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Research Article

Studies on the Effects of Treatments of Methyl Methanesulphonate and Sodium Azide on Induction of Variability in *Hordeum vulgare* L.

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Abstract: Cereals constitute an important source of food in India. India depends mostly on cereals and pulses for food. Cereals contain high amounts of starch along with considerable amounts of proteins, lipids and minerals. The present experiment was conducted to induce the variability in *Hordeum vulgare* L. by methyl methanesulphonate and sodium azide. The seeds were sown to raise the M_1 generation. Seed germination of mutagen treated seeds in M_1 and cytology in PMC's of M_1 generation plants were observed. Seed germination decreased with increasing concentrations of MMS but it showed irregular trend in case of SA treated seeds. Seeds obtained from M_1 generation plants were sown to raise the M_2 generation. Lower concentrations of both mutagens produced less frequency of meiotic abnormalities than higher concentrations but MMS produced more frequency of meiotic abnormalities than SA. Different quantitative characters were studied in M_2 generation. Quantitative characters of a plant are the most important characters associated with yield. Data on quantitative characters did not show regular trend but followed an irregular trend. But lower concentrations of MMS treated plants showed more positive response than SA treated plants.
Keywords: *Hordeum vulgare* L., methyl methanesulphonate, sodium azide.

INTRODUCTION

Food insecurity and malnutrition are threatening the population worldwide. India ranks 63rd according to Global Hunger Index [1]. More than 40% of the population in India has the highest prevalence to be underweight specially in children under five years old. The current increase in population is also posing a great threat to the nation. Due to more urbanization in India, less land is available for producing sufficient food to cope these problems. Scientists throughout the world are busy in ameliorating to solve these problems. Different methodologies are applied worldwide, of which mutation breeding forms an important part. Induced mutation is a fast method of increasing the variability and so the yield of plants and hence reducing the food insecurity and malnutrition of the fast growing population.

Barley, an important nutritious plant is used mostly in Asia and North Africa. Barley contains an abundant amount of starch (65-68%) along with considerable amount of proteins (10-17%) and minerals (1.5-2.5%) [2]. It belongs to family Poaceae and is an annual herb. The diploid chromosome number of barley is 14 (2n=14). Thus it can be said that it is an important nutritional plant.

Therefore, the present experiment was conducted to induce the variability in the plant and increasing the yield of plant. The mutagens used were methyl methanesulphonate and sodium azide.

MATERIALS AND METHODS

Fresh and healthy seeds of *Hordeum vulgare* L. were used for the experiment. 1% Stock solutions of both the mutagens were first prepared. For methyl methanesulphonate the stock solution was prepared in phosphate buffer of pH 7 while that of sodium azide was prepared in phosphate buffer of pH 3. From this stock solution, different concentrations (0.01%, 0.02%, 0.03% and 0.04%) of both mutagens were prepared. Nine sets of seeds each containing about 30 seeds (3 replications of each treatment) were used for this experiment. Those seeds were pre-soaked in double distilled water for 9 hours. Then the seeds were put in different concentrations of both mutagens for 6 hours while one set was kept in double distilled water to act as control. After the mutagenic treatment, the seeds were washed in running tap water to remove any residual effects of mutagens. The seeds were sown in to raise the M_1 generation. For cytology, flower buds of mutants and control were fixed in Carnoy's fluid (1 part Glacial acetic acid : 3 parts Chloroform : 6 parts Alcohol) for 24 hours. Traces of ferric chloride were added to improve the stainability of chromosomes. The flower

buds were washed with and preserved in 70% alcohol. Anthers were smeared in 1% acetocarmine solution. Meiosis of PMC's was observed in M_1 generation plants. The M_1 seeds were sown to raise the M_2 generation. Different quantitative characters were studied in M_2 generation plants.

