IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
ISSN-0973-1547 Volume **15** | Issue 2 | July, 2020 | 107-112

DOI: 10.15740/HAS/IJPS/15.2/107-112
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RESEARCH ARTICLE

Study on mutagenic effectiveness and efficiency of mutagens in inducing chlorophyll mutations in m_2 generation in sorghum [*Sorghum bicolor* (L.) Moench]

■ **S.M. Surashe, H.V. Kalpande and S.B. Borgaonkar**

SUMMARY

An investigation was carried out to create the variability generated through induced mutation in two sorghum populations *viz*., 296 B (*Kharif*) and Parbhani Moti (*Rabi*). Two mutagens *viz*., gamma irradiation (10 kR, 20kR, 30kR and 40kR) and EMS (0.1%EMS, 0.2%EMS, 0.3%EMS and 0.3%EMS) and their combination were used M_2 generation. Mutagenic sensitivity in M_2 generation on the basis of reduced germination and plant survival revealed a dose dependent reaction and differential response of the populations. In general, chlorophyll mutation frequency expressed on M_2 seedling basis increased linearly with doses of three the mutagens in 296 B and Parbhani Moti. The frequency was more in 296 B followed by Parbhani Moti of three mutagens. The spectrum of chlorophyll mutations comprised albina, xantha, viridis, xanthaviridis, chlorina. The most frequently occurred mutant was viridis type followed by chlorina in all the populations. The population 296 B had expressed largest frequency of chlorophyll mutants followed by Parbhani Moti.

Key Words : Mutation, Segregants, EMS, LD_{50}, Genetic variability, Albina, Xantha, Chlorina

How to cite this article : Surashe, S.M., Kalpande, H.V. and Borgaonkar, S.B. (2020). Study on mutagenic effectiveness and efficiency of mutagens in inducing chlorophyll mutations in m_2 generation in sorghum [*Sorghum bicolor* (L.) Moench]. *Internat. J. Plant Sci.*, **15** (2): 107-112, **DOI: 10.15740/HAS/IJPS/15.2/107-112,** Copyright@ 2020: Hind Agri-Horticultural Society.

Article chronicle : Received : 08.03.2020; **Revised :** 21.05.2020; **Accepted :** 08.06.2020

Sorghum (*Sorghum* spp.) is cultivated predominantly in USA, China, India and Africa for both human and livestock consumption. In India, sorghum is cultivated over of 4.10 million ha with an annual production of 4.17 million tonnes of grain with a productivity of 1018 kg/ha (Ministry of Agriculture Government of India, 2018). In India, the productivity of *Rabi* sorghum is very low, and highly variable from year to year mainly due to post flowering drought but *Rabi* sorghum is highly valued because of its good grain quality. This crop is usually affected by water stress at both pre and post-flowering stages of development and has the most adverse effect on yield (Kebede *et al*., 2001). Drought tolerance is defined as the relative yield of a

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genotype compared with other genotypes subjected to same drought stress (Hall, 1993). Drought tolerance depends on the plant developmental stage at the onset of the stress condition, which may happen in sorghum during the early vegetative seedling stage, during panicle development and in post-flowering, in the period between grain filling and physiological maturity. Genetic variability for economic traits is the pre-requisite for any successful breeding programmers as the degree of response to selection depends on the quantum of variability. Mutation breeding is recognized as one of the driving force of evolution. Mutation breeding is relatively quicker method for improvement of various crop species. It is an important tool to create variability for quantitatively inherited traits in different plants and is considered as an alternative method to increase genetic variability in plant breeding programs. It is often used to correct defects in a cultivar which has a set of good agronomic characteristics. Among various physical mutagens such as x-rays, fast neutrons, thermal neutrons, ultraviolet and beta radiation, gamma rays in particular are well known with their effect on the plant growth and development by inducing cytological, physiological and morphological changes in cell and tissues (Thapa, 2004). Gamma radiation is an important tool for inducing the genetic variability, enhancing yield and yield contributing traits. However, the changes brought about by mutations were not very successful in simultaneous improvement of all yield-contributing characters. It is suggested that the application of mutagenic treatments to hybrids may be one of the mean of adding the variability inherent in the cross to that induced by mutation. Complementing the conventional methods, mutation breeding can play a unique role in crop improvement which provides a novel approach to plant breeder for improving the productivity of crop plants. Proves to be the method of choice for obtaining quicker results, when it is desired to rectify small defects in any crop variety. The present investigation was taken up to study mutagenic effectiveness and efficiency of mutagens in inducing chlorophyll mutations in m_2 generation in sorghum in M_2 generation

