Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 3, Issue, 11, pp.037-040, October, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STUDIES ON INDUCED CHEMICAL MUTAGENESIS IN MAIZE (*Zea mays* (L.)

Gnanamurthy, S., *Dhanavel, D. and Girija, M

Division of Cytogenetics and Plant Breeding, Department of Botany, Annamalai University, Annamalainagar-608002, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 5th July, 2011
Received in revised form
8th August, 2011
Accepted 17th September, 2011
Published online 15th October, 2011

Key words:
EMS, DES, Sodium azide, Maize, Chlorophyll, Viable, Effectiveness and efficiency.

ABSTRACT

The present study was undertaken in maize *Zea mays* (L.) var. JKMH-1001. Seeds were treated with different concentration of ethyl methane sulphonate, diethyl sulphate and sodium azide in treated level for 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM which induced chlorophyll mutation and morphological mutation in maize. Four different types of chlorophyll mutants' viz., Albino, Viridis, Xantha and Chlorina were identified in the treated population. The morphological mutation consisted of tall, dwarf, early maturity, late maturity, triangular leaf, bold size seed, long ear, short ear and male sterility in all the level. EMS was found to be more effective than DES and SA in producing chlorophyll and viable mutants on M_1 and M_2 plant basis as well as efficient on lethality and injury bases. While the mutagenic effectiveness and efficiency generally decreased and increased in higher doses of mutagens.

INTRODUCTION

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetics, biotechnology and molecular biology etc. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement Acharya *et al.* (2007) and an efficient means supplementing existing germplasm for cultivar improvement in breeding program's Dubinin, (1961). Mutation is a sudden heritable change in organism generally the structural change in gene. The term mutation was first introduced by Hugo de Vries in (1901). Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al*., 2002). These mutations provide beneficial variation for practical plant breeding purpose. During the fast seven decades, more than 2252 mutant varieties have been officially released in world (Maluszynski *et al.,* 2000). Corn belongs to the grass family and is a cross-pollinated, monoecious plant in which the male and female flowers are located in different inflorescences on the same stalk. Maize is a tall, annual grass with overlapping sheaths and broad conspicuously distichous blades. Maize is chiefly used as food for man and livestock. The grain is very nutritious, with a high percentage of carbohydrates, fats and proteins. Not only is the grain valuable as a stock feed, but the plant as a whole forms an important fodder crop. The immature cobs are largely eaten after roasting. The grains are also used in making corn starch and industrial alcohol. The glucose is also manufactured from the grain. The corn oil is prepared which is used for soap making, lubrication and as salad oil. Corn flakes make a good breakfast food. In general micro mutants play an important role in plant breeding as it may lead to the evolution of new genotypes Swaminathan (1996). Chlorophyll mutants are extremely used to judge the optimum dose/concentration of which maximum mutations achieved. The effectiveness of mutagenesis as a measure of frequency of mutation induced by a unit dose of mutagens where as efficiency gives on idea of the proportion of mutations relation to other associated undesirable biological effects such as injury, lethality and sterility induced by mutagen Konzak *et al*. (1965). Selection of efficient and effective mutant is very essential to recover high frequency of desirable mutations for breeding program Gautam *et al*. (1998). Therefore, the present investigation was made in chlorophyll, On the frequency of chlorophyll and viable mutations of EMS, DES and sodium azide on maize.

MATERIALS AND METHODS

The dry and dormant seeds of maize were collected from Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu. The mutation treatment in 200 well filled seeds were selected and presoaked in double distilled water for six hours. The excess moisture in the seeds was removed by pressing in the fold of filter paper. They were treated with required mM concentrations of EMS, DES and sodium azide solution for six hours at room temperature (26±2°C) with intermittent shaking. After that the

