ENHANCE THE GROUNDNUT VARIETIES THROUGH IN VITRO MUTAGENIC APPROACHES

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ABSTRACT

Groundnut is an important global food and oil crop that underpins agriculture-dependent livelihood strategies meeting food, nutrition, and income security. Aflatoxins, pose a major challenge to increased competitiveness of groundnut limiting access to lucrative markets and affecting populations that consume it. Other drivers of low competitiveness include allergens and limited shelf life occasioned by low oleic acid profile in the oil. Thus grain off-takers such as consumers, domestic, and export markets as well as processors need solutions to increase profitability of the grain. There are some technological solutions to these challenges and this review paper highlights advances in crop improvement to enhance groundnut grain quality and nutrient profile for food, nutrition, and economic benefits. Significant advances have been made in setting the stage for marker-assisted allele pyramiding for different aflatoxin resistance mechanisms in vitro seed colonization, pre-harvest aflatoxin contamination, and aflatoxin production which, together with pre- and post-harvest management practices, will go a long way in mitigating the aflatoxin menace.

***Key words**: aflatoxin, allergens, Arachis hypogaea, crop improvement, groundnut*

INTRODUCTION

In vitro and especially in the Northern Region, groundnuts are the most cultivated crops for their oil and protein content. Groundnut is a self-pollinated, annual leguminous crop that is fairly drought resistant and mainly cultivated in dry tropical areas. The crop increases the nitrogen content of the soil through its nodulation and biomass production. Therefore, subsequent crops that are cultivated after the harvest of the groundnut crop, benefit a lot from the residual nitrogen especially, when groundnut residues are incorporated into the soil during ploughing. Despite the high local demands for groundnuts, yields in Ghana continue to be low, averaging 0.1 t ha–1 of dry shelled seeds. Groundnut is an important oil, food and feed legume crop grown in over 100 countries in Sub-Saharan Africa. It covered 24 million ha area worldwide with a total production of 38 million tons in 20103. Groundnut is the 6th most important oilseed crop in the world. It contains 48-50% oil and 26-28% protein and is a rich source of dietary fibre, minerals and vitamins.

It has been demonstrated in many studies that genetic variability for several desired characters can be induced successfully through mutations and the practical value of mutation in plant improvement programmers has been well established4. Mutation breeding is the process of generating mutants with desirable traits when plants are exposed to chemicals or radiation. It is sometimes referred to as "variation breeding". Plants created using mutagenesis are sometimes called mutagenic plants or mutagenic seeds. Induced mutations can provide beneficial variations to breed high yielding groundnut.

More than 2252 mutant varieties of different crops have been officially released in the world5. There has been fruitful gamma irradiation for the development of new mutant varieties. Previously8 reported beneficial use of grain legume mutation breeding for the development of improved cultivars. Mutation breeding serves as a source of creating variability and could confer specific improvement in a crop without significantly altering its phenotype. The successful utilization of gamma rays to generate genetic variability in plant breeding has been reported in groundnut9. From 1930-2014 more than 3200 mutagenic plant varietals have been released that have been derived either as direct mutants (70%) or from their progeny (30%). Crop plants account for 75% of released mutagenic species with the remaining 25% being ornamentals. There are different kinds of mutagenic breeding, a few of such includes: The use of chemical mutagens, radiation and also transposons are used to generate mutants.

LITERATURE REVIEW

Sugey Vásquez-Hernández (2023) Biotechnological techniques provide a viable alternative to help improve and increase the production of plant species of agricultural and economic importance, which have been affected over the years by climate change, increasing their susceptibility to pests and/or diseases, generating losses in production as well as a decrease in their regenerative and genetic diversity. The application of biotechnological techniques such as in vitro mutagenesis offers a viable option for the generation of crops that are resistant to the different factors caused by abiotic and biotic stress. In vitro mutagenesis has been used in an efficient way to generate genetic changes in different plant species.

