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In vitro Analysis of Seedling Parameters in Groundnut under Induced EMS Mutagenesis

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ABSTRACT: The present in vitro analysis of seedling parameters of groundnut was carried out with three genotypes (ICG2106, ICG5236 and ICG76) at Department of Genetics, Osmania University, Hyderabad. The main aim of the present work is to observe the impact of seed germination and seedling traits under induced EMS in groundnut. The seeds were treated with three different concentrations of EMS *viz.*, 0.3%, 0.4%, and 0.5%, and the control, and kept in petri plates. The germination (%) was measured on the seventh day following treatment, while the shoot, root length and number of leaves of the seedlings were measured on the fifteenth day. The ANOVA revealed that significant differences between treatments for all three traits under study. The results also revealed that ICG76 showed highest seed germination at 0.4% and ICG 5236 recorded highest shoot length, root length and number of leaves at 0.3% EMS concentration in our study.

Keywords: Groundnut, EMS, germination% and seedling traits.

INTRODUCTION

Groundnut (*Arachis hypogeae* L.) is an important food legume and oil seed crop grown worldwide. The cultivated groundnut originated in the southern Bolivian region of South America. Groundnut is a self-pollinated, annual leguminous crop that is fairly drought resistant and mainly cultivated in dry tropical areas. A typically among legume crop plants, peanut pods develop underground rather than above ground. The peanut belongs to the botanical family *Fabaceae*, commonly known as the legume family. The genome size of cultivated groundnut is estimated to be 2.7 GB (Zhuang *et al.,* 2019). Groundnut is the 6th most important oil seed crop in the world. It is recognized for its high caloric content, which comes from oil (48-50%), Protein (26-28%) and Carbohydrate (10-20%) in the kernals, providing 564 kcal of energy and 8.50 g of dietary fiber per 100 g of kernals (Jambunathan, 1991) and rich source of calcium, iron, minerals and vitamins. It was grown globally on an area 31 million hectares with a production of 53.60 million tones and productivity of 3.674kg/ha during 2020 (FAOSTAT, 2020). Over 60 per cent of world groundnut production is utilized to extract oil, with the remaining 40 per cent being consumed as food. Groundnut has been identified as a nutrient-dense food with high capacity to deliver nutrition and income outcomes to producers and consumers. Mutation breeding serves as a source of creating variability and could confer specific improvement in a crop without significantly altering its phonotype (Mohamad *et al*., 2005). The successful utilization of induced EMS to generate genetic variability in plant breeding has been reported in groundnut (Asif and Khalil 2019). There are different kinds of mutagenic breeding, a few of such include. The use of chemical mutagens, radiation and also transposons are used to generate mutants. The exposure of plant materials to induced EMS has been reported to give rise to morphological, physiological and biochemical mutants (Green *et al*., 2003). Induced EMS improved germination, plant height, seed per plant and seed yield per plant in groundnut. It has been demonstrated that genetic variability for several desired characters can be induced successfully through mutations and the practical value of mutagenesis in plant improvement programmes has been established (Bera *et al.*, 2018). The objective of the study was to create genetic variation in groundnut through mutagenesis and hence select desirable mutant genotypes for multi locational traits. Plant breeding requires a genetic variation of useful traits for crop improvement. Often, however the desired variation is lacking. Consequently, the extent to which groundnut cultivars may be improved through conventional breeding methods is limited. Therefore, the current study focuses on how to treat EMS in a groundnut in relation to germination %, seedling height (cm), root length (cm) and effect on quantitative characters under study.

MATERIAL AND METHODS

The present investigation entitled with '*In vitro* analysis of seedling parameters in groundnut under induced EMS mutagenesis' was carried out with three groundnut lines *viz.*, ICG2106, ICG5236 and ICG76 at

experimental farm, Department of Genetics, Osmania University, Hyderabad. Initially healthy and uniform size seeds from these three selected groundnut genotypes were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for about one minute, washed thoroughly and soaked for 5 hrs in distilled water. The groundnut 12 seeds of each genotype (50) were pre-soaked in distilled water for 11 hours. These soaked seeds treated with three different concentration of EMS (LD 50) *viz.*, 0.3%, 0.4%, and 0.5% and control (untreated), keep in incubator shaker for 6hrs. After treatment, carefully remove the EMS solution and treated seeds were washed under running tap water to remove the traces of mutagen sticking to the seed coat 12 treated seeds of each concentration were placed in Petri plates in three replication along with untreated seeds for germination test. The germination percentage (%) was observed on the 7th day after treatment while shoot, root length and number of leaves of seedlings was recorded on the 15 day after sowing.

RESULT AND DISCUSSIONS

The analysis of variance (ANOVA) results showed that highly significant variability among treatments *viz*, shoot length, Root length, number of leaves per plant for all the three varieties (ICG2106, ICG5236, ICG76) except germination percentage due to treatment in our study (Table 1-ABC).

