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Studied on Effect of Mutagenesis in Groundnut to Induce Variability in Seed Quality Parameters (Arachis Hypogaea L.)

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Abstract: *The experiment consisting induced mutation in groundnut were performed by exposing the healthy and dry seeds to gamma rays viz.,20, 30 and 40 Kr doses and ethyl methane sulphonate (EMS) viz.,40mM and 60mM. The observations were made for seed quality parameters such as root length, shoot length, seedling length and vigour index, in the treated plants The experimental material were evaluated in CRD design. The experimental results revealed that the percentage of germination had decreased after irradiation and the effect become stronger with increase of gamma dose. Parameters such as germination percentage, speed of germination, mean daily germination, peak value and germination value had significantly decreased with increased irradiation doses. Similarly seedling parameters viz., Root length, Shoot length, Vigour index and Root/Shoot length ratio expressed higher reduction at higher doses as compared to no irradiated control. Based on the variation in seed quality parameters of gamma rays and EMS treated plants, superior strain will be screened by PCR-RAPD marker and published in the near future.*

Key words: *Ethyl Methane Sulphonate, Gamma rays, Induced mutagenesis, Groundnut*

I. Introduction

Groundnut (Arachis hypogaea L.) is grown in majority of countries of the world and plays an important role in world economy. It is known by several vernaculars as peanut, monkey-nut or goober nut etc. (Reddy, 1988). It is the single largest source of edible oils and constitutes roughly about 50 per cent of the total oil seeds production. Artificially induced mutation is one of method to enhance genetic variability within short time. Earlier experiment in this field have indicated that ionizing radiation could cause permanent genetical effects, lethal or beneficial mutation, morphological modification and others effects in plants. The genetic variability is highly desirable for developing new cultivars, which is induced by mutagen treatments and natural spontaneous changes. The spontaneous mutation rate is pretty low and can't be exploited for breeding; therefore artificial mutations are induced with physical and chemical mutagen treatment. Quite many useful genetic changes have been induced by mutagen treatment including high yield, flower colour, disease resistance, early maturation etc. in crop, vegetables, medicinal herbs, fruit and ornamental plants.

II. Materials And Methods

The experimental material consists effect of physical and chemical mutagenesis on groundnut genotype CTMG-6-1. Physical mutagene included gamma radiation treatment and chemical mutagene included Ethyl methane sulphonate treatment. In gamma radiation treatment seeds were irradiated with gamma radiation at Bhabha Atomic Research Centre, Mumbai, India. The gamma radiation was derived from a Cobalt-60 (60Co) source with a measured dose rate of 4.07 Gy/min. A completely randomized design was used. LD50 was determined at maturity of plants by preliminary experiments. There were 3 treatments with 200 seeds per treatment. The seeds were treated with gamma radiation at 20, 30 and 40 kR. In chemical mutagenesis The alkylating substances ethyl methanesulphonate was used as a chemical mutagen. LD50 was determined at maturity of plants by preliminary experiments. Seeds were initially soaked in double distilled water for 6 h at 4°C. Then the seed surface was blotted free of water with filter paper. There were 2 treatments with 100 seeds per treatment. The seeds were treated with 40 and 60 mM solutions of EMS. The EMS solutions in phosphate buffer (0.1 M, pH 7), were prepared just before use. Seeds were left in the mutagen solution for 24 h at 4°C.Then seeds were washed for 4 h with tap water to completely remove EMS and seeds were surface blotted. Both gamma irradiated and EMS treated seeds were sown in experimental field at Dr. PDKV, Akola in randomized-block design to raise the M_1 generation. The M1 plants were harvested individually and M_2 generation as plant-to-row progeny were produced. The treated genotype were subjected to laboratory experiments by using Completely Randomized Design for determination the effects of the physical and chemical mutagen on seed quality parameters for M_1 and M_2 generation.

