Indian Res. J. Genet. & Biotech. 9(1) : 111-119 (2017)

Mutagenesis for Oligogenic Traits with Gamma rays and EMSin Groundnut(*Arachishypogaea* L.)

S.K.BURGHATE[1], N.J. CHIKHALE[2], M.N. MISHRA[3] and A.M. MAHALLE[4]

[1] Department of Agril. Botany, ShriShivaji College of Horticulture, Amravati-440603 (MS)

[2] ShriShivaji Agriculture College, Amravati-444603 (MS)

[3] Department of Plant Breeding and Genetics, R.B.S. College, Bichpuri, Agra (UP)

[4] Gram Sevak Training Center, Amravati-444603 (MS)

(Received : December, 2016 : Revised : January, 2017; Accepted : January, 2017)

Abstract

Induced qualitative mutation in TAG-24 groundnut cultivar consisted of chlorophyll and viable mutants. The combination treatments exhibits wide spectrum and frequency on both M_1 family and M_2 plant basis for both. Increased in doses and concentrations of mutagens showed increased in spectrum and frequency. Chlorophyll mutants viz. albino, albo-viridis, xantha, xantha- viridis and viridis and viable mutants viz; plant type, leaf, growth habit, flowering, pod, kernel and economic mutation were observed. Desirable mutants like bold poded, early maturing, high yielding were also isolated.

(**Key Words** :TAG-24, Spectrum, Frequency, Gamma Rays and EMS)

INTRODUCTION

Mutation breeding has contributed to increase genetic resources and has a valuable tool for plant breeding. Genetic variations induced by mutation represent a more efficient source of genetic variability than gene pools conserved by nature (Brock, 1977). The advantage of mutation breeding is that it can be applied to altering specific characters in other wise good varieties by incorporating some useful changes such as earliness, high oil content and high yielding ability in a comparatively shorter time than the conventionsbreeding methods. So, the induced mutations supplement plant breeding method and confer specific improvements on a genotype

Corresponding author's e-mail :drsagar311@gmail.com
Published by Indian Society of Genetics, Biotechnology Research and Development,
5, E Biotech Bhawan, Nikhil Estate, Mugalia Road, Shastripuram, Sikandra, Agra 282007 Online management by www.isgbrd.co.in , www.irjgbt.org

without significantly altering its otherwise acceptable phenotype. Since the induction of mutation has been accepted as a useful tool in plant breeding, a systematic study of the induced mutagenesis in crop like groundnut appears to be essential specially in the light of the growing need for food feed, fuel and fertilizer.

Both radiations and chemical mutagens have been employed to generate the desired variability invarious crop species. Though their effect on quantitative characters, investigations involving chemical mutagens are mere in groundnut.Even these investigations were directed to assess the physical sensitivity of varieties in groundnut (Gregory,1956; Ashri and Goldin,1966;Shchori and Ashri,1970; Levy and Ashri,1975 and Levy et.al., 1979) Intensive studies on the effect of physical and chemical mutagens and their combinations inducing viable mutations in groundnut cultivars are required to derive the maximum benefits from these tools. Therefore, the present investigation was undertaken to study the effect of physical and chemical mutagenson induction of viable mutation.

MATERIALS AND METHODS

Two mutagens viz; gamma rays, etylmethanesulphonate and their combination treatments were administered. The breeder seeds of TAG-24 variety of groundnut were procured from Oilseed Research Unit, Dr.PDKV, Akola (MS) India and seeds of uniform size were treated with the details given in **Table-1**

Table - 1 Treatment details

Treatment code	Treatment details
Gamma rays	
T_1 – 200gy	Irradiation of seeds with 200 Gy Gamma-rays
T_2 – 300gy	Irradiation of seeds with 300 Gy Gamma-rays
T_3– 300gy	Irradiation of seeds with 400 Gy Gamma-rays
EMS	
T_4 – 0.05% EMS	Presoaking of seeds for 6 hrs. followed by 6hrs. soaking in 0.05% EMS
T_5 – 0.1% EMS	Presoaking of seeds for 6 hrs. followed by 6hrs. soaking in 0.1% EMS
T_6 – 0.2% EMS	Presoaking of seeds for 6 hrs. followed by 6 hrs. soaking in 0.2%EMS
Combinations	

T_7 – 200 Gy + 0.2% EMS	Irradiation of seeds with 200Gy, presoaking for 6 hrs. followed by 6 hrs. soaking in 0.2% EMS
T_8– 300 Gy + 0.1%EMS	Irradiation of seeds with 300 Gy, presoaking for 6 hrs. followed by 6 hrs. soaking in 0.1% EMS
T_9 – 400 Gy + 0.05% EMS	Irradiation of seeds with 400Gy, presoaking for 6 hrs. followed by 6hrs. soaking in 0.05% EMS
Control	
T_{10} – Dry	Control (Dry seeds)
T_{11}– Presoaked	Control (Seeds presoaked in distilled water only)

Hundred seeds of each treatment were sowned in augmented block design with spacing of 30 X 15cm. The M_1 plants were harvested on single plant basis. The seeds harvested fromrandomly selected 50 M_1 plantswere sown to raise M_2generation. It was expected that in each treatment should consisted 50 plants but due to lethality in some higher doses the number of plant progenies were obtained less than fifty. These all plant progenies were raised on progeny row basis along with two controls with 45 X 20 spacing.

