$\text{Effectiveness (Chemical mutagen)} = \frac{\text{Mutation rate on the basis of M1 plant progenies (Mp) or M2 population (Ms)}}{\text{Concentration of mutagen (\%)} + \text{Time of treatment (hr)}}$$

$$\text{Effectiveness (Physical + Chemical)} = \frac{\text{Mutation rate on the basis of M1 plant progenies (Mp) or M2 population (Ms)}}{\text{Dose of physical mutagen (kR)} + \text{Concentration of chemical mutagen (mM)} + \text{Time (hr)}}$$

Mutagenic Efficiency:

$$\text{Efficiency} = \frac{\text{Mutation rate on the basis of M1 plant progenies (Mp) or M2 population (Ms)}}{\text{\% pollen sterility (Ps) or \% ovule sterility (Os)}}$$

To evaluate the effect of combination treatments on mutation frequency the data were analyzed using the formula adopted by [6]:

$$(a)+(b) = 1/k\,(a+b)$$

Where *a* and *b* stands for two treatments and k is a hypothetical interaction coefficient. The value of *k* should be one; if the interaction is additive. Any deviation from this value should show synergistic or less than additive effects.

RESULTS AND SISCUSSION

The data presented in Table 1 revealed that pollen and ovule sterility showed direct relation ship with gamma rays and SA doses. The higher doses of gamma rays, sodium azide and their combination treatments exhibited the maximum pollen and ovule sterility. Whereas in case of combination treatments highest pollen and ovule sterility was observed at 40kR + 20mM combination treatments. Thus, post irradiation treatment with sodium azide (SA) produced additive effects. Effectiveness of gamma rays increased with increase in dose from 5kR to 40kR but showed declination from 50kR to 60kR. Among the different mutagenic treatments, 40 kR gamma rays were found most effective in case of both M_1 plant progenies and M_2 plant basis.

Whereas, in case of SA, the most effective treatment was 40 mM. On the other hand, in case of combination treatments, the lower dose i.e. 10kR (gamma rays) + 20 mM (SA) dose was the most effective in both in M_1 and M_2 plant progenies. In general, gamma rays treatments were more effective than SA and combination treatments. Similar results were also reported by [7,8].

Table 1: Effect of different doses of mutagens on pollen and ovule sterility

Mutagenic treatment	Pollen sterility (%)	Ovule sterility (%)
Control	1.0	2.0
Gamma rays (kR)		
5kR	13.0	11.0
10kR	23.0	16.0
20kR	24.0	30.0
30kR	42.0	46.0
40kR	67.0	69.0
50kR	73.0	78.0
60kR	79.0	82.0
Sodium Azide (mM)		
10mM	9.5	10.0
20mM	11.9	12.5
30mM	18.8	18.8
40mM	24.6	21.4
50mM	46.7	40.7
60mM	55.8	60.4
70mM	65.0	67.0
Gamma rays + Sodium Azide		
10kR + 20mM	24.5	18.6
20kR+ 20mM	30.6	32.4
30kR+ 20mM	46.5	42.0
40kR+ 20mM	70.9	72.5

Mutagenic efficiency, calculated on the basis of both percentage pollen sterility and percentage ovule sterility, 40kR gamma ray treatment was most efficient among gamma rays treatments and in SA 40mM concentration was found to be most efficient on the basis of M_2 plant mutated for both pollen sterility and ovule sterility. Among combination treatments, 20kR + 20 mM SA was the most efficient on the basis of pollen sterility and 10kR + 20mM SA on the basis of ovule sterility. In general SA was most efficient than gamma rays. The above findings are in accordance to the observations of several other workers [9,2].

Mutation frequency calculated on the basis of M_1, plant progenies and M_2 plants mutated showed dose dependence increase. A gradual increase in mutation frequency was associated with obtained with the increased in combination treatments, being highest at 40kR gamma rays + 0.20 mM SA combination treatments.

Similarly, in other combination treatments of gamma rays with SA, the rate of mutation frequency increased in comparison to individual mutagen treatments of gamma rays and sodium azide. In combination treatments, the 10kR gamma rays with 20mM SA and 20kR gamma rays with 20mM SA produced synergistic effect in increasing mutation frequency calculated on the basis of both

Table 2: Effectiveness, efficiency of gamma rays, sodium azide and their combination treatments in M_2 generation of urd bean c.v. T9

					Mutation rate		Effectiveness		Efficiency		
Gamma rays	M_1	M_2	Mm_1	Mm_2	M_1plants progenies (Mp)	M_2 Plants (Ms)	M_1 Plants progenies (Mp/ dose)	M_2 Plants (Ms/ dose)	Ms/Ps	Ms/Os	Synergism on the % M_2 plants mutated
Control	200	4132	-	-	--	-	-	-	-	-	--
5kR	192	3954	18	25	6.77	0.63	1.35	0.126	0.048	0.057	--
10kR	185	3784	25	34	13.66	0.82	1.36	0.80	0.038	0.055	--
20kR	147	3603	35	96	23.80	2.66	1.19	0.133	0.11	0.088	--
30kR	118	3200	58	117	49.15	3.65	1.63	0.121	0.086	0.079	--
40kR	105	2930	85	158	80.95	5.39	2.02	0.134	0.080	0.078	--
50kR	96	2700	90	160	93.75	5.92	1.87	0.118	0.081	0.075	--
60kR	80	2100	72	150	90.0	7.14	1.50	0.119	0.090	0.087	--
Sodium Azide										--	
10mM	200	3700	17	19	8.5	0.51	0.146	0.0025	0.053	0.051	--
20mM	190	3624	19	46	10.0	1.26	0.083	0.0105	0.105	0.100	--
30mM	174	3400	25	83	14.36	2.44	0.079	0.0135	0.129	0.129	--
40mM	104	2200	41	123	39.40	5.59	0.164	0.0232	0.227	0.261	--
50mM	96	1750	47	117	48.90	6.6	0.163	0.022	0.141	0.162	--
60mM	90	1800	56	130	62.22	7.2	0.172	0.02	0.129	0.119	--
70mM	70	1600	50	120	71.42	7.5	0.170	0.078	0.115	0.111	--
Gamma Rays+SA											
10kR+20mM	125	2800	35	97	28.00	3.46	0.0231	0.002	0.141	0.186	1.7
20kR+20mM	112	3200	48	143	42.80	4.47	0.0171	0.001	0.146	0.138	1.2
30kR+20mM	96	2500	40	112	41.6	4.48	0.011	0.001	0.098	0.106	1.0
40kR+20mM	56	1200	48	100	85.70	8.33	0.017	0.001	0.117	0.115	1.2

M_1=M_1 plants progenies (no.), Mm_1=M_1 progenies mutated (no.), Mm_2= M_2 plants mutated (no.), Ps = pollen sterility, Os = ovule sterility

M_1, plant progenies as well as M_2 plants basis. On the other hand, at higher gamma rays (40kR) dose + 20mM SA it was additive when expressed as percent M_1 plant progenies but a synergistic effect was observed when calculated as percent of M_2 plants mutated (Table 2). Synergistic effects of physical and chemical mutagen have been also reported by other workers [10-12].

The synergism among two mutagens may be firstly because of first mutagen treatment making accessible otherwise non-available sites for reaction to the second mutagen; and secondary pre-mutational lesions induced by the first mutagen becomes fined due to an inhibitory effect of the second mutagen on repair enzyme [4, 13]. Both these pathways should yield a frequency of mutations higher than the total of two mutagens applied individually.

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