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DIGITONIN AS AN ENHANCER OF MUTAGENICITY OF ETHYL METHANE SULPHONATE IN BLACKGRAM (*VIGNA MUNGO* L.)

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ABSTRACT

In an induced mutation study in blackgram digitonin was applied as a pretreatment chemical just before the treatment with the mutagen, ethyl methanesulphonate (EMS) to enhance the mutagenic effect of EMS through greater uptake of the mutagen into the cells. The M_2 populations were screened for macro- and micro- mutations. The frequency and spectrum of total macro-mutations were substantially increased by digitonin-EMS treatment compared to EMS treatment alone. There was also increase in micro- mutations by digitonin pretreatment for almost all the quantitative characters except seeds per pod and single-plant yield.

Key words : *Vigna mungo*, blackgram, Digitonin, EMS, enhancer, mutagenicity.

In induced mutagenesis, dimethyl sulfoxide (DMSO) has been used as a pretreatment chemical because it enhances absorption of chemical mutagen through biological membranes acting as a carrier [1, 2]. In the present study, digitonin ($C_{56}H_{92}O_{29}$) was used as a pretreatment chemical with two broad objectives: (i) to promote entry of the chemical mutagen because of its detergent activity [3, 4]. (ii) to induce cytoplasmic mutations by disintegrating the plastids [5-7] and making the organelle DNA more vulnerable to the mutagen.

MATERIALS AND METHODS

A blackgram cultivar, B 3-8-8 was presoaked for 6 hours and 12 hours followed by pretreatment with three doses of digitonin (200 ppm, 350 ppm and 500 ppm) for three hours. The pretreated seeds were immediately treated with two doses of ethyl methanesulphonate (0.075% and 0.125%). In all, there were 24 treatment combinations including controls. The macromutants (both chlorophyll and morphological) were isolated in M_2 generation. Observations were recorded on twenty randomly chosen normal looking plants from each treatment on seven quantitative traits for polygenic variation (micro-mutation). The frequency and spectrum of

macro-mutants was worked out for each treatment, and for micro-mutations, coefficient of variation was calculated for each character under each treatment. To examine the effect of Digitonin pretreatment in enhancing the mutagenicity of EMS in inducing both macro- and micro-mutations, only sixteen treatments (with and without Digitonin followed by EMS) were considered and the data were pooled over presoakings and EMS treatments under each digitonin pretreatment.

RESULTS AND DISCUSSION

Although Digitonin alone did not induce mutations, Digitnonin-EMS combined treatments increased both chlorophyll and morphological mutation frequency as compared to only EMS treatment. The increase over no Digitonin pretreatment (D_0) was 3.85 times by D_1 (200 ppm Digotonin), 3.98 times by D_2 (350 ppm Digitonin) and 3.23 times by D_3 (500 ppm Digitonin) for chlorohyll mutation frequency, and 2.97, 6.15 and 6.22 times by D_1, D_2 and D_3, respectively for morphological mutation frequency (table 1). The increase in chlorophyll mutation frequency was well marked in D_1 (3.85 times) over D_0, but further increase in D_2 over D_1 was very little, rather it decreased in D_3 over D_1 and D_2. For morphological mutation frequency, the increase over D_0 was very well pronounced in D_2 (6.15 times) and D_3 (6.22 times) as compared to D_1 (2.97 times).

The macro-mutations spectrum also increased by about two times when Digitonin pretreatments were followed by EMS treatment. But no differences in spectrum were found by different doses of Digitonin pretreatment (Table 1). The enhancement of frequency and spectrum of macro-mutations by Digitonin-EMS treatments clearly indicated that the mutagen might have reached both the nuclear and cytoplasmic genetic material, by disintegration of the membrane system caused by Digitonin.

Table 1. Macromutation frequency in M_2 generation of blackgram

Digitomin treatment (ppm)	Total population screened	Chlorophyll mutation frequency(%)	Morphological mutation frequency(%)	Total macromutation frequency (%)	Macro-mutation spectrum (no. of different kinds)
0 + EMS*	2652	0.189	0.490	0.679	11
200 + EMS*	2611	0.728	1.455	2.183	21
350 + EMS*	1592	0.754	3.015	3.769	20
500 + EMS*	1802	0.610	3.052	3.663	21

*Pooled over EMS treatments

The coefficient of variation (CV) for almost all the characters except seeds per pod and single-plant yield increased following digitonin-EMS treatment indicating more of micro-mutational changes by pretreatment with digitonin than EMS alone (Table 2).

Table 2. Coefficient of variation of quantitative traits in M_2 generation of blackgram

Digitonin treatment (ppm)	Plant height (%)	Branches/ plant (%)	Branches/ plants (%)	Pods/ plant (%)	Seeds/ pod (%)	100-seed weight (%)	Single-plant yield (%)
0 + EMS*	8.59	45.64	16.18	12.66	21.63	20.38	76.45
200 + EMS*	9.56	77.67	23.16	15.27	18.45	27.36	72.89
350 + EMS*	10.94	54.90	21.41	14.38	14.51	30.56	48.48
500 + EMS*	13.13	51.77	18.35	13.22	18.54	35.25	48.55

*Pooled over EMS treatments

The present investigation in blackgram indicates that digitonin could be used in enhancing the mutagenicity of chemical mutagen for broadening the frequency and spectrum of both macro-mutations and micro-mutations.

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