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THE INFLUENCE OF COPPER ON THE MUTAGENIC EFFICIENCY OF EMS IN MUNG BEAN

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Two inbreds of Mung bean were treated with different doses of copper sulphate (0.005%, 0.01% and 0.05%) in combination with EMS (0.2%) indicate that irrespective of the varieties involved, in general, the combination treatment (EMS + copper sulphate) reduced the germination percentage, seedling height, pollen fertility, seed fertility, mitotic index and increased the chromosomal abnormalities in M_1 generation and chlorophyll and viable mutations in M_2 generation as compared to EMS treatment alone.

Key Words : EMS, Copper, Chlorophyll and viable mutations.

Metallic ions have been reported to alter the mutagenic efficiency of chemical mutagens when administered in combination treatment. Ramanna and Natrajan (1965) reported the reduction in chlorophyll mutation frequency in barley by metallic ions. However, Bhatia and Narayanan (1965) observed the increased frequency of chlorophyll and viable mutations in combination treatment of EMS with copper. Pusztal (1987) treated the barley seeds with copper sulphate and ethylimine indicated that the presence of copper enhanced the mutagenic effect of ethylimine but copper sulphate treatment alone caused no effect on barley seeds. Hence, the present investigation was undertaken to study the effect of copper on mutagenic efficiency of EMS in two inbreds of mung bean.

MATERIALS AND METHODS

Two inbreds of mung bean (*Vigna radiata* (L) Wilczek) viz., PDM-116 and PDM-11 obtained from Pulse Directorate, Kalyanpur, Kanpur, were presoaked for twelve hours at room temperature (26°C) in distilled water. The presoaked seeds were treated with aqueous solution of 0.2% EMS and 0.2% EMS solution made with different concentration of copper sulphate (0.005%, 0.01% and 0.05%) at pH 7 for six hours with intermittent shaking of seeds. After the termination of chemical treatment the seeds were thoroughly washed in running tap water and sown in the field. Seeds from each M_1 plant were collected on the individual plant basis and sown in the field in randomized block single row design as M_2 generation. The seedling height was measured on 15 days old seedlings and pollen fertility was determined by staining the pollen with 2% acetocarmine chlorophyll mutations were observed in 10-15 days old seedlings and mutation detection was done as per classification of Jana (1963).

RESULTS

M_1 generation : Irrespective of the varieties involved out of the three different doses of copper used with EMS, the 0.2% EMS treatment administered with 0.05% copper reduced the M_1 parameters i.e. seed germination, seedling height, pollen fertility and seed fertility (only in variety PDM-116) significantly as compared to the 0.2% EMS treatment alone (Table 1). Besides, the post copper sulphate treatment with EMS increased the reduction in the mitotic index and enhanced cytological damages in both the varieties as compared to EMS treatment alone. The maximum cytological damage was observed at 0.05% copper sulphate treatment in combination with EMS treatment in both the varieties (Table 1).

M_2 generation

Chlorophyll mutation : The four main types of chlorophyll mutations observed in M_2 generation are as under.

(i) *Chlorina :* The seedling had light green or yellowish leaves of the size comparable to xantha type. The seedling usually survived for 10-12 days.

(ii) *Albina :* The seedling had first pair of white leaves. The albina mutant died within 15 days after germination.

(iii) *Xantha :* The seedling had yellowish first pair of leaves which were larger than those of albino type and

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Table 1: Effect of copper with EMS on M_1 plant characteristics

Treatment	Germination percentage	Seedling height	Pollen fertility	Seed fertility	Mitotic index	Chromosomal+ abnormalities (%
Variety PDM-116						
02% EMS+0.005% $CuSO_4$	70	12.62 ± 0.70	71.88 ± 1.2	9.50 ±0.35	7.6	0.179
0.2% EMS + 0.01% $CuSO_4$	65	11.23* ± 1.00	58.40** ± 1.76	8.78 ±0.38	6.8	0.212
0.2% EMS + 0.05% $CuSO_4$	65	11.09** ± 0.67	48.40** ± 3.77	8.18 ±0.33	6.1	0.225
0.2% EMS	70	13.92 ± 0.27	12.60 ± 2.97	9.40 ±0.54	7.9	0.162
Control	90	17.39 ± 0.52	84.60 ± 1.86	9.80 ±0.69	10.7	0.030
Variety PDM-11						
0.2% EMS + 0.005% $CuSO_4$	60	13.62 ± 1.13	57.70 ± 2.83	8.98 ±0.46	7.9	0.182
0.2% EMS + 0.01% $CuSO_4$	60	12.70 ± 1.15	55.20 ± 2.03	8.40 ±0.40	7.6	0.198
0.2% EMS + 0.05% $CuSO_4$	55	11.40** ± 1.42	51.70** ± 2.51	8.18 ±0.21	7.1	0.236
0.2% EMS	65	13.79 ± 0.33	61.80 ± 3.57	8.70 ±0.33	8.3	0.184
Control	85	17.82 ± 0.32	82.30 ± 1.70	9.58 ±0.37	9.88	0.020

* Significant at 5% level
** Significant at 1% level
\+ Binucleate, Micronuclei, Fragments, Disturbed metaphase, Disturbed anaphase

Table 2: Spectrum and frequency of chlorophyll and viable mutations in M_2 generation.

