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EVALUATION OF CYTOLOGICAL VARIATIONS IN HYDROXYLAMINE HYDROCHLORIDE AND X-RAY-INDUCED M_1 AND M_2 GENERATIONS OF GROUNDNUT (*Arachis hypogea* L.) GENOTYPES

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ABSTRACT
It is pertinent to concede the transmissible portion of polymorphism as it provides heterogeneity component measure for the transmission of traits to next generation. The research is aim at evaluating cytological variations in Hydroxylamine hydrochloride and X-ray-induced M_1 and M_2 generations of groundnut (Arachis hypogea L.) genotypes. Groundnut production in Nigeria is constrained by multiple factors including low genetic variability. Pot trials were carried out during 2024/2025 dry seasons at the teaching and research field, Department of Plant Science and Biotechnology, Bayero University, Kano. Two improved groundnut genotypes (SAMNUT-24, SAMNUT-25) were collected from Kano State Agricultural and Rural Development Authority (KNARDA) and the two Local varieties ('YAR-GINGO and 'YAR-FARA) were collected from Dawanau Market, Dawakin Tofa Local Government Kano State. The experimental design used was Complete Randomized Design (CRD) with a total of nine hundred seeds of each variety. The seeds were soaked in different concentrations of Hydroxylamine hydrochloride (0.00mM, 1.0mM, 2.0mM, 3.0mM and 4.0mM) for three (3) hours. Seed irradiation was carried out at Radiography department, Specialist Hospital Dutsin-Ma, Katsina State at 0, 100, 200, 300 and 400 Gy, with non-irradiated bag as the control. The results revealed significant variation among the genotypes. YARGINZO treated with maximal dose of Hydroxylamine Hydrochloride at 3.0 Mm and SAMNUT-25 at 4.0mM produced highest frequency of severely aneuploidy cells when compared to control and the remaining treatments. It is recommended that, farmers should adopt the use of genotypes with mild hyper-aneuploidy and near-aneuploidy best for genetic improvement due to relative stability.

Keywords: Groundnut, Cytological Variations, Chromosomal Number, Euploidy

INTRODUCTION
Groundnut (*Arachis hypogea* L.) the oldest cultivated plant in the world also known as peanut belongs to the sub-family Papiodonoiceae under the family leguminoceae. It is firstly found in South America and India but originated from Bolivia (Janila *et al.,* 2016). Ground nut is one of the major food crops in the tropics especially Asia and African continent. Groundnut productivity significantly varies among regions with Africa having the lowest mean yield of around 965 kg/ha. The productivity has significantly increased over the last five decades with a global yield average increasing from 989 kg/ha in 1998 to 1955 kg/ha in 2018, which is attributed to significant advances in genetics, genomics, breeding and crop management (FAOSTAT, 2018). Jetsada *et al*. (2025) reported that, the world production of groundnuts in 2023 was approximately 54 million metric tons, with China leading as the largest producer at 18.3 million metric tons, followed by India at 6.3 million tons. Other significant producers include Nigeria, Senegal, and the United States, contributing to the global supply of this essential oil seed crop. The production of groundnuts in Africa varied across different regions. In South Africa, there was a downward trend in groundnut production due to insufficient production to meet local demand, with a 20.5% decrease in intended groundnut hectares annually (Samah *et al*., 2022). In Nigeria the annual production of groundnut was decreased to around 4.45 lakh tonnes (445,000 metric tonnes) due to low genetic variability which impact the development of high-yielding varieties. The productivity of groundnut cultivation in African countries is poor due to factors such as unreliable rains, traditional small-scale farming with little mechanization, outbreaks of pests and diseases, use of low-yielding varieties, and limited extension services (Jetsada *et al*., 2025).
Hydroxylamine hydrochloride is a chemical mutagen serving as a plant mutagen in breeding program to induce genetic variations and potentially improve crop characteristics (Rizwan *et al*., 2007). It works by causing changes in DNA, which can lead to mutations in plants, affecting traits like yield, flowering time, and plant height. Exposure of plant to Hydroxylamine hydrochloride enhances genetic gain and improve the productivity of the crop (Girma *et al*., 2023). Hydroxylamine hydrochloride is commonly used in plant breeding to induce polyploidy, which involve doubling the number of chromosome in a plant leading to larger plants with bigger organs like flowers, fruits, seeds, leaves, stems, and roots (Konzak and Wagner, 2015). Crop improvement by mutagenesis has been applied in a number of crops for incorporation of desirable traits in existing plant varieties or for creation of new cultivars. The primary goal of mutation breeding is increasing genetic variation inducing beneficial mutations in a number of crops (Nada *et al*., 2023). They work by interfering with one of the stages in the mitosis process, causing an increase in ploidy that affects the morphological characteristics of plants (Janila *et al.,* 2016). This research