MATERIAL AND METHODS

The material for the present study was undertaken at sorghum research station, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The pure seed of two commercial varieties of sorghum [*Sorghum bicolar* (L.) Moench] *viz*., 296 B and Parbhani Moti were selected for mutagenic treatment. Selfed seeds (1200) with about 10 ± 1 per cent moisture for each of the two varieties *viz*., 296 B and Parbhani Moti were exposed to 10, 20, 30 and 40 kR dose of gamma rays (CO^{60}) with a dose rate of 2.39 kR per minute at Nuclear Agriculture and Biotechnology Division, B. A. R. C. Trombay, Mumbai, and the same number of untreated seeds of each variety served as control. Seeds treated with chemical mutagens were thoroughly washed in tap water remove the traces of mutagens and were immediately sown in the field of Sorghum research station, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani on dated 12st January 2016 (Summer, 2016). By dibbling one seed hill^{-1} at a distance of 45 cm between rows and 15 cm between seeds. The experiment was laid in a Randomized Block Design (RBD) with three replication. Twelve hundred seeds of each treatment including control (untreated seeds) were sown in a plot size of 6.75 m x 4 m (15 rows of 4 m length) in each replication. Recommended package of practices was followed to raise a good crop. The mean values recorded for various traits in M_1 generation were used for further statistical analysis. The chlorophyll mutations were carefully scored based on M_2 plant basis. Such mutations were counted and recorded upto 15th day after sowing. They were classified according to method suggested by Gustafsson (1940). Mutagenic effectiveness is defined as a measure of frequency of mutation induced by a unit of mutagen while mutagenic efficiency is the proportion of mutation in relation to the other associated undesirable effects such as lethality injury and pollen sterility. The statistical analysis was carried out as per standard method of Analysis of variance for Randomized Block Design (Panse and Sukhatme, 1954).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Studies in M_2 generation:

Studies in M_2 generation:

Chlorophyll mutations:

The scoring of chlorophyll mutations in M_2 generation has been estimated as one of the most dependable indices for evaluation of genetic effects of mutagenic treatments. The mutagenic effect can be measured in both the terms of quantity and quality by studying the spectrum and the frequency of chlorophyll mutations. He postulated that the use of different mutagens under appropriately modifying treatment conditions could alter relative proportions of different types of mutations and enhance the mutations frequency. The results are shown in Table 1. The spectrum of chlorophyll mutation induced by mutagenic treatments was found to vary according to the dose and genotypes used. The albino, xantha, viridis, xanthaviridis and chlorina mutations were of common occurrence in both populations. The different mutagens used, differed significantly from each other for inducing chlorophyll mutations. However, combination treatments in both populations were found most effective in inducing chlorophyll mutations than gamma rays and ethyl methane sulphonate. All mutagens *viz.*, gamma rays, EMS and their combination induced maximum chlorophyll mutations namely albina, xantha, alboviridis, xanthaviridis and chlorophyll. Similar spectrum of chlorophyll mutations was also reported by Amarnath and Prasad (2000) and Cheng and Chandlee (1999). The frequency of chlorophyll mutation on M_2 family basis were found highest in 20kR+0.2%EMS, 0.4%EMS and 40kR gamma rays concentration in all the populations. Similar results were also reported by Usharani and Kumar (2015) and Goyal *et al.* (2019).

