

**IMPACT OF ETHYL METHANE SULPHONATE ON M₁ ATTRIBUTES OF
GROUNDNUT WITH SPECIAL EMPHASIS OF AMINO ACID PROFILING**

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ABSTRACT

The present investigation was undertaken to know the effect of EMS on groundnut cultivar TMV-7 particularly for M₁ generation with special reference to amino acids. The healthy and viable seeds of groundnut cultivar TMV-7 were exposed to different concentrations of EMS. The chemical mutagens were screened against groundnut ranged from 10 to 50mM and sown in the field along with control. The various growth, morphological parameters like percentage of seed germination, survival percentage plant height, number of branches, days taken for flower initiation, total height at time of harvest, root length, number of lateral roots, number of pods per plant and 100 seeds weight were decreased with increasing concentrations of EMS. The amino acid analysis also carried out in control, EMS derived M₁ plants grown in field. The amino acid contents were recorded in both positive and negative tendency.

KEY WORDS: *Arachis hypogaea*.L, Chemical mutagens, Amino acids.

INTRODUCTION

The peanut or groundnut (*Arachis hypogaea* L.) is a species in the legume family (Fabaceae). It is an important oilseed legume grown worldwide and is known by many other local names such as earthnut, peanut, gooberpea, monkeynut and pignut [McDonald, 1968]. It is grown both for domestic market and for export. The world groundnut production was estimated to be 35.367 million metric tons in 2011-2012. The world leading producers are China, India, and USA followed by Nigeria the fourth position and the largest producer in Africa.

Groundnut is a nutritive crop with approximately 25% protein and about 45 – 50% oil. The skin of groundnut is rich in vitamin B and it is used as a base ingredient for cosmetics. It also provides important ingredients in numerous industries for Confectionery and bakery products. Groundnut proteins contends is of high biological value than other proteins. The residue of the extraction process is used as commercial groundnut cake which is a concentrate feed for livestock and poultry. The nuts are eaten raw or after roasting as snacks. The green leaves or shoot makes excellent fodder and hay for animals [National Peanut Council (NPC), 1990; Anon, 1977].

Chemical mutagenesis, is a simple approach to create mutation in plants for the improvement of potential agronomic traits. Mutations are the tools and being used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu *et al.*,(2007). Mutation methodology has been used to produce many cultivars with improved economic value and study genetics and plant developmental phenomena (Van, Den-Bulk *et al.*,(1990); Bertagne – Sagnard *et al.*,(1996).

Amino acids are the basic constituents of proteins, The qualitative and quantitative analysis of amino acid composition of hydrolyzed samples of pure proteins or peptides is used to identify the material and to directly measure its concentration. Amino acids are also intermediates in metabolic pathways , often not directly involving proteins. The amino acids are measured as elements of physiological and nutritional studies. This has proven particularly important in monitoring the growth of cells in cultures, as used in the production of biopharmaceuticals. Analysis of amino acids is required in several areas of research, and it is fundamental tool in product analysis. So the present study was aimed to know the effect of

EMS on growth and synthesis of amino acids in treatment with EMS.

MATERIALS AND METHODS

Seed material

Mature,. healthy and uniform seeds of groundnut variety TMV-7 was obtained from Tamil Nadu Agricultural University, Coimbatore and used as an experimental material to carry out the mutagenic studies using Ethyl Methane Sulfonate as a chemical mutagen.

Preparation of phosphate buffer:

0.2M solution monobasic sodium phosphate (27.8g in 100mL) 0.2M solution of dibasic phosphate (53.65g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ or 71.7g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in 1000mL) xml of solution-1 and ml of solution -2 were taken and diluted.

Determination of imbibition period

For the purpose of chemical mutagen treatment, the total imbibitions period was calculated. The total imbibition period was six hours. For the purpose of mutagenic treatment, the total imbibition period divided in to (1) pre-soaking period -4 hrs and EMS treatment period of 2 hrs.

Treatment with chemical mutagens :

For chemical mutagen treatment ,the groundnut seeds were placed in perforated polyethylene bags (with 3 replica each containing 100 seeds in separate polyethylene bags)and pre soaked in distilled water for 4 hrs. The different concentrations of EMS (10 to 50mM) were prepared using phosphate buffer. Then the pre soaked seeds were immersed in various concentrations EMS for 2 hrs. After mutagenic treatment the seeds were carefully washed with tap water to remove the traces of chemical mutagens present on the surface of the seeds. Then the seeds were immediately sown in the field for further study.

M₁ Characteristics studied:

The following M₁ characteristics were studied in the field. The various growth, morphological parameters like percentage of seed germination, survival percentage plant height, number of branches, days taken for flower initiation , total height at time of harvest, root length, number of lateral roots, number of pods per plant and 100 seeds weight were calculated. All the above M₁ parameters were carefully taken in field itself in all the 3 replica for each EMS treatment (R₁,R₂ and R₃).

