

Chlorophyll and Morphological Mutations Induced by Chemical Mutagens EMS, DES in (*Setaria italica(L.)Beauv.)Var .CO(Te)7* in M₂ Generation

I. Anittha¹, L. Mullainathan^{2*}

^{1,2}Department of Botany, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India

* Corresponding Author: vishnumullai@gmail.com Phone No. +91 9965553849

Available online at: www.isroset.org

Received: 19/Nov/2018, Accepted: 16/Dec/2018, Online: 31/Dec/2018

Abstract- The assessment of mutagen concentration is typically evaluated by the morphological and chlorophyll mutants. *Setariaitalica* var. CO(Te)7 Fox tail millet seeds were treated with the chemical mutagens namely EMS in the concentration 20, 30,40 mM and DES in the concentration 30, 40 and 50 mM. The present investigation reveals chlorophyll mutants like Albino, Chlorina, Xantha , Straita and various morphological mutants like Tall plant, Dwarf plant, Long spike, Short spike, multiple spike, Long awn spike, Thumb branch spike, Bold seed, Sterile spike, Tip sterile spike, Anthocyanin pigment in spike, Helical spike, Brachytic, Tiller, Zebra striped, Early and late maturity mutants were observed in M₂ generation. The primary objective of the research work was to increase the availability of our traditional millet variety Tenai which was a balanced nutricereale and can be cultivated in large scale with the superior quality .They can be screened and enhanced through mutation breeding for enormous yield. The effect of EMS shows more chlorophyll and morphological mutants than DES in fox tail millet.

Keywords: Chlorophyll mutants, Morphological mutants, EMS and DES, Setaria italica, M2 Generation

I. INTRODUCTION

Setaria italica is a member of sub family panicoideaceae and is a grass known to be the important food crop with a good background of nutritional value with rich source of protein. fiber, minerals and vitamins[1]. This nutricereale is the crop of poor. They are considered to have short life span and was suitable to be a second food and fodder crop after wheat and barley. Furthermore, it is an agronomical important crop[2,3]. In addition the health promising quality is found to be additional back up energy quality for Setaria italica. The fox tail millet is considered as a good therapeutic diet. Such valuable crop need to be cultivated in large scale production with better characteristics by using improved methodology like mutation breeding. The Setaria italica is an ecofriendly plant suitable for vulnerable ecosystem, utilized as energy rich feed for love birds, grained flakes are available as quick food cereal, malt based product and for the preparation of puttu .The crop is of therapeutic potential in low glycemic index [4], reducing colon cancer [5]. It has an short life span [6] alert the productivity in limited duration in yield. Records of implementation as Bioenergy crop through mutation breeding and genetic manipulation is a criterion for commercial usage [7]. Novel protein in stress tolerance identification [8] was highlighted.

Mutation act as a raw material in indicating the genetic variability for crop. The genetic modulation established through mutation breeding is used to improve the economic value in the crop and resolve themselves as genetic marker in upcoming field of plant breeding technology [9]. Among the various plants breeding technique induced mutation using chemical mutagens play a great achieving role in current days in improving economic value of the crop with genetic modification. The efficient tool considered for analyzing the morphological difference, micro and macro mutants is chlorophyll and morphological mutation in M₂ generation and macro mutants were recorded in grain density cm⁻¹ and yield plantcm⁻¹ in variety of finger millet [10].Which correlated the work for exclusive improvement of the crop Setaria italica. The occurrence of different chlorophyll mutation and morphological changes have been reported in 30mM concentration of EMS, 40 mM in DES. In various aspects a meager or large change over in the phenotype will produce more significant modification in the plant .The viable and desirable phenotypical changes exhibited through induced macro mutant [11] mediate as raw material for genetic modification which can be applicable through mutation breeding. The present investigation has been shown broad spectrum of mutants in M₂ generation of Setaria italic var .CO(Te)7[12]. The previous work initiated with M₁ generation prevailed in decline phase due to the

stress factor of chemical mutagen which later progressed in M_2 generation with recurrent mutants [13].