Bekele Hundie Kotu (2022) This study was conducted to assess the potential impact of applying a new groundnut planting density on welfare of smallholder farmers in northern Ghana. We used data from on farm experiments, focus group discussions, and a household survey. We followed three steps in our analysis. First, we conducted cost-benefit analysis in which we showed the economic advantage of the new technology over the farmers' practice. Second, we predicted adoption rates along timeline using the Adoption and Diffusion Outcome Prediction Tool (ADOPT). Third, using the results of the first and the second steps, we estimated the potential impact of the technology on poverty at household level using a combination of methods such as economic surplus model and econometric model.

Lalit Agrawal (2021) In several crop breeding programs, high selection pressure has been applied since its domestication which resulted in narrowing in the genetic variability. Therefore, obtaining new crop cultivars has become a difficult task for breeders. Development of strategies to increase the genetic variability has now become the prime area of research in crop breeding for several research groups. Mutation breeding is able to create lot of genetic diversity in the crops naturally as well as through induced mutagenesis. Mutation breeding is an important tool in plant breeding which has proven highly successful in improving crop varieties globally to feed an ever increasing and nutritionally demanding human population.

Chris O. Ojiewo (2020) Groundnut is an important global food and oil crop that underpins agriculture-dependent livelihood

strategies meeting food, nutrition, and income security. Aflatoxins, pose a major challenge to increased competitiveness of groundnut limiting access to lucrative markets and affecting populations that consume it. Other drivers of low competitiveness include allergens and limited shelf life occasioned by low oleic acid profile in the oil. Thus grain off-takers such as consumers, domestic, and export markets as well as processors need solutions to increase profitability of the grain.

Induced Mutagenesis

Since the discovery of X-ray induced mutations and the first mutants developed into bacco and apple, the field of mutagenesis has expanded tremendously in the past several decades for developing superior plant cultivars in several crop plants. Spontaneous mutation frequency is very low, on the order of one in a million. Both physical and chemical mutagens have been used to enhance the mutation rate by several folds and increase genetic variability in crop plants for a wide range of traits, including yield, plant stature, flowering, salt/drought/heat stress tolerance, disease resistance, high yield, plant architecture, and nutritional quality.

In Vitro Culture and Mutagenesis

The development of efficient in vitro culture method has facilitated the use of mutation technique for crop improvement. The haploid system can be successfully used for induction of genetic variation through mutagenesis. It has advantages like immediate fixation of mutated genotypes in the first generation, in vitro selection, and increased selection efficiency. Mutagenic treatment can be given to spikes, buds, anthers, microspores, and haploid calli; embryos or

Physical mutagenesis

In the past 80 years, physical mutagens, mostly ionizing radiations, have been used widely for inducing hereditary aberrations and more than 70% of mutant varieties were developed using physical mutagenesis (reviewed in [Citation15,Citation24]). Radiation is defined as energy travelling through a distance in the form of waves or particles. These are relatively high-energy levels of electromagnetic (EM) spectrum that are capable of dislodging electrons from the nuclear orbits of the atoms that they impact upon. The impacted atoms, therefore, become ions.

Breeding methods of groundnut

Groundnut improvement and cultivar development in SSA mainly depended on conventional breeding including pure line selection, mass selection, pedigree breeding and backcross breeding methods. For example, Serenut 5R ahigh yielding, early maturing, resistant to groundnut rosette disease and late leaf spot was released in Uganda using bulk selection. Babile-1 with the accession number ICGV-98412, released in Ghana and Ethiopia, is high yielding, medium maturing and moderately resistant to late leaf spot. It was bred at the International Crop Research Institute for Semi-Arid Tropics, Patancheru, India. Genetic variability available in cultivated and wild Arachis have been extensively exploited through conventional breeding to develop improved varieties.

New and emerging tools for groundnut breeding

Plant phenotypic data collection with sufficient resolution and accuracy remains a major limiting factor for the effective use of genomic data for crop improvement. In developing countries where groundnut yield is low, the breeding focus is to

improve yield and tolerance to biotic and abiotic stress factors. Selection of groundnut genotypes using pod yield has been slow and yielded highly variable results as yield is affected by genotype by environment interactions, which causes difficulties in selecting genotypes with wide adaptation resulting in delayed cultivar release.

