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REGULAR ARTICLE

MUTAGENIC EFFICIENCY AND EFFECTIVENESS IN CLUSTER BEAN (*CYAMOPSIS TETRAGONOLOBA* (L.)TAUB.)

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SUMMARY

Mutagenic effectiveness and efficiency of EMS, SA and Gamma rays were studied in the two varieties of cluster bean (GE-36 and HR). The mutagenic treatments seeds were tested for lethal dose 50% for all mutagens, separately and the dose at which 50% of seed germination was considered as LD_{50} values. EMS, SA and Gamma rays produced high frequency as well as a wide spectrum in mutation. The frequency of mutation was high at lower concentration %/dose of mutagen. The mutagenic effectiveness and efficiency was calculated based on biological damage. In M_1 generation based on seed lethality and pollen sterility and M_2 generation was carefully screened for various chlorophyll and viable mutation. Mutagenic effectiveness and efficiency reduced with the increase in dose or concentration. In present investigation SA proved to be effective in two varieties and EMS proved to be more efficient.

Key words: Cluster bean, EMS, SA, Gamma rays, chlorophyll mutation
Abbreviations: SSP- Seed storage proteins, TDF-total dietary fibre, EMS- Ethyl methanesulphonate, SA- Sodium azide, GE-36-Golden Early-36 and HR-Harit Rani

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1. Introduction

Cluster bean is also called as guar. The word "GUAR" represents a derivation from the Sanskrit word "GAUAAHAR" which means cow fodder or fodder of live stock. Basically cluster bean is a drought hardy, deep rooted annual legume.

The crop is mainly grown in the dry habitats of Rajasthan, Haryana, Gujarat and Punjab. In addition to its major cultivation in India, the crop is also grown as a cash crop, although to limited extent in other parts of the world like Australia, Brazil and South Africa. The crop is known for its exceptionally high adaptation towards poor and erratic rains, multiuse in cropping system, in industrial use in many ways besides other social and dietary uses. These qualities have made it most the favored crop of marginal farmers in arid areas. In cluster bean, seed storage proteins and galactomannan accumulate to high amount in mature seeds, representing 23% - 31% of the seed dry weight (Kays *et al*., 2006)[1]. Seed storage proteins (SSPs) are a set of proteins that accumulate to high levels in seeds during the later stages of development. During germination, seed storage proteins are degraded and the resulting amino acids are utilized by the developing seedlings as a nutritional source (Patrik and Offer[2], 2001, Jang[3] et al., 1997). Anderson[4] (1949) reported that in 42% of the cluster bean seed endosperm the predominant portion is mucilage or gum (guar gum). The crude fiber was measured in seeds of several cultivars and was found to be 7.8% - 8.8% of the seed weight (Elsheikh and Ibrahim[5], 1999), the range in total dietary fibre (TDF) was 52.4% - 57.7% of seed dry weight (Kays[1] et al., 2006). Khatta[6] et al., (1988) examined four cultivars of guar and found 24.5%-32.9% crude protein, 2.4%- 3.3% crude fat, 3.2% – 4.0 % crude fiber in oven dried seeds. Carbohydrate content (calculated by difference) was 50.2%-59.9 %. If the carbohydrate and crude fiber values

are added to give total carbohydrate (Khatta et al., 1988), the range becomes 59.9%-69.2%.

Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Acharya[7], 2007) also it acts as an efficient means supplementing existing germplasm for cultivar improvement in breeding program's (Dubinin[8], 1961). Mutation is a sudden heritable change in organism generally the structural change in gene. It's produced by change in the base sequence of genes and it can be induced either spontaneously or artificially both in seed and vegetatively propagated crops. Induced mutation has recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Induced mutations provide beneficial variation for practical plant breeding purpose. In India still today there is only one mutant variety of cluster bean released by both physical and chemical mutagens (Chopra[9], 2005). Hence mutation breeding programme has proved to be a successful tool in bringing amelioration in self pollinated crops.

