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Isolation of macromutations and mutagenic effectiveness and efficiency in lentil (*Lens culinaris* Medik.)

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Abstract

Dry and healthy seeds of a *macrosperma* cv., LH90-54 were treated with three doses each of ethyl methane sulphonate (0.2, 0.4 and 0.8%) and sodium azide (0.02, 0.04 and 0.08%). EMS induced higher frequency of macromutations, i.e. 40.0 and 10.2% against 23.91 and 3.63% mutated progenies and plants of SA, respectively. Majority of the progenies (45.24%) segregated for mutation of one type. Among macromutations, the frequency of chlorophyll mutations obtained was higher (61.9%) than the morphological mutations (38.1%). Four kinds of chlorophyll (albina, chlorina, xantha and viridis) and twelve kinds of morphological mutations included changes for growth habit (compact, bushy, prostrate), foliage (narrow, broad, rogue, tendrillar), plant height (tall, dwarf) and maturity and flowering behaviour (early, late, sterile). EMS induced albina, chlorina and xantha, whereas SA induced viridis with higher frequency. The mutations for growth habit (56.2%) and foliage (59.1%) were induced with higher frequency by EMS, whereas those for plant height (61.5%) and maturity and flowering behavior (51.1%) were induced with higher frequency by SA. On the basis of sterility, SA was found 4.24 and 4.28 times more effective and efficient, respectively, than EMS.

Key words: Lentil, induced mutagenesis, chemical mutagens, macromutations, mutagenic effectiveness, mutagenic efficiency

Introduction

In lentil (*Lens culinaris* Medik.), out of the two groups of varieties, i.e. *microsperma* and *macrosperma*, the ones belonging to the latter group are found to be more mutable [1]. The study of induced viable mutation frequency in M_2 generation is the most dependable index for evaluating the effectiveness of mutagenic treatments [2-8]. The chlorophyll mutation frequency in M_2 generation is the most dependable index for evaluating the genetic effects of mutagenic treatments [9, 10]. Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics for their either direct or indirect exploitation in the breeding programme is an important goal of mutation research. Morphological mutations affecting different plant parts can be of immense practical utility and many of them have been released directly as crop varieties [11, 12].

The usefulness of a mutagen in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagen), but also on its mutagenic efficiency (mutations in relation to undesirable changes/damage like sterility, injury, lethality, etc.). The selection of effective and efficient mutagen(s) is very essential to recover a high frequency and spectrum of desirable mutations [13-15]. The present investigation, thus, was undertaken to study the frequency and spectrum of macro mutations along with the mutagenic effectiveness and efficiency of ethyl methane sulphonate (EMS) and sodium azide (SA) in lentil.

Materials and methods

Well filled, uniform and dry seeds (12% moisture content) of an extra large seeded cultivar, LH90-54, were treated with three doses each of ethyl methane sulphonate (EMS) and sodium azide (SA). The doses of EMS were 0.2, 0.4 and 0.8% (only few plants survived in 0.8% EMS treatment and, therefore, the observations recorded were not included) and that of SA were 0.02, 0.04 and 0.08%. The seeds were treated with freshly prepared aqueous solutions (pH 5.2) of EMS and SA at different concentrations for 6h at 20±2°C with intermittent shaking. The treated seeds were thoroughly washed in running water for 30 minutes to leach out the residual chemicals and then dried on blotting paper. Seeds wetted for 6h were used as control. The treated seeds along with control were sown immediately in the field 10cm apart in 4 m long rows spaced 30cm from each other to raise the M_1 generation. The experiment was conducted at the Pulses Research Area, Department of Plant Breeding, CCS HAU, Hisar during *rabi*, 1999-2000.

Data on seedling height and leaf number on all the seedlings in a particular treatment were recorded 40 days after sowing. The averages were computed and expressed as percentage of control. Plant survival was recorded at the time of maturity and also expressed

as percentage of control. The biological damage (lethality/injury) was computed as the reduction in plant survival (L), seedling height (I_1) and leaf number (I_2) for each treatment. Surviving plants with sufficient seed in different treatments including control were harvested and threshed individually, and their seed yield recorded. Average seed yield per plant in different treatments was estimated by dividing the total seed yield of all the plants by the total number of plants in a particular treatment. Finally, relative seed fertility was computed by dividing the average seed yield per plant in a particular treatment by that of control multiplied by 100. Consequently, seed sterility (S) for each treatment was expressed as the reduction in seed fertility in relation to control.

