



## Biochemical Investigation of 24 Prominent Mutants of *Arachis Hypogaea* (L.)

### KEYWORDS

Mutation, Oil quality and *Arachis hypogaea*.

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**ABSTRACT** *Groundnut (Arachis hypogaea L.) is an important oil seed crop and grain legume worldwide. It is self pollinating and possesses limited variability. Thus this study investigates the improvement of groundnut oil quantitatively and qualitatively by induced mutations with Gamma rays and EMS mutagens. Pertaining to the oil quantity 20 kR and 10 kR doses of gamma rays is more effective in variety AK-159 and TAG-24 respectively. While in improving oil quality the 0.15% concentration of EMS and 0.10% concentration of EMS are more potent in variety AK-159 and TAG-24 respectively. This work has enabled us to conclude that the oil content, fatty acid composition and oil stability could get alter in positive direction through the application of mutagen.*

### Introduction:

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop and grain legume worldwide. As an oilseed crop, groundnut rank fourth in world production. Although groundnut in India is primarily used as an oil seed, it is also consumed directly as food because of its palatability. Besides, it contain oil (48-52 %) and protein (22-30%) seed contain carbohydrates (10%), minerals (3%) and B complex vitamins especially thiamin (Rajgopal, et al., 2000). Groundnut oil contains 51% of mono-saturated oil and 30% polyunsaturated and 19% saturated oil (Amarican heritages, 2007). It also has neutral flavor and odour. It does not absorb odours from other foods (Passera, 1981). This makes it the most preferred oil in India.

India is the third largest consumer after China and EU. It was observed that the area under cultivation, production and productivity of groundnut in Maharashtra and India have revealed continuous decreasing trend (Patil et al., 2009 and Talwar, 2003). However per capita consumption of edible oils is around 11 Kg per year. (Ramesh P & Murughan M. 2008). Oil quality and its stability are therefore very important for the consumers and application industries (Jambunathan et al., 1993). However, it is self pollinating and possesses limited variability. Thus this study investigate the improvement of groundnut oil quantitatively and qualitatively by induced mutations with physical (Gamma rays) and chemical (EMS) mutagens.

Mutation breeding offers the possibility of inducing desired attributes that either cannot be found in nature. There are examples where mutants have been identified for yield and quality traits in groundnut (Patil 1975).

Generally in groundnut, mutation breeding has done for the evolving species / varieties which are high yielding and resistant to leafy diseases. However, pertaining efforts has been made to incline secondary importance to the compositional and nutritional aspects of the final crop. In case of groundnut it would be desirable to evolve a variety that has not only high oil content but also nutritionally desirable fatty acid composition. Fatty acid composition of groundnut oil is an important trait with reference to human nutrition and oil stability during storage.

### Materials and Methods:

The germplasm was obtained from the Department of Crop Research Unit (Oilseeds), Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola-444 104. (M.S.) was used in present study. Seeds having moisture content 7%, treated with different concentrations of Ethyl methylsulphonate (EMS) viz. 0.05%, 0.10% and 0.015% and irradiated to Co<sup>60</sup> at three different doses viz. 10 kR, 15 kR and 20 kR in the gamma chamber of

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Treated seeds of each variety were sown in field for raising M<sub>1</sub> generation following randomized block design (RBD) with three replicate having spacing of 15 X 35 cm. An untreated seed serves as a control. The experimental field situated at Latitude 19° 48' 23" N and longitude 75° 50' 42" E at an altitude of 507 meter above sea level. The experimental area for M<sub>2</sub> and M<sub>3</sub> and spacing are same as those used in M<sub>1</sub> generations.

Critical screening was done though the M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> generation and viable mutants were carefully screened from the population.

#### 1. Oil content (%):

The seed oil percentage of 24 prominent mutants was estimated by soxhlet method according to pearson's (1981) 5 gm of each finely ground and sample was extracted with hexane (boiling point 65 -70 ).