III. Results And Discussion

The present investigation seed quality parameters studies in groundnut mutants was carried out to study the effect of different mutagenic treatments of gamma rays and EMS on seed quality parameters and induced variability in polygenic characters in groundnut genotype CTMG-6-1 in M_1 and M_2 generations.The observations were made for seed germination percentage, growth and yield characters such as root length, shoot length, seedling length and vigour index, plant height, petiole length, days to first flowering, days taken for flowering to fruiting, days taken for fruiting to maturity, in the treated plants. The result obtained from physical mutagenesis were discussed as given below. Analysis of variance for different seedling parameters in M_1 and M_2 generation exhibited significant statistical differences among treatments for all nine parameters as showed in Table 1 and 2. Indicating substandard magnitude of mutagenic effects in M_1generation and effectiveness of mutagenic treatments on the seedling qualitative traits of treated material in M_2 generation. The effect of gamma rays and EMS treatments on germination percentage percentage (Table-3) in M_1 generation was ranged from 69.50 to 86.00 per cent and in M_2 generation was from 79.12 to 91.25 per cent. Germination percentage was found statistically significant in all the treatments in both M_1 and M_2 generations. maximum germination percentage of 86.00 per cent was recorded in T_4 followed by T_5 (82.00%) which were statistically superior to that of control (74.00%). However, germination percentage was adversely affected by doses of gamma rays. In M_2 generation, it was observed that the germination percentage decreases with increasing the doses of mutagenic agents. Maximum germination percentage was observed in T_1 (91.25%) followed by T_4 (89.50%) and T_2 (85.75%) which showed the statistical significance over control (82.00 %). The lowest germination percentage was observed in T_5 (79.12).

The speed of germination index in M_1 and M_2 generations indicates the progressive performance of the seed for germination over period (Table 4).Speed of germination index provides good seed vigour which facilitate in categorizing strong and weak seedling and represent potential of seedling for successful establishment. The range of speed of germination index was observed in M_1 generation from12.19 to 22.31 and from 19.18 to 26.32 in M_2 generation. Differences among the treatments in respect of speed of germination index were statistically highly significant. However, the maximum speed of germination index recorded in treatment T_4 (22.31) followed by T_5 (19.70) representing effect due to EMS (40 mM) and (60 mM) respectively as compared to control (13.60) in M_1 generation. the lowest magnitude of speed of germination index was recorded in T_3 (12.20) which was due to gamma rays (40 kR) in M_1 generation. In M_2 generation, the maximum value for speed of germination index recorded in T_1 (26.32) followed by T_2 (23.33) and T_3 (22.73) as compared to control (19.50). Krishnaswamy and Seshu (1989) opined that the rate of germination was positively correlated with oxygen uptake, dehydrogenase activity by providing energy to the germinating embryo and interfering with integrity and overall capacity of the metabolic machinery of the young germinating primordia. Speed of germination index decreases with increased doses of gamma rays. Similar results reported by Aparna et al. (2013) in groundnut. For Electrical Conductivity ($Dsm^{-}1$) in M_1 and M_2 Generations The effect of gamma rays and EMS on electrical conductivity showed (Table-5). The wide range for electrical conductivity in M_1 generation (0.54 - 0.98 dSm^{-1}) and in M_2 generation from 0.27 to 0.52 dSm^{-1} it revealed that the increase in the doses of mutagenic treatments of gamma rays and EMS, electrical conductivity was increased. Differences due to treatments was statistically significant in respect of electrical conductivity both in M_1 and M_2 generations**.** In M_1 generation, the maximum EC was observed in T_3 (0.98 dSm^{-1}) due to the gamma rays (40kR), while minimum electrical conductivity was recorded in T_4 (0.56 dSm^{-1}) followed by T_5 (0.59 dSm^{-1}) due to EMS (40 mM) and EMS (60 mM) respectively over its control (0.81 dSm^{-1}).