The M_2 generation was examined up to 15^{th}day after germination for chlorophyll mutation mutations. The spectrum and frequency was estimated and expressed as percentage on both M_1 family and M_2 plant basis. The mutant and normal seedlings were counted separately to determine the segregation ratio i.e., percentage of mutants to total progenies. The chlorophyll mutations were classified according to the system proposed by Gustafsson (1940) and Blixt (1961).The viable mutants were observed periodically from seedling stage to maturity in M_2generation and its frequency and spectrum was also estimated as like chlorophyll mutants.

RESULT AND DISCUSSION

ChlorophyllMutations: The spectrum of chlorophyll mutation induced by mutagenic treatment was found to vary according to the mutagen doses or concentrations. The xantha-viridis and viridis mutations were of common occurrence in most of the mutagenic treatments. The different mutagenic treatments used, differed significantly from each other for inducing chlorophyll mutations. However, combination treatments of gamma rays with ethylmethanesulphonate were found most effective in inducing chlorophyll mutations and sole treatments of ethylmethanesulphonate were found least effective. Generally all the mutagenic treatment induced maximum chlorophyll mutations namely albina, albo-viridis, xantha, xantha-viridis, viridis and chlorina. Similar spectrum of chlorophyll mutations was also reported by Chopde (2009), in groundnut and Venkateshwarlu*et al.* (1978) in pigeonpea.

Table- 2 Frequency and spectrum of the chlorophyll mutations in M2 generation.

Treatments	Total No. of M1 families sown	No. of M1 families segregating	Total No. of M2 plants	Spectrum of chlorophyll mutations						Total No. Mutations	Mutation frequency	
				Albina	Albo ir-dis	Xantha	Xantha-viridis	Viridis	Chloroti-na		M1 Family basis	M2 plant basis
Gamma rays												
T_1 – 200Gy	50	3	728	0.41	0.14	-	-	0.27	-	0.82	6	0.11
T_2 – 300Gy	50	4	539	0.37		0.19	0.19	0.74	-	1.48	8	0.28
T_3 – 400Gy	32	2	384	-	0.26	-	0.52	-	0.26	1.04	6.25	0.27
EMS												
T_4 – 0.05% EMS	50	1	960	-	-	-	0.21	0.10	-	0.31	2	0.03
T_5 – 0.1% EMS	50	2	805	-	0.25	0.12	0.50	-	-	0.87	4	0.11
T_6 – 0.2% EMS	50	1	742	-	0.40	0.27	0.40	0.13	-	1.21	2	0.16
Combination												
T_7 – 200 Gy + 0.2% EMS	46	4	276	0.36	-	-	0.36	-	0.72	1.45	8.70	0.53
T_8 – 300 Gy + 0.1% EMS	24	2	131	-	-	0.76	-	0.76	-	1.53	8.33	1.17
T_9 – 400 Gy + 0.05% EMS	16	2	110	1.82	-	-	-	-	-	1.82	12.5	1.65
Control												
T_{10} – Dry	50	-	1120	-	-	-	-	-	-	-	-	-
T_{11} – Presoaked	50	-	1050	-	-	-	-	-	-	-	-	-

The mutation frequency on both M_1 family basis and M_2 plant basis was found maximum (12.5 percent on M_1 family basis and 1.65 percent on M_2 plant basis) in 400 Gy gamma rays + 0.05percent EMS. It was indicated from the results that increased doses or concentrations of the mutagens showed increased both frequency and spectrum of the chlorophyll mutations.

The differential response obtained in the present investigation to various mutagenic treatments could be due to the reaction of specific genes to mutagens, which could be responding differentially to physical, chemical mutagens or their combinations. It is also believed that these mutations seemed to be brought about by genes located on different chromosomes. Swaminathan (1965) has suggested that the genes controlling the chlorophyll characters may be located near the centromere and proximal system is responsible for the high incidence of chlorophyll mutations data from linkage analysis in barley (Robetson, 1963 and Nilan, 1964) and maize (Neuffer, 1966) and the chromosomes aberrations studies (Natrajan and Upadhyaya, 1964) have provided evidence in support of this view. Ramanathan and Rathinam (1983) have reported high frequency of chlorophyll mutations in M_2 generation

by combined dose of gamma rays and EMS. The more or less similar results were also reported by Chopde (2009), Venkatachalam*et al.* (1999), Levy and Ashri (1975), Shivasubramaniam (1978) and Patil and Bora (1963).