METHODOLOGY

During the Kharif season of 2021 two varieties of groundnut ICGS-76 and Rajashree were treated with individual doses of 0.2% NaN3, 0.4% NaN3 and 0.6% NaN3. The treated seeds were sown as M1 generation. The Seeds were harvested from all the treatments of M1 generation including controls of both varieties. The M2 generation was raised from the bulk seeds of selected M1 plants and was laid out in FRBD in three replications along with control at the Experimental Field of the Department of Genetics & Plant Breeding, College of Agriculture, Central Agricultural University, Imphal during kharif season of 2022. All the recommended package of practices was followed as and when necessary to raise a good crop of groundnut during the period of investigation. The biological damage (injury and lethality) was computed as the percentage reduction in seedling height and survival respectively. The respective control and treatment progenies were screened several times for chlorophyll and morphological mutations throughout the crop season. Mutation frequency was calculated as percentage of mutated M2 progenies for both chlorophyll and morphological mutations in each treatment.

RESULTS

Effect of Ethyl Methane Sulphonate on analysis of individual amino acids:

The amount of individual amino acids (µg g-1) for control and EMS treatment derived multiple shoots from whole embryonal axes was presented in Table - 1, graph -1. In EMS derived multiple shoots totally 19 amino acids have been observed. The individual amino acids showed increasing and decreasing trend based on the concentrations of EMS. The amino acids like, Lysine, Histidine, Methionine, Tyrosine, Isoleucine, Tryptophan, Leucine, Phenyl alanine, Glutamine were increased with increasing concentrations of EMS up to 20mM. The content of Cystine, Aspartic acid, Alanine increased up to 30mM EMS. The content of Proline, Serine, Asginine, Glycine showed increasing trend up to 40mM EMS. The content of Glutamic acid, Threonine, Valine were totally decreased in all the five concentrations of EMS when compared to control. In 40 mM EMS treatment except few amino acids (Proline, Serine, Asginine, Glycine) and all the 19 amino acids in 50mM EMS treatment indicating the negative trend i.e. all the amino acids content significantly decreased when compared to control.

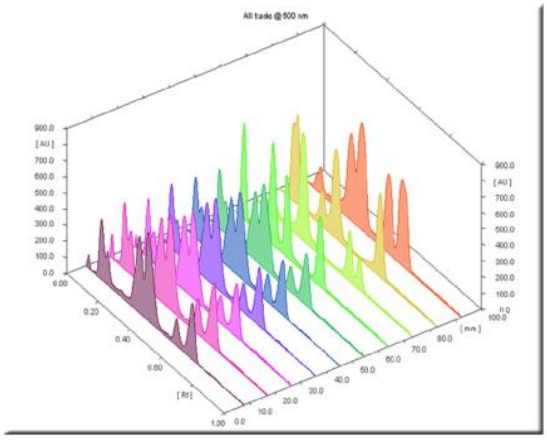
Table 1: Effect of EMS on amino acid contents in whole embryonal axes derived multiple shoots of groundnut Cv.TMV-7.

S. No	Name of the amino acids	Concentrations in µg					
		Control	10mM	20mM	30mM	40mM	50mM
1	Lysin	0.	0	0	1	0	0

	e	75	.86	.97	.07	.72	.56
2	Histidine	1.46	1.88	1.90	1.98	1.57	1.25
3	Proline	3.11	3.31	3.60	3.77	3.35	3.19
4	Serine	2.18	2.44	2.65	2.98	2.66	2.40
5	Arginine	0.73	0.88	0.96	0.99	0.80	0.61
6	Glycine	1.35	1.65	1.68	1.75	1.47	1.29
7	Glutamine	0.75	0.80	0.85	0.92	0.85	0.79
8	Cystine	0.83	0.88	0.93	0.99	0.83	0.77
9	Aspartic acid	2.19	2.31	2.48	2.76	2.18	2.02
10	Alanine	0.83	0.89	0.93	1.01	0.93	0.86
11	Glutamicacid	1.15	1.23	1.35	1.57	1.24	1.17