Each M_1 fertile plant was harvested individually and M_2 progenies were raised during 2000-01 in separate rows of 4m length spaced 30cm apart. The control and treated progenies were screened for lethal chlorophyll mutations from emergence till the age of four week, whereas viable chlorophyll and morphological mutations were scored several times throughout the life period of the plants. Different kinds of chlorophyll mutations (albina, chlorina, xantha and viridis) and morphological mutations affecting different features of the plant [growth habit (compact, bushy and prostrate), foliage (narrow, broad, rogue and tendrillar), plant height (tall and dwarf), and maturity and flowering behaviour (early, late and sterile)] were grouped according to the modified classification proposed by Bixit [16]. Mutation frequency was calculated as the percentage of mutated progenies and plants. Both mutagenic effectiveness and efficiency were determined as per the formulae suggested by Konzak *et al.* [17].

Results and discussion

High frequency of macromutations, 29.58 and 5.40% mutated M_2 progenies and plants was induced by both the chemical mutagens, EMS and SA, respectively (Table 1). Compared to 23.91 and 3.63% mutated progenies and plants, respectively, of SA, EMS treatments induced much higher frequency of mutations (40.00 and 10.24%, resp.). Dose dependent relationship was observed for SA, i.e. with the increase in the dose of mutagen, the macromutation frequency increased. Analogous results have been reported in lentil [2, 3, 15, 18]. In case of EMS, the mutation frequency decreased with the increase in dose. Similar results have been reported in chickpea [6], but in lentil, dose-dependent increase in mutation frequency has been reported [4]. The decrease in mutation frequency at higher doses may be attributed to chromosomal aberrations or saturation in the mutational events which may result in the elimination of mutant cells during growth [18, 19].

Table 1. Frequency of macromutations in M_2 generation in lentil

Treatment	Plants			Progenies			Progenies segregating for macromutations (%) of					
	Total	Mutants	% mutated	Total	Segregating	% mutated	1 type	2 types	3 types	4 types	5 types	6 types
Control	975	0	-	50	0	-	-	-	-	-	-	
Sodium azide												
0.02%	1495	49	3.28	70	14	20.00	50.00	21.43	14.29	7.14	7.14	-
0.04%	1106	40	3.62	62	14	22.58	50.00	21.43	28.57	-	-	-
0.08%	816	35	4.29	52	16	30.77	50.00	37.50	6.25	6.25	-	-
Pooled	3417	124	3.63	184	44	23.91	50.00	27.27	15.91	4.54	2.27	-
Ethyl methane sulphonate												
0.2%	746	95	12.73	57	25	43.86	32.00	20.00	16.00	12.00	16.00	4.00
0.4%	504	33	6.55	43	15	34.88	53.33	13.33	20.00	6.67	6.67	-
Pooled	1250	128	10.24	100	40	40.00	40.00	17.50	17.50	10.00	12.50	2.50
Overall	4667	252	5.40	284	84	29.58	45.24	22.62	16.67	7.14	7.14	1.19

A majority of the segregating progenies yielded only one kind of macromutation in all the treatments and with an increase in the number of mutational events (two or more kinds of macromutations), the frequency of segregating progenies decreased (Table 1). Most probably, it may be due to the action of mutagen on several genes distorting the activity of many enzymes/proteins which have a definite bearing on growth, reproduction and survival. As a result of this, most of the mutants with multimutational events might be lethal in M_1, reducing their frequency in M_2 onwards [10, 18].

Among the macromutations (Table 2), the induced frequency of chlorophyll mutations was higher (61.9%) than the morphological mutations (38.1%). The ratio of chlorophyll to morphological mutations observed was 1.6: 1. Nerkar [18] in *Lathyrus* also reported this ratio ranging from 1.5 to 2.1. Different SA and EMS treatments induced almost equal spectrum of chlorophyll (62.10 and 61.72%) and morphological (37.90 and 38.28%) mutations. The lowest dose of EMS (0.2%) and medium dose of SA (0.04%) yielded the widest spectrum of macromutations with 15 and 12 types, respectively. A

Table 2. Relative spectrum of macromutations (%) induced by chemical mutagens in M_2 generation in lentil