Studies in M_1 generation

Seed germination

Germination (%) =

$$\frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Inhibition

Inhibition (%)

$$= \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Frequency of meiotic abnormality (%) =

$$\frac{\text{Total no. of abnormal PMC's of particular treatment}}{\text{Total no. of PMC's observed in that particular treatment}} \times 100$$

RESULTS AND DISCUSSION

Sodium azide is one of the most powerful mutagens and its mutagenecity is mediated through the production of azide compound. On the other hand, methyl methanesulphonate is an alkylating agent and alkylates DNA on N^7-deoxyguanosine and N^3-deoxyadenosine predominantly. The methyl methanesulphonate was found to induce more variability than sodium azide in both generations.

M_1 GENERATION STUDY

Seed Germination and Inhibition

Data recorded on seed germination and inhibition is shown in Table-1. As is clear from the Table, the seed germination decreased with increasing concentrations of MMS. Similar results were obtained by Ambreen *et al* [3], but followed an irregular trend in case of SA.

Cytology was performed in buds of M_1 generation plants. The control plants showed normal meiosis while the mutagen treated plants showed abnormal meiosis. The MMS treated plants showed higher frequency of meiotic abnormalities than SA treated plants (Table-2). Gulfishan *et al* [4] observed similar results for MMS but in comparison with diethyl sulphate. The different meiotic abnormalities observed were disturbed metaphase, dispolarity at telophase-I, multivalent, laggard formation at anaphase-I. (Plate-1). Dose dependent abnormalities of both mutagens were observed.

The laggards and fragments which fail to reach to the poles results ultimately into the formation of micronucleus[5-6]. Ring formation of chromosomes has also been observed. It may be due to the breaking of chromosome ends[7].

Disturbed metaphase was also observed in treated plants. Disturbed metaphase has also been already reported in *Allium cepa* L. by Maslam [8] after treating it with some medicinal extracts. Disturbed metaphase has been observed due to the effect on proteins that constitute the spindle. Laggard formation occurs due to the improper organization of spindle[9]. Multivalents as observed may occur due to the translocation and inversion[10].

M_2 GENERATION STUDY

The M_2 generation plants were screened for different quantitative traits like plant height, number of fertile branches per plant and plant yield. (Table-3)

PLANT HEIGHT

The mean plant height in control plants was 69.16 cms while as in MMS treated plants, the plant height ranged between 78.38-96.56 cms and in SA treated plants the height ranged between 74-80.14 cms. The maximum height was observed in 0.04% MMS concentration. All treated plants showed increase in height of plants compared to control.

FERTILE BRANCHES PER PLANT

Mean fertile branches in control plants weas observed as 3 while as in MMS treated plants, it ranged between 1.80-5.40 and in SA treated plants it ranged between 1.40-4.80.

100-SEEDS WEIGHT

Mean 100-seeds weight in control plants was 3.69 gms while as in MMS treated plants, it was observed between 2.34-4.70 gms and in SA treated plants it was observed as 2.84-4.01 gms.

Quantitative characters of a plant are the most important characters associated with yield. Data on quantitative characters did not show regular trend but followed an irregular trend. But lower concentrations of MMS treated plants showed more positive response than SA treated plants.

The lower concentrations inducing growth promoting effects has already been reported by many workers in different plants[11-12]. The different variable response has already been reported by Wani and Khan in *Vigna radiate*[13]. Waghmare and Mehra [14] also reported increase in number of branches in *Lathyrus sativus* after mutagenesis.