Table 1A: The Effect of EMS Mutagenesis on germination and seedling parameters in Groundnut accession-ICG-2106, Analyzed using analysis of variance (ANOVA).

Source of Variation	Df	"MSSQ Characters			
		Germination (%)	Shoot length(cm)	Root length (cm)	Number of leaves per plantlet
Replications	**2**	5.583	0.276	0.256	0.083
Treatments	**3**	1.639(NS)	28.256**	3.959**	5.417**
Mean		96.083	3.633	2.383	1.917
Error		2.472	0.038	0.121	0.083
SEd		1.284	0.159	0.284	0.236
CV%		1.64	5.37	14.62	15.06

Table 1B: The Effect of EMS Mutagenesis on germination and seedling parameters in Groundnut accession-ICG-5236, Analyzed using analysis of variance (ANOVA).

Source of Variation	Df	"MSSQ Characters			
		Germination (%)	Shoot length(cm)	Root length (cm)	Number of leaves per plantlet
Replications	**2**	3.583	0.227	0.381	0.250
Treatments	**3**	5.111(NS)	25.036**	2.669**	5.556**
Mean		96.667	4.275	2.833	2.000
Error		2.028	0.033	0.033	0.139
SEd		1.163	0.148	0.148	0.304
CV%		1.47	4.25	6.42	18.63

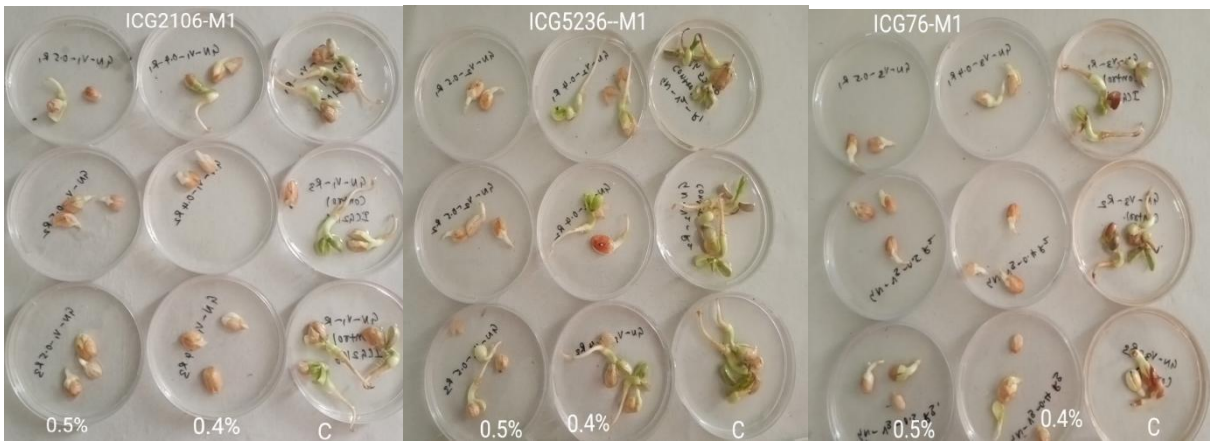
Table 1C: The Effect of EMS Mutagenesis on germination and seedling parameters in Groundnut accession-ICG-76, Analyzed using analysis of variance (ANOVA).

Source of Variation	Df	"MSSQ Characters			
		Germination (%)	Shoot length(cm)	Root length (cm)	Number of leaves per plantlet
Replications	**2**	0.250	0.381	0.566	0.083
Treatments	**3**	12.667(NS)	19.936**	6.410**	0.667*
Mean		96.000	3.333	1.942	2.333
Error		3.583	0.013	0.008	0.083
SEd		1.546	0.093	0.073	0.236
CV%		1.97	3.43	4.62	12.37

The mean performance of germination percentage has recorded highest in control (98.33%) by ICG5236 followed by ICG2106 (96.33%) and ICG76 (93.00%). Whereas the genotype ICG76 grown under 0.3% EMS concentration has recorded highest germination percentage (96.67%) followed by ICG5236(96.00%) and ICG2106 (95.33%) respectively (Table 2).

The genotype ICG76 observed highest germination percentage at 0.4% EMS concentration (97.37%) followed by the genotype ICG5236 (97.00%) and ICG2106 (95.67%) respectively (Fig. 1).

At 0.5% EMS concentration the highest germination percentage recorded by ICG2106 (97.00%) followed by ICG76 (96.67%) and ICG5236 (95.33%) respectively (Table 2). It was observed that ICG76 recorded highest seed germination % (97.67%) at 0.4% in our study. It was observed that no significance variability in germination % in the selected groundnut genotypes due to EMS induction in our study.



