Induction of Chlorophyll Mutations in Trigonella foenum - graecum Linn.

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Abstract

Dry and healthy seeds of *Trigonella foenum - graecum Linn.* were irradiated with 200,400,600,800Gy gamma rays. Seeds were also treated with sodium azide (SA) (0.001%,0.002%, 0.003%, 0.004%). All the two mutagens were found to induce chlorophyll mutations. They were *chlorina, xantha, viridis* and *albino*. The chlorophyll mutants survived for 10-15 days and then died. The gamma rays were found to produce maximum chlorophyll mutations than sodium azide (SA).

KEYWORDS: *Trigonella foenm-graecum,* chlorophyll mutations, Gamma rays, Sodium azide

INTRODUCTION

Mutagenesis is extremely useful in creating new variations. The spectrum of chlorophyll mutations is essentially a parameter for the index of mutation frequency and for studying the biological effect (Gustaffson, 1954). Muller (1927) demonstrated that mutation could be induced by ionizing radiations, in *Drosophila melanogastor*. The great breakthrough towards the use of artificial mutagenesis came when Stadlar (1928) demonstrated the induction of mutation in barley and maize, using x-rays, thus arousing the hope of plant breeders to new heights, sodium azide (SA), which is reported to be more potent mutagen than alkylating agents, is relatively safe, non-persistant and inexpensive (Nilan et al. 1973). Azide, in acidic solution (pH-3), was found to be very effective in inducing chlorophyll deficient as well as morphological mutations in barley, which in alkaline solution, it seemed to be ineffective (Kleinhofs et al, 1974). In Peas (Hadwiger et al. 1976, Sander and Muehlbauer, 1977), Soyabean (Vig, 1975) and maize (Conger and Carebia 1977).

The present investigation deals with an analysis of different chlorophyll mutations after the independent treatments of Gamma rays and SA in *Trigonella foenm-graecum L.*

MATERIALS AND METHODS

Dry seeds of uniform size and shape of *Trigonella foenum - graecum Linn.* Were treated in gamma cell with ^{60}Co as gamma source @ 100 Gy per minute at the Department of Chemistry, Rashtrasant Tukdoji Maharaj Nagpur University Camps, Nagpur. They were irradiated with 200,400,600 and 800 Gy does of gamma rays. Seeds without irradiation served as control. Chemical mutagen, sodium azide (E. Merck, Germany) was used for inducing mutations. Seeds were presoaked for 5 and 10 hours in distilled water surface dried with blotting paper and subjected to chemical mutagen.

The dry as well as pre-soaked seeds of *Trigonella foenum - graecum Linn.* seeds were treated with 25 ml aqueous solutions of 0.0, 0.001%, 0.002%, 0.003% , and 0.004% sodium azide (SA) for 18 hours with uniform and continues shaking on orbital shaker, at $22 \pm 1°C$ in

50ml flask. All other conditions were similar. Treated seeds were thoroughly washed in running tap water soaked in 50 ml of glass distilled water for 2 hours, again washed in running tap water and surface dried. One hundred seeds of each treatment were sown in earthen pots of medium size. The seedlings were observed from seedlings stage to maturity and chlorophyll mutations such as *chlorina, xantha, viridis* and albino were recorded.

OBSERVATION AND RESULT

The treated population was screened for the chlorophyll chimeras from the seedling stage to the flowering initiation. Chlorophyll chimeras were classified according to Gustafsson (1954). They were of four types viz., *viridis, xantha, chlorine* and *albino*.

The *viridis* was represented by light green colour in early stages of growth. This colour gradually changes to the normal green colour during the subsequent period of growth of the plants.

The majority of the *xantha* mutants were lethal at the seedling stage. The colour of this mutant varied from light yellow to dark yellow. Only a few of them survived in the early period of growth. The *chlorina mutants* were yellowish green in colour. The *albino* mutants were completely white in colour found to be completely lethal. Due to lack of chlorophyll, the seedlings collapsed after 2-3 days.

The frequency of induced mutations was calculated per 100 M_2 seedling (Gaul, 1964). The mutagens were found to induce chlorophyll mutations.

