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Comparative biological sensitivity and mutability of chemo-mutagens in lentil (*Lens culinaris* Medik)

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ABSTRACT

Present investigation was carried out using three different categories of chemical mutagens *viz*., ethylmethane sulphonate (EMS)- an alkylating agent, hydrazine hydrate (HZ)- a base analogue and sodium azide (SA)- a respiratory inhibitor on two varieties *viz*., Pant L-406 and Type-8 of lentil to study the immediate biological damage induced by the mutagens and to determine the sensitivity of biological material in question. Biological damage induced in M_1 generation was estimated in terms of seed germination, seedling height and pollen fertility. A dose dependent reduction with increasing concentrations of the mutagens for all these parameters was observed in both the varieties. The inhibition was more severe at the highest concentration of all the three mutagens under study. Variety Type-8 was found to be more sensitive than the var. Pant L-406 with respect to the mutagens utilized. Reduction in seed germination, seedling growth and pollen fertility in M_2 generation was reasonably less as compared to M_1 generation.

Key words: Biological damage, Chemical mutagens, Induced mutagenesis, Lentil, Variability.

INTRODUCTION

Pulses thrive well under varied environmental conditions due to their inherent genetic potential and soil ameliorative properties and have become the most important component of sustainable agriculture (Wani *et al.* 2011). Although India is the largest pulse producing country in the world, yet the share of pulses to total food grain production is only 6-7% in the country. With the increase in infrastructural and irrigation facilities, the cereal crops are preferred by the farmers and the pulses in contrast get marginalized treatment and pushed to poor and marginal lands. While efforts have been geared up to bring additional area under the cultivation of pulses, more important is to increase the production by exploiting the yield potential of existing varieties through genetic manipulations induced by mutations.

Induced mutations are one of the most important approaches for broadening the genetic base of lentil genotypes to circumvent the bottleneck conditions (Taziun *et al.,* 2018). The major advantage of induced mutation is its ability to correct one or few negative traits of a cultivar, without altering the major part of its otherwise acceptable genetic makeup. Thus, mutation breeding technique has a greater role to play in crops like pulses where large part of natural variability has been exhausted in the process of evolution and adaptation to the environmental stresses. In recent years, a lot of work has been undertaken on induced mutagenesis through physical and chemical mutagens in various crop plants (Khan *et al.,* 2006a,b; Shekar and Pushpendra, 2017; Wani, 2017, 2018; Laskar *et al.,* 2018; Ramachander *et al.,* 2018; Verma *et al.,* 2018; Das and Kundagrami, 2018; Amin *et al.,* 2019).

Alkylating agents are the most successful mutagens in producing new mutants. They are characterized by one or more alkyl groups which react with DNA by alkylating the phosphate groups as well as the purine (A, G) and pyrimidine (C, T) bases of the DNA. When the organic bases are alkylated, the formation of 7-alkyl guanine is the most frequently occurring event (van Harten, 1998). Base analogues occasionally can be incorporated into DNA because they are sufficiently similar to the DNA bases. They are able to replace the normal bases, without hindering the replication, but pairing errors may occur. Base analogues induce transitions in both directions and as a consequence, mutations induced by a base analogue can also be reverted.

The other chemical mutagen in addition to alkylating agents and base analogues is sodium azide (NaN_3) which has been used on a relatively large scale. This compound was originally employed as a respiration inhibitor to study the effects of irradiation in barley. Sodium azide was introduced as a mutagen in early 1970's at Washington State University, Pullman, USA (Sideris *et al.,* 1973; Nilan *et al.,* 1973). The word 'azide' refers to both inorganic and organic azides. For mutation breeding purpose, the simple inorganic metallic azides like sodium azide are of greater interest (van Harten, 1998). According to Kleinhofs *et al.* (1984), organic azides in fact may be better mutagens from practical point of view as they are not volatile and unlike inorganic azides,

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do not require low pH for effective uptake. Azide often has been described as a 'super mutagen' in the sense that the ratio of mutations to gross chromosomal changes or large deletions is very high. Further, the toxicity of this compound is very low and the mutagenic effectiveness is quite higher.

Keeping in view the mechanism of action and efficiency of alkylating agents, base analogues and respiratory inhibitors, an attempt has been made to study the comparative biological damage induced by EMS, HZ and SA with respect to seed germination, seedling growth and pollen fertility in M_1 and M_2 generations of lentil.