#### 2. Fatty acid profiling by GLC:

The fatty acid analysis was carried out by using Gas Liquid Chromatography (GLC) at quality control Laboratory, Teena oils and chemicals Ltd. MIDC, Latur (MS)

#### a) Preparation of Fatty acid methyl esters:

300 mg of oil was mixed with 6 ml of methanolic NaOH in standard volumetric flask of 50 ml. Saponified it on water bath till no oil globules could remain in flask. After cooling the 8 ml of Boron trifluoride (BF<sub>3</sub>) was added for esterification and heated with continuous shaking for 2 minutes carefully, because BF<sub>3</sub> is toxic and explosive. Cooled the flask and 2 ml of petroleum ether was added. Then final volume (50 ml) was made with saturated NaCl. (AOAC 1990).

#### b) Determination of fatty acid composition of oil :

A layer of hexane was given on the fatty acid methyl ester solution and mixed well. Then the solution was allow to settle for 15 minutes and from clear supernatant, 0.6 of this solution was injected by sample syringe in gas chromatography. The fatty acid composition of oil was determined by gas liquid chromatography (GLC) performed on model Chemito GC-1000, armed with flame ionization detector (FID) and Polar capillary column BPX-70 (manufacturer-SEG capillary columns) having pore size 0.25 , Internal diameter 0.32mm and length 50 m (Part No. 054607). The oven, injector and detector temperatures was maintained at 100 , 250 and 280 respectively. Nitrogen was used as carrier gas, Split ratio

was 1/36 and the run time was upto 18.00 minutes. Individual peak of fatty acid methyl ester (FAME) was identified by comparing their retention times with those of standards (Sigma Aldrich). Fatty acid composition of each sample was encoded by software (Chemito chrome card A/D for windows Reversion A-1.) in the form of peak in chromatogram.

#### Result and Discussion:

The oil content in prominent mutant ranged from 48.10% to 54.34% in variety AK-159 and 47.20% to 52.76% in variety TAG-24. The highest oil percentage was recorded 54.34% and 52.76% in Early maturing with dwarf Mutant (irradiated to 20 kR dose of gamma rays) of variety AK-159 and Faint seed coat mutant (10 kR of gamma rays) of var.TAG-24 respectively. Nadaf and et al., 2009 also recorded increased oil content after treating seeds with gamma rays and EMS

in groundnut. Giriraj, et.al., (1990) have observed higher variability and shift in mean towards positive direction in M3 generation after EMS and gamma ray treatments in sunflower. Similar results were recorded by Badere and Choudhary (2004) after using SA and EMS in linseed.

Generally high oleic content oil is preferred. It is a mono-unsaturated fatty acid. It lowers the heart attack risk and atherosclerosis and aids in cancer prevention (Rotella, 2004). David, T.W. (2005) examined the major role of oleic acid in prevention of breast cancer. The evidence that long-chain fatty acids e.g. arachidic and behenic, in groundnut oil may be implicated in heart disease has been noted (Kies et al., 1978). The oil produced from high oleic acid groundnuts had a substantially higher resistance to oxidation than that from conventional groundnuts (Talcott et al 2005a)

**Table 1: Oil content, Fatty acid profile and Quality parameters of selected viable mutants of *Arachis hypogaea* (L.) of variety AK-159.**

Sr. No.	Mutant *	Oil %	Fatty acid profile										Quality parameters	
			C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 20:0	C 22:0	C 22:1 n 6	C 24:0	OSI #	NQI #	
01	GN-01	51.08	13.174	0.459	3.020	41.869	35.263	1.458	0.753	3.090	0.815	1.1873	4.00	
02	GN-02	52.44	13.264	0.374	3.016	39.631	36.924	1.579	0.796	3.311	1.011	1.0733	3.88	
03	GN-03	52.86	13.459	0.314	2.998	40.574	36.439	1.434	0.720	3.300	0.670	1.1134	3.98	
04	GN-04	49.64	12.234	0.523	3.187	41.573	35.875	1.608	0.765	3.277	0.857	1.1588	4.13	
05	GN-05	52.64	12.283	1.243	2.475	40.978	36.119	1.606	0.796	3.252	1.141	1.1345	4.19	
06	GN-06	49.64	13.147	0.342	3.199	41.853	35.166	1.521	0.703	2.985	0.982	1.1901	3.88	
07	GN-07	51.52	12.696	0.388	2.504	41.528	36.252	1.340	0.855	3.200	1.109	1.1455	4.18	
08	GN-08	49.12	13.399	0.321	2.965	39.743	36.291	1.494	0.794	3.709	1.173	1.0950	3.82	
09	GN-09	52.92	12.941	0.357	2.722	42.254	35.308	1.473	0.765	3.072	1.025	1.1966	4.09	
10	GN-10	54.34	13.256	0.299	2.230	40.519	36.940	1.490	0.819	3.243	1.102	1.0969	4.08	
11	GN-11	50.48	14.115	0.350	2.256	40.519	36.922	1.310	0.711	2.900	0.810	1.0974	4.02	
12	GN-12	48.10	12.932	0.432	2.820	39.854	37.563	1.486	0.686	3.199	0.922	1.0609	4.08	