In gamma ray treated M_2 Population, the frequency of chlorophyll mutations was recorded to be 0.84, 0.78, 0.82, 0.03 for 200, 400, 600, 800 Gy respectively. All types of mutations were induced by gamma rays. It was observed that total frequency of chlorophyll mutations increases as the dose of the gamma rays increases except 0.78 (200Gy). Gamma radiations induced *xantha* type mutations followed by *viridis*, *chlorina* and *albino*(Table-1). The frequency of chlorophyll mutations declined in M_3 generation (Table-2)

All the concentrations of SA were to be effective in inducing chlorophyll mutations. Maximum frequency (0.57%) of chlorophyll mutations was recorded with 0.004% in dry set while minimum frequency (0.16) was recorded at 10h PSW 0.001%. In case of SA treatment, mutagen induced *viridis* type mutations with maximum frequency followed by *chlorina*, *xantha* and *albino*. In case of SA, dry seed treatment of mutagen yield more chlorophyll mutations as compared to 5h PSW set and 10h PSW set (Table-3). In M_3.generation, the frequency of chlorophyll mutations declined (Table-4).

The overall result reveals that gamma rays produced maximum chlorophyll mutations than sodium azide (SA).

DISCUSSION

Many investigators have reported the induction of chlorophyll mutations in plants by physical and chemical mutagen treatments e.g. Gual (1964) in barely with gamma rays and EMS, Fuji(1962) and Yamaguchi (1969) in rice with x-rays, Burton and Powell (1966) in *Pennisetum typehoides* with EMS and thermal neutrons, Lather and Chaudhary (1972), Yadava and Chaudhary (1974), Sato(1966) in rice with EI, DES AND X-rays, Sree Ramulu (1970) in Sorghum with gamma ray, X-rays, DES, EMS and other chemicals, Kothekar (1978) in *Solanum nigrum* with gamma rays and EMS and Deshmukh (1979) in *Anethum sowa* in gamma rays and EMS.

Ryan and Heslot (1964) have clearly demonstrated the randomness in the action of physical mutagens and specificity of EMS to certain loci in the barley for the production of chlorophyll mutations. Natarajan and Upadhya (1964) have stated that the chlorophyll development seems to be controlled by many genes located on several chromosomes. The

high spectrum of chlorophyll mutations in EMS and diethyl sulfate (EMS) treatment might be due o its specificity to affect certain regions of the chromosomes in *Vicia faba*. Varughese and Swaminathan (1968) have stated that the chlorophyll chimeras produced in wheat by EMS treatment are mainly due to the chlorophyll DNA as EMS in more specific to the quinine and cytosine.

The results obtained by Ehrenberg and Gustafsson (1957), Ehrenburg (1960 a & b), Nilan (1964), Konzak et al. (1965), Ehrenberg et al. (1966), and Wagner et al. (1968) indicate that the frequency of mutations is directly related to the concentrations of the mutagen. Increase in concentration and duration of EMS treatment enhances the frequency of mutations upto a certain level. A decrease in the number of mutations and increase in lethality are observed with further increase in doses (Ehrenberg and Gustafsson, 1957), Similarly Sree Ramulu (1970) in *Sorghum* observed, while critically comparing chlorophyll mutations, that the mutation rate in general increased with an increase in the dose upto a certain level beyond which it decreased.

Natarajan and Shivashankar (1965) noted that in barley, the chlorophyll mutations frequency was very high with 24 hour presoaking followed by 0.3% EMS treatment where as 0.6% EMS treatment with 16 hour presoaking yielded the maximum mutation rate. Savin et al. (1960) also noted that the treatment of barley seeds presoaked in water for different periods ranging from 8 to 40 hours with EMS and NMU showed that the seeds were most sensitive to mutagenic treatments at 16 and 28 hours.

D' Amato et al. (1962) observed more chlorophyll mutations in EMS-treatment than in dES-treatment. Monti (1968) found that the average effectiveness of dES was 3-4 times greater than X-rays, when either chlorophyll or morphological mutations or both were considered in pea seeds.

Auerback (1967) has suggested that in nucleotide bases mutations do not occur at random but tend to attack preferential sites whose position within a given gene depends on the mutagen. Nilan (1964) believes that 250 to 300 loci are involved in chlorophyll anthesis in barley while Gustafsson (1954) is of the opinion that 125 to 150 loci may be concerned with *albino*, 125 with viridis, 10 to 15 with a rare type. Nasare and Choudhary (2010) observed that in *Ocimum sanctum*. chlorophyll mutation frequency was found to be higher in the presoaked sets, than in the dry set. In case of SA and of EMS treatments mutants were recorded only in 5hrs presoaked set.