MATERIALS AND METHODS

Uniform and healthy seeds of two lentil varieties *viz.,* Pant L-406 and Type-8 were presoaked in distilled water for 9 hours, prior to treatment with 0.1% to 0.4% of EMS ($C_3H_8O_3S$) and 0.01% to 0.04% of HZ ($NH_2NH_4H_2O$) and SA (NaN_3) for 6 hours. The solutions of EMS and HZ were prepared in phosphate buffer of pH 7, whereas SA solution was prepared in phosphate buffer adjusted to pH 3. For each treatment, 350 seeds were used. The treatments were given at a temperature of 27±1°C. For each variety, 350 pre-soaked seeds were again soaked in phosphate buffer for 6 hours to serve as controls. During treatment, flasks containing the solution and seeds were frequently shaken to ensure sufficient aeration. After treatment, the seeds were thoroughly washed in running tap water to remove the residual mutagen from seed surface.

Three replications of 100 seeds each were sown for every treatment and control in each variety in a complete randomized block design (CRBD) to raise M_1 generation. The distance between the seeds in a row and between the rows was kept at 30 x 60 cms, respectively. Recommended agronomic practices were employed for preparation of field, sowing and subsequent management of the crop. The remaining lot of 50 seeds was used for determining the percentage of seed germination and seedling height. Seeds of each treatment and the controls of both the varieties were spread over moist cotton in petri-plates. Finally, the petri-plates were kept in BOD incubator at 27±1°C temperature.

The percentage of seed germination was calculated on the basis of total number of seeds sown in petri-plates and the number of seeds germinated. Seedling height was recorded after 10 days of sowing the seeds in petri-plates by measuring the root and shoot length for each treatment and the control. Seedling injury was measured in terms of reduction in seedling height with respect to control. Pollen fertility was determined from 30 randomly selected M_1 plants (10 from each replicate) from each treatment and control at the time of flowering. Pollen grains were stained with 1% aceto-carmine solution. Pollen grains which took stain and had regular outline were considered as fertile, while shrunken, empty and unstained ones were counted as sterile.

The M_1 plants were harvested separately and the seeds sown in next season in plant progeny rows to raise M_2 generation. From each treatment and controls of M_2 plants in both the varieties, 20 to 30 young flower buds were collected for the studies of pollen fertility. Moreover, 50 M_1 harvested seeds from each treatment and control were spread over moist cotton in petri-plates and kept in BOD incubator for determining the percentage of seed germination and seedling growth. After 10 days of sowing the seeds in petri-plates, germination counts and growth observations were recorded on shoot and root lengths of seedlings for M_2 generation. The following formula was used to calculate the percentage inhibition or injury in seed germination, seedling growth and pollen fertility in both M_1 and M_2 generations.

$$\text{Percentage injury} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

In the present study, data recorded on seed germination were presented in Table 1. A gradual decrease in seed germination with increasing concentrations of mutagens was observed in both the varieties of lentil. However, the extent of decrease in seed germination differed with respect to the mutagens as well as the varieties. Seed germination was affected more adversely in the variety Type-8 than Pant L-406. In Type-8, the percentage inhibition was 12.37 and 32.98 with 0.01% and 0.04% SA, respectively. However, it was 6.31% and 23.16% with the same concentrations in Pant L-406. Sodium azide treatments were found to cause maximum reduction in seed germination as compared to HZ and EMS treatments.

Reduction in germination by mutagenic treatments could be due to delay or inhibition of physiological and biological processes including enzymatic activity (Kurobane *et al.,* 1979), imbalances of hormones (Chrispeels and Varner, 1967) and hampering of mitotic processes (Ananthaswamy *et al.,* 1971). Usuf and Nair (1974) stated that gamma irradiation accelerated the degradation of existing enzymes involved in the formation of auxins, thus reduces the seed germination. High reduction in germination percentage in SA treatments may be due to weakening and disturbances of growth processes. Greater lethality at higher mutagenic concentrations was observed in both the varieties. The larger sensitivity at higher doses of mutagens has been attributed to various factors such as change in the metabolic activity of the cells and imbalance between the promoters and inhibitors of growth regulators (Natarajan and Shiva Shankar, 1965; Meherchandani, 1975). In M_2 generation, although a dose dependent decrease in seed germination persisted, however the percentage inhibition in seed germination was less and showed a good proportion of recovery as compared to M_1 generation.

Data recorded for seedling height of 10 days old seedlings raised in petri-plates were presented in Table 2. The results showed that all the mutagenic treatments brought dose dependent reduction in seedling height. In Pant L-406, the total seedling height was 11.40 cm in control. Seedling injury ranged from 4.38 to 21.66 per cent in EMS treatments,

Table 1: Effect of mutagens on seed germination and pollen fertility in M_1 and M_2 generations of lentil varieties Pant L-406 and Type-8.