assesses the manipulation of genetically important characteristics by integration genomic tools using chemical mutagens to enhance the genetic gain and improve the productivity of the crop.

Statement of Problems

Although groundnut ranks second among oil seed crops in the world after sun flower and also an excellent source of protein, carbohydrates minerals and antioxidant, the production in Nigeria is constrained by multiple factors including low genetic variability which impact the development of high-yielding varieties (FAOSTAT, 2018). This is due to its allotetraploidy nature with poor genetic variability, poor agronomic factors, narrow genetic bases of the cultivar, low genetic variability for the characteristics of importance, polyploidization nature, limited utilization of wild species' potential nature hindering improvement of the groundnut production (Wang *et al*., 2021). Other factors contributing to the low yields include the erratic nature of rainfall in sub-saharan areas, the excessive drought, extreme moisture that leads to flooding and lodging as well as the low soil fertility are some of the major problems causing low variability with total crop failure. This research will deploy the genotypic variability for agronomic traits in chemically mutagenized groundnut to develop groundnut improve variety.

MATERIALS AND METHODS

Study Area

Pot trials were carried out during 2025/2026 dry season under screen house at the teaching and research field in the Department of Plant Science and Biotechnology, Bayero University, Kano which lies between latitude 11^0 58N and longitude $8^0$30E with altitude of 440m above sea level. The site is typical Sudan Savanna ecological zone of Nigeria.

Selection of Study Plants

The seeds of the two improved groundnut genotype varieties (SAMNUT-24, SAMNUT-25) were collected from Kano State Agricultural and Rural Development Authority (KNARDA) and the two Local varieties ('YAR-GINGO and 'YAR-FARA) were collected from Dawanau Market, Dawakin Tofa Local Governent Kano State.

Experimental Design

The experimental design used was Complete Randomized Design (CRD) with a total of nine hundred seeds of each variety sorted out and surface sterilized in 2.0% Sodium hypochloride for 1minute. The seeds were then rinsed three (3) times in sterile distilled water. The seeds were soaked in different concentrations of Hydroxylamine hydrochloride (0.00mM, 1.0mM, 2.0mM, 3.0mM and 4.0mM) for three (3) hours. The seeds were then rinsed with distilled water to remove the excess chemicals and exudate from the seeds. Another seeds were irradiated with different X-ray radiation at 0, 100, 200, 300 and 400 Gy, with non-irradiated bag as the control. Top soil was mixed with compost manure in a ratio of 3:1 and placed in a polypots of 30 x 30 x 40cm in diameter laid out at 0.5 X 0.5m, having the same soil combinations, each at the rate of 7kg per polypot. Five (5) treated seeds were sown at the depth of 3cm by dibbling with two fingers and a thumb, covered with soil and moderately watered every other day. The seed produced from M_0 generation were replanted to produce the M_1 and M_2 generation.

Treatment of Groundnut Varieties

Various doses of X-ray radiations were used in the study. Irradiation was carried out at Radiography department, Specialist Hospital Dutsin-Ma, Katsina State. The total number of 25 seeds per genotype were placed in 15 × 22 cm zip-lock plastic bags, each containing approximately 225 groundnut seeds replicated three times. The bags were irradiated with different X-ray radiation at 0, 100, 200, 300 and 400 Gy, with non-irradiated bag as the control. Another seeds were soaked in different concentrations of Hydroxylamine Hydrochloride (0.00mM, 1.0mM, 2.0mM, 3.0mM and 4.0mM) for three (3) hours.

