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INDUCED MUTATIONS IN HORSEGRAM: MUTAGENIC EFFICIENCY AND EFFECTIVENESS

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Abstract

Mutation breeding is one of the most reliable techniques in improving crop plants. Physical and chemical mutagens are used to bring the variability. Among chemical mutagens, to identify the efficient mutagen that can bring broad spectrum of variability, it is therefore necessary to study the efficiency and effectiveness of various mutagens. In present study, two varieties of horsegram, viz., SINA (K-42) and KS-2 were treated with three concentrations of ethyl methane sulphonate (EMS), N- nitroso N- ethyl urea (NEU) and sodium azide (SA). Identification of effective mutagen could be possible by studying different parameters. In M2 generation studies were made on the mutagenic efficiency, mutagenic effectiveness and mutagenic rate. In the present study, the results revealed that efficiency of mutagens was variable, sodium azide was most effective mutagen and showed highest mutagenic rate.

Keywords: Sodium azide, Mutation, Efficiency, Effectiveness, Mutagenic rate

Introduction

The legumes are the group of plants that stand next in the importance to the cereals. They occupy unique position in world agriculture being very rich in protein and the nitrogen fixing capacity of them. The cultivation of legumes is the best and quickest way of production of food proteins especially in the developing countries.

Horsegram (*Macrotyloma uniflorum* Lam.) is an important legume crop but under exploited. The induction of physical or chemical mutations is the quickest way to produce the variations to develop new varieties. The mutagens have ability to induce variability. The effective and efficient mutagens could be providing a large number of variable plants. The study of efficient and effective mutagens could be possible in early generations. In the present contexts, the attempts were made to find out the effective and efficient mutagens.

Materials and Methods

The two varieties of horesegram namely SINA (K-42) and KS-2 and three of the chemical mutagens i.e. Ethyl methane sulphonate (EMS), N-nitroso, N-ethyl urea (NEU) and Sodium Azide (SA) were used in present investigation.The different concentration used for chemical mutagenic treatments were 0.05%, 0.1%, 0.125%, for EMS, 0.001%, 0.002%, and 0.003% for SA and 0.001%, 0.003% and 0.005% for NEU.

The effectiveness and efficiency of different mutagens were calculated as per the formulae of Konzak et.al [1]. The mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen i.e. time x concentration where as mutagenic efficiency gives an idea about the damage caused by mutagen like lethality, mitotic aberrations, pollen sterility, seedling injury etc.

$$\text{Mutagenic effectiveness} = \frac{\text{Factor mutation}}{\text{Dose or (time x concentration)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Factor mutation}}{\text{Biological damage}}$$

= MF/L, MF/S,MF/MI

Where,

MF = % of chlorophyll mutations in M_2 generation
L = % of lethality in M_1 generation
S = % of pollen sterility in M_1 generation
MI = % of mitotic abnormalities in M_1 generation.

It gives idea about mutations induced by a particulars mutagen irrespective of dose. The formula used to calculate mutation rate was

$$\text{Mutation rate} = \frac{\text{Sum values of effectiveness or efficiency of a particular mutagen}}{\text{Number of treatments of that particular mutagen}}$$

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Results and Discussions

The mutagenic effectiveness is a measure of factor mutations induced by unit dose of mutagen. The major trend pertaining to this parameter as influenced by different mutagens can be understood through a critical perusal of table 1 & 2.

Table 1: The relative efficiency of mutagens in M_2 generation of horsegram

Mutagen	Dose /Concentration (%)	Chlorophyll Mutations (M.F)		%Lethality (L)		Efficiency (Mf / L)		Pollen Sterility (S)		Efficiency (Mf / S)		Mitotic aberrations (MIA)		Efficiency Mf / MIA	
		SINA	KS2	SINA	KS-2	SINA	KS2	SINA	KS-2	SINA	KS-2	SINA	KS-2	SINA	KS-2
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EMS	0.05	3.15	2.71	15.67	22.67	0.2010	0.1195	6.27	5.20	0.5023	0.5211	2.23	3.55	1.4125	0.7633
	0.10	4.10	2.95	16.34	24.34	0.2509	0.1211	9.21	8.21	0.4451	0.3593	4.26	6.07	0.9624	0.4859
	0.125	5.23	3.70	16.67	24.67	0.3137	0.1499	13.34	11.22	0.3920	0.3297	4.63	7.46	1.1295	0.4959
SA	0.001	4.21	4.25	17.34	26.67	0.2427	0.1593	4.29	5.03	0.9813	0.8449	4.59	9.28	0.917	0.4579
	0.002	6.15	6.51	17.67	29.00	0.3480	0.2244	6.49	8.10	0.9476	0.8037	4.37	10.98	1.4073	0.5928
	0.003	7.20	9.10	19.67	29.67	0.3660	0.3067	7.73	10.92	0.9314	0.8333	6.52	11.62	1.1042	0.7831
NEU	0.001	3.20	4.15	18.00	27.67	0.1777	0.1499	6.47	6.86	0.4945	0.6049	2.73	8.11	1.1721	0.5117
	0.003	4.70	4.80	19.67	29.34	0.2389	0.1635	12.93	9.20	0.3634	0.5217	3.98	8.62	1.1809	0.5584
	0.005	6.51	7.00	22.00	30.67	0.2795	0.2282	17.33	15.33	0.3548	0.4566	4.09	9.52	1.5036	0.7352