12	Threonine	1.06	1.11	1.38	1.41	0.99	0.94
13	Valine	1.09	1.08	1.02	0.96	0.94	0.89
14	Methionine	0.67	0.67	0.71	0.60	0.63	0.63
15	Tyrosine	2.79	2.78	2.96	2.49	2.64	2.61
16	Isoleucine	0.58	0.58	0.61	0.52	0.55	0.54
17	Tryptopham	1.11	1.20	1.21	1.07	0.97	0.95
18	Leucine	1.21	1.31	1.32	1.17	1.06	1.03
19	Phenylalanine	0.75	0.81	0.81	0.72	0.66	0.64

Figure.10: View of amino acids chromatogram in multiple shoots obtained from various concentrations of EMS



Effect of Sodium Azide on analysis of individual amino acids:

The individual amino acids levels were analyzed in control and SA treatment derived multiple shoots of whole embryonal axes were summarized in Table -2, graph - 2. In SA derived multiple shoots totally 19 amino acids have been recorded. The individual amino acids showed a marginal increasing and decreasing tendency with respect to concentrations of SA. The amino acids such as Proline, Arginine, Histidine, Glycine, Tryptopham, Glutamine, Cystine, Glutamic acid, Theronine, Valine were increased with increasing concentrations of SA up to 30mM. The levels of Aspartic acid, Alanine, Aspargine, Lysine, Methionine, Isoleucine, Leucine, Tyrosine, Phenyl alanine, increased up to 40mM SA. The levels of all the amino acids were totally reduced in 50mM SA treatment showed a negative trend i.e. all the amino acids content significantly decreased when compared to control.

Effect of mutagenic agents on callus induction and plantlet regeneration from whole embryonal axes:

The pre cultured whole embryonal axes were exposed to 1.0 to 5.0 Kr gamma irradiation and 1.0 to 5.0 mM EMS and SA introduced callus induction medium with 2.0 mg/l of IAA + 1.0mg/l of BAP. An increasing percentage of callus induction was noticed upto 3.0Kr gamma rays and 3.0mM EMS and SA. Then there was a reduction in 4.0, 5.0Kr and 4.0, 5.0mM EMS and SA. When compared to untreated embryonal leaflets, the mutagen treated embryonal leaf explants responded earlier. The treated population had higher percentage of callusing. The maximum percentage of callusing was observed in 3.0Kr gamma rays in 97.54 followed by 3.0mM EMS and SA in 95.82 and 94.65. The higher dose/ concentrations 4.0, 5.0Kr / mM of mutagenic agents were inhibitory (Table-3).

Like that of callusing ability, the fresh and dry weight of the callus increased over control up to 3.0mM concentrations of EMS and SA and 3.0 Kr gamma rays. The maximum fresh weight and dry weight of the calli were 3.55g, 0.310g in gamma rays, 3.18g, 0.289g in EMS and 2.82g, 0.235g in SA. The lower doses/

Table 2: Effect of Sodium azide on amino acid contents in whole embryonal axes derived multiple shoots of groundnut Cv.TMV.7.

	Name of the amino aci	

	d						
		Control	10 mM	20 mM	30 mM	40 mM	50 mM
1	Lysine	0.98	1.08	1.10	1.34	1.02	1.00
2	Histidine	1.56	1.72	1.73	1.76	1.63	1.44
3	Proline	2.12	2.42	2.57	2.84	2.25	2.21
4	Asginine	1.07	1.22	1.30	1.43	1.13	1.11
5	Aspargine	4.07	4.57	4.64	5.44	4.32	4.25
6	Glycine	1.08	1.28	1.51	1.57	1.22	1.17
7	Glutamine	0.54	0.63	0.69	0.72	0.57	0.47
8	Cystine	1.00	1.17	1.29	1.48	1.06	1.00
9	Asparticacid	0.47	0.49	0.51	0.71	0.50	0.39
	Alanine	0.41	0.44	0.47	0.55	0.44	0.34
	Glutamicacid	1.67	1.77	1.80	2.05	1.65	1.41
	Theronine	1.20	1.32	1.40	1.58	1.19	1.03
	Valine	0.93	1.09	1.20	1.34	0.91	0.75
	Methionine	0.63	0.73	0.85	0.96	0.70	0.62
	Tyrosine	0.85	1.34	1.55	1.29	0.94	0.83
	Isoleucine	0.30	0.48	0.51	0.66	0.63	0.30
	Tryptopham	1.21	1.34	1.68	2.15	1.62	0.93
	Leucine	1.09	1.27	1.45	1.65	1.12	0.96
	Phenylala	0.6	0.7	0.8	0.9	0.6	0.5