Viable Mutations: The various types of viable mutations with altered plant habit were isolated in M_2 generation and important ones were confirmed in M_3 generation. The frequency of macro mutations expressed on M_2 plant basis as well as M_1 family basis was found increased as the dose of mutagens increased. Similar results were reported in ground nut by Venkatachalam and Jayabalan (1997), Ramanathan and Rathinam (1983) and Ashri and Levy (1972).

Study of spectrum of viable mutations showed that numbers of viable mutations were induced for plant type, leaf modifications, growth habit, flowering, pod characters, seed and economic mutants. The mutations confirmed in M_3 generation.

The maximum mutation rate on both M_1 family basis and M_2 plant basis was recorded in 400 Gy gamma rays and combined dose of 400 Gy gamma rays + 0.05 percent EMS respectively. It was predicted that increased mutation rate responsible for increased doses or concentrations of the mutagens. The frequency of viable mutations was recorded higher under gamma ray treatment in comparison with ethyl methane sulphonate. Gregory (1968 found 11,502 visible mutations in M_2 out of 84, 213 plants following the treatment with 18.5 kR of X-rays.Ashri and Levy (1972) found that gamma rays gave a higher mutation rate than EMS in groundnut. In present study higher mutation on M_2 plant basis was obtained in combined treatment might be due to higher dose of 400 Gy gamma rays.

Table :3 Frequency and spectrum of macromutation in M2 generation.

Treatment	Total No. of M1 families sown	No. of M1 families segregating	Total No. of M2 plants	Plant type mutants				Leaf Modifications mutants						Growth habit Mutants		
				Dwarf	Tall	Bushy	Total	Oval leaves	Narrow leaves	Small leaves	5-6 leaves	Miniature leaves	Total	Erect	Spreading	Total
Gamma rays																
T_1 – 200Gy	50	2	728	0.41	0.1	-	0.54	-	-	-	-	-	-	-	-	-
T_2 – 300Gy	50	3	839	0.23	0.2	0.11	0.47	-	0.11	-	-	-	0.11	0.11	-	0.11
T_3 – 400Gy	32	5	384	1.04	0.3	0.26	1.56	0.26	-	0.26	-	-	0.52	-	0.26	0.26
EMS																
T_4 – 0.05% EMS	50	2	960	-	0.10	-	0.10	-	-	0.10	-	-	0.10	-	-	-
T_5 – 0.1% EMS	50	3	805	0.24	0.1	0.13	0.62	-		-	-	-	-	0.12	-	0.12
T_6 – 0.2% EMS	50	2	742	0.13	-	-	0.13	0.13	-	-	-	-	0.13	-	-	-
Combination																
T_7 – 200 Gy + 0.2% EMS	46	4	276	0.36	-	0.36	0.72	-	0.36	-	0.72	-	1.09	0.72	-	0.72
T_8 – 300 Gy + 0.1% EMS	24	3	131	0.76	-	-	0.76	-	-	-	-	1	0.76	-	-	-
T_9 – 400 Gy + 0.05% EMS	16	2	110	-	-	-	-	-	-	0.90	0.90	-	1.8	-	-	-
Control																
T_{10} – Dry	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_{11} – Presoaked	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table :3 Frequency and spectrum of macromutation in M2 generation.

Flowering mutants			Pod character mutants				Kernel mutants						Mutants of economic interest						Total number of mutations	Mutation rate	
Early flowering	Late flowering	Total	Bold	With constriction	Without Construction	Total	Shriveled	Round bold	Elongated	Violet beak	Red seed testa	Total	Early maturing	Late maturing	High yielding	Profuse flowering	Profuse peg forming	Total		M1 family basis	M2 plant basis
Gamma rays																					
-	-	-	0.13	-	-	0.13	-	0.3	0.27	-	-	0.54	0.27	0.1	-	-	-	0.4	12	4	1.64
-	0.11	0.11	-	-	-	-	0.11	0.1	0.11	-	-	0.33	-	-	0.1	0.11	-	0.22	12	6	1.43
-	-	-	-	-	-	-	-	-	0.78	0.26	-	1.04	-	0.3	-	-	0.26	0.52	15	15.62	3.9
EMS																					
-	0.10	0.10	0.2	-	-	0.2	-	0.3	0.2	-	-	0.51	0.1	-	-	-	-	0.1	11	4	1.14
-	-	-	-	0.13	-	0.13	-	0.1	0.37	-	0.13	0.62	0.37	-	0.1	-	0.13	0.62	17	6	2.11
-	-	-	0.27	0.27	-	0.54	-	0.3	-	0.13	-	0.4	-	0.1	0.3	0.13	0.40	0.94	16	4	2.15
Combination																					
0.36	-	0.36	0.72	0.36	-	1.08	-	0.7	0.36	-	-	1.08	-	0.7	0.4	-	-	1.08	15	8.69	5.43
0.76	-	0.76	-	-	0.76	0.76	-	-	-	-	-	-	-	-	-	0.76	-	0.76	5	12.5	3.81
-	0.90	0.90	-	0.9	-	0.9	0.9	-	-	-	0.9	1.8	-	-	0.9	-	-	0.9	22	12.5	6.36
Control																					
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