Treatment	Var. Pant L-406				Var. Type-8			
	Seed germination (%)	Seed inhibition (%)	Pollen fertility (%)	Pollen reduction (%)	Seed germination (%)	Seed inhibition (%)	Pollen fertility (%)	Pollen reduction (%)
				M_1 generation				
Control	95.00	-	95.16	-	97.00	-	94.83	-
0.1% EMS	90.00	5.26	84.18	11.54	89.00	8.24	84.18	11.23
0.2% EMS	85.00	10.52	75.92	20.22	83.00	14.43	72.91	23.11
0.3% EMS	81.00	14.73	72.07	24.26	81.00	16.49	70.42	25.74
0.4% EMS	77.00	18.94	69.37	27.10	75.00	22.68	68.56	27.70
Average	**83.25**	**12.36**	**75.38**	**20.78**	**82.00**	**15.46**	**74.01**	**21.94**
0.01% HZ	91.00	4.21	83.21	12.55	89.00	8.24	81.61	13.94
0.02% HZ	81.00	14.73	78.16	17.86	77.00	20.61	76.27	19.57
0.03% HZ	77.00	18.95	75.19	20.98	75.00	22.68	72.91	23.11
0.04% HZ	75.00	21.05	72.91	23.38	69.00	28.86	72.07	24.00
Average	**81.00**	**14.73**	**77.36**	**18.69**	**77.50**	**20.09**	**75.71**	**20.15**
0.01% SA	89.00	6.31	86.65	8.94	85.00	12.37	86.50	8.78
0.02% SA	77.00	18.95	84.10	11.62	73.00	24.74	83.36	12.09
0.03% SA	75.00	21.05	77.14	18.93	67.00	30.92	77.94	17.81
0.04% SA	73.00	23.16	75.31	20.85	65.00	32.98	75.19	20.71
Average	**78.50**	**17.37**	**80.80**	**15.08**	**72.50**	**25.25**	**80.74**	**14.84**
				M_2 generation				
Control	96.00	-	95.65	-	96.00	-	96.10	-
0.1% EMS	94.00	2.08	90.90	4.96	93.00	3.12	90.05	6.29
0.2% EMS	91.00	5.20	87.50	8.52	90.00	6.25	85.93	10.58
0.3% EMS	90.00	6.25	85.71	10.39	88.00	8.33	83.80	12.79
0.4% EMS	87.00	9.37	80.95	15.36	85.00	11.45	81.81	14.87
Average	**90.50**	**5.72**	**86.26**	**9.80**	**89.00**	**7.28**	**85.39**	**11.13**
0.01% HZ	93.00	3.12	91.30	4.54	91.00	5.20	90.20	6.13
0.02% HZ	90.00	6.25	88.89	7.06	88.00	8.33	86.33	10.16
0.03% HZ	86.00	10.41	86.36	9.71	84.00	12.50	84.60	11.96
0.04% HZ	84.00	12.50	84.21	11.96	82.00	14.58	82.26	14.40
Average	**88.25**	**8.07**	**87.69**	**8.31**	**86.25**	**10.15**	**85.84**	**10.66**
0.01% SA	91.00	5.20	94.44	1.26	89.00	7.29	92.90	3.32
0.02% SA	89.00	7.29	90.47	5.41	87.00	9.37	89.75	6.60
0.03% SA	85.00	11.45	89.47	6.46	84.00	12.50	87.80	8.63
0.04% SA	83.00	13.54	86.23	9.84	81.00	15.62	84.90	11.65
Average	**87.00**	**9.37**	**90.15**	**5.74**	**85.25**	**11.19**	**88.83**	**7.55**

whereas it ranged from 6.66 to 26.93 percent in HZ treatments. The injury was more drastic in SA treatments ranging from 9.12 to 31.14 per cent. In Type-8, the seedling height was 11.85 cm in control. Seedling injury ranged from 13.92 to 37.55 percent with 0.01% to 0.04% treatments of SA. Compared to M_1 in both the varieties, the percentage injury in seedling height was reduced to a large extent in M_2 generation. The reduction in seedling height after mutagenic treatments is mainly due to the uneven damage caused to the meristematic cells resulting from genetic injury. Variation in auxin level (Goud and Nayar, 1968) and changes in the specific activity of enzymes (Cherry *et al.,* 1962) were correlated with reduction in seedling height after mutagenic treatments.