***Corresponding author:** *gnanabot@gmail.com*

seeds were thoroughly washed with running tap water for 8-10 times. Non-treated dry seeds were pre-soaked in distilled water for 6 hours and then used as control. The mutagenic treatment seeds were immediately sown in the field along with the control in a randomized block design with three replications. A total of 200 seeds were sown in each treatment. All the treatments including the control were raised adopting a spacing of 45 cm in between rows and 20 cm in between plants. All the recommended cultural measures namely, irrigation, weeding and plant production methods were carried out during the growth period of the crop. From the remaining treated seeds, 100 seeds were placed in the moist germination paper and replicated twice for the purpose of laboratory analysis. The seeds harvested from M_1 generation were taken from individual treatments and were used to raise M_2 generation. The M_2 generation was grown in triplicate in randomized block design. 200 plants were maintained for each treatment in each replication. Biometric observations were recorded and individual plant data's were calculated using statistical analysis. The M_2 seedlings were screened from 15th to 20th day to record the various chlorophyll mutants was periodically the classification and identification of the chlorophyll mutant was done. Based on the nomenclature adopted by Gustafsson (1940), the mutation frequency was estimated on M_2 seedling basis.

mutation in relation to undesirable changes like lethality and injury. The effectiveness and efficiency of the mutagens namely, EMS, DES and sodium azide were worked out by using the formulae suggested by Konzak *et al*. (1965).

Mutagenic effectiveness = $M \times 100 / C \times T$
Mutagenic efficiency (Lethal) = $M \times 100 / L$
Mutagenic efficiency (Injury) = $M \times 100 / I$

Where

M: Mutation frequency for 100M_2 plants
T: Period of treatment with chemical mutagen in hours
C: Concentration of mutagen in mM in percent
L: Percentage of lethality or survival reduction
I: Percentage of injury or reduction in seedling size

RESULT AND DISCUSSION

The present study was undertaken in maize to study the effect of chemical mutagens namely, EMS, DES and sodium azide through the biological changes in M_1 generation and the frequency and spectrum of chlorophyll and viable mutants in M_2 generation. It was also aimed to find out the economic potentialities of the viable mutants and the nature of induced

Table 1. Frequency of chlorophyll and Viable mutants in maize

Mutagens (Conc. mM)	EMS (mM)			DES (mM)			SA (mM)		
	40	50	60	30	40	50	30	40	50
No. of plants studied	137	140	135	141	138	136	140	142	139
Chlorophyll mutants									
Albino	1	2	1	-	1	1	1	1	1
Viridis	2	1	1	1	2	1	-	2	-
Xantha	1	2	2	1	1	1	-	1	1
Chlorina	1	1	1	1	2	2	1	1	1
Viable mutants									
Tall	1	2	1	-	1	-	1	-	2
Dwarf	2	2	2	1	1	1	1	2	-
Early maturity	1	1	1	1	1	1	1	1	1
Late maturity	1	4	2	1	1	-	-	1	1
Triangular leaf	1	2	2	2	1	1	1	2	2
Long ear	1	2	1	2	2	2	1	1	-
Short ear	2	4	2	1	1	1	1	2	2
Male sterility	2	2	1	-	1	1	1	1	1
Total	17	26	19	12	16	14	10	15	13
Frequency	12.40	18.57	14.07	8.51	11.59	10.29	7.14	10.56	9.35

Table 2. Mutagenic Effectiveness and Efficiency in Maize

Treatment (Conc. mM)		Survival Reduction (L) 30th day	Height Reduction (I) 30th day	Mutation Frequency	Effectiveness $\frac{M \times 100}{C \times T}$	Efficiency $\frac{M \times 100}{L}$	Efficiency $\frac{M \times 100}{I}$
EMS	40	26.68	21.94	12.40	10.33	46.47	56.51
(Conc. mM)	50	47.05	26.51	18.57	12.38	39.46	70.04
	60	58.04	33.14	14.07	7.81	24.24	42.45
DES	30	32.87	17.80	8.51	9.45	25.88	47.80
(Conc. mM)	40	51.07	22.46	11.59	9.65	22.69	51.60
	50	64.29	30.21	10.29	6.86	16.00	34.06
SA	30	31.42	14.89	7.14	7.93	22.72	47.95
(Conc. mM)	40	49.29	20.08	10.56	8.80	21.42	52.58
	50	58.46	28.12	9.35	6.25	15.99	33.25

