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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(1): 285-287

www.thepharmajournal.com
Received: 21-11-2020
Accepted: 24-12-2020

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Evaluation of mutagenic effects on frequency and spectrum of chlorophyll mutation in sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract
A comparative study of frequency and spectrum of chlorophyll mutations induced by physical (gamma rays) and chemical mutagens (EMS) in relation to the effect of mutations in M_2 generation plant of *kharif* sorghum (*Sorghum bicolor* L.) genotype 'PVK 801'. The experimental material comprised of different mutagenic treatments treated with different concentrations *viz*., 0.1%, 0.2%, 0.3% were EMS treatments and 10 kR + 0.1% EMS, 20 kR + 0.1% EMS, 30 kR + 0.1% EMS and 40 kR + 0.1% EMS were combination treatments and dry and wet control treatments. All these treatments were grown in randomized block design with three replications during *kharif* 2019. Results showed induction of broad spectrum of chlorophyll mutations which included albino, xantha, chlorina, viridis and xanthaviridis. Among these albino type was predominant in all the mutagenic treatments. Highest frequency of chlorophyll mutants were found under 20 kR + 0.1% EMS combination treatment. Based on the chlorophyll mutation frequency and spectrum in combination treatment were most effective followed by EMS treatments. The frequencies and spectrum of five different kind of induced chlorophyll mutations was in the order; albino > xantha > chlorina = viridis = xanthaviridis.

Keywords: Sorghum, mutation, chlorophyll, frequency, spectrum

Introduction
Sorghum [*Sorghum bicolor* (L.) Moench] is often cross pollinated, diploid (2n = 2x = 20) and belongs to the family Poaceae. Sorghum is the fourth most important cereal crop followed by rice, wheat and maize. Cultivated sorghums originated about 5000-7000 years ago or earlier in Northern East Africa, probably in Sudan or Ethiopia. India is a major sorghum growing country in the world, ranks first in area and second in production followed by United States of America. In India sorghum is grown in areas receiving 500 to 1000 mm annual rainfall and temperature varying from 26 to 32 ºC. Mutation breeding can play a unique role in improving crops which offers a new way for plant breeders to increase crop productivity as well as other quantitative traits. In mutation breeding, chlorophyll mutations have great significance for methodological investigation. Frequency of chlorophyll mutations in M_2 generation following the treatment are serves as a guideline to evaluating the effectiveness of mutagenic treatments. (Gustafsson, A. 1954) [2] reported that the mutation approach was superior to other methods of crop improvement. The scoring of chlorophyll mutations in M_2 generation has been proved to be most accurate index for determining the genetic effects of mutagenic treatments. Induction of chlorophyll mutations act as the most reliable indices for evaluating the efficiency of various mutagens, besides inducing the genetic changes for crop development. In induced mutation studies various types of chlorophyll mutation have often been reported in different crop plants including sorghum. Chlorophyll mutants are used as markers in genetic, physiological and biochemical investigations. They are the most frequently observed and easily identified factorial mutations in M_2 generation.

Material and Method
The present study was undertaken at the field of Department of Agricultural Botany, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The pure seed of PVK 801 variety of sorghum (*Sorghum bicolor* (L). Moench) was selected for mutagenic treatment. Seven different mutagenic treatments of PVK 801 *viz*., T1 (0.1%), T2 (0.2%), T3 (0.3%) EMS treatments and T4 (10kR+0.1% EMS), T5 (20kR+0.1% EMS), T6 (30kR+0.1% EMS) and T7 (40kR+0.1% EMS) combination treatments obtained from B.A.R.C. Trombay, Mumbai, along with two

control treatments *viz*., T8 (dry control) and T9 (wet control) were sown in Randomized Block Design with 3 replications, at spacing of 15 cm within plants and 45 cm between plants in M_2 generation. For M_2 generation seed selected from M_1 generation that treat with physical and chemical mutagen. The M_2 populations were screened for both frequency and spectrum of different types of chlorophyll mutations such mutations were counted and recorded upto 15th day after sowing. They were classified according to method suggested by Gustafsson (1940) [2] and are presented as albino mutant are white in colour, xantha chlorophylls are yellow colour, chlorina are light green to yellow green and bacame to normal plant, viridis have light yellow colour patches, Xantha/viridis are green with yellow apex. Mutations frequency was worked out by the following methods given by (Gaul 1958) [4]:

$$\text{Mutation frequency} = \frac{\text{No. of mutated plants}}{\text{Total number of plants}} \times 100$$