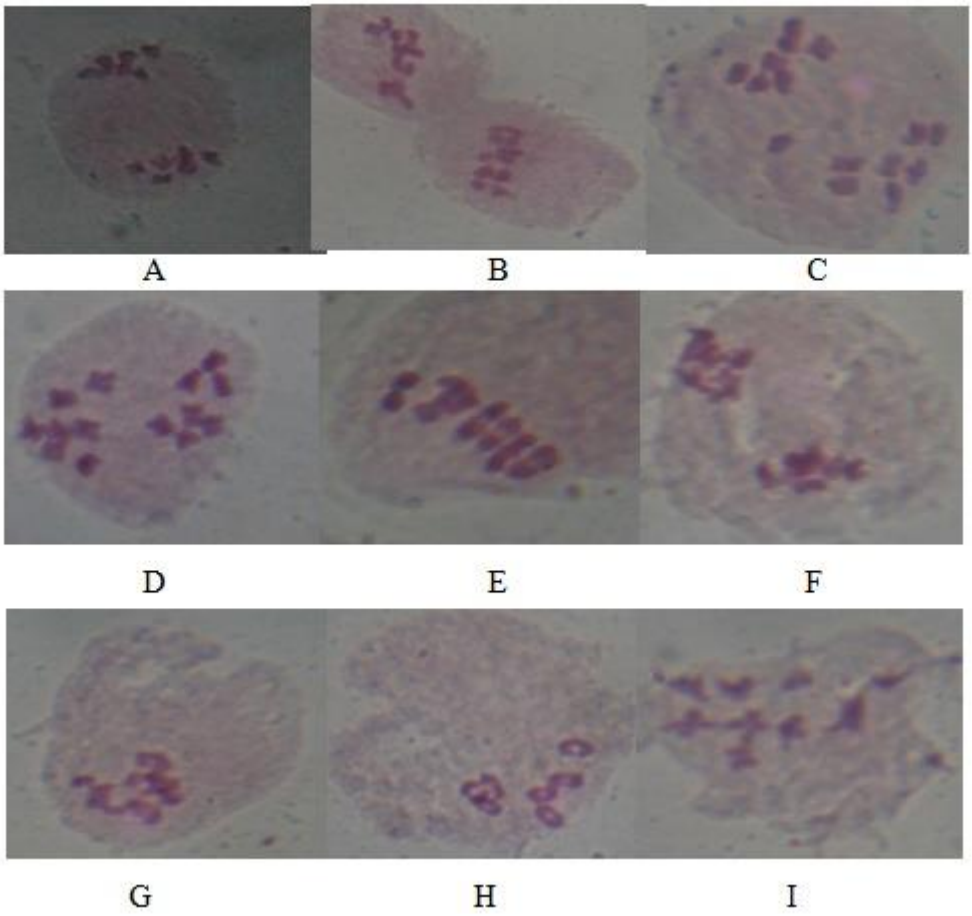

Plate-1

A: Late anaphase (Control), B: Cytomixis, C: Laggard formation at anaphase-I, D: Early anaphase, E: Disturbed metaphase-I, F& G: Disturbed telophase-I, H: Ring bivalents, I: Multivalents

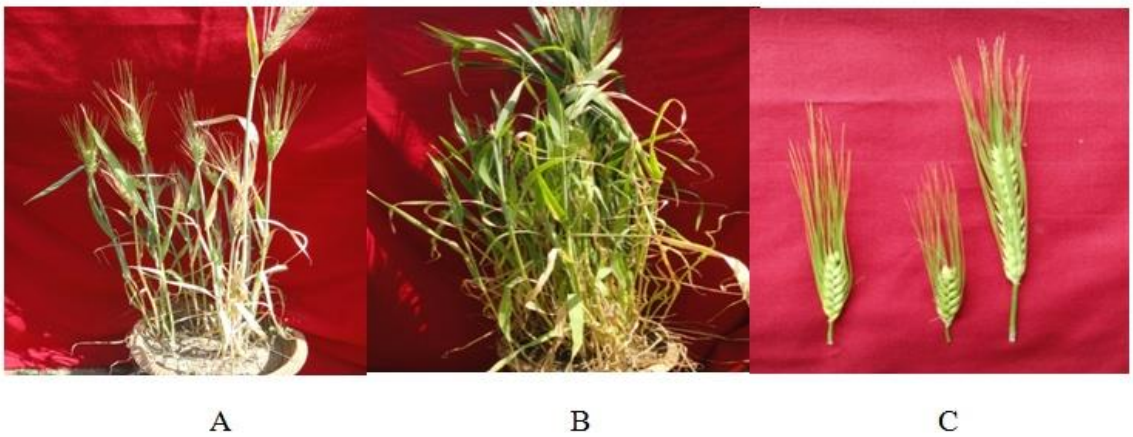

Plate-2

A: Control Plant, B: High Yielding Bushy Plant (0.03% MMS), C: Spike Length (Control, 0.04% MMS, 0.02% MMS)

Table 1: Effect of different concentrations of MMS and SA on seed germination of *Hordeum vulgare* L. in M_1 generation.