RESULTS AND DISCUSSION

Chromosome Counting and Numerical Variations of M_2 Generation in Groundnut (*Arachis hypogaea* L.) Genotype Induced by Hydroxylamine Hydrochloride

The number of chromosomes in the M_2 generation were assessed using the squashing method in which plant samples from each treatment were selected and the root tips were cut between the hours of 0.00 - 11.00am, the period in which the metaphase cells are optimally active for observing the number of chromosomes in groundnut during the mitosis phase. Chromosome calculations were carried out manually with the help of the Image J application. The cytological observation was carried out using the procedure of Carnoy's fixative and Giemsa staining as present in Fig 1. The result obtained revealed significant variation in chromosome numbers ranging from 17 to 25 chromosomes, whereas the normal diploid chromosome number for cultivated groundnut is n = 20. In somatic metaphase, spreads where approximately 20 chromosomes visibly observed and the cells regarded as euploid, indicating genomic stability of groundnut. This confirmed proper mitotic division and chromosomal stability obtained in untreated genotype.

In plant treated with Hydroxylamine hydrochloride, variation in chromosomal number distribution showed differential mutagenic effect in Hydroxylamine Hydrochloride-treated plants. SAMNUT-24 Plant treated with moderate dose of 3.0mM produced less frequent (19-chromosomes) showing common chromosomes loss. YARGINZO Plant treated with maximal dose of Hydroxylamine Hydrochloride at 3.0 mM and SAMNUT-25 at 4.0mM produced highest frequency of severely aneuploidy cells (25 chromosome number) when compared to control and the remaining treatments. The result also indicated that, cells with 17-18 chromosomes observe under hypo-aneuploidy, show chromosome loss during cell division. The occurrence of 21-23 chromosomes indicated mild hyper-aneuploidy, while 24-25 chromosomes show severe hyper-aneuploidy.

Figure 1: Hydroxylamine Hydrochloride
HHCL-0.00=20,V1-HHCL-1.0=23, V1HHCL-2.0=22, V1-HHCL-3.0=19, V2-HHCL-1.0=23, V2-HHCL-2.0=23, V2-HHCL-4.0=25, V3-HHCL-1.0=20, V3-HHCL-2.0=24, V3-HHCL-4.0=25, V4-HHCL-1.0=24, V4-HHCL-2.0=22, V4-HHCL-3.0=2

Chromosome Counting and Numerical Variations of M_2 Generation in Groundnut (*Arachis hypogaea* L.) Genotype Induced by X-ray Radiation

Chromosome counting and numerical variations of M_2 generation in groundnut (*Arachis hypogaea* L.) genotype induced by x-ray radiation were presented in Fig 2. The counting of chromosome number remains a fundamental cytogenetic approach for assessing genome stability, ploidy level, and chromosomal abnormalities in plants. The result obtained from Fig 2.0 showed that cells exhibiting 20 chromosomes were considered euploid, while deviations from this number indicated chromosomal numerical aberrations. The observation on number of chromosomes in the M_2 generation were carried out on nine plant selected genotypes from each treatment as present in plate 2.0. Mitotic chromosomes counting was produced in x-ray radiation treated plants. The variation in chromosomal number distribution showed differential mutagenic effect in X-ray radiation treated plants. SAMNUT-24, SAMNUT-25 and YARGINZO Plant treated with higher dose of 400GY produced higher frequency of severely aneuploidy cells (24 and 25 chromosome number) when compared to control and the remaining treatment. The chromosomal number observed in SAMNUT-25 plant with moderate dose of x-ray radiation at 300GY were less frequent (17 chromosome number) indicating multiple chromosomes losses. The observations showed that the chromosome that ranged from 17 to 25, indicated the occurrence of aneuploidy induced by X-ray radiation. Cells that observed 17-18 chromosomes were grouped as hypo-aneuploid, indicating loss of chromosome.

The occurrence of 21-23 chromosomes was categorized as mild hyper-aneuploidy, leading to chromosome gain through nondisjunction and chromatid non-separation. This may be due to the fact that x-ray radiation interfere with attachment of kinetochore leading to unequal chromosome distribution. Singh (2014) reported that low to moderate doses of radiation often produce mild hyper-aneuploidy rather than complete polyploidy in crop plants. More pronounced deviations were produced in cells with 24–25 chromosomes, grouped as severe hyper-aneuploidy showing extensive genomic instability and chromosomal imbalance. Severe hyper-aneuploid cells were associated with abnormalities in the morphology of cell and reduction of mitotic index.