In M_2 generation, minimum EC was recorded in T_5 (0.27 dSm^{-1}) followed by T_1 (0.30 dSm^{-1}), T_2 (0.33 dSm^{-1}) while maximum EC recorded in T_3 (0.52 dSm^{-1}) and T_4 (0.45 dSm^{-1}) as compared to control (0.41 dSm^{-1}). As per the studies the electrical conductivity was negative related to seed quality aspects in M_1 generation, the treatments of gamma rays shown higher magnitude of for electrical conductivity whereas, EMS treatment shows lower values of electrical conductivity than the control (0.81 dSm^{1}).In M_2 generation all the treatments shown lower magnitude than M_1 generation for each treatment. In T_1 (0.30 dSm^{-1}), T_2 (0.33 dSm^{-1}) and T_5 (0.27 dSm^{-1}) treatment shown lower magnitude than control (0.41 dSm^{-1}) Electrical conductivity of the seeds exposed to gamma rays higher than that of unirradiated seed (control) finding by Amjad and Anjum (2002) in onion.

The effect of gamma rays and EMS on moisture content of seed in M_1 and M_2 generation (Table 6) ranged 6.9 to 7.5 per cent in M_1 and in M_2 generation ranged between 6.1 to 6.5 per cent. The lowest moisture content was found in T_4 (6.9%) due to EMS whereas the maximum moisture content was found in T_2 (7.5%) due to gamma rays (30 kR) which was statistical at par over its control (7.2%) in M_1 generation. In M_2 generation, the higher magnitude of moisture content recorded in all the treatments in comparison to its control (6.1%). Moisture Content (%) in M_1 and M_2 generations was statistically significant. The results revealed that the moisture content in M_1 generation was higher than those of M_2 generation for all treatments. The effect of gamma rays and EMS on seedling dry weight (Table -7) Seedling dry weight (g) in M_1 and M_2 generations **(**Table-7) ranged the magnitude of seedling dry weight in M_1 generation from 0.31 to 0.43 g. and from 0.27 -

0.33g in M_2 generation. seedling dry weight recorded in T_4 (0.43 g) followed by T_5 (0.39g) while lowest seedling dry weight in T_1 (0.33g) and T_3 (0.33 g) over its control (0.36 g). In M_2 generation, the maximum seedling dry weight of 0.33g recorded in T_1 and T_4.

It can concluded that the increase in the doses of gamma rays and EMS decreases the seedling dry weight. Seedling dry weight differs statistically significant in all the treatments. Increase in the doses of gamma rays and EMS, seedling dry weight was decreased concluded negative relation with increase mutagenic doses. Borzouei et al. (2010) reported that seedling dry weight decreased with increasing radiation doses in wheat. The effect of gamma rays and EMS on seedling shoot length (Table.8) .The range for seedling shoot length in M_1 generation was found in between 5.45 cm to 7.50 cm whereas range of 10.72 cm to 12.75 cm seedling shoots length in M_2 generation. It showed that in M_1 generation decrease shoot length was observed with increase in doses of mutagens viz. gamma rays and EMS respectively. The treatment differences in both M_1 and M_2 generations were statistically significant. The significantly reduction in shoot length was observed in T_3 (5.45 cm) over control increased 5.87 cm whereas at par with 5.70cm the highest length of shoot of 9.82cm observed in T_4 treatment in which EMS was used with concentration of 40 mM. In M_2 generation maximum shoot length observed in T_1 (12.75 cm) followed by T_2 (12.41cm), T_4 (11.76cm), T_5 (11.14cm) as compared to its control (11.14 cm) whereas T_3 exhibited decrease in shoot length. Seedling shoot length was decreased with increasing concentration and doses of and gamma rays and EMS respectively.

The effect of gamma rays and EMS on seedling root length (Table 9) The range for seedling root length in M_1 generation was recorded in range of 8.20 cm to 14.37 cm whereas range of 14.91 cm to 16.95 cm was found in M_2 generation.The trait, seedling root length shown statistically significant differences for all the treatments in both M_1 and M_2 generations. It indicated the decrease root length was observed with increase dose of both mutagens gamma rays and EMS in M_1 and M_2 generations. In M_1 generation, the significant reduction in root length was observed in T_3 (8.20 cm) followed by T_2 (8.60 cm) over its control (10.45 cm). The maximum root length observed in T_1 (14.37cm) followed by T_4 (13.62cm) and T_5 (11.95 cm) which were statistically significant over control. In M_2 generation, maximum root length was observed in T_4 (17.61 cm) followed by T_5 (17.06 cm) and T_1 (16.95 cm) significant as compared to control (15.55 cm). Seedling root length was decreased with increasing concentration and doses of both gamma rays and EMS respectively.