	nine	2	4	2	7	4	4

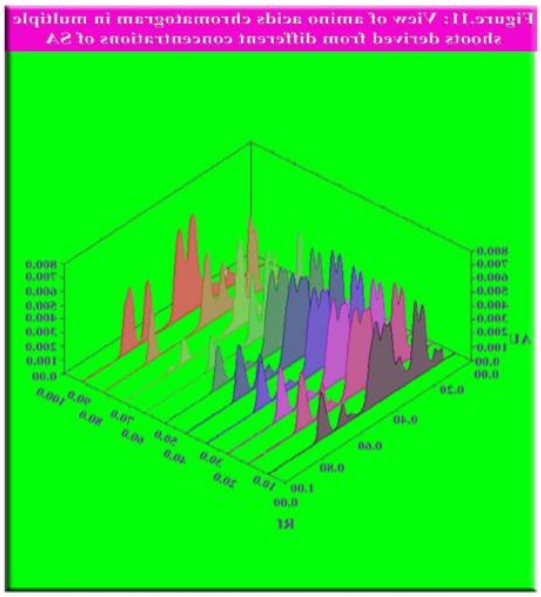


Table: 3. Effect of mutagenic agents on callus induction and callus growth of whole embryonal axes of groundnut Cv.TMV-7 (Mean±SD)

S. No	Treatment in Kr/m M		Percentage of callus induction	fresh weight(g)	Dry weight(g)
1	Gamarays	0	87.18±1.92^{d}	2.00±0.55^{d}	0.180±0.04^{d}
2		1	92.85±0.74^{bc}	2.69±0.88^{bc}	0.236±0.05^{bc}
3		2	95.33±1.44^{ab}	3.07±0.96^{b}	0.254±0.01^{b}
4		3	97.54±0.98^{a}	3.55±0.33^{a}	0.310±0.07^{a}
5		4	84.60±2.06^{de}	1.87±0.44^{de}	0.158±0.03^{de}
6		5	79.00±2.04^{f}	1.57±0.48^{ef}	0.128±0.02^{ef}
7	EMS	1	91.61±1.14^{bc}	2.41±0.42^{bc}	0.208±0.05^{bc}
8		2	94.35±1.22^{ab}	2.76±0.66^{ab}	0.222±0.01^{b}
9		3	95.82±1.44^{a}	3.18±0.12^{a}	0.289±0.03^{a}
10		4	76.44±1.68^{d}	1.50±0.29^{d}	0.132±0.02^{d}
11		5	75.32±0.98^{de}	1.31±0.12^{de}	0.120±0.03^{de}
12	SA	1	90.66±1.24^{bc}	2.14±0.59^{bc}	0.188±0.09^{bc}
13		2	93.80±0.74^{ab}	2.48±0.69^{ab}	0.203±0.02^{ab}
14		3	94.65±0.81^{a}	2.82±0.81^{a}	0.235±0.01^{a}
15		4	74.37±1.	1.32±0.6	0.103±

			52^{d}	6^{d}	0.04^{d}
16		5	71.86±1.04^{de}	1.00±0.21d^{e}	0.089±0.01^{de}

CONCLUSION

The study might emphasize the importance of continued research to fully exploit the potential of in vitro mutagenesis in groundnut improvement. This could include further elucidation of the molecular mechanisms underlying mutagenesis, optimization of mutagenesis protocols, and comprehensive phenotypic and genotypic characterization of mutant populations. Conclusions may also address considerations related to biosafety, risk assessment, and regulatory frameworks governing the release of mutagenesis-derived crops. Ensuring the safety of novel mutant varieties and adherence to regulatory guidelines is crucial for their successful deployment in agriculture. Further research should focus on optimizing mutagenesis protocols to maximize the efficiency of inducing desirable mutations while minimizing unwanted side effects. This includes determining optimal doses and exposure times for different mutagenic agents.

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