II. MATERIALS AND METHODS

In this research the seeds of (*Setaria italica* (L.)Beauv.)var. CO(Te)7 collected from Tamil Nadu Agriculture University Coimbatore have been selected to induce variation.

1. Chemical mutagen treatment:

Ethyl methane sulphonate (EMS) in the concentration 20, 30,40mM and Diethyl sulphate (DES) in the concentration 30,40and 50mM.

2. Experimental design:

The seeds of fox tail millet harvested from M_1 generation is proceeded to M_2 generation in complete Randomized block design. The field was fertilized with organic manure and better agricultural practice was performed.

3. Growth strategies:

Seeds of M_2 generation were germinated from respective concentrations of EMS and DES on 15^{th} day the chlorophyll and morphological mutants were isolated.

4. Isolation of mutants:

In M_2 generation from EMS, DES concentration at 15th day the following chlorophyll mutants such as chlorina, Albino, xantha, varigata and viridis various morphological mutants were observed like long, short spike, Tall, Dwarf, early and late maturity of seed and sterile tip. The mutation frequency was calculated with M_2 seedlings.The chlorophyll and morphological mutants were identified and sorted out based on their classification (Table 1).

III. RESULT AND DISCUSSION

Micro and Macro mutants play an important role to assess the concentration of mutagens, almost all the mutagenic treatment showed different degree of mutants in respective concentration. Among the mutagens EMS produced more number of chlorophyll and morphological mutants than DES. Albino is pale dull white in colour and are relatively shorter than normal and lifespan of the seedling is within 10 - 20 days[14], viridis is with whitish tips of leaves leads to lethal effect, xantha is straw coloured yellow leaves, striata with normal growth mutants of EMS in M_2 generation [15]. The type of chlorophyll mutation in pearl millet, black gram, (Mungbean) [16], chickpea [17], cow pea [18], the variation found to be controlled by the genes present on several chromosome which could be located near to the centrosome and proximal segment of chromosome [19]. The present investigation albino, chlorina, xantha mutants in M₂ plants is based on the intensity of pigmentation at seedling stage in Setaria italica, var.CO(Te)7 and the above result was correlated with the present studies (Figure 1). The morphological mutants observed are long and short spike,

tall and dwarf mutant, Barren stalk, Brittle stalk, Tiller, Tip sterility, Bushy mutant, Bold seed, late and early maturity [20]. The various chlorophyll mutants in *Setaria italica* was found to be more in response and efficient in EMS than DES. The maximum frequency of mutants was observed in 30mM(28.4%) concentration in EMS and 40 mM (25.3%) in DES, EMS was found to the more effective mutagen as reported in rice [21,22,23]. The minimum mutations were observed in lesser concentrations like 20mM (13.4%) in EMS, and 30 mM (17.2%) in DES, greater number of variations was obtained as the concentration increased similar reports was proved in *pennisetum typhoides*[24].

IV. CONCLUSION

In this investigation the frequency of chlorophyll and morphological mutants found in *Setaria italica* var.CO(Te)7 act as an indicator in analyzing the response of the chemical mutagen EMS, DES. The occurrence of mutation in the pigmentation gene led to various chlorophyll mutants. The morphological mutants obtained were essential and useful in breeding technology to obtain suitable gene coding character for more yield in the foxtail millet. The frequency of variants obtained in M_2 generation is a reflection factors observed as a result of mutagens which induced changes during M_1 generation. Thus the mutants obtained can be applied in the field of plant improvement and their varieties can be applicable in developing beneficial productive products in current years through mutation breeding in crop improvement.