In the M_2 generation, morphological and physiological mutants observed were classified based on the stature, duration and leaf shape. Mutation frequency was estimated on M_2 plant basis. Mutagenic effectiveness is a measure of the frequency of mutation induced by unit mutagen, where as mutagenic efficiency gives an indication of the proportion of variability in the quantitative traits in M_2 generation. In all, the parameters were reduced in M_1 generation at maturity time. Jana (1964) reported that the effect of mutagen and dry seeds of *Phaseolus mungo* showed reduction in growth. Fehr (1987) reported that differences exist among the species and among the genotype within a species for sensitivity in mutagen treatments. The growth and other quantitative traits proportionately decreased with increased concentration of chemical mutagens.

Chlorophyll mutants

The frequency of chlorophyll mutants in M_2 generation is mainly used as a dependable measure of genetic effects in mutagens Nilan and Konzak, 1961. The mutation frequency showed a decrease with increase in the concentration of mutagens. In the present investigation, the spectrum of chlorophyll mutant's *viz.,* albino, chlorina, viridis and xantha were observed in all mutagenic treatments.

Albino

These seedlings were characterized by their dull white color and were devoid of chlorophyll, carotenoid and other pigments. Albino seedlings are smaller in height and survive to a maximum of 20 days after germination and then die.

Viridis

The seedlings are dark green in the early stages of development and turn normal green in the later stages. The mutants produce normal looking flowers and also set seeds.

Xantha

Colors of the mutants vary from deep yellow to yellowish white. Growth of mutants is retarded and most of them die within 17 to 20 days after emergence.

Chlorina

Normally chlorine mutants do not survive. These mutant seedlings have light yellowish/ yellowish green leaves and culm with yellowish cobs. The mutants breed true for the altered characters. Similar observations were made by Girija and Dhanavel (2009) in cowpea; Thilagavathi and Mullainathan (2009) and Arulbalachandran (2006) in black gram; Solanki (2005) in lentil; Jayakumar and Selvaraj (2003) in sunflower; Pavadai (2006) in soybean; Mensah and Obadoni (2007) in *Arachis hypogaea.*

Viable mutants

Gaul (1964) classified viable mutations as macro and micro mutations, while Swaminathan, (1964) grouped them as macro mutations are systematic mutations. The mutational event may be accompanied by a large or small change in phenotype. Such changes have the highest significance in plant breeding and have been stressed by Sigurbjornsson, (1972). Some of the viable mutants were observed in M_2 generation with different concentration of mutagen. Increase the concentration of EMS, DES and sodium azide an increase in the number of viable mutants were realized up to certain level was 50mM of EMS produced more number of viable mutants than DES and sodium azide (Table 1). Tall and dwarf mutants were observed in different mutagenic treatments. The maximum numbers of mutants were recorded at 50mM of EMS. Similar results were observed by Thilagavathi (2007) in black gram, Yadava *et al.,* (2003) in kodo-millet, Pavadai (2006) in soybean, and Girija (2008) in cowpea. Physiological mutants such as early and late maturity were observed in all the mutagenic treatments. The maximum numbers of early and late maturity mutant were observed at 50mM of EMS. Early maturity mutants were reported by Kumar and Dubey, (1998) in *Lathyrus sativus,* Yadava *et al.* (2003) in Kodo-millet, Sasi (2004) in bhendi and Pavadai (2006) in soybean.

Effectiveness

The mutagenic effectiveness and efficiency were estimated on the basis of chlorophyll and viable mutants. In comparing EMS, DES and sodium azide mutagens reduction in plant survival and plant height were observed (Table -2). In the present study, EMS was found to be more effective and efficient than DES and sodium azide. While the mutagenic effectiveness and efficiency generally decreased with increase in the higher concentration of mutagens up to a certain level. EMS was found to be more effective than DES and sodium azide in inducing mutation. The maximum mutagenic effectiveness was observed at 50mM of EMS (12.38) while the minimum was observed at 50mM of sodium azide (6.25). Similar results were recorded by Solanki (2005) in lentil; Sassikumar *et al*. (2003) in Lima bean, Thilagavathi and Mullainathan (2009) and Arulbalachandran (2006) in black gram.

