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A STUDY ON MUTAGENIC INTERVENTIONS IN GROUNDNUT CULTIVATION

MATHE ANIL KUMAR
Research Scholar
Shri JJT University

DR. DINESH KUMAR SINGH
Professor
Shri JJT University

DR. KORPOLE ANURADHA
Asst. Professor
Government Degree College, Sadasivpet.
Telangana

ABSTRACT

Mutagenesis has been a crucial tool in crop improvement programs aimed at enhancing yield, quality, and resilience to biotic and abiotic stresses. This study investigates the potential of in vitro mutagenesis for groundnut (Arachis hypogaea L.) improvement. Groundnut is an important oilseed crop globally, facing challenges such as diseases, pests, and environmental stresses, which hinder its productivity. In this research, various mutagenic agents, including chemical mutagens like ethyl methane suffocate (EMS) and physical mutagens like gamma radiation, were employed to induce genetic variations in groundnut genotypes. In vitro mutagenesis offers advantages such as controlled mutagen exposure, rapid generation of mutants, and efficient screening methods. The study evaluates the mutagenic effectiveness and efficiency by assessing the frequency and spectrum of induced mutations in key agronomic traits such as yield, oil content, disease resistance, and drought tolerance. Molecular techniques such as DNA markers are employed to characterize the induced mutations and understand their genetic basis.

Key words: Mutagenesis, crop improvement, groundnut, vitro mutagenesis, chemical mutagenesis

INTRODUCTION

Genetic variations are the basic tools in the hands of breeders to develop new cultivars with better traits, like tolerance against various environmental stresses, resistance against pests and diseases, and improved yield and quality. Thus, the mutagens is technology has been applied to plant breeding comprehensively, which allowed crops to produce beneficial varieties with good traits. Mutant varieties database reveals that 2,541 varieties derived from mutagenesis are currently registered online, among them the oilseed crops and oil-seed rape are comprised of 63 and 25 varieties, respectively.

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Tissue culture techniques have been used to induce genetic variability to improve crop plants. Various in vitro techniques are available for most crops, although optimization is still needed for some of them. In vitro techniques of protoplast, microspore, anther, ovule, and embryo culture have been used to create soma clonal and game to clonal variation. Now, we have better and efficient techniques like in vitro mutagenesis by combining both issue culture techniques and induced mutation strategy. Tissue culture techniques are utilized to create in vitro alterations because they have a number of advantages, ike a number of plant materials (e.g., in vitro axillary buds, organs, tissues, and cells) can be treated and handled easily. Easy handling of large populations for mutagenic treatment, selection, and cloning of selected variants and the rapid execution of the propagation cycles of subculture aimed to separate mutated from non-mutated sectors (dissolving a chimera to obtain homo-histones)are further significant contributions of tissue culture techniques.

In recent years, in vitro mutagenesis technology has been applied more frequently to the development of quality and to improve resistance traits, which has accelerated crop improvement and germplasm innovation. Plant regeneration through cotyledons explants is one of the best in vitro regeneration systems, which could yield many sterile explants in a short time. Moreover, the experiment will not be limited to factors such as growing season and site. Therefore, under the in vitro condition, mutagenesis through cotyledons explants has substantial potential. The use of mutation techniques in combination with in vitro culture technology can be regarded as “an ideal system” for crop improvement because of the following reasons: First, the plants produced through culturing techniques, whether haploid or doubled haploid, can express all mutations, recessive or dominant; thus, screening of recessive mutants is also possible in first generation, and mutants can be fixed rapidly. Second, it provides a large population available for mutation processes and, therefore, increases the probability to identify the beneficial mutants. Third, during he production process of mutants, chimeras is avoided.

LITERATURE REVIEW

Aras Türkoğlu (2023) Wheat (Triticum aestivum L.) is highly rich in nutrients and is an important staple food for humankind. Mutation breeding offers a relatively quick method for crop improvement and it provides variation for selective breeding programs and functional gene studies. In vitro mutagenesis, coupled with in vitro regeneration procedure, can offer a wide variety of plant materials for mutagenesis; enable generation of large mutant populations in a relatively short period. Present experiments were conducted to investigate potential use of conventional chemical mutagenesis technique through ethyl methanesulfonate (EMS) for mature embryo culture in wheat. EMS mutagenesis was experimented with 4 treatment durations and 5 treatment concentrations.

BabuN Motagi (2022) about 94% of the world groundnut (Arachis hypogaea L.) production comes from the rainfed crop grown largely by resource-poor farmers. Several biotic and abiotic stresses limit groundnut productivity, together causing annual yield losses of over US $ 3.2 billion, and probably half of this could be recovered through genetic enhancement in groundnut. Cultivated species and the wild Arachis species do carry novel genes which could be employed for improvement of both seed yield and quality in addition to imparting resistance

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to diseases and insect pests. Many of the wild Arachis species are not cross compatible with the cultivated groundnut. However, the efforts to overcome incompatibility in wide crosses have been successful in transferring the novel genes through interspecific progenies.