(a) **(b)** **(c)**

Fig. 1. Performance of seed germination in (a) ICG 2106, (b) ICG 5236 and (c) ICG 76 under EMS induced mutagenesis.

(a) **(b)** **(c)**

Fig. 2. Performance of seedling shoot length, root length and number of leaves in (a) ICG 2106, (b) ICG 5236 and (c) ICG 76 under EMS induced mutagenesis.

Table 2: Mean performance of Seed germination % in Groundnut genotypes under EMS induced mutagenesis.

Genotypes	Germination%					
	Control	**0.3%**	**0.4%**	**0.5%**	**SEd**	**Error**
ICG2106	96.33	95.33	95.67	97.00	1.284	2.472
ICG5236	98.33	96.00	97.00	95.33	1.163	2.028
ICG76	93.00	96.67	97.67	96.67	1.546	3.583

Among three genotypes ICG5236 showed higher shoot length, Root length, and number of leaves at control (6.07cm, 3.13cm and 2), at 0.3% EMS (6.00cm, 4.03cm, and 2) respectively.

Where as the genotype ICG76 showed higher shoot length (1.17 cm), root length (1.27cm), and number of leaves (2.0) even at 0.5% EMS concentration (Table 3). Similar results were reported in groundnut by Kharade *et al*. (2016) and Aparna *et al*. (2013). Similar results also found by Borzouei *et al*. (2010) in wheat, Muralidharan and Rajendran (2011) in Okra, Dehapour *et al*. (2011) in rice (*Oryza sativa*), Talebi *et al*. (2012) in paddy, Lukanda *et al*. (2012). It was also observed that the reveled that the seedling treatments, viz. Germination percentage, shoot length, root length, number of leaves, observed significant variation for all three genotypes except seed germination for the variety ICG2106 in our study (Fig.1 and 2). The results also reveled that among three genotypes ICG76 recorded highest seed germination, shoot length, number of leaves at 0.4% EMS concentration and it is recorded higher shoot length, root length, number of leaves even at 0.5%EMS concentration over other two genotypes (Table 2).

Table 3: Mean performance of seedling shoot length, root length and number of leaves in Groundnut genotypes under EMS induced mutagenesis.

Control			
Genotypes	**Root length(cm)**	**Shoot length(cm)**	**No. of leaves**
ICG2106	4.03 ± 0.45	7.03 ± 7.03	2.00 ± 2.00
ICG5236	3.13 ± 0.42	6.07 ± 6.07	2.00 ± 2.00
ICG76	4.13 ± 0.42	7.03 ± 7.03	2.00 ± 2.00
0.3% EMS			
Genotypes	**Root length(cm)**	**Shoot length(cm)**	**No. of leaves**
ICG2106	2.10 ± 0.36	5.07 ± 0.40	2.00 ± 0.00
ICG5236	4.03 ± 0.35	6.00 ± 0.20	2.00 ± 0.00
ICG76	1.17 ± 0.29	3.00 ± 0.20	2.00 ± 0.00
0.4% EMS			
Genotypes	**Root length(cm)**	**Shoot length(cm)**	**No. of leaves**
ICG2106	2.03 ± 0.35	2.43 ± 0.40	2.00 ± 0.00
ICG5236	2.17 ± 0.15	5.03 ± 0.35	2.00 ± 0.00
ICG76	1.20 ± 0.35	2.13 ± 0.42	2.00 ± 0.00
0.5% EMS			
Genotypes	**Root length(cm)**	**Shoot length(cm)**	**No. of leaves**
ICG2106	1.37 ± 0.40	0.00 ± 0.00	0.00 ±0.00
ICG5236	2.00 ± 0.40	0.00 ± 0.00	0.00 ± 0.00
ICG76	1.27 ± 0.46	1.17 ± 0.29	2.13 ± 042

CONCLUSION

The results can be concluded that significant variability were observed for shoot length, root length, and number of leaves among three genotypes under various EMS concentration. The results also concluded that among three genotypes ICG76 showed highest seed germination at 0.4% and ICG 5236 recorded highest shoot length, root length and number of leaves at 0.3% EMS concentration.

FUTURE SCOPE

The mutant ICG 76 grown under 0.3% EMS and ICG 5236 (0.4% EMS) will be carried out for further generation for improvement of yield parameters.

Authors contribution

Yugandhar A.- Conducted experiment, observed and recoreded the data in lab.

Srinivas Naik K.- Suggested the preparation of EMS concentration and precautions during seedling observation in the lab.

Bhattu Rajesh Nayak- Helped in the data analysis, compilation of data and writing part of the article.

Kumara Joshi- Helped in data analysis, taking pics and and writing part of the article.

Vijay Kumar G. - Guided in conduction of whole experiment and involved in completion of writing part of the experiment.

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