Induction of particular type of mutation depends on the reaction of the mutagen with the specific locus/loci. It is well known that various types of physical and chemical mutagens have differential action on genetic material. In *Trigonella foenum- graecu*m, it might be possible that many loci, present on different chromosomes, are responsible for production of chlorophyll mutations. Due to differential penetrating powers and the differences in actions of physical and chemical mutagens on the genetic material, different locus/loci, might have got affected by various mutagens resulting in the production of various types mutation spectra and frequency.

In the present investigation, chlorophyll mutation frequency was found to be higher in gamma rays treated population than in SA treatment population.

ACKNOWLEDGEMENT

Auhor express heartfelt thanks to Dr. A. D. Choudhary, Dr. K. H. Makde, Professor and Ex-Head, Department of Botany, RTM Nagpur University Campus, Nagpur. Dr. R. P. Thakre for providing the green house facilities. Professor Dr. A. P. Joshi, Head, Department

of Chemistry for making available gamma cell facility for irradiation of *Trigonella foenum – graecum* seeds and for their valuable guidance and cooperation.

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Table : 1 : ***Frequency of Chlorophyll Mutations induced by gamma rays in M_2 generation.***

PARAMETERS	Frequency of				Total Frequency of Mutations (%)
TREATMENTS	Albino	Xantha	Chlorina	Viridis	
Control	----	----	----	----	0.00
200 Gy	0.12	0.32	0.21	0.19	0.84
400 Gy	---	0.11	0.45	0.22	0.78
600 Gy	0.09	0.39	----	0.34	0.82
800 Gy	----	----	0.03	----	0.03

Table : 2: ***Frequency of Chlorophyll Mutations induced by gamma rays in M_3 generation.***

PARAMETERS	Frequency of				Total Frequency of Mutations (%)
TREATMENTS	Albino	Xantha	Chlorina	Viridis	
Control	----	----	----	----	0.00
200 Gy	----	0.17	----	0.13	0.30
400 Gy	----	----	-----	----	0.00
600 Gy	----	----	----	----	0.00
800 Gy	----	----	----	----	0.00

Gy = Grey

Table : 3 : ***Frequency of Chlorophyll Mutations induced by Sodium azide in M_2 generation.***

PARAMETERS	Frequency of				Total Frequency of Mutations (%)
TREATMENTS	Albino	Xantha	Chlorina	Viridis	
Dry Set Control	----	----	----	----	0.00
Dry Set Control 0.001%	----	----	----	0.51	0.51
Dry Set Control 0.002%	----	0.31	----	0.42	0.73
Dry Set Control 0.003%	----	----	0.24	0.28	0.52
Dry Set Control 0.004%	----	----	0.57	----	0.57
5h PSW Control	----	----	----	----	0.00
5h PSW Control 0.001%	----	----	0.28	----	0.28
5h PSW Control 0.002%	----	0.17	----	----	0.17
5h PSW Control 0.003%	----	----	----	0.32	0.32
5h PSW Control 0.004%	0.12	0.31	-----	0.11	0.54
10h PSW Control	----	----	----	----	0.00
10h PSW Control 0.001%	----	0.16	----	----	0.06
10h PSW Control 0.002%	----	----	0.39	----	0.39
10h PSW Control 0.003%	----	0.14	----	0.33	0.47
10h PSW Control 0.004%	----	0.30	0.22	----	0.52

Table : 4 : ***Frequency of Chlorophyll Mutations induced by Sodium azide in M_3 generation.***

PARAMETERS	Frequency of				Total Frequency of Mutations (%)
TREATMENTS	Albino	Xantha	Chlorina	Viridis	
Dry Set Control	----	----	----	----	0.00
Dry Set Control 0.001%	----	0.21	----	0.12	0.33
Dry Set Control 0.002%	----	----	----	0.09	0.9
Dry Set Control 0.003%	----	0.24	----	0.12	0.36
Dry Set Control 0.004%	----	----	0.24	----	0.24
5h PSW Control	----	----	----	----	0.00
5h PSW Control 0.001%	----	----	----	----	0.00
5h PSW Control 0.002%	----	0.19	----	----	0.19
5h PSW Control 0.003%	----	----	0.21	----	0.21
5h PSW Control 0.004%	----	0.27	-----	----	0.27
10h PSW Control	----	----	----	----	0.00
10h PSW Control 0.001%	0.12	----	----	----	0.12
10h PSW Control 0.002%	0.03	----	0.13	----	0.16
10h PSW Control 0.003%	----	----	----	0.21	0.21
10h PSW Control 0.004%	----	----	----	27	0.27

h = hours, PSW = Presoaked in Water