Oliyad Sori (2021) Groundnut production is important for consumption, income generation and improves food security of smallholder farmers in Western Oromia. Unlike its importance its production has less concern and it's marketing is challenged by the amount produced. The study aimed to analyze determinants of groundnut market supply in western Oromia region, Ethiopia. In order to do this, both primary and secondary data were used to collect qualitative and quantitative data. A multi-stage sampling technique was used to select samples of groundnut producers from the study area. Primary data were collected from randomly select 400 sampled groundnut producers through a semi-structured questionnaire.

Jing-shan WANG (2020) Peanut (Arachis hypogaea L.) is an important oil crop globally and high oil content is one of the major targets in peanut breeding programs. Previous studies indicated that the osmotic pressure (OP) of the leaves of peanut plants subjected to drought stress was negatively correlated with kernel oil content. Based on this knowledge, we established a practical and reliable method for creating new peanut varieties with high oil content using in vitro mutagenesis and directional OP-based selection. Using embryonic leaflets of peanut variety Huayu 20 as explants, pingyangmycin (PYM) as the mutagen, and hydroxyproline (HYP) as the OP regulator, we developed 15 HYP-tolerant regenerated plants.

Mutagenesis

Mutagenesis is a process by which the genetic information of an organism is changed by the production of a mutation. It may occur spontaneously in nature, or as a result of exposure to mutagens. It can also be achieved experimentally using laboratory procedures. A mutagen is a mutation-causing agent, be it chemical or physical, which results in an increased rate of mutations in an organism's genetic code. In nature mutagenesis can lead to cancer and various heritable diseases, and it is also a driving force of evolution. Mutagenesis as a science was developed based on work done in the first half of the 20th century. Mutagenesis is the process by which an organism's deoxyribonucleic acids (DNA) change, resulting in a gene mutation.

Adaptive mutagenesis mechanisms

Adaptive mutagenesis has been defined as mutagenesis mechanisms that enable an organism to adapt to an environmental stress. Since the variety of environmental stresses is very broad, the mechanisms that enable it are also quite broad, as far as research on the field has shown. For instance, in bacteria, while modulation of the SOS response and endogenous prophage DNA synthesis has been shown to increase Acineto bacter baumannii resistance to ciprofloxacin. Resistance mechanisms are presumed to be linked to chromosomal mutation un transferable via horizontal gene transfer in some members of family Enterobacteriaceae, such as E. coli, Salmonella spp., Klebsiella spp., and Enterobacter spp. Chromosomal events, specially gene amplification, seem also to be relevant to this adaptive mutagenesis in bacteria.

Site-directed mutagenesis

Site-directed mutagenesis is a molecular biology method that is used to make specific and intentional mutating changes to the DNA sequence of a gene and any gene products. Also called site-specific mutagenesis or oligonucleotide-directed mutagenesis, it is used for

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investigating the structure and biological activity of DNA, RNA, and protein molecules, and for protein engineering. Site-directed mutagenesis is one of the most important laboratory techniques for creating DNA libraries by introducing mutations into DNA sequences.

Cassette mutagenesis

Unlike other methods, cassette mutagenesis need not involve primer extension using DNA polymerase. In this method, a fragment of DNA is synthesized, and then inserted into a plasmid. It involves the cleavage by a restriction enzyme at a site in the plasmid and subsequent ligation of a pair of complementary oligonucleotides containing the mutation in the gene of interest to the plasmid. Usually, the restriction enzymes that cut at the plasmid and the oligonucleotide are the same, permitting sticky ends of the plasmid and insert to ligate to one another. This method can generate mutants at close to 100% efficiency, but is limited by the availability of suitable restriction sites flanking the site that is to be mutated.

Previous studies on groundnut mutagenesis

The groundnut or peanut is one of the important legume crops of tropical and semiarid tropical countries, where it provides a major source of edible oil and vegetable protein. Groundnut kernels contain 47-53% oil and 25-36% protein. The crop is cultivated between 40ºN to 40ºS of the equator. Groundnut is a self-pollinated crop whereby flowers are produced above ground and, after fertilization, pegs move towards the soil, and seed-containing pods are formed and developed underneath the soil.