REFERENCES

- X. Diao, J. Schnable, J. Bennetzen, J. Li. "Initiation of Setaria as a model plant", Frontiers of Agricultural Science and Engineering, Vol. 1, Issue., 1 pp. 16-20, 2014.
- M. Brink PROTA "Plant Resources of Tropical Africa / Ressourcesvegetales de' Afriquetropicale". Wageningen, Netherlands. 2006.
- [3] L. Mlcochova, O. Chloupek, R. Uptmoor, F. Ordon and W. Friedt, "Molecular analysis of the barley cv. 'Valticky' and its X-ray derived semi dwarf mutant 'Diamant'', Plant Breeding, Vol. 123. pp. 421-427, 2004.
- [4] M. V. Jali, M. Y. Kamatar, S. M. Jali, M. B. Hiremath and M. B Naik, "Efficacy of value added foxtail millet therapeutic food in the management of Diabetes and Dyslipidamea in type 2 diabetic patients", Recent Research in Science and Technology, Vol. 4, pp. 03-04, 2012.
- [5] S. Shan, J. Shi, Z. Li, H. Gao, T. Shi, Z. Li, "Targeted anti-colon cancer activities of a millet bran-derived peroxidase were mediated by elevated ROS generation". Food and Function, Vol. 6, pp. 2331-2338, 2015.
- [6] A. N. Doust, E. A. Kellogg, K. M. Devos, J. L. Bennetzen, "Foxtail millet: A sequence-driven grass model system". Plant Physiology, Vol. 149, pp. 137–14, 2009.
- [7] B. Christopher, P. Antony, Martin, P. L Christopher, "The application of Fourier transform mid-infrared (FT IR) spectroscopy to identify variation in cell wall composition of Setariaitalica ecotypes", Journal of Integrative Agriculture, Vol. 16, Issue., 6, pp. 1256–1267, 2017.

- [8] A. K. Mistra, S. Puranik, R.P. Bahadur and M. Prasad, "The DNAbinding activity of an AP2 protein is involved in transcriptional regulation of a stress-responsive gene, SiWD40, in foxtail millet" Genomics, Vol.100, pp. 252–263, 2012.
- [9] V. P. Singh and R. D. S. Yadav. "Induced mutations for quantitative and qualitative traits in green gram (Vigna radiate L. Wilczwk.) cv. T44", Journal of Genetics and Breeding, Vol. 45, pp. 1-6, 1991.
- [10] A.R. Ambavane, S.V Sawardekar, S.A. Sawantdesai, N.B. Gokhale, "Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (Eleusinecoracana L. Gaertn)", Journal of Radiation Research and Applied Sciences, Vol. 8, 120-125, 2015.
- [11] S.K. Sharma and B. Sharma, "Induced chlorophyll mutation in *Lentil*". Indian Journal of Genetics, Vol. 41, pp. 328-333, 1981.
- [12] A. Gustafsson, The mutation system of the chlorophyll apparatus-Lund- UnivArasks, N. P. Adv. Vol.36, pp. 1- 40, 1940.
- [13] I. Anittha and L.Mullainathan, "Mutagenic effect of EMS and DES on tenai (*Setaria italica*) in M₁ generation" Journal of phytology, Vol. 10, pp.06-08, 2018
- [14] I. S. Solanki, "Isolation of Macromutations and mutagen effectiveness and efficiency in lentil (Lens culinarisMedik.)" Indian Journal of Genetics, Vol. 4, Issue., 10, pp. 264-268, 2005.
- [15] D. Arulbalachandran and L. Mullainathan, "Chlorophyll and morphological mutants of Black gram (Vignamungo (L.) hepper) derived by gamma rays and EMS" Journal of Phytology. Vol. 1, Issue., 4, pp. 236–241, 2009.
- [16] V.P. Singh, and P. Singh, "Cytomorphological changes induced in breadwheat following seed treatment with pesticides and

mutagenic chemicals". Indian Journal of Genetics, Vol. 3, pp. 341-349, 1989.