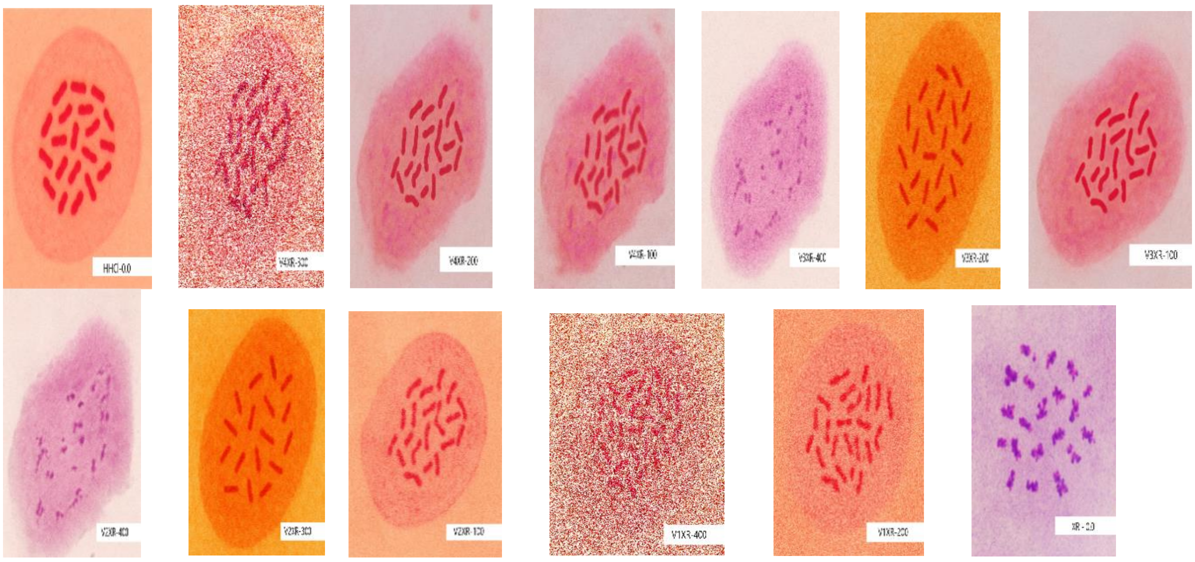

Figure 2: X-ray Radiations
Key = XR-0.0=20, V1-XR-200GY=23, V1-XR-400GY=25, V2-XR-100GY=21, V2-XR-300GY=17, V2-XR-400GY=25, V3-XR-100GY=20, V3-XR-200GY=23, V3-XR-400GY=24, V3-XR-100GY=20,V3-XR-200GY=21,V3-XR-300GY=24.

The Estimation of Chromosomal Number of Three Cells in some Selected Hydroxylamine Hydrochloride and X-ray Radiation-Treated Groundnut Genotypes

Table 1 presents the chromosomal number variations observed in selected groundnut genotypes treated with Hydroxylamine hydrochloride. The table shows the chromosomal counts recorded from three metaphase cells, namely First Cell Arrested at Metaphase (FCAM), Second Cell Arrested at Metaphase (SCAM), and Third Cell Arrested at Metaphase (TCAM), together with their mean performance and chromosomal number (CN). The results indicated substantial cytological variations among the treated genotypes, showing that chemical mutagen effectively induces chromosomal abnormalities.

The control plants under X-ray treatment maintained the normal diploid chromosome number of 2n = 20, which was classified as euploidy. The mean chromosome number for the control was 20.00 ± 0.51 under Hydroxylamine hydrochloride treatment and 20.00 ± 0.54 under X-ray treatment. The chromosomes appeared normal and uniformly arranged during metaphase, indicating cytological stability and normal mitotic activity in untreated plants. Under Hydroxylamine hydrochloride treatment, the genotype SAMNUT-24 treated with 1.0 mM produced 23 chromosomes with a mean performance of 23.00 ± 0.61, which was classified as mild hyper-aneuploidy. At 2.0 mM, chromosome number slightly reduced to 22 chromosomes with a mean of 22.00 ± 0.55, but still remained within the mild hyper-aneuploid category. However, treatment with 3.0 mM caused a reduction to 19 chromosomes with a mean of 19.00 ± 0.49, indicating near-euploidy. This suggests that moderate mutagen concentration may induce chromosome loss or partial stabilization of chromosome segregation.