Treatments	Chlorophyll mutaton spectrum Xantha	Albina	Chlorina	Variagated	Frequency (Total)	Total viable* frequency
Var. PDM - 116						
0.2% EMS + 0.005% $CuSO_4$	4	-	3	2	7.50	1.66
0.2% EMS + 0.01% $CuSO_4$	-	5	5	2	10.00	1.66
0.2% EMS + 0.05% $CuSO_4$	3	4	6	1	11.66	2.50
0.2% EMS	2	3	1	3	6.66	0.83
Var. PDM-11						
0.2% EMS + 0.005% $CuSO_4$	1	-	5	4	8.33	0.83
0.2% EMS + 0.01% $CuSO_4$	4	4	3	2	10.83	1.66
0.2% EMS + 0.05% $CuSO_4$	5	1	5	4	12.59	2.50
0.2% EMS	3	-	3	2	6.66	1.66

Synchronous maturing mutant, Long pod mutant, Bigger grain size mutant, High yielding mutant, Errect plant type mutant

Table 3: Segregating pattern of the viable mutants in M_3 generation.

Mutants	Treatments	Total plants	Normal plant	Mutant plants	X^2 value
Var. PDM-116					
A	0.2% EMS + 0.01% $CuSO_4$	20	14	06	0.266
B	0.2% EMS + 0.005% $CuSO_4$	30	19	11	2.107
C	0.2% EMS + 0.05% $CuSO_4$	22	16	06	0.060
	0.2% EMS	30	19	11	2.107
Var. PDM-11					
D	0.2% EMS	30	19	11	2.107
	0.2% EMS + 0.005% $CuSO_4$	20	12	08	2.400
E	0.2% EMS + 0.01% $CuSO_4$	22	14	08	1.410
B	0.2% EMS + 0.05% $CuSO_4$	28	17	11	2.309

Table value of x^2 at 1 degree of freedom and 5% level of significance is 3.814
A - Synchronous maturing mutant
B - Long pod mutant
C - Bigger grain size mutant
D - High yielding mutant
E - Errect to be mutant.

survived for 10-15 days.

(iv) *Variegated* : The seedling were dark green in colour. The leaves were generally dotted or patched. The trifoliate leaves were white or light yellow.

To assess the role of copper in combination with EMS, a comparison was made between EMS vs EMS with different doses of copper sulphate treatment. Copper markedly increase the frequency of chlorophyll mutation induced by EMS in both the varieties. Chlorophyll mutation frequency increases with increase in doses of copper with EMS. Mutation spectrum was also enlarged in combination treatment of copper (0.05%)with EMS in variety PDM-116 and 0.01% and 0.05% copper in combination with EMS treatment in variety PDM-11 as compared to the spectrum induced by the chemical mutagen alone in both the varieties (Table 2).

Viable mutations : Any visible morphological change either in the general architecture of the plant or maturity or in the pod number or their size were kept in this group. The present counts are concerned only with the true breeding morphological mutations. The details of morphological distinguishable viable mutants have already been described in a separate communication (Sharma and Singh, communicated).

A comparison made between EMS treatment Vs EMS in combination with different doses of copper indicate that the higher frequency and enlarged spectrum of viable mutations was reported in combination treatment. The increase in mutation frequency increases with the increase in doses of copper and the highest frequency and enlarged spectrum of viable mutations was reported in 0.2% EMS+0.05% copper treatment, in both the varieties, as compared to EMS treatment (Table 2). The mutants were found bred true in M_3 generation (Table 3).

DISCUSSION

In the present investigation the role of copper in combination with chemical mutagen i.e. EMS, indicate that the administration of copper with EMS causes reduction in germination percentage, seedling height, pollen fertility, seed fertility and mitotic index and increases the induction of chromosomal abnormalities in M_1generation. Moreover, the treatment of copper with EMS increases the frequency of chlorophyll and viable mutation in M_2 generation. The spectrum of viable mutation was also enlarged in combination treatment of EMS with copper sulphate. Mutants segregated into 1:3 ratio in M_3 generation suggesting that mutant character is governed by recessive gene.

There are contradictory reports regarding the effects of metal ions to influence the mutagenic efficiency of chemical mutagens. It has been reported that metal ions reduced the chlorophyll mutation frequency of barley (Ramanna and Natarajan, 1965) but enhances it in Anapidopsis thaliava (Bhatia and Narayanan, 1965). Patrick and Haynes (1964) have observed that the repair system in yeast is extremely sensitive towards metabolic inhibitors of various kinds and is inhibited by trace quantities of metallic cations such as iron, copper and zinc. Since probably the same primarily lesions can give rise to chromosome aberration or mutation the biological end result in higher organisms may depend upon successful repair of the damage.

The findings of the present investigation indicate that the copper with EMS increases the mutagenic efficiency of the EMS. Similarly the increased mutagenic efficiency of chemical mutagens by copper have earlier been reported by Bhatia and Narayanan (1965); Aldiev (1981); Somashekhar and Arekal (1983) and Pusztal (1987). Thus, the copper can be used to increase the mutagenic efficiency of the chemical mutagen i.e. Ethyl methanesulphonate.

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