Type of mutation	Control	Sodium azide				Ethyl methane sulphonate			Pooled mutagens (%)
		0.02%	0.04%	0.08%	Pooled	0.02%	0.04%	Pooled	
Total mutants	-	49	40	35	124	95	33	128	252
Chlorophyll mutations									
Albina	-	6.12 (3)*	7.50 (3)	-	4.84 (6)	8.42 (8)	3.03 (1)	7.03 (9)	5.95 (15)
Xantha	-	20.41 (10)	15.00 (6)	5.71 (2)	14.52 (18)	18.95 (18)	12.12 (4)	17.19 (22)	15.87 (40)
Chlorina	-	38.78 (19)	22.50 (9)	20.00 (7)	28.22 (35)	33.68 (32)	27.27 (9)	32.03 (41)	30.16 (76)
Viridis	-	16.33 (8)	12.50 (5)	14.28 (5)	14.52 (18)	6.32 (6)	3.03 (1)	5.47 (7)	9.92 (25)
Pooled	-	81.63(40)	57.50 (23)	40.00 (14)	62.10 (77)	67.37 (64)	45.45 (15)	61.72 (79)	61.90 (156)
Morphological mutations									
Growth habit									
Compact	-	-	-	2.86 (1)	0.81 (1)	1.05 (1)	-	0.78 (1)	0.79 (2)
Bushy	-	2.04(1)	5.00 (2)	8.57 (3)	4.84 (6)	4.21 (4)	6.06 (2)	4.69 (6)	4.76 (12)
Prostrate	-	-	-	-	-	1.05 (1)	3.03 (1)	1.56 (2)	0.79 (2)
Pooled	-	2.04 (1)	5.00 (2)	11.43 (4)	5.64 (7)	6.32 (6)	9.09 (3)	7.03 (9)	6.35 (16)
Foliage									
Narrow	-	2.04 (1)	5.00 (2)	5.71 (2)	4.03 (5)	6.32 (6)	9.09 (3)	7.03 (9)	5.56 (14)
Broad	-	-	2.50 (1)	2.86(1)	1.61 (2)	1.05 (1)	-	0.78(1)	1.19 (3)
Rogue	-	2.04 (1)	-	-	0.81 (1)	1.05 (1)	6.06 (2)	2.34 (3)	1.59 (4)
Tendrillar	-	-	2.50 (1)	-	0.81 (1)	-	-	-	0.40 (1)
Pooled	-	4.08 (2)	10.00 (4)	8.57 (3)	7.26 (9)	8.42 (8)	15.15 (5)	10.16 (13)	8.73 (22)
Plant height									
Tall	-	-	-	-	-	2.11 (2)	-	1.56 (2)	0.79 (2)
Dwarf	-	2.04 (1)	7.50 (3)	11.43 (4)	6.45 (8)	2.11 (2)	3.03 (1)	2.34 (3)	4.37 (11)
Pooled	-	2.04 (1)	7.50 (3)	11.43 (4)	6.45 (8)	4.21 (4)	3.03 (1)	3.91 (5)	5.16 (13)
Maturity and flowering behaviour									
Early	-	4.08 (2)	5.00 (2)	2.87 (1)	4.03 (5)	4.21 (4)	6.06 (2)	4.69 (6)	4.37 (11)
Late	-	4.08 (2)	7.50 (3)	14.29 (5)	8.06 (10)	2.11 (2)	9.09 (3)	3.91 (5)	5.95 (15)
Sterile	-	2.04 (1)	7.50 (3)	11.43 (4)	6.45 (8)	7.37 (7)	12.12 (4)	8.59 (11)	7.54 (19)
Pooled	-	10.20 (5)	20.00 (8)	28.57 (10)	18.55 (23)	13.68 (13)	27.27 (9)	17.19 (22)	17.86 (45)
Pooled morphological mutations	-	18.37 (9)	42.5 (17)	60.00 (21)	37.90 (47)	32.63 (31)	54.55 (18)	38.28 (49)	38.10 (96)
Pooled macromutations	-	19.44 (49)	15.87 (40)	13.89 (35)	49.21 (124)	37.70 (95)	13.10 (33)	50.79 (128)	100.00 (252)

*Figures in parentheses indicate the number of mutants

brief account of different macromutations, i.e. chlorophyll and morphological is given below.

The spectrum of chlorophyll mutations obtained in the present study include four different types, viz. albina, chlorina, xantha and viridis (Table 2). These four types of mutations in lentil [20] and mungbean [21]; albina, chlorina and xantha in chickpea [9] and grasspea [10], and xantha and viridis in lentil [22] have been reported earlier. Sodium azide induced mutations in the order, chlorina (28.22%) > xantha and viridis (14.52) > albina (4.84), whereas the order of different mutations observed in case of EMS was, chlorina (32.03) > xantha (17.19) > albina (7.03%) > viridis (5.47%). Except viridis that was induced with higher frequency by SA treatments, those of EMS induced the remaining three kinds of mutations more frequently. Overall mutation spectrum for both the mutagens showed that chlorina occurred with the highest frequency, followed by xantha, viridis and albina. Similar results for chlorina, xantha and albina mutations have been reported in grasspea [10]. However, Sarker and Sharma [20] and Mehraj-ud-din *et al.* [23] reported that mutations of xantha type were induced more often than chlorina. Among all the mutations, albina appeared with the lowest frequency. The relatively poor induction of this mutation has been reported earlier [20, 23].