The utilization of new mutagenic agents in several plant species has played an important role in mutation breeding (Silva and Barbosa[10], 1996). Among the chemical mutagens, EMS is reported to be the most effective and powerful mutagen (Minocha[11], 1962 and Hajra[12], 1979). In plants EMS usually causes point mutations (Okagaki[13], 1991). Sodium azide is marginally mutagenic in different organisms (Jones[14], 1980, Arenaz[15], 1989). SA has been reported to induce high frequency of point mutation (base substitutions) and no detectable chromosomal aberrations (Nilan[16] et al., 1973). As compared to other mutagen SA is relatively safe to handle, inexpensive and no carcinogenic (Nilan[17] et al., 1977). Similarly Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cell and tissue (Gunckel and Sparrow[18], 1961). The usefulness of mutagens in mutation breeding depends not only on its mutagenetic effectiveness (mutation per unit dose of mutagens), but also on its mutagenic efficiency (mutation in relation to undesirable changes like sterility, lethality, injury and other). The present investigation was undertaken to study the effectiveness and efficiency of mutagenic treatments of EMS, SA and Gamma rays.

2. Materials and Methods

The experimental seeds of cluster bean varieties (GE-36 and HR) were treated with physical and chemical mutagens like Gamma rays (5kR, 10kR and 15kR), EMS (0.05%,0.10% and 15%) and SA (0.01%, 0.02% and 0.03%). Gamma irradiation was carried out at Government institute of Science, Aurangabad). Similarly in case of EMS and SA prior to chemical mutagenic treatments, seeds were immersed in distilled water for 6hrs. The presoaking enhances the rate of uptake of the mutagen through increase in cell permeability and also initiates metabolism in the seeds. Such presoaked seeds were later immersed in the mutagenic solution for 6hrs with regular shaking. Seeds soaked in distilled water for 6hrs served as control. Immediately after the completion of treatment, the seeds were washed thoroughly under tap water. Later on seeds with chemical mutagenic treatment were kept for post soaking in distilled water. The seeds which were given physical mutagenic treatment were sown in field immediately. For each treatment a batch of 300 presoaked seeds was used. 50 seeds from each treatment were dried between the folds of filter paper and germinated in petridishes to record germination percentage. The remaining 250 seeds from each treatment were sown in field following randomized block design (RBD) with three replications along with control as the M1 generation. The seeds were sown at a distance of 40cm between the plants and 60cm between the rows. All the recommended cultural measures namely, irrigation, weeding and plant production methods were carried out during the growth period of the crop.

Seed germination and lethality, seedling height, leaf morphological changes, chlorophyll deficient sectors, pollen sterility and plant survival at maturity were recorded

in M1 generation. Chlorophyll deficient sectors were recorded as informed by Stadler[19] (1930). The plant survival (L) was calculated as percentage of plants surviving till maturity. The biological damage (lethality) was calculated as the reduction in plant survival. At maturity all the surviving M1 fertile plants were harvest separately and seeds were sown in next season in plant to row basis to raise M2 generation. The two control varieties and treatment progenies were screened several times for morphological mutations throughout the crop duration. Different kinds of chlorophyll mutants (*xantha, viridis* and *chlorina*) were scored from emergence till the age of four week in M2 generation by using modified classified by Kharkwal[20] (1998). Mutation frequency was calculated as percent of mutated M2 progenies for both chlorophyll and morphological mutation in each treatment. The mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak[21] et al., (1965).

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Time X Concentration}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological damage}} = \text{MF/L, MF/I, MF/S,}$$

Where

MF=% chlorophyll mutations in M_2 generation.

L = % of lethality in M_1 generation.

I = % of seedling injury in M_1 generation.

S = % of pollen sterility in M_1 generation.

3. Results

Mutagenic effectiveness :- (Tables 1-2)

The mutagenic effectiveness is a measure of factor mutations induced by a unit dose of mutagen (Konzak et al., 1965). The major trend pertaining to this parameter influenced by different mutagens can be understood through a critical perusal of tables 1 and 2.

In M2 generation of cluster bean, the numerical values of effectiveness gradually reduced with an enhancement in concentration/dose of all the three mutagens in GE-36 and HR of cluster bean except the 0.02% SA treatment in variety HR. At the 0.01% concentration of SA the highest effectiveness value (33.33) could be seen in variety GE-36. While in HR the highest value (29.83) was shown by 0.02% SA. The lowest effectiveness values (0.174 and 0.222) could be noted at 15kR dose, in both the varieties. In EMS treatment the effectiveness values decreased with the increasing concentration, and they ranged from 5.76 to 3.33 and 8 to 4.44 in varieties GE-36 and HR, respectively. After SA treatment the values ranged from 33.33 to 15.88 and 29.83 to 22.16 in the two varieties of cluster bean. While in Gamma ray treatments they ranged from 0.332 to 0.174 and 0.322 to 0.222 in GE-36 and HR varieties of cluster bean.