Viable mutations:

In the present study, the frequency of viable mutations expressed in M_2 generation was found to

Table 1: Spectrum of chlorophyll mutations in M_2 generation of sorghum genotypes

Genotype	Treatments	Chlorophyll mutants					Total
		Albina	Xantha	Chlorina	Viridis	Xanthviridis	
296-B	10 kR	-	2	3	3	1	9
	20 kR	2	3	2	3	1	11
	30 kR	2	2	4	5	-	13
	40 kR	3	4	5	4	-	15
	0.1 % EMS	-	2	3	2	-	8
	0.2 % EMS	1	2	2	4	1	10
	0.3 % EMS	2	3	2	3	2	12
	0.4% EMS	2	4	3	4	3	16
	10kR+0.1EMS	2	3	4	3	4	12
	10kR+0.2EMS	3	2	3	4	3	15
	20kR+0.1EMS	3	4	4	4	2	17
	20kR+0.2EMS	4	3	6	5	2	19
	Wet control	-	-	-	-	-	-
	Dry control	-	-	-	-	-	-
Parbhani Moti	10 kR	-	1	4	2	1	8
	20 kR	-	3	4	2	1	10
	30 kR	2	3	3	2	2	12
	40 kR	2	6	1	4	1	14
	0.1 %EMS	-	2	3	2	-	7
	0.2 %EMS	-	3	4	3	1	10
	0.3 %EMS	1	4	3	4	1	13
	0.4%EMS	3	5	4	3	-	15
	10kR+0.1EMS	-	2	1	5	2	11
	10kR+0.2EMS	1	4	5	3	1	14
	20kR+0.1EMS	2	3	4	5	2	16
	20kR+0.2EMS	3	5	5	4	1	18
	Wet control	-	-	-	-	-	-
	Dry control	-	-	-	-	-	-

increase in doses for both the mutagens. Frequency and spectrum of viable mutations were high in combination treatments than gamma rays and EMS. This is in contradiction to the earlier reports of Veenakumari (1994) who recorded a greater frequency and wider spectrum of viable mutations in populations treated with combination than gamma rays and chemical mutagens alone. However, superiority of combination treatments over radiations and chemical causing functional alterations in genes has been described by Makeen *et al*. (2013) in urd bean. The various types of viable mutations with altered plant habit were isolated in M_2 generation and important ones were confirmed in the M_3 generation of in both sorghum genotypes. Study on spectrum of viable mutations showed that number of viable mutations were observed for growth habit. Among the population, Parbhani Moti was able to express higher frequency and wider spectrum followed by 296 B. Similar finding have been reported by Veenakumari (1994), Amarnath and Prasad (2000); Wakode *et al*. (2000) this suggests that, mutation production and recovery of desirable mutations seems to be mostly governed by genotypic background of the material treated. The mutations confirmed in M_3 generation are described below.

Narrow leaf mutant :

The mutant isolated for leaf character in which the leaf size was reduced upto one third of the control. The leaf shape was found to be elongated with narrow blade. Similar kinds of mutants were also recorded earlier by Veenakumari (1994) in soybean.

Broad leaf mutant :

These mutants were having broad leaf size. The leaf area of these mutants was observed from 9 cm to 13 cm in 296 B and from 10 cm to 12.10 cm in Parbhani Moti, panicle bearing of these mutants was medium size. Similar type of mutants were also noted by Sharma (2005) and Pavadai *et al*. (2010).

White midrib mutant :

These mutants were isolated in maximum frequency in 0.3 per cent EMS in 296 Band 40kR and 10kR+ 0.2% EMS in Parbhani Moti populations. The reports are in agreement with Maheshwari *et al*. (2003).

Brown midrib mutant :

Characteristic feature of these mutants were brown midrib leaves was found with 0.2 per cent concentration of EMS.

Shrinkage leaf :

These mutants were high isolated in20kR+0.1% EMS of 296 B and 0.4% EMS and 10kR+0.1%EMS in Parbhani Moti populations. The size of these leaf mutant was great reduced. The leaf look like leaf mottling.

Dwarf mutant:

These mutants were having reduced plant height in 0.2% EMS and 20kR+0.2% EMS in Parbhani Moti. The insect attack was found very negligible on these mutants. Sharma (2005) has reported such type of mutants.