Studies of pollen fertility in mutagen treated population forms a reliable index in assessing any internal change in the plants as well as in determining the efficiency of a particular mutagen. The pollen fertility was dose dependent as evident from proportionate decrease with increasing concentrations of EMS, HZ and SA in both the varieties of lentil (Table 1). The dose dependent decrease in pollen fertility was earlier reported in *Nigella sativa* (Mitra and Bhowmik, 1998), *Gossypium hirsutum* (Muthusamy and Jayabalan, 2002), *Cicer arietinum* (Barshile *et al.,* 2006), *Vigna radiata* (Khan and Wani, 2006) and *Vigna mungo* (Sharma *et al.,* 2006; Bhosale *et al.,* 2013). The highest percentage of reduction 27.10 and 23.38 in pollen fertility was observed with 0.4% of EMS and 0.04% of HZ treatments

Table 2: Effect of mutagens on seedling growth (root and shoot) in M_1 and M_2 generations of lentil varieties Pant L-406 and Type-8.

Treatment	Var. Pant L-406				Var. Type-8			
	Root length (cm) Mean ± S.E.	Shoot length (cm) Mean ±S.E.	Total length (cm) Mean ± S.E.	% injury	Root length (cm) Mean ± S.E.	Shoot length (cm) Mean ±S.E.	Total length (cm) Mean ± S.E.	% injury
M_1 generation								
Control	2.60 ± 0.09	8.80 ± 0.12	11.40 ± 0.21	-	2.75 ± 0.06	9.10 ± 0.10	11.85 ± 0.16	-
0.1% EMS	2.35 ± 0.16	8.55 ± 0.40	10.90 ± 0.56	4.38	2.65 ± 0.15	8.27 ± 0.38	10.92 ± 0.53	7.85
0.2% EMS	2.15 ± 0.20	8.28 ± 0.37	10.43 ± 0.57	8.50	2.05 ± 0.13	8.25 ± 0.33	10.30 ± 0.46	13.08
0.3% EMS	2.03 ± 0.15	7.77 ± 0.39	9.80 ± 0.54	14.03	1.90 ± 0.12	7.80 ± 0.37	9.70 ± 0.49	18.14
0.4% EMS	1.95 ± 0.13	6.98 ± 0.35	8.93 ± 0.48	21.66	1.82 ± 0.15	6.93 ± 0.40	8.75 ± 0.55	26.16
Average	**2.12 ± 0.16**	**7.89 ±0.37**	**10.01 ± 0.53**	**12.14**	**2.10 ± 0.14**	**7.81 ± 0.37**	**9.92 ± 0.51**	**16.31**
0.01% HZ	2.28 ± 0.15	8.36 ± 0.30	10.64 ± 0.45	6.66	2.18 ± 0.17	8.44 ± 0.36	10.62 ± 0.53	10.38
0.02% HZ	2.16 ± 0.17	8.15 ± 0.33	10.31 ± 0.50	9.56	1.95 ± 0.14	7.99 ± 0.29	9.94 ± 0.43	16.11
0.03% HZ	1.87 ± 0.19	7.42 ± 0.36	9.29 ± 0.55	18.50	1.84 ± 0.16	7.61 ± 0.32	9.45 ± 0.48	20.25
0.04% HZ	1.83 ± 0.22	6.50 ± 0.35	8.33 ± 0.57	26.93	1.75 ± 0.15	6.26 ± 0.34	8.01 ± 0.49	32.40
Average	**2.03±0.18**	**7.60 ± 0.33**	**9.64 ± 0.52**	**15.41**	**1.93 ± 0.15**	**7.57±0.33**	**9.50±0.48**	**19.78**
0.01% SA	2.13 ± 0.18	8.23 ± 0.43	10.36 ± 0.61	9.12	1.89 ± 0.14	8.31 ± 0.41	10.20 ± 0.55	13.92
0.02% SA	2.07 ± 0.15	7.75 ± 0.37	9.82 ± 0.52	13.86	1.82 ± 0.18	7.58 ± 0.43	9.40 ± 0.61	20.67
0.03% SA	1.80 ± 0.14	7.01 ± 0.35	8.81 ± 0.49	22.72	1.70 ± 0.15	6.75 ± 0.45	8.45 ± 0.60	28.69
0.04% SA	1.75 ± 0.20	6.10 ± 0.38	7.85 ± 0.58	31.14	1.35 ± 0.17	6.05 ± 0.34	7.40 ± 0.51	37.55
Average	**1.93 ± 0.17**	**7.27 ± 0.38**	**9.21 ± 0.55**	**19.21**	**1.69 ± 0.16**	**7.17 ± 0.41**	**8.86 ± 0.57**	**25.21**
M_2 generation								
Control	2.62 ± 0.08	8.86 ± 0.10	11.48 ± 0.18	-	2.70 ± 0.10	9.10 ± 0.12	11.80 ± 0.22	-