Efficiency

The mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen Konzak *et al.* (1965). The mutagenic efficiency was worked out based on injury and lethality. On the basis of injury, the maximum mutagenic efficiency was observed at 50 mM of EMS (70.04) and the minimum was observed at 50mM of sodium azide (33.25). On the basis of lethality, the highest mutagenic efficiency was recorded at 50mM of EMS (39.46), while the lowest mutagenic efficiency was observed at 50 mM of sodium azide (15.99). Generally, 50mM of EMS was found to be highly efficient for induced chlorophyll and viable mutants. Therefore, the present study revealed that EMS is highly efficient than DES and sodium azide. Similar results were recorded by Deepalakshmi and Anandakumar (2003) and Sharma *et al.,* (2005) in urdbean; Jabee and Ansari (2005) in chickpea; Girija and Dhanavel (2009) in cowpea.

Conclusion

In the present study revealed that the effectiveness and efficiency of EMS, DES and sodium azide. Among these chemical mutagens, EMS was more effective particularly in 50 mM concentration inducing chlorophyll and viable mutants than DES and sodium azide. Therefore, morphological mutants induced in the present study induced in agronomical desirable traits which may be possibly utilized in future breeding program.

Acknowledgement

The authors are thankful to the Head of the Department of Botany and authorities of Annamalai University for providing the necessary facilities and extend our heartful thanks to University Grants Commission, New Delhi for their financial assistance and for having provided the necessary facilities to carry out this work.

REFERENCES

Acharya SN, Thomas JE, Basu SK. 2007. Improvement in the medicinal and nutritional properties of fenugreek (*Trigonella foenum-graecum* L.). In: Acharya SN, Thomas JE (eds) Advances in medicinal plant research, Research Signpost, Trivandrum, Kerala, India.

Arulbalachandran, D. 2006. Effect of physical and chemical mutagenesis in black gram (*Vigna mungo* (L.) Hepper.Var.VPN-1). Ph.D. Thesis, Annamalai University, Annamalainagar, Tamil Nadu.

Deepalakshmi, A.J. and C.R. Anandakumar, 2003. Efficiency and effectiveness of physical and chemical mutagens in urdbean (*Vigna mungo* (L.) Hepper). *Madras Agric. J.,* 90(7-9): 485-489.

Dubinin NP. 1961. Problems of radiation genetics. Oliver & Boyd, London.

Fehr, L. 1987. Genetics of Vigna. P. 311-394. In. J. Janick (ed). Horti culture Review. A VI, Westport, Connecticut.

Gaul, H., 1964. Mutation in plant breeding. *Rad. Bot.,* 4: 155-232.

Gautam, A.S., K.C. Sood and R.K. Mittal, 1998. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in rajmash (*Phaseolus vulgaris* L.). *Legume Res.,* 21(3&4): 217-220.

Girija,M., 2008. Studies on induced mutagenesis in cowpea (*Vigna unguiculata* (L.) Walp) M.Phil. Thesis, Annamalai University, Annamalainagar, Tamil Nadu.

Girija, M. and D. Dhanavel, 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatment in cowpea (*Vigna unguiculata* L. Walp). *Glo. J. Mol. Sci.,* 4 (2): 68-75.

Gustafsson, A., 1940. The mutation system of the chlorophyll apparatus. Lond Univ. Arsskr., 36: 1-40.

Hugo de Vries, J. 1901. Study on mutation and its use in various aspects. Journal of the Agricultural association of China No. 140, 22-29 (Ench: 9 ref). Dep. Agron. Nat., Chung- Hsing Univ., Taichung, Taiwan.

Jabee, F. and M.Y.K. Ansari, 2005. Mutagenic effectiveness and efficiency of hydrazine sulphate (HS) in inducing cytomorphological mutation in *Cicer arietinum* L. var. K. 850. *J .Cytol. Genet.,* 6(2): 161-166.