A relatively mild mutagenic specificity was observed in relation to the morphological mutations (Table 2). EMS induced wider spectrum of morphological mutations with 11 out of 12 mutation types accounting for 38.28% mutations, whereas 10 different types accounting for 37.9% of total mutations were induced by SA. The morphological mutations affecting maturity and flowering behaviour (17.86%) and foliage (8.73%) occurred more frequently than other types. The sterile mutations appeared most frequently (7.54%), followed

by late (5.95%) and narrow leaved (5.56%). Tendrillar (0.4%), and compact, prostrate and tall mutants (0.79%) appeared with the lowest frequency. Similar results on different morphological mutations have been reported earlier in lentil [2, 4, 5] and *Lathyrus* [18].

Relative mutagen dependent differences in the specificity of induction of morpho-mutations were also noted. Numerically, the mutations affecting foliage (10.16%) and growth habit (7.03%) appeared more frequently with EMS, whereas SA induced higher frequency of mutations for maturity and flowering behaviour (18.55%) and plant height (6.45%). The more frequent induction of certain mutations by a particular mutagen may be attributed to the fact that genes for these characters are probably more responsive to it. Different mutagens and treatment procedures may also change the relative proportion of different mutation types. Differences in the frequency of various macromutations have been reported in literature [2, 4-7].

were estimated on the basis of relative proportion of families segregating for macromutations. The mutagenic effectiveness (mutations per unit dose) decreased with the increase in dose of both the mutagens, indicating that the proportionate increase in the mutations rate (Mf) was much lower than the proportionate increase in the dose of the mutagen. SA was 4.24 and 2.57 times more effective than EMS on the basis of the mean values of all the treatments and the biologically comparable doses, respectively. Similar results have been reported earlier in lentil [3], mungbean [13] and *Lathyrus* [24].

Mutagenic efficiency (mutation rate in relation to M_1 damage) of both the mutagens was the highest at the lowest dose and it decreased with the increase in dose. Similar results have been reported by Nerkar [24] in *Lathyrus*. Higher efficiency at lower concentration of the mutagen appears mainly due to the fact that lethality, injury and sterility increase with an increase in the mutagen concentration than the actual mutations

Table 3. Mutagenic effectiveness and efficiency in *macrosperma* lentil

Treatment	Biological damage (%)				Per cent		Mutagenic efficiency			
	Plant survival reduction (L)	Seedling height reduction (l_1)	Seedling leaf no. reduction (l_2)	Seed sterility (S)	Mutated families (Mf)	Mutagenic effective-ness (Mf/tc)	Mf/L	Mf/l_1	Mf/l_2	Mf/S
Control	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
Sodium azide										
0.02%	22.68	8.70	3.30	4.20	20.00	166.67	0.88	2.30	6.06	4.76
0.04%	32.99	17.90	11.30	18.50	22.58	94.08	0.68	1.26	2.00	1.22
0.08%	44.33	25.30	21.10	32.00	30.77	64.10	0.69	1.22	1.46	0.96
Pooled	33.33	17.30	11.90	18.23	23.91	108.28	0.75	1.59	3.17	2.31
Ethyl methane sulphonate										
0.2%	38.14	21.40	10.10	64.50	43.86	36.55	1.15	2.05	4.34	0.68
0.4%	53.61	32.30	16.80	88.40	34.88	14.53	0.65	1.08	2.08	0.39
Pooled	45.88	26.85	13.45	76.45	40.00	25.54	0.90	1.56	3.21	0.54

Some definite patterns regarding the biological damage, i.e. reduction in plant survival, seedling height, number of leaves per seedling and seed fertility were observed (Table 3). The EMS treatments were found much more damaging than those of SA. Dose-dependent relationship for biological damage was observed in case of both the mutagens, i.e. with the increase in dose, there was corresponding increase in damage. Except seed fertility, other three parameters showed that the medium dose (0.04%) of SA was biologically comparable with the lowest dose (0.2%) of EMS. EMS treatments induced higher seed sterility ranging from 64.5 to 88.4% as compared to the SA treatments (4.2 to 32.0%). The biologically comparable doses are most appropriate for comparing the effectiveness and efficiency of different mutagens at comparable levels of damage.

Effectiveness and efficiency of both the mutagens

[17]. The mean efficiency of SA computed on the basis of seed sterility was 4.28 times higher than that of EMS, whereas the one computed on the basis of other parameters of biological damage was almost at par with that of EMS. At the biologically comparable doses, on the basis of different bjological damages, except seed sterility, EMS was found almost twice as efficient as SA. Similar results in lentil have been reported by Sharma [3].

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