HPTLC Analysis of Samples for Aminoacids

Procedure: Sample digestion

The given samples each 100mg were weighed in an electronic balance and transferred into labeled glass test tubes (BOROSIL). 1ml of 6M Hydrochloric acid solution was added with sample in specified test tubes. These test tubes were sealed at the top under vacuum by high temperature gas flame, conducted triplicates of samples. All the sealed tubes were kept in a hot-air oven at 110°C for 48hrs continuously.

Test solution preparation

After completion of digestion, broken the tubes at the top and transferred the digest into glass beaker (BOROSIL), rinsed the tubes 5 times with distilled water. The acid in the digest was evaporated to core dry under vacuum using Roto-vac evaporator. The residual content was dissolved with distilled water and made-up to 2.4ml in a centrifuge tubes. This solution contains 41.6µg raw sample in 1µl distilled water and used as test solution for amino-acid profile analysis by HPTLC technique.

RESULTS

The effect different concentrations of EMS on morphological characters in M₁ generation were collected and presented here.

Germination percentage:

The various concentrations of EMS decreased the germination percentage with increasing concentrations. In control the germination percentage was 98.00 whereas it was reduced to 29.00 % in 50 mM. There was a gradual reduction in germination percentage with increasing concentrations of EMS. The LD 50 value was observed in 40mM concentration of EMS. The survival percentage also decreased with increasing concentrations of chemical mutagens. In EMS the survival percentage was recorded from 97.0 % to 14 % . the number branches ranged from 5.0 to 3.6. When compared to control, the chemical mutagens delayed the flower initiation with increasing concentrations of chemical mutagens. The yield characteristics were studied in terms of number of pods/plant and weight of 100 seeds. The yield parameters were increased over control in lower concentrations of chemical mutagens. The decreasing trend was noticed in 40 and 50mM treatments. After harvest, the total root length and number of lateral roots were counted individually for each chemical mutagen treatment and control. The root length and number of lateral roots have been increased with increasing doses of mutagenic agents but reduced in higher concentrations.

Effect of EMS on Amino acid content (in %).

In 10mM treatment of EMS, recorded decreasing tendency of few amino acid like Histidine, Proline, Serine, Arginine and Glycine, Remaining amino acids are increased in their content in comparison with 10mM treatment. In 20mM treatment of EMS exhibited decreasing trend in only the amino acids such

as Histidine, Aspartic acid, and Threonine. All other amino acid are increased its content. 30mM treatment all the amino acids were showed increasing like then control. In 40mM treatment of EMS only four amino acids are noticed increasing over control. But all the amino acids contents were reduced when compared to control. 50mM treatment all the 19 amino acids were showed decreasing trend over control.

Sl. No	EMS in mM	Germination %	Survival %	Plant height (30 th day) (cm) Mean±SD	Number of branches (30 th day) Mean±SD	Days of first flowering Mean±SD	Number of mature pod/plant Mean±SD	100 seeds weight(g) Mean±SD	Root length Mean±SD	No of lateral root Mean±SD
1	Control	98.0±1.89	97±1.56	9.2±0.97	6.3±0.22	33.0±1.12	18.1±1.44	26.1±1.14	12.1±0.97	32.0±1.03
2	10mM	77.0±1.22	65±1.32	7.0±0.56	4.6±0.33	33.1±1.24	20.1±1.20	30.1±1.37	12.4±0.78	30.2±2.68
3	20mM	75.0±1.33	61±1.02	6.5±0.10	4.6±0.56	33.5±1.57	22.6±1.02	32.1±1.30	13.2±1.02	28.2±2.35
4	30mM	55.0±1.02	49±1.45	6.0±0.78	4.3±0.12	34.7±1.45	24.1±1.92	38.7±2.28	15.2±0.87	20.4±1.32
5	40mM	48.0±1.45	36±1.02	5.2±0.98	4.0±0.74	34.1±1.22	20.7±1.41	31.0±1.87	11.9±0.67	14.1±1.14
6	50mM	29.0±1.11	14±1.12	4.5±0.45	4.0±0.11	36.1±1.02	15.4±1.87	24.1±1.47	10.3±0.88	12.0±1.78

Table : 1 Effect of Ethyl methane sulphonate on morphological characteristics of M₁ generation in Groundnut

DISCUSSION:

The Present discussion is mainly focused on the effect of chemical mutagen EMS on several morphological and yield characteristics and amino acid profiling in groundnut. Seed germination is an important parameter to estimate the effect of mutagens on plants. In the present investigation the seed germination gradually decreased with increasing concentration of all three mutagens. seed germination was highly affected by EMS . Germination of seeds after breaking dormancy period is a process of resumption of active metabolism manifested in visible growth. Inhibition in seed germination, after the treatment of seeds with different mutagens is a convenient technique for studying their effects of mutagens in plants. Like that of our study, the reduction in germination