METHODOLOGY

For treatment with EMS and SA, the known quantity of EMS and SA were mixed in MS basal medium and dissolved thoroughly. The pH of the medium was adjusted to 6.0 for EMS and pH 3.0 for SA prior to mutagenic treatment. The solutions were then brought in to the laminar air flow chamber and sterilized in millipore filter unit with the help of vacuum pump and collected in sterilized conical flasks for the treatment of seeds. Based on the imbibitions period, two hours duration was fixed for the treatment of EMS and SA. Before mutagenic treatment the seeds were pre-soaked in water for four hours. From EMS and SA treated seeds, the WEC and WEA explants used for direct organogenesis and apical portion of mature embryo were used for somatic embryogenesis, immature leaflet and cotyledonary segments explants were used callus induction and biochemical analysis.

RESULTS

Callus induction from cotyledonary segments:

Calli were induced from cotyledonary segments excised from aseptically grown seedlings of groundnut (7- days old) and cultured on callus induction medium supplemented with five different concentrations of NAA +KIN and NAA+BAP (1.0 to 3.0 mg/l and 0.5 to 1.5 mg/l) for callus induction. The cotyledonary segments of all concentrations swelling up after six days of inoculation and callus formed afterwards. The callus growth was observed in all the concentrations of NAA at different levels. The rate of callus induction, fresh and dry weight of the calli were increased with increasing concentrations of NAA+KIN. In NAA+KIN combinations, the callus induction rate was noticed from 60.00 to 82.00. Like this the relative fresh weight of the calli differed from significantly among the various hormonal

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concentrations. The callus production was strongly influenced by NAA. The fresh weight ranged from 1.02 to 1.81 g and the dry weight recorded from 0.122 to 0.195g.

The cotyledonary segments also cultured on medium containing NAA+BAP like that of NAA+KIN. When compared to NAA+KIN combinations all the growth characteristics were significantly increased in different concentrations of NAA+BAP. The percentage of response was recorded from 63.33 to 90.66.Like that, the fresh weight was noticed from 1.45 to 2.09g and the corresponding dry weights were 0.155 to 0.213g .

Table: 1. Effect of IAA with KIN and BAP on callus induction from hypocotyl segments of groundnut cultivar TMV-7 (Mean±SD)

S.No	Hormones(mg/l)		Percentage of callus induction	Callus fresh weight(g)	Callus dry weight(g)
	IAA	KIN			
1	1.0+0.50		62.10±3.00^{e}	0.95±0.036^{e}	0.057±0.005^{de}
2	1.5+0.75		75.24±1.30^{cd}	1.64±0.030^{cd}	0.069±0.006^{cd}
3	2.0+1.00		89.33±1.87^{a}	2.33±0.024^{a}	0.141±0.007^{a}
4	2.5+1.25		86.12±3.00^{ab}	1.81±0.022^{bc}	0.117±0.009^{b}
5	3.0+1.50		78.62±1.41^{c}	1.87±0.024^{b}	0.087±0.004^{bc}
	IAA	BAP			
1	1.0+0.50		66.22±2.28^{e}	0.95±0.009^{de}	0.067±0.004^{e}
2	1.5+0.75		75.81±1.92^{d}	1.74±0.011^{cd}	0.094±0.003^{cd}
3	2.0+1.00		95.26±1.30^{a}	2.54±0.024^{a}	0.171±0.008^{a}
4	2.5+1.25		87.50±1.37^{b}	2.06±0.014^{bc}	0.111±0.003^{bc}
5	3.0+1.50		86.12±1.47^{bc}	1.99±0.198^{b}	0.128±0.006^{b}

Values with the same superscript are not significantly different at the 0.5% probability level according to Duncan‟s Multiple Range Test.

Table: 2. Effect of NAA along with KIN and BAP on callus induction of cotyledonary segments in groundnut Cv.TMV-7 (Mean±SD)

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S.No	Hormones(mg/l)	Percentage of Callus induction	Callus fresh weight(g)	Callus dry weight(g)
	NAA+KIN			
1	1.0+0.50	60.00±1.12^{e}	1.02±0.096^{de}	0.122±0.020^{de}
2	1.5+0.75	68.01±1.42^{cd}	1.26±0.154^{cd}	0.141±0.010^{cd}
3	2.0+1.00	73.33±0.57^{bc}	1.43±0.124^{bc}	0.153±0.020^{bc}
4	2.5+1.25	77.12±1.15^{b}	1.58±0.115^{b}	0.172±0.011^{b}
5	3.0+1.50	82.00±1.00^{a}	1.81±0.088^{a}	0.195±0.015^{a}
	NAA+BAP			
1	1.0+0.50	63.33±1.52^{e}	1.45±0.076^{de}	0.155±0.004^{de}
2	1.5+0.75	73.33±0.57^{cd}	1.53±0.069^{cd}	0.165±0.005^{cd}
3	2.0+1.00	80.00±1.52^{bc}	1.59±0.070^{bc}	0.171±0.009^{bc}
4	2.5+1.25	83.22±2.11^{b}	1.76±0.046^{b}	0.187±0.001^{b}
5	3.0+1.50	90.66±0.57^{a}	2.09±0.002^{a}	0.213±0.002^{a}

Values with the same superscript are not significantly different at the 0.5% probability level according to Duncan‟s Multiple Range Test.