Table 1: Effectiveness of mutagens in *Cyamopsis tetragonoloba* (L.)Taub. variety-GE-36

Treatments	Concentration (%) /Dose	% Chlorophyll mutants	Effectiveness MF/T*C
Control	--	--	--
EMS	0.05	1.73	5.76
	0.10	2.59	4.31
	0.15	3.00	3.33
SA	0.01	2.00	33.33
	0.02	3.07	25.5
	0.03	2.86	15.88
Gamma rays	5 kR	1.66	0.332
	10kR	2.21	0.221
	15kR	2.61	0.174

Table 2: Effectiveness of mutagens in *Cyamopsis tetragonoloba* (L.)Taub. variety-HR

Treatments	Concentration (%) /Dose	% Chlorophyll mutants	Effectiveness MF/T*C
Control	--	--	--
EMS	0.05	2.40	8
	0.10	3.70	6.16
	0.15	4.00	4.44
SA	0.01	1.33	22.16
	0.02	3.58	29.83
	0.03	4.54	25.22
Gamma rays	5 kR	1.61	0.322
	10kR	2.69	0.269
	15kR	3.33	0.222

Mutagenic efficiency: (Tables -3 -4)

The mutagenic efficiency is the ratio of frequency of chlorophyll mutants induced in M2 generation to various biological damages induced in M1 generation such as lethality and pollen sterility. The tables-3 and 4 present the data on efficiency of mutagens in relation to various biological damages.

Table 3: The relative efficiency of mutagenic treatments in M_2 generation of *Cyamopsis tetragonoloba* (L.)Taub. variety GE-36

Mutagens	Concentration (%) /Dose	Chlorophyll Mutation Frequency(MF)	Lethality	MF/L	Pollen Sterility	MF/S	Total ME
EMS	0.05	1.73	39	0.04	20	0.08	0.12
	0.10	2.59	41	0.06	10	0.25	0.31
	0.15	3.00	48	0.06	6	0.5	0.56
SA	0.01	2.00	37	0.05	18	0.11	0.16
	0.02	3.07	42	0.07	16	0.19	0.26
	0.03	2.86	49	0.05	14	0.20	0.25
Gamma rays	5 kR	1.66	36	0.04	18	0.09	0.13
	10kR	2.21	44	0.05	14	0.15	0.20
	15kR	2.61	50	0.05	12	0.21	0.26

Table 4: The relative efficiency of mutagenic treatments in M_2 generation of *Cyamopsis tetragonoloba* (L.)Taub. variety HR

Mutagens	Concentration (%) /Dose	Chlorophyll Mutation Frequency(MF)	Lethality	MF/L	Pollen Sterility	MF/S	Total ME
EMS	0.05	2.40	37	0.06	18	0.13	0.19
	0.10	3.70	42	0.08	10	0.36	0.44
	0.15	4.00	47	0.08	8	0.5	0.58
SA	0.01	1.33	32	0.04	16	0.08	0.12
	0.02	3.58	40	0.08	14	0.25	0.33
	0.03	4.54	47	0.09	10	0.45	0.54
Gamma rays	5kR	1.61	38	0.04	18	0.08	0.12
	10kR	2.69	45	0.05	16	0.16	0.21
	15kR	3.33	49	0.06	10	0.33	0.39

In both the varieties of cluster bean, the efficiency of mutagens showed variable trend with rising concentration/dose in regard to lethality and pollen sterility. Among the chemical mutagens, the EMS showed lowest efficiency value (0.04) at 0.05% concentration and highest value (0.06) at 0.10% and 0.15% EMS treatments in variety GE-36 pertaining to lethality. In variety HR the EMS treatment showed lowest efficiency (0.06) at 0.05% in regard to lethality and the highest efficiency (0.08) at EMS 0.10% and 0.15% treatments. The efficiency of mutagens indicated lowest value (0.08) at 0.05% EMS in regard to pollen sterility in GE-36 and showed the highest efficiency (0.5) at 0.15% EMS. Where as in variety HR, the efficiency of mutagens demonstrated highest value (0.5) in regard to pollen sterility at 0.15% EMS and showed lowest efficiency value (0.13) at 0.05% EMS treatment. In SA treatment, the efficiency values indicated increasing trend with increasing concentration in respect of pollen sterility in both the varieties GE-36 and HR. In GE-36 the lowest efficiency (0.11) at 0.01% and highest efficiency (0.20) at 0.03% SA treatments could be seen. In variety HR the lowest efficiency (0.08) was at 0.01% SA while the highest value (0.45) could be observed at 0.03% SA in respect of pollen sterility.