Table 2: Effectiveness of mutagens in M2 generation of horsegram

Mutagen	Dose / Concentration (%)	%Chlorophyll Mutations (M.F)		Effectiveness MF/Dose or MF/TXC	
		SINA	KS-2	SINA	KS-2
Control	-	-	-	-	-
EMS	0.05	3.15	2.71	10.50	90.33
	0.10	4.10	2.95	6.83	4.91
	0.125	5.23	3.70	3.92	4.93
SA	0.001	4.21	4.25	701.66	708.33
	0.002	6.51	6.51	512.50	542.50
	0.003	7.20	9.10	400.00	505.55
NEU	0.001	3.20	4.15	533.33	691.66
	0.003	4.70	4.80	261.11	266.66
	0.005	6.15	7.00	205.00	233.33

Table 3: The mutation rates based on efficiency in M2 generation of horsegram

Mutagens Used	Mutation Rates Based On					
	Lethality		Sterility		Mitotic Aberrations	
	SINA	KS-2	SINA	KS-2	SINA	KS-2
EMS	0.2552	0.1301	0.4464	0.4033	1.681	0.5817
SA	0.3189	0.2301	0.9534	0.8273	1.1428	0.6112
NEU	0.2320	0.1805	0.4042	0.5277	1.2855	0.6017

In the M_2 generation of horsegram, the sodium azide mutagen indicated the highest value effectiveness followed by NEU in both the varieties. In variety SINA, it was found that the highest effectiveness (701.66) could be seen at 0.001 % SA treatment and the lowest value (3.92) could be observed at 0.125% concentration of EMS. In case of variety KS-2, the highest effectiveness value (708.33) could be recorded at 0.001% SA concentration and lowest value (4.91) could be observed at 0.10% concentration of EMS.

Efficient mutagenesis is the production of the desirable changes (mutations) free from associated undesirable changes. Mutagenic efficiency is the ratio of chlorophyll mutations induced in M_2 generation to the various biological damages induced in M_1 generation such as lethality, pollen sterility and chromosomal aberrations. The data on efficiency of mutagens in relation to various biological effects indicated in table 3&4.

In case of SINA variety, all the mutagens showed variable total efficiency values. Whereas in case of the variety KS-2 increasing total efficiency values were obtained in SA treatment and variable values obtained in EMS and NEU. The total efficiency values could be found higher in variety SINA as compared with KS-2.

In case of variety SINA, the mutagenic efficiency values for lethality could be increasing with increasing concentrations of mutagens. However, in contrast of this, decreasing values could be obtained for pollen sterility and variable for mitotic aberrations. In case of the variety KS-2, mutagenic efficiency values for lethality could be increasing with increasing concentration of mutagens. The mutagenic efficiency for pollen sterility could be observed increasing in EMS and NEU but variable in SA. The values of mitotic aberrations could be decreasing in EMS, increasing in SA and NEU concentration.

From the data of total mutagenic efficiency for the cultivar SINA, it could be deduced that SA treatment at 0.002% concentration in particular has been the most efficient (2.7026) followed by 0.003% treatment of SA (2.4014). In variety KS-2, it was observed that SA treatment at 0.003% concentration has been most efficient (1.9231) followed by 0.002% concentration of SA (1.6209). The order of mutagen with respect to efficiency values indicated that SA was more efficient than EMS and NEU in both the varieties of horsegram.