Treatment	Seed germination (%)		Inhibition (%)	
	In Pots	In Petriplates	In Pots	In Petriplates
Control	100	100	-	-
0.01% MMS	92.89	96.87	7.11	3.13
0.02% MMS	90.30	93.75	9.70	6.25
0.03% MMS	86.47	90.62	13.53	9.38
0.04% MMS	79.51	93..75	20.49	6.25
0.01% SA	91.36	96.87	8.64	3.13
0.02% SA	82.91	84.37	17.09	15.63
0.03% SA	85.25	93.75	14.75	6.25
0.04% SA	90.30	96.87	9.70	3.13

Table 2: Frequency of meiotic abnormalities in various mutagenic treatments of MMS and SA of *Hordeum vulgare* L. in M_1 generation.

	Total No.of PMC's observed	Metaphase			Anaphase	Telophase	
		Dm	Rb	Mt	Lg	Dp	Ct
Control	444	-	-	-	-	-	-
0.01% MMS	438	0.22	-	-	0.45	0.45	0.68
0.02% MMS	436	0.45	0.22	-	0.68	-	0.45
0.03% MMS	440	0.68	-	0.45	0.22	0.45	0.22
0.04% MMS	435	0.91	0.45	0.22	-	-	0.68
0.01% SA	434	-	-	-	0.46	0.23	0.69
0.02% SA	432	0.23	-	-	0.69	0.46	0.23
0.03% SA	437	0.45	0.45	0.45	0.22	0.22	-
0.04% SA	430	-	0.69	0.46	0.23	-	0.69

Dm: Disturbed metaphase, Rb: Ring bivalents, Mt: Multivalents, Lg: Laggard, Dp: Disturbed polarity, Ct: Cytomixis

Table 3: Effect of different concentrations of MMS and SA on quantitative characters of *Hordeum vulgare* L. in M_2 generation.

Treatment	Plant height(cm) $\bar{x} \pm$ S.E	Fertile branchs/plant $\bar{x} \pm$ S.E	100-seeds weight(g) $\bar{x} \pm$ S.E
Control	69.16 ± 1.10	3.0 ± 0.76	3.69 ± 0.26
0.01% MMS	86.96 ± 1.52	3.20 ± 0.33	4.17 ± 0.007
0.02% MMS	78.38 ± 0.53	4.00 ± 0.63	4.14 ± 0.006
0.03% MMS	80.26 ± 3.80	5.40 ± 0.82	2.34 ± 0.011
0.04% MMS	96.56 ± 0.46	1.80 ± 0.17	4.70 ± 0.013
0.01% SA	74.00 ± 1.81	1.40 ± 0.21	3.91 ± 0.014
0.02% SA	76.60 ± 3.21	4.80 ± 0.76	2.84 ± 0.010
0.03% SA	80.14 ± 0.45	3.40 ± 0.35	3.91 ± 0.009
0.04% SA	79.76 ± 0.55	3.80 ± 0.33	4.01 ± 0.012

CONCLUSION

The present experiment was conducted to induce variability in *Hordeum vulgare* L. The mutagens used were methyl methanesulphonate and sodium azide. MMS at lower concentrations produced less frequency of meiotic abnormalities in M_1 generation that can be tolerated by plant while M_2 generation plants showed more positive response in quantitative characters at lower concentrations of MMS than that of SA. So, it is better to use MMS at lower concentrations than SA to increase the favourable mutations in plant.

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