- [17] A. A. Wani and M. Anis, "Spectrum and frequency of chlorophyll mutations induced by gamma rays and EMS in Cicerarietinum L", Journal of Cytology and Genetics, Vol. 5, pp. 143-147, 2004.
- [18] M.S. Hood. R. S. Dhillon and K. S. Bangarwa, "Albinism in jojoba (Simmondsiachinensis)", National Journal of Plant Improvement, Vol. 1, pp. 69-70, 2004.
- [19] R. E. Chandola, M. P. Bhatnagar, I. Totuka, "Chlorophyll mutants of Pennisetumtyphoides (Bajra) induced by gamma rays", Current Science, Vol. 32, pp. 179–180, 1963.
- [20] S. K Sharma and B. Sharma, "Pattern of induced macro and micro •i. mutations with gamma rays and NMU in lentil". Environment Experimental Botany, Vol. 24, Issue., 4, pp. 343-351, 1984.
- [21] M.L.H. Kaul and A. K. Bhan, "Mutagenic Effectiveness and Efficiency of EMS, DES and Gamma-rays in Rice", Theory of Applied Genetics, Vol. 50, pp. 241-246, 1977.
- [22] C.S. Reddy and J.D. Smith, "Mutagenic Effectiveness and Efficiency of Hydrazine and Ethyl Methane sulphonate in Sorghum Bicolor", Indian journal of Genetics, Vol. 44, pp. 49-54, 1984.
- [23] M.S. Swaminathan, M.S Naik, M.S. Kaul, and A. Austin, "Improving plant protein by nuclear techniques. In; Rice breeding with induced mutations", IAEA, Vienna, Vol. 165, 1970.
- [24] K. Ambli and L. Mullainathan, "Chlorophyll and morphological mutants of Pearl millet (Pennisetumtphoides(Burn.) stapf. Var. CO(cu)9", European Journal of Experimental Biology, Vol. 5 Issue., 3, pp. 72-77, 2015.

MUTAGEN	EMS			DES		
Concentration	20mM	30mM	40mM	30mM	40mM	50mM
Number of plants studied	290	284	263	278	256	239
Xantha	2	5	6	5	6	4
Albino	1	4	3	1	1	1
Viridis	2	2	3	2	1	2
Straita	3	7	4	2	3	2
Tall	1	6	3	2	2	3
Dwarf	1	6	3	3	2	2
Bushy	2	6	4	2	4	2
Brittle	1	3	2	1	2	2
Tiller	2	5	3	2	3	2
Narrow leaf	3	8	2	3	2	2
Zebra striped	1	2	3	1	2	1
Brevis	2	3	2	2	1	1
Barren stalk	-	4	1	1	2	2
Sterile spike	1	2	2	1	1	2

Table 1. Frequency of chlorophyll and viable mutants in M₂ generation

Int. J. Sci. Res. in Biological Sciences

Nano dwarf	-	1	2	1	2	2
Crinkly leaf	1	2	2	1	2	2
Thumb branch	1	2	2	-	1	1
Internodal spike	-	2	2	1	2	2
Multiple spike	1	2	2	2	4	2
Brachytic	1	2	3	2	3	3
Early maturity	4	6	3	4	2	2
Late maturity	3	5	3	1	4	2
Short spike	2	2	3	2	3	2
Long spike	1	7	2	2	3	2
Bold seed	1	5	2	1	5	2
Small seed	2	3	2	3	2	1
Total	39	100	68	48	65	52
Percentage of mutation frequency	13.4	28.4	25.8	17.2	25.3	21.75



Albino(40mM) EMS



Xantha 30 (mM) DES



Sterile tip 30 (mM) DES



Thumb branch spike 40 mM EMS



Long awn 50 (mM) DES

Int. J. Sci. Res. in Biological Sciences

Vol. 5(6), Dec. 2018, ISSN: 2347-7520



Helical spiklet 30(mM) EMS



Multiple spike 30(mM) DES



Tiller 30(mM) DES



Anthocyanin Pigment 40(mM) EMS



Bushy mutant 50(mM) DMS

Figure 1. Chlorophyll and Morphological Mutants



Long spike 40 mM DES