Sterile mutant:

Sterile mutants were more pronounced at higher doses and with less frequency in 296 B than Parbhani Moti. The characteristic feature of these mutants having more branches and luxuriant vegetative growth without converting to reproductive growth. Flowers on these mutant plants were found to be of rudimentary types. The number of spikelets and anthers in inflorescence were found to be minimum. Similar results were reported by Dhole (1999); Wakode *et al*. (2000) and Basavaraja (2002) in soybean these types of mutants.

Economic and other panicle mutants:

On the basis of earliness, small seeded and bold seedness the mutations are classified in to early and late maturing. Early maturity mutant was recorded in all the populations, matured in 16 to 18 days earlier than control and were of short stature. Late maturing recorded 15 to 18 days delayed maturity as compared to control. High yielding mutants were medium tall, healthy, with increase grain bearing panicle and grain yield. Some mutants were long and small panicle, high branching, small and bold seeded, verigated seed shape and colour isolated in all the populations in M_2 generation. Similar results reported by Makeen *et al*. (2013) and Bhatnagar and Tiwari (1997).

Mutagenic effectiveness and efficiency:

The usefulness of any mutagen in plant breeding programme depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. Efficient mutagenesis is the production of desirable changes with minimum undesirable changes. The results are shown in Table 2. The efficiency of a mutagenic agent is a complex question as it not only depends on the agent with the material and its applicability to the biological system but also on the degree to which physiological damage, chromosomal aberrations and sterility is induced by the mutagens. In the present investigation, in general lower doses of both and also its combination the mutagens were effective in producing chlorophyll mutations indicating that increase in chlorophyll mutations frequency was not proportional to the increase in dose. Among the three mutagens tried, combination was highly effective as compared to gamma rays and EMS. This is in conformity with the reports of Suthakar and Mullainathan (2015) in sorghum. The estimates of mutagenic efficiency in general indicated that the efficiency of combination was more as compared to gamma rays in all two population.

A maximum mutation frequency (2.96% and 2.05%) was recorded in 296 B populations with 20kr+0.2% EMS and 0.4 per cent of EMS treatment, respectively. The results are shown in Table 3. Whereas in case of Parbhani Moti was highest mutation frequency observed with 20kR+0.2% EMS and 0.4 per cent EMS mutagenic treatment. In general, the chlorophyll mutation frequency was higher in 296 B populations for both the mutagens. Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen while mutagenic efficiency is the proportion of mutations in relation to other associated undesirable changes such as lethality and sterility induced by a mutagen.

Table 2: Mutagenic effectiveness and efficiency of mutagens in inducing chlorophyll mutations M_2 generation of sorghum genotypes

Genotype	Treatments	Mutagenic effectiveness (%)	Mutagenic efficiency (%)
296 B	10 Kr	8.80	3.52
	20 kR	5.70	5.70
	30 kR	4.86	4.17
	40 kR	4.92	4.92
	0.1 % EMS	9.50	3.80
	0.2 % EMS	6.37	4.08
	0.3 % EMS	5.87	3.80
	0.4% EMS	6.40	5.85
	10kR+0.1EMS	15.25	5.30
	10kR+0.2EMS	10.75	8.19
	20kR+0.1EMS	12.18	7.50
	20kR+0.2EMS	9.25	6.57
	Wet control	-	-
	Dry control	-	-
Genotype	Treatments	Mutagenic effectiveness (%)	Mutagenic efficiency (%)
Parbhani Moti	10 kR	7.60	3.16
	20 kR	5.15	3.21
	30 kR	4.70	3.52
	40kR	4.52	4.11
	0.1 % EMS	8.62	2.87
	0.2 % EMS	6.75	3.27
	0.3 % EMS	6.20	3.82
	0.4% EMS	6.25	4.76
	10kR+0.1EMS	14.37	4.10
	10kR+0.2EMS	10.50	5.09
	20kR+0.1EMS	13.12	5.38
	20kR+0.2EMS	8.90	6.47
	Wet control	-	-
	Dry control	-	-

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