S.No	Substance	Control	10mM	20mM	30mM	40mM	50mM
1	Lysine	1.80	2.06	2.09	2.55	1.73	1.62
2	Histidine	3.71	4.90	4.56	5.02	3.77	3.47
3	Proline	7.95	8.43	8.65	9.0	8.10	7.22
4	Serine	5.86	6.20	6.37	6.63	6.11	5.25
5	Arginine	2.21	2.13	2.23	2.40	2.30	2.16
6	Glycine	3.96	4.20	4.59	4.22	4.03	3.52
7	Glutamine	2.04	2.07	2.16	2.21	2.01	1.89
8	Cystine	1.99	2.01	2.11	2.16	1.89	1.85
9	Aspartic acid	5.26	5.24	5.55	5.67	5.24	4.85
10	Alanine	2.23	2.23	2.37	2.42	2.20	2.06
11	Glutamic acid	3.24	3.43	3.48	3.70	2.98	2.81
12	Theronine	2.42	2.59	2.62	2.78	2.37	2.25
13	Valine	2.30	2.59	2.45	2.62	2.25	2.13
14	Methionine	1.61	1.65	1.70	1.51	1.44	1.23
15	Tyrosine	6.70	6.75	7.11	7.41	6.35	6.27
16	Isoleucine	1.39	1.42	1.46	1.52	1.32	1.29
17	Tryptophan	2.66	2.88	2.90	2.01	2.33	2.28
18	Leucine	2.90	3.14	3.17	2.81	2.54	2.47
19	Phenyl alanine	1.80	1.94	2.02	2.15	1.58	1.53

Table:2. Effect of EMS on Amino acid contents in(%) field grown plants of Groundnut (*Arachis hypogaea* L.)

percentage was reported in groundnut and other legumes. Dehydrogenase activity by providing energy to the germinating embryo and interfering with integrity and overall capacity of the metabolic machinery of the young germinating primordial. Speed of germination index decreases with increased doses of gamma rays. Similar results reported by Aparana *et al.*, (2013) in groundnut.

It can concluded that the increase in the doses of EMS decreases the seedling dry weight. Seedling dry weight differs significantly in all the treatments with chemical mutagens. In the present study, increasing doses of EMS

decreased the seedling dry weight. Similar study was reported by Borzouei *et al.*, (2010) in wheat.

In the present investigation, lower doses of EMS treatments reduced the several growth parameters. There are several earlier studies confirming our report. The decrease in seedling emergence, seedling height, root length, and seedling survival, height and maturity and fruits per plant with increasing mutagenic concentration has been reported in mutagenic studies of Adamu *et al.*,(2002). when groundnut seeds were treated with gamma rays. Similar results was obtained by Sheeba *et al.*,(2005) when gamma rays and

EMS were used to treat *Sesamum indicum* L. where seed germination, seedling survival, plant height and pollen fertility were reduced significantly with an increase in doses levels of chemical mutagens .

The impact chemical mutagens on germination percentage, plant height and number of branches, number of pods per plant were decreased with increasing concentrations of EMS have been well understood in our study. Such a inhibitory effect of various mutagens were reported in several other crops by several earlier workers (Reddy *et al.*, 1992, 1992a). Jana (1964) reported that effect of mutagens on dry seeds of *Phaseolus mungo* showed reduction in growth.

Like that root length, shoot length and number of branches were decreased with increase in concentrations of EMS. Similar results from earlier study of Heringa (1964). He reported that 50% reduction in germination at the doses of 40mM EMS. Constantin (1976) and Singh (1998) observed linear relationship between dose and reduction in survival of field grown plant of bhendi treated with EMS and DES. Higher mutagenic efficiency at the lower doses of the mutagens were also reported in mungbean (Khan and Hashim.,1979), Cowpea (Gnanamurthy *et al.*,(2012).

In the present investigation the reduction was noticed in seedling height, number of pods ,100 seed weight, number of root , root length , lateral roots and days to first flowering. The decreasing trend in mutagenic effect was also found in quantitative characters of soybean (Pepo.,1989).

The inhibitory effect of chemical mutation was supported by Burghate *et al.*, (2013). Impact of mutagens its efficiency and effectiveness in groundnut (*Aradhis hypogaea* L). Gamma rays, EMS and combination in M₁ generation, the germination percentage was reduced due to various mutagenic treatments under field as well as laboratory reduction in germination was found in maximum in higher dose and/or concentration of the mutagens. The progressive decreased in seedlings growth via. root and shoot length with corresponding increased in dose or concentration of gamma rays, ethyl methane sulphonate and their combinations was observed in M₁ generation.

In this part of research work, amino acids were analyzed in EMS derived M₁ populations of groundnut. The distribution pattern of amino acids were highly influenced by chemical mutagens. This type of research work was almost rare in crop plants based on available literature. The increasing and decreasing trend

of amino acids were reported by Mehrian *et al.*, (2015) in tomato plants treated with silver nanoparticles.

CONCLUSION

The mutation methodology has been used to produce many cultivars with improved economic value and useful to studied the genetics, plant developmental phenomena. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops. The present study is useful to understand the morphological and physiological changes induced by chemical mutagens particularly in M₁ generation.

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