REFERENCES

Ashri, A. and Goldin, E. 1965.The mutagenic activity of diethyl sulphate in peanuts.*Radiat*. *Bot.*,**5**: 431-441.

Ashri, A. and Levy, A. 1972. Mutation yield and types obtained in peanuts (*Arachishypogaea*L.)by treating mature seeds with EMS and gamma rays and developing embryos with EMS. Mutations in Plant Breeding, IAEA, Vienna, pp. 1-12.

Blixt, S. 1961.Quantitative studies of induced mutations in Peas, Chlorophyll MutationsAgric. Hort. Gen., 18:216-227.

Brock,R.D. 1977.Prospetus and perspectives in mutation breeding.177-232.In:Genetic Diversity in Plants,Edg.Muhammed,A.R.,Akseland Von-BorsteR.,Pennum Press, New York.

Chopde, V.L. 2009.Mutation breeding in groundnut. M.Sc. (Agri.) Thesis,JunagadhAgril. University, Gujarat, India.

Gregory, W.C. 1956.Induction of useful mutations in the peanut.*Brookhaven Symp. Biol*., **9**: 177-190.

Gregory, W.C., 1968.A radiation breeding experiment with peanuts.*Radiat*. *Bot.*,**8**:81-147.

Gustafsson,A. 1940.A mutation system of chlorophyll apparatus.Lund.Univ.Arsskr. N.F. Adv., **36**: 1-40

Levy, A. and Ashri, A. 1975.Ethidium bromide-an efficient mutagen in higher plants.*Mutation Res*., **28**: 397-404.

Levy, A., Ashri, A. and Rubin, B. 1979.Ethidium bromide uptake by peanut seeds and its relationship to varietal sensitivity and mutagenic efficiency.*Eniron. Expt. Bot*., **19**: 49

Shchori, Y. and Ashri, A. 1970 Inheritance of several macromutations induced by diethyl sulphate in peanuts *Arachishypogaea. Radiat*. *Bot*., **10**:551-555.

Natrajan, A.T. and Upadhya, M.D. 1964. Localizes chromosomes breakage induced by EMS and HI in *Viciafeba*.,*Chromosoma*, **15**: 156-169.

Neuffer, M.G. 1966. Linkage maps of maize chromosomes. *Maize Genet. Coop. Newsletter*, **40**: 167-172.

Nilan, R.A. 1964.*The cytology and genetics of barley* 1951-1962.Washington State University press, Washington, p. 53-57.

Patil, S.H. and Bora K.C. 1963.Radiation inducedmutations in groundnut-Chlorophyll mutations.*Ind. J. Genet.and Plant Breed*., **23**: 47-49.

Ramnathan, T. and Rathinam, M. 1983.Induced qualitative mutations in groundnut.*Madras Agric. J*., **70** (7): 427-432.

Robertson, D.W. 1963.New genes in barley with their related to linkage groups and chromosomes.Proc.1st International Barley Genet.,Wageningen, Netherland, p. 150-167.

Shivasubramaniam, S. 1978. Studied on induce mutations in peanut, (*Arachishypogaea*L.).Ph.D. (Agri.) Thesis, Tamilnadu Agricultural University, Coimbatore, India.

Swaminathan, M.S. 1965.A comparison of mutation production in diploids and Polyploids.*Radiat*. *Bot*., **5**: 619-641.

Venkatachalam, P. and Jayabalan, N. 1997.Frequency and spectrum of viable

mutations in groundnut induced by physical and chemical mutagens.*Crop Res.*, **14** (1): 61-75.

Venkatachalam, P., Geetha, N. and Jayabalan, N. 1999. Frequency and spectrum of chlorophyll mutations in groundnut (*Arachishypogaea* L.), *Bangladesh J. Bot.*, 28 (1): 17-25.

Venkateshwarlu, S., Singh, R.M., Singh, R.B. and Singh, B.D. 1978.Radio sensitivity and frequency of chlorophyll mutations in pigeon pea.*Indian J. Genet.*,**38**(1): 90-95.