The genotype SAMNUT-25 exhibited chromosome gains at all mutagen concentrations. Treatments with 1.0 mM and 2.0 mM both produced 23 chromosomes with mean performances of 23.00 ± 0.61, indicating mild hyper-aneuploidy. At 4.0 mM, chromosome number increased markedly to 25 chromosomes with a mean performance of 25.00 ± 0.72, representing severe hyper-aneuploidy. This indicates that higher mutagen concentration intensified chromosomal imbalance and genomic instability. Similarly, the genotype YAR'GINZO maintained normal euploidy at 1.0 mM with 20 chromosomes and a mean value of 20.00 ± 0.73, suggesting resistance to low mutagen concentration. However, increasing the concentration to 3.0 mM and 4.0 mM induced 24 and 25 chromosomes, respectively, with mean performances of 24.00 ± 0.64 and 25.00 ± 0.72, both classified as severe hyper-aneuploidy. This demonstrates that higher Hydroxylamine hydrochloride concentrations progressively disrupted normal chromosome segregation. The genotype YAR'FARA also showed marked chromosomal abnormalities under Hydroxylamine hydrochloride treatment. At 1.0 mM, chromosome number increased to 24 chromosomes with a mean value of 24.00 ± 0.64, indicating severe hyper-aneuploidy. Treatment with 2.0 mM reduced chromosome number slightly to 22 chromosomes with a mean performance of 22.00 ± 0.55, classified as mild hyper-aneuploidy. However, at 3.0 mM, chromosome number again increased to 24 chromosomes with a mean of 24.00 ± 0.64, suggesting severe chromosomal imbalance at higher mutagen concentration.

Table 1: The Estimation of Chromosomal Number of Three Cells in some Selected Hydroxylamine Hydrochloride and X-ray Radiation-Treated Groundnut Genotypes

Sodium Azide Genotypes	Aneuploidy Rating	FCAM	SCAM	TCAM	Mean Performance	CN
Hydroxylamine						
CONTROL	Euploidy	20	20	20	20.00±0.51	2n = 2x = 20
SAMNUT-24,1.0 Mm	Mild Hyperaneuploidy	23	23	23	23.00±0.61	2n = 2x = 23
SAMNUT-24,2.0 mM	Mild Hyperaneuploidy	22	22	22	22.00±0.55	2n = 2x = 22
SAMNUT-24,3.0 mM	Near-euploidy	19	19	19	19.00±0.49	2n = 2x = 19
SAMNUT-25,1.0 Mm	Mild Hyperaneuploidy	23	23	23	23.00±0.61	2n = 2x = 23
SAMNUT-25,2.0 Mm	Mild Hyperaneuploidy	23	23	23	23.00±0.61	2n = 2x = 23
SAMNUT-25,4.0 mM	Severe Hyperaneuploidy	25	25	25	25.00±0.72	2n = 2x = 25
YAR'GINZO, 1.0 Mm	Euploidy	20	20	20	20.00±0.73	2n = 2x = 20
YAR'GINZO, 3.0 Mm	Severe Hyperaneuploidy	24	24	24	24.00±0.64	2n = 2x = 24
YAR'GINZO, 4.0 Mm	Severe Hyperaneuploidy	25	25	25	25.00±0.72	2n = 2x = 25
YAR'FARA, 1.0 mM	Severe Hyperaneuploidy	24	24	24	24.00±0.64	2n = 2x = 24
YAR'FARA, 2.0Mm	Mild Hyperaneuploidy	22	22	22	22.00±0.55	2n = 2x = 22
YAR'FARA, 3.0Mm	Severe Hyperaneuploidy	24	24	24	24.00±0.64	2n = 2x = 24

KEY; ± Standard Error, FCAMS = First Cell Arrested at Metaphase Stage, SCAMS = Second Cell Arrested at Metaphase Stage, TCAMS= Third Cell Arrested at Metaphase Stage, CN = Chromosomal Number

The result in table 2 indicated substantial cytological variations among the treated genotypes, showing that physical mutagen effectively induced chromosomal abnormalities in groundnut. The control plants under X-ray treatment was observed normal diploid chromosome number of 2n = 20, which was classified as euploidy. The genotype SAMNUT-24 exposed to 100GY produced 21 chromosomes with a mean value of 21.00 ± 0.58, indicating