A mutagen is useful only if it is effective as well as efficient. Efficient mutagenesis is the production of desirable changes with minimum undesirable effects. In mutation breeding programme, a high mutation rate accompanied by minimal deleterious effects is desirable. But, generally the mutagen dose that gives the highest mutation rate also induces a high degree of lethality, sterility and other undesirable effects [1]. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of mutagen and mutagenic efficiency gives an idea of mutation frequency in relation to biological damage such as lethality, injury, sterility and chromosomal aberrations etc. caused as a result of mutagenic treatment. Hence, a mutagen is useful only if it is effective as well as efficient since it results in the production of desirable changes that is mutations with minimum undesirable effects. In present investigation, the most effective mutant was SA followed by NEU in both the varieties of horsegram.

In the present study, Sodium Azide and NEU mutagens were used first time to bring the variability through mutagenesis in horsegram. Earlier the reports indicate that only EMS was used. However, in legume crops like lentil similar type of results were obtained by Reddy and Annadurai [2]. Some researchers found that alkylating agents are more effective and efficient in inducing mutation than gamma rays, [1, 3, 4, 5, 6, 7, 8, 9, 10 and 11] in different plant systems.

Higher mutagenic effectiveness and efficiency at lower dose have been by reported by Siddiqi *et al* [12], Prasad [6], Nerkar [7], Bhamburkar [10], Farook [13], Chary [14], Sudha rani [11], Reddy and Annadurai [2] and Solanki and Sharma [15]. Lower concentrations are more efficient as the injury, lethality or sterility increases with mutagen concentration at faster rate than mutations [1].

In present study, SA was more effective and efficient than NEU and EMS. The second effective and efficient mutagen was NEU. Maximum effectiveness was observed at 0.001% SA treatment whereas 0.003% SA treatment was observed to be more efficient in both the varieties.

The mutation rates were calculated using a mutagen is useful only if it is effective as well as efficient. Efficient mutagenesis is the production of desirable changes with minimum undesirable effects. In mutation breeding programme, a high mutation rate accompanied by minimal deleterious effects is desirable. But, generally the mutagen dose that gives the highest mutation rate also induces a high degree of lethality, sterility and other undesirable effects [1]. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of mutagen and mutagenic efficiency gives an idea of mutation frequency in relation to biological damage such as lethality, injury, sterility and chromosomal aberrations etc. caused as a result of mutagenic treatment. Hence, a mutagen is useful only if it is effective as well as efficient since it results in the production of desirable changes that is mutations with minimum undesirable effects. In present investigation, the most effective mutant was SA followed by NEU in both the varieties of horsegram. This gives an idea about the average rate

of mutation induction per mutagen. The mutation rates of mutagens based on the efficiency are presented in table 5 & 6.

If we consider the mutation rates based on efficiency, the order of mutagens changes as the mutagens have different values in relation to lethality, pollen sterility and chromosomal aberrations.

The mutation rates for lethality, the values were 0.2552 (EMS), 0.3189 (SA) and 0.2320 (NEU) in the variety SINA. In the variety KS-2 the values for mutation rates were 0.1301(EMS), 0.2301 (SA) and 0.1805 (NEU). Thus, with respect to lethality the order of mutagens in both the varieties has been:

In the variety SINA: NEU< EMS< SA

In the variety KS-2: EMS < NEU < SA

When the mutation rate for pollen sterility was considered, the values were 0.4464(EMS), 0.9534(SA) and 0.4042 (NEU) in variety SINA. The values for variety KS-2 were 0.4033 (EMS), 0.8273 (SA) and 0.5277(NEU). In respect of the pollen sterility, the sequence of mutagens in both the varieties was as follows:

In variety SINA : NEU< EMS< SA

In variety KS-2: EMS< NEU< SA

With reference to the parameter of mitotic aberrations, the values of mutation rates were 1.1681(EMS), 1.1428(SA) and 1.2855(NEU) in variety SINA. While in variety KS-2, the values were 0.5817 (EMS), 0.6112 (SA), 0.6017 (NEU). The sequence of mutagens in the increasing order pertaining to parameter of mitotic aberrations was as follows:

In variety SINA: SA< EMS< NEU

In variety KS-2: EMS< NEU< SA

When mutation rates based on efficiency were compared, SA was found to be most efficient as far as lethality and sterility in both the varieties of horsegram are concerned. However, mutation rate based on efficiency scored for mitotic aberrations was different i.e. EMS was more efficient in variety SINA and SA in variety KS-2. Nilan (1972) has opined that physical and chemical agents can be helpful in recovery of new mutants and possibly is an important step towards the goal of directed mutagenesis.

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