Callus induction from immature leaflets:

The immature leaflets were collected from aseptically grown seedlings of groundnut (7- days old) and evaluated for callus induction. The callus induction medium containing five different concentrations of 2,4-D +KIN and 2,4-D +BAP (1.0 to 3.0 mg/l and 0.5 to 1.5 mg/l). The immature leaflets of all concentrations swelling up after four days of inoculation and callus started appearing after8 to 10 days. The callus growth was observed in all the concentrations of 2,4-D at different levels. The percentage of callus induction, fresh and dry weight of the calli were increased with increasing concentrations of 2,4-D +KIN. In 2,4-D +KIN combinations the percentage of callus induction was found from 62.25 to 85.31. Like this the relative fresh weight of the calli differed significantly among the different hormonal concentrations. The callus production was highly influenced by 2,4-D. The fresh weight ranged from 0.91 to 2.21g and the dry weight recorded from 0.067 to 0.157g . The immature leaflets also transferred on medium containing 2,4-D +BAP like that of 2,4-D +KIN. When compared to 2,4-D+KIN

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combinations all the growth parameter were interestingly increased in different concentrations of 2,4-D +BAP. The percentage of response was noted from 63.12 to 88.65. Like that the fresh weight was noticed from 0.95 to 2.66g and the corresponding dry weights were 0.67 to 0.198g (Table-3).

Somatic Embryogenesis from apical region of the mature embryo:

The direct somatic embryogenesis were observed using auxins alone. The addition of cytokinins like BAP or KIN did not influence either the percentage of embryogenic cultures nor number of somatic embryos per culture. The somatic embryos were induced only in solid medium. The apical portion of the mature embryos were cultured on somatic embryo induction medium containing 2,4-D and NAA individually. The auxinconcentrations was ranged from 10.0 to 50.0mg/l. The apical portion of the mature embryos directly formed somatic embryos without intermediate callus phase when inoculated on auxin

Table: 3. Effect of 2,4-D in combination with KIN and BAP on callus induction from immature leaflets of groundnut Cv.TMV-7 (Mean±SD)

S.No	Hormones mg/l		Percentage of Callus induction	Callus	
	2,4-D	KIN		Fresh weight(g)	Dry weight(g)
1	1.0+0.50		62.25±3.00^{e}	0.91±0.036^{de}	0.067±0.005^{de}
2	1.5+0.75		71.83±1.30^{d}	0.95±0.030^{cd}	0.071±0.006^{d}
3	2.0+1.00		78.62±1.41b^{c}	1.26±0.024^{bc}	0.097±0.004^{bc}
4	2.5+1.25		81.50±1.87^{b}	1.53±0.024^{ab}	0.111±0.007^{b}
5	3.0+1.50		85.31±1.47^{a}	2.21±0.022^{a}	0.157±0.009^{a}
	2,4-D	BAP			
1	1.0+0.50		63.12=2.28^{e}	0.95±0.009^{de}	0.067±0.004^{de}
2	1.5+0.75		75.24±1.92^{d}	1.04±0.021^{cd}	0.074±0.001^{cd}
3	2.0+1.00		82.54±1.3^{7bc}	1.29±0.014^{c}	0.091±0.003^{bc}

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4	2.5+1.25	84.33±1.30[ab]	1.84±0.021[b]	0.131±0.008[b]
5	3.0+1.50	88.65±3.00[a]	2.66±0.198[a]	0.198±0.006[a]

Values with the same superscript are not significantly different at the 0.5% probability level according to Duncan‟s Multiple Range Test. containing medium. The apical portion of the mature embryos were cultured on somatic embryo induction medium with different concentrations of NAA. The percentage of response and mean number of somatic embryos per culture was increased upto 30.0mg/l of NAA and then there was a reduction in responsive culture and number of somatic embryos per culture.

CONCLUSION

The study may evaluate the efficiency of mutagenesis techniques in generating desired genetic variation within groundnut populations. This assessment could include the frequency of induced mutations, the spectrum of mutations generated, and the heritability of induced traits. Conclusions may be drawn regarding the phenotypic diversity observed among mutant populations generated through in vitro mutagenesis. This could include variations in plant morphology, growth habits, flowering time, seed size, oil content, and resistance/tolerance to biotic and abiotic stresses. The study could identify promising mutant lines exhibiting desirable agronomic traits for further evaluation and breeding purposes. These mutants may demonstrate improved yield potential, nutritional quality, disease resistance, or adaptation to specific environmental conditions.

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