\* GN 1- Control, GN 2- Multi branched, GN 3- Dwarf, GN 4-Bold seeded, GN 5- Large leaf, GN 6- Tall, GN 7- High yielding GN 8- Pink, GN 9- Late flowered, GN 10-Early maturing with dwarf, GN 11- Late maturing with bold seeded and GN12- Faint.

# OSI-Oxidative stability index #NQI-Nutritional quality index

The final quality of groundnut oil depends on its fatty acid composition and in present investigation the acid composition among the mutants revealed significant alternation in the linoleic and oleic acid and in regard to other fatty acids only slight change could be noticed in both the varieties of groundnut as shown in table 1. The major fatty acid component in groundnut

seed oil is Palmitic(C 16:0), Stearic (C 18:0), Oleic (C 18:1), Linoleic (C 18:2), Arachidic(C 20:0) and Erucic (C 22:1 n6).

Highest oleic acid value (42.254%) was recorded in late flowered mutant (0.15% of EMS) it range from 39.631% to 42.254%. linoleic acid value was found highest (37.563%) in Faint seed coat colour mutant (0.05% of EMS). It range from 35.166% to 37.563% in variety AK-159, while in variety TAG-24 the highest oleic (44.485%) and lowest linoleic (32.532%) was demonstrated by Large leaf mutant (0.10% of EMS) and lowest oleic (41.275%) and highest linoleic (35.952%) values are found in faint seed coat colour mutant (10 kR of Gamma rays). Oleate and linoleate together constitute 76.03% to 77.78% of the total fatty acids; a desirable mutational trait.

**Table 2: Oil content (%), Fatty acid profile and Quality parameters of selected viable mutants of *Arachis hypogaea* (L.) of variety TAG-24.**

Sr. No.	Mutant *	Oil %	Fatty acid Profile										Quality parameters	
			C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 20:0	C 22:0	C 22:1 n 6	C 24:0	OSI #	NQI #	
01	GN-13	48.20	13.13	0.424	3.833	41.783	35.246	1.519	0.587	2.677	0.709	1.185	3.88	
02	GN-14	50.58	12.98	0.426	3.012	42.303	35.33	1.599	0.732	3.034	0.759	1.197	4.05	
03	GN-15	50.24	12.65	0.518	3.317	42.160	34.764	1.655	0.688	3.089	1.054	1.212	3.96	

04	GN-16	47.20	13.08	0.331	3.57	41.341	35.852	1.532	0.644	2.758	0.78	1.153	3.92
05	GN-17	47.94	13.66	0.368	3.982	41.308	34.389	1.685	0.686	2.961	0.836	1.201	3.61
06	GN-18	52.64	13.34	0.225	3.132	41.293	34.923	1.536	0.678	2.872	0.888	1.182	3.88
07	GN-19	51.30	12.70	0.421	4.060	43.116	34.135	1.488	0.549	2.480	0.707	1.263	3.90
08	GN-20	51.24	13.03	0.256	3.981	44.485	32.534	1.552	0.622	2.622	0.879	1.367	3.82
09	GN-21	49.62	13.41	0.279	3.607	41.436	35.803	1.451	0.583	2.588	0.731	1.157	3.89
10	GN-22	52.76	13.36	0.273	3.002	41.275	35.952	1.487	0.715	3.072	0.767	1.148	3.98
11	GN-23	48.38	13.72	0.242	4.416	42.137	33.737	1.679	0.598	2.679	0.668	1.248	3.58
12	GN-24	49.52	13.26	0.180	4.034	43.169	33.947	1.608	0.500	2.499	0.684	1.271	3.82

\*GN 13- Dwarf, GN 14-Bold seeded, GN 15-Pink, GN 16-Tall, GN 17-Multi branched, GN 18-Late flowered, GN 19-Early maturing, GN 20-Large leaf, GN 21-High yielding, GN 22-Faint, GN 23-Tall with early maturing and GN 24-Control.