As far as Gamma rays are concerned, in variety GE-36, the 10kR and 15kR doses were found to be most efficient in regard to lethality (0.05) and for pollen sterility it was 15kR dose which displayed that feature with value of 0.21. In variety HR the dose 15kR was found most efficient in regard to lethality and pollen sterility revealing efficiency values as 0.06 and 0.33.

From the data on total mutagenic efficiency values, it could be noted that 0.15% EMS, 0.02% SA and 15kR of Gamma rays were the most efficient in case of GE-36. In HR it could be observed that the 0.15% EMS, 0.03% SA and 15kR Gamma rays were the most efficient.

4. Discussion

The mutagenic effectiveness is a measure of the factor mutations induced by a unit dose of mutagen. In the present study an attempt has been made for gathering the details of the mutagenic effectiveness and efficiency values in two varieties of cluster bean.

Many workers have recorded the effectiveness/efficiency values to be higher at lower dose of gamma rays, EMS and HZ (Gaul[22], 1962; Siddiq and Swaminathan[23], 1968; Nerkar[24], 1977; Hakande[25], 1992, More[26] 1992, Satpute[27] 1994). Panchabhaye[28] (1997), Kashid[29] (2004) and Khadke[30] (2005) proposed that the relative higher efficiency at lower concentration/dose of the mutagen could be ascribed to the lesser percentage of injury at such doses.

General decrease in effectiveness with increasing doses of Gamma rays has been reported in foxtail millet (Gupta & Yashvir[31] 1975), lentil (Sharma[32] 1990) and mungbean (Solanki[33] 1999).

In present study it was observed that effectiveness reduced with an increase in concentration in both the varieties of cluster bean except in SA treatments in variety HR. Higher mutagenic effectiveness and efficiency were observed in *Lathyrus sativus* at lower concentrations of EMS than in Gamma ray treatments by Waghmare and Mehra[34](2001) and Kumar[35] et al., (2003). Such difference in effects of mutagen on different materials might be due to seed metabolism and onset of DNA synthesis. Kundi[36] et al., (1997) reported differential sensitivity within crop and even genotype. It was opined that the sensitivity depends upon the genetic architecture and mutagens employed (Blixt[37], 1970) besides the amount of DNA, its replication time in initial stages and degree of heterochromatin.

In present study, in variety GE-36, in most of its treatments the lower concentration/dose of EMS (0.05%), SA (0.01%) and Gamma rays (5kR) were more effective. Besides this in variety HR it

indicated that EMS (0.05%), SA (0.02%) and Gamma rays (15kR) were most effective. Highest efficiency could be noted on the basis of chlorophyll mutations and other biological parameters like lethality and pollen sterility in GE-36 treated by EMS (0.15%), SA (0.02%) and Gamma ray (15kR) while in variety HR the pertinent treatments were EMS (0.15%), SA (0.03%) and Gamma rays (15kR). It is supported by the work done in chickpea by Tariq[38] (2008) and Cheema[39] et al., (2003).

The efficiency of a mutagenic agent is of complex nature, as it not only depends on reactivity of agent with the material and on its applicability through which physiological damage, chromosomal aberrations and pollen sterility gets induced in addition to mutation (Tariq[39] 2008).

5. Conclusion

From the data on biological damage in M_1 generation and chlorophyll mutation frequency in M2, the relative effectiveness and efficiency values of the three mutagens used were assessed. It was SA (0.01%) in GE-36 and SA (0.02%) in HR of cluster bean which showed the maximum effectiveness values. The order of mutagenic efficiency varied with different biological parameters studied. The total mutagenic efficiency revealed highest value at the higher concentration/dose in both the varieties of cluster bean in majority of the treatments.

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