Results and Discussion

Frequency of chlorophyll mutation: The chlorophyll mutations were carefully scored based on M_2 plant basis. Chlorophyll mutation frequency in M_2 generation is one of the most reliable measures for evaluating the mutagen-induced genetic alterations. Most of the chlorophyll mutant types (albina, chlorina and xantha) were lethal and survived to seedling stage only. Such mutations were counted and recorded up to 15th day after sowing. They were classified according to method suggested by Gustafsson (1940) [2]. The data on frequency of chlorophyll mutations in M_2 generation of sorghum genotype is presented in Table 1. A maximum mutation frequency (1.60%) was recorded in 20kR+0.1% EMS and followed by 0.2% EMS (1.26) treatment and the minimum mutation frequency was recorded in the 0.3% EMS (0.83) and 0.1% EMS (0.87) mutagenic treatment. Similar results were also reported by Usharani *et al.*, (2015) [7]. The chlorophyll mutation acts not only as a scale for evaluating effectiveness and efficiency of mutagens, but also as indicators to predict the size of vital factor mutations.

Table 1: Frequency of chlorophyll mutations in M_2 generation

Treatment	No. of seedlings scored	No. of chlorophyll mutants	Frequency of chlorophyll mutations (%)
T1 (0.1% EMS)	684	6	0.87
T2 (0.2% EMS)	710	9	1.26
T3 (0.3% EMS)	715	6	0.83
T4 (10kR + 0.1% EMS)	780	9	1.15
T5 (20kR + 0.1% EMS)	810	13	1.60
T6 (30kR + 0.1% EMS)	760	8	1.05
T7 (40kR + 0.1% EMS)	680	8	1.17

Spectrum of chlorophyll mutation

Various types of chlorophyll mutations were induced by different mutagens *viz*., ethyl methane sulphonate, gamma rays and ethyl methane sulphonate combination those obtained during M_2 generation immediately after emergence. Table 2. The spectrum included albino, xantha, chlorina, viridis and xanthaviridis.

1. **Albino:** The albino seedling lacked chlorophyll pigment completely and were found almost white as well as relatively smaller in size. These types survived about 7-8 days and there after ultimately die.
2. **Xantha:** The seedling leaves were distinctly yellowish to white in colour. In these mutants carotenoides were present but chlorophyll was absent. They survived for about 10-12 days and were found lethal in nature.
3. **Viridis:** The leaves of these mutants were yellow green to pale green in colour and changed to normal green.
4. **Chlorina:** The leaves of these type of mutants were golden yellowish to green colour and had shown lethal reaction after 15-20 days.
5. **Xanthaviridis:** The leaves of xanthaviridis mutant were green with yellow apex.

These mutants were viable.

The above described mutants *viz*., albino, xantha, viridis, chlorina and xanthaviridis were found to be prominent in all the populations. These mutants were studied on the basis of pooled frequencies of doses over populations, treatments 20kR+0.1% EMS in the population. The mutagenic treatment 20kR+0.1%EMS had shown wider spectrum (all types) of chlorophyll mutations. Albino types were not noticed in 10kR+0.1% EMS treatment. Xantha appeared in all the doses/treatments. Chlorina and viridis mutant appeared in all the treatments. Albino mutants were found in large number as compared to other types. Xanthaviridis type were not noticed in 40kR+0.1% EMS treatment. Albino type were not noticed in 10kR+0.1% EMS treatment.

The different mutagens used, differed significantly from each other for inducing chlorophyll mutations. However, combination treatments in population was found most effective in inducing chlorophyll mutations than ethyl methane sulphonate. All mutagens *viz*., gamma rays, EMS and their combination induced maximum chlorophyll mutations namely albina, xantha, alboviridis, xanthaviridis and chlorina. Similar spectrum of chlorophyll mutations was also reported by Harb (1990) [5], Plesnik (1991) [6], Amarnath and Prasad (2000) [1], Wakode *et al*., (2000) [8].

Induced mutations have been recognized as an important tool for crop improvement, and have sufficient scope in pulses. Chlorophyll mutants inferred in this investigation, how chlorophyll gene response to mutagen. Mutations in these chlorophyll genes are expressed in the M_2 and coming after generations in the form of various types of mutants.

Table 2: Spectrum of chlorophyll mutations in M_2 generation

Treatment	Chlorophyll mutants					Total
	Albino	Xantha	Chlorina	Viridis	Xantha/viridis	
T1 (0.1% EMS)	2	1	1	1	1	6
T2 (0.2% EMS)	3	2	1	2	1	9

T3 (0.3% EMS)	2	1	1	1	1	6
T4 (10kR + 0.1% EMS)	-	3	2	1	3	9
T5 (20kR + 0.1% EMS)	4	3	2	2	2	13
T6 (30kR + 0.1% EMS)	2	1	2	1	2	8
T7 (40kR + 0.1% EMS)	3	2	1	2	-	8

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