Several high oleate groundnut varieties were reported by Chu et al., (2009). Wang et al., (2011) screened the varieties of groundnut with elevated oleate content by sodium azide mutagenesis. Wang et al., (2007) noticed that EMS was an effective chemical mutagen for improving oleic and linoleic acid content in 3 cultivar of groundnut. Mondal and Badiganavar (2010) reported the variation in fatty acid composition in groundnut after mutagenesis.

The seed oil stability is again important parameter regarding the shelf-life of products made from oil. It can be determine from the ratio of 18:1/18:2 in groundnut (Young et al 1974). Higher this ratio, the higher is the oil stability and longer is the shelf-life of groundnut products. In present investigation the oil stability among 24 prominent mutants of groundnut is highest in late flower mutant (0.15% of Ems) and Large leaf mutant (0.10% EMS) of variety AK-159 and variety TAG-24 respectively. However higher unsaturation in an oil affects seed oil stability and lowers the shelf life of such oil derived products (Lea, 1962).

**Table 3: Variability in fatty acid composition and quality parameters in viable mutants of *Arachis hypogaea* (L.) variety AK-159.**

Fatty acid/ quality parameter	Mean	Range	S.D.	S.E.	C.V.
Palmitic C16:0	13.08	12.23-14.12	0.52	0.15	3.95
Steric C 18:0	2.78	2.23-3.20	0.34	0.10	12.29
Arachidic C 20:0	1.48	1.31-1.61	0.09	0.03	6.26
Erucic C 22:1	3.21	2.90-3.71	0.20	0.06	6.33
Oleic C 18:1	40.91	39.63-42.25	0.90	0.26	2.21
Linoleic C 18:2	36.26	35.17-37.56	0.76	0.22	2.09
Nutritional quality index (NQI)	4.03	3.82-4.19	0.12	0.03	3.00
Oil stability index (OSI)	1.13	1.06-1.20	0.05	0.01	4.14

**Table 4: Variability in fatty acid composition and quality parameters in viable mutants of *Arachis hypogaea* (L.) variety TAG-24.**

Fatty acid/ quality parameter	Mean	Range	S.D.	S.E.	C.V.
Palmitic C16:0	13.19	12.65-13.73	0.33	0.10	2.52
Steric C 18:0	3.66	3.00-1.41	0.46	0.13	12.66

Arachidic C 20:0	1.57	1.45-1.69	0.08	0.02	5.00
Erucic C 22:1	2.78	2.48-3.03	0.22	0.06	7.97
Oleic C 18:1	42.15	41.28-44.49	1.00	0.29	2.37
Linoleic C 18:2	34.72	32.53-35.95	1.02	0.29	2.94
Nutritional quality index (NQI)	3.86	3.58-4.05	0.14	0.04	3.55
Oil stability index (OSI)	1.22	1.15-1.37	0.06	0.02	5.20

Groundnut oil is a source of valuable nutrients. The ratio of unsaturated fatty acid to saturated fatty acid indicates NQI of oil. These health parameters have been recommended by WHO for the dietary use of vegetable oils. It was found that, majority of the mutants possessed the NQI above 4. In variety AK-159 the highest NQI value was recorded in the large leaf and high yielding mutant, while in case of variety TAG-24 the highest NQI value was demonstrated by bold seeded mutant.

#### Conclusion:

This work has enabled us to conclude that the oil content, fatty acid composition and oil stability could get alter in positive direction through the application of physical (Gamma rays) and chemical (EMS) mutagen. Pertaining to the oil quantity 20 kR and 10 kR doses of gamma rays is more effective in variety AK-159 and TAG-24 respectively. While in improving oil quality the 0.15% concentration of EMS and 0.10% concentration of EMS are more potent in variety AK-159 and TAG-24 respectively. In conclusion, Induced mutation can enhance the oil yield, nutritional value and stability of groundnut (*Arachis hypogaea* L.)

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