0.1% EMS	2.45 ± 0.13	8.75 ± 0.35	11.20± 0.48	2.44	2.55 ± 0.12	8.95 ± 0.34	11.50± 0.46	2.54
0.2% EMS	2.39 ± 0.17	8.51 ± 0.34	10.90 ± 0.51	5.05	2.40 ± 0.17	8.70 ± 0.31	11.10 ± 0.48	5.93
0.3% EMS	2.30 ± 0.13	8.20 ± 0.31	10.50 ± 0.44	8.53	2.20 ± 0.14	8.50 ± 0.32	10.70 ± 0.46	9.32
0.4% EMS	2.26 ± 0.11	8.09 ± 0.38	10.35 ± 0.49	9.84	2.16 ± 0.17	8.39 ± 0.37	10.55 ± 0.54	10.59
Average	**2.35 ± 0.13**	**8.39 ± 0.34**	**10.74 ± 0.48**	**6.46**	**2.33 ± 0.15**	**8.63 ± 0.33**	**10.96 ± 0.48**	**7.09**
0.01% HZ	2.40 ± 0.13	8.69 ± 0.31	11.09 ± 0.44	3.39	2.46 ± 0.15	8.90 ± 0.34	11.36 ± 0.49	3.73
0.02% HZ	2.34 ± 0.19	8.46 ± 0.29	10.80 ± 0.48	5.92	2.20 ± 0.12	8.80 ± 0.32	11.00 ± 0.44	6.77
0.03% HZ	2.19 ± 0.16	8.21 ± 0.34	10.40 ± 0.50	9.40	2.05 ± 0.14	8.55 ± 0.31	10.60 ± 0.45	10.17
0.04% HZ	2.15 ± 0.20	8.15 ± 0.32	10.30 ± 0.52	10.27	1.90± 0.16	8.50 ± 0.31	10.40 ± 0.47	11.86
Average	**2.27 ± 0.17**	**8.38 ± 0.31**	**10.65 ± 0.48**	**7.24**	**2.15 ± 0.14**	**8.69 ± 0.32**	**10.84 ± 0.46**	**8.13**
0.01% SA	2.41 ± 0.19	8.64 ± 0.37	11.05 ± 0.56	3.74	2.45 ± 0.12	8.85 ± 0.44	11.30 ± 0.56	4.23
0.02% SA	2.37 ± 0.15	8.15 ± 0.34	10.52 ± 0.49	8.36	2.20 ± 0.14	8.55 ± 0.47	10.75 ± 0.61	8.89
0.03% SA	2.30 ± 0.14	8.05 ± 0.32	10.35 ± 0.46	9.84	2.00 ± 0.13	8.50 ± 0.50	10.50 ± 0.63	11.01
0.04% SA	2.20 ± 0.17	8.00± 0.33	10.20 ± 0.50	11.14	1.80 ± 0.19	8.45 ± 0.30	10.25 ± 0.49	13.13
Average	**2.32 ± 0.16**	**8.21 ± 0.34**	**10.53 ± 0.50**	**8.27**	**2.11 ± 0.14**	**8.58 ± 0.42**	**10.70 ± 0.57**	**9.31**

±S.E. =Standard error.

in Pant L-406, whereas in Type-8, it was 27.70 and 24.00 percent with the highest concentration of EMS and HZ, respectively. Among the various mutagens, EMS caused more drastic effects on pollen fertility than the other two mutagens. In majority of the cases, meiotic abnormalities are responsible for pollen sterility (Gaul, 1970; Sinha and Godward, 1972; Gottschalk and Klein, 1976; Kumar and Singh, 2003). The sterility induced by SA seems to be more genic and less chromosomal. Reduction in pollen fertility in M_2 generation was fairly less as compared to M_1 generation, indicating that recovery mechanism had operated in the intervening generations.

CONCLUSION

Seed germination, pollen fertility and seedling growth were proportionately inhibited with increasing concentrations of the mutagens. However, lower doses of the mutagens comparatively induced lesser damage than those of higher ones, which could be successfully exploited for enhancing genetic variability in subsequent generations. Moreover, the immediate information obtained from M_1 biological parameters may be helpful in the initial rejection of less important mutagen concentrations and mutagenized population which may save time and efforts of plant breeders in taking further the mutation breeding research with better precision and more chances of getting desired results.

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