The effect of gamma rays and EMS on seedling length (Table -10) The seedling length in M_1 generation was ranged from 13.65 cm to 23.45 cm and was found 25.24 cm to 29.71 cm in M_2 generation. Seedling shoot length differs statistically significant in all treatments in M_1 and M_2 generations. Table 4.10, showed that decreased seedling length with increasing dose of gamma rays and EMS. The significant reduction of seedling length observed in T_3 (13.65 cm) followed by T_2 (14.30 cm) over its control (16.32 cm) while the maximum seedling length observed in T_4 (23.45 cm) in M_1 generation.Maximum seedling length was observed in T_1 (29.71 cm) followed by T_4 (29.37 cm) and T_5 (28.74 cm) which was statistical significant over control (26.69 cm) in M_2 generation. Seedling length was decreased with increasing concentration and doses of EMS and gamma rays respectively. Similar results found by Lukanda et al. (2012) and Aparna et al. (2013) in groundnut, Borzouei et al. (2010) in wheat, Muralidharan and Rajendran (2011) in okra, Talebi et al. (2012) in paddy. The effect of mutagenic treatments of gamma rays and EMS on seedling vigour index(Table -11)

The range for seedling vigour index in M_1 generation was observed between 984.00 to 2017.50 and 2063.00 to 2709.58 in M_2 generation. Seedling vigour index decreases with increasing the dose of gamma rays and EMS. Differences due to treatments were statistically significant in M_1 and M_2 generations in respect to seedling vigour index. It revealed that in M_1 generation, maximum seedling vigour index observed in T_4 (2017.50) of EMS (40 mM) followed by T_1 (1663.25) due to 20 kR gamma rays over its control (1208.75) which were statistically significant. In M_2 generation, maximum seedling vigour index recorded in T_1 (2709.58) followed by T_4 (2627.98), T_2 (2348.32), T_5 (2291.48) as compared to its control (2186.93). The gradual reduction in root and shoot length with increase in gamma dose also resulted in corresponding decrease in seedling vigour index from T_1 (1663.25) due to 20 kR to T_3 (984.00) due to 40 kR of gamma rays. Seedling vigour index decreased with increase the doses and concentration of gamma rays and EMS. It indicates the relative sensitivity of groundnut for varying dose of mutagenic treatments include radiation (Gamma rays) and chemical (EMS) agents resulting on overall vigour of seedling and subsequent establishment of mutants Similar results reported by Aparna et al. (2013) in groundnut, Muralidharan and Rajendran (2011) in okra.

IV. Conclusion

Groundnut is a self pollinated crop and artificially induced mutation is the one method to enhance genetic variability within a short time for a desirable traits. Speed of germination index decreases with increasing the doses and concentrations of gamma rays and EMS in M_1 and M_2 generations. Seedling length and seedling vigour index observed higher in M_2 generation as compared to M_1 generation.

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Table -1 Analysis of variance for different seedling parameters in different mutagenic treated groundnut in M_1 generation

Sources of variation	d.f.	GP (%)	SGI	EC (dSm^{-1})	MC (%)	SDW (g)	SSL (cm)	SRL (cm)	SL (cm)	SVI
Treatment	5	156.96**	70.07**	0.13**	0.16*	6.87 E-03**	10.84**	26.75**	64.36**	671688.80**
Error	18	13.72	2.70	5.62 E-04	0.052	5.61 E-04	0.085	0.13	0.31	8755.11

**** and * indicates significant at 1% and 5%, respectively**

Table - 2 Analysis of variance for different seedling parameters in different mutagenic treated groundnut in M_2 generation

Sources of varation	d.f.	GP (%)	SGI	EC (dSm^{-1})	MC (%)	SDW (g)	SSL (cm)	SRL (cm)	SL (cm)	SVI
Treatment	5	87.46**	28.13**	3.55 E-02**	0.11**	2.39 E-03**	2.29**	7.13**	12.30**	252860.80**
Error	18	3.55	0.22	6.98 E-04	0.027	3.63 E-04	0.52	1.06	1.01	18375.11