Jana, M.K. 1964. Effects of X-ray and neutron irradiation on seeds of *Phaseolus mungo* L. Genetia Agraria, XVIII (4): 617-628.

Jayakumar, S. and R. Selvaraj, 2003. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in sunflower (*Helianthus annuus* L.). *Madras Agric. J.,* 90(7-9): 574-576.

Konzak, C.F., R.A. Nilan, J. Wagner and R.J. Foster, 1965. Efficient chemical mutagenesis. The use of induced mutations in plant breeding Rept. FAO/IAEA Tech. Meet. Rome.

Kumar, S. and D.K. Dubey, 1998. Induced morphological mutations in *Lathyrus sativus*. *J. Cytol. Genet.,* 33(2): 131-137.

Lee, Y. I., I.S. Lee and Y.P. Lim, 2002. Variation in sweet potato regenerates from gamma-rays irradiated embryogenic callus. *J Plant Biotech., 4*: 163-170.

Maluszynski, K.N., L.V. Zanten and B.S. Ahloowalia. 2000. Officially released mutant varieties. The FAO/IAEA Database. *Mut. Breed. Rev.,* 12: 1-12.

Mensah, J.K. and B. Obadoni, 2007. Effects of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.) *Afric. J Biotech.,* 6(6): 668-671.

Nilan, R.A., C.F. Fonzak J. Wagner & R.R. Legault. 1961. Effectiveness and efficiency of radiation for inducing genetic and cytogenetic changes. *Rad. Bot.,* 5: 71-89.

Pavadai, P. 2006. Studies on induced mutagenesis in soybean (*Glycine max* (L.) Merr.). Ph.D. Thesis, Annamalai University, Annamalainagar, Tamil Nadu.

Ramesh, D.V. and T.V.V. Seetharami Reddi, 2002. Induced morphological variability among three genotypes of rice. *J. Cytol. Genet.,* 3(2): 115-120.

Sasi, A., 2004. Effect of chemical mutagenesis in bhendi (*Abelmoschus esculentus* (L.) Moench.). M.Phil. Thesis, Annamalai University, Annamalainagar, Tamil Nadu.

Sassikumar, D., T. Nepolean and A. Gopalan, 2003. Effectiveness and efficiency of the mutagens gamma rays and ethyl methane sulphonate on lima bean (*Phaseolus lunatus* L.). *Indian J. Agric. Res.,* 37(2): 115-119.

Sharma, S.K., R. Sood and D.P. Pandey, 2005. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (*Vigna mungo* (L.) Hepper). *Indian J. Genet.,* 65(1): 20-22.

Sigurbjornsson, B., 1972. Breeding with natural and induced variability. Induced mutations and plant improvement, 3-5 IAEA, Vienna.

Solanki, I.S., 2005. Isolation of macromutations and mutagenic effectiveness and efficiency in lentil (*Lens culinaris* Medik.). *Indian J. Genet.,* 65(4): 264-268.

Swaminathan, M.S., 1964 A comparison of mutation induction in diploid and polyploidy. The use of induced mutation in plant breeding. Rad Bot.,5:619.

Swaminathan, M.S., 1996. Use of induced mutations. Indian Fmg., 16: 34-35.

Thilagavathi, C., 2007. Effect of Ethyl methane sulphonate and Diethyl sulphonate in black gram (*Vigna mungo* (L.) Hepper. M.Phil. Thesis, Annamalai University, Annamalainagar, Tamil Nadu.

Thilagavathi, C. and L. Mullainathan, 2009. Isolation of macro mutants and mutagenic effectiveness, efficiency in black gram (*Vigna mungo* (L.) Hepper). *Glo. J. Mol. Sci.,* 4 (2): 76-79.

Yadava, H.S., A.N. Tikle and D.V. Bhagat, 2003. Effect of induced mutation through gamma rays on growth and yield parameters of kodo-millet. *J. Soil and Crops,* 13(1): 25-28.

****