**** and * indicates significant at 1% and 5%, respectively**

Table .3 Germination percentage in M_1 and M_2 generations

Treatments	Germination percentage	
	M_1	M_2
T_1	76.00	91.25
T_2	69.50	85.75
T_3	72.00	81.25
T_4	86.00	89.50
T_5	82.00	79.12
Control	74.00	82.00
F test	Sig.	Sig.
SE(m)±	1.85	0.94
CD at 5 %	5.50	2.80

Table .4 Speed of germination index in M_1 and M_2 generations

Treatments	Speed of germination index	
	M_1	**M_2**
T_1	14.11	26.32
T_2	12.76	23.33
T_3	12.19	22.73
T_4	22.31	21.65
T_5	19.70	19.18
Control	13.60	19.50
F test	Sig.	Sig.
SE(m)±	0.82	0.23
CD at 5 %	2.44	0.69

Table .5 Electrical conductivity (dSm^{-1}) in M_1 and M_2 generations

Treatments	Electrical conductivity(dSm^{-1})	
	M_1	**M_2**
T_1	0.88	0.30
T_2	0.92	0.33
T_3	0.98	0.52
T_4	0.54	0.45
T_5	0.59	0.27
Control	0.81	0.41
F test	Sig.	Sig.
SE(m)±	0.011	0.013
CD at 5 %	0.035	0.039

Table .6 Moisture content (%) in M_1 and M_2 generations

Treatments	Moisture content (%)	
	M_1	**M_2**
T_1	7.3	6.2
T_2	7.5	6.2
T_3	7.3	6.5
T_4	6.9	6.3
T_5	7.1	6.5
Control	7.2	6.1
F test	Sig.	Sig.
SE(m)±	0.11	0.08
CD at 5 %	0.33	0.24

Table .7 Seedling dry weight (g) in M_1 and M_2 generations

Treatments	Seedling dry weight (g)	
	M_1	**M_2**
T_1	0.33	0.33
T_2	0.31	0.30
T_3	0.33	0.27
T_4	0.43	0.33
T_5	0.39	0.31
Control	0.36	0.32
F test	Sig.	Sig.
SE(m)±	0.011	0.0095
CD at 5 %	0.035	0.028

Table .8 Seedling shoot length (cm) in M_1 and M_2 generations

Treatments	Seedling shoot length (cm)	
	M_1	**M_2**
T_1	7.50	12.75
T_2	5.70	12.41
T_3	5.45	10.72
T_4	9.82	11.76
T_5	6.62	11.67
Control	5.87	11.14
F test	Sig.	Sig.
SE(m)±	0.14	0.36
CD at 5 %	0.43	1.07

Table .9 Seedling root length (cm) in M_1 and M_2 generations

Treatments	Seedling root length (cm)	
	M_1	**M_2**
T_1	14.37	16.95
T_2	8.60	14.62
T_3	8.20	14.51
T_4	13.62	17.61
T_5	11.95	17.06
Control	10.45	15.55
F test	Sig.	Sig.
SE(m)±	0.18	0.51
CD at 5 %	0.54	1.53

Table .10 Seedling length (cm) in M_1 and M_2 generations

Treatments	Seedling length (cm)	
	M_1	**M_2**
T_1	21.87	29.71
T_2	14.30	27.03
T_3	13.65	25.24
T_4	23.45	29.37
T_5	18.57	28.74
Control	16.32	26.69
F test	Sig.	Sig.
SE(m)±	0.27	0.50
CD at 5 %	0.82	1.49

Table .11 Seedling vigour index in M_1 and M_2 generations

Treatments	Seedling vigour index	
	M_1	**M_2**
T_1	1663.25	2709.58
T_2	994.92	2348.32
T_3	984.00	2063.30
T_4	2017.50	2627.98
T_5	1523.67	2291.48
Control	1208.75	2186.93
SE(m)±	Sig.	Sig.
F test	46.78	67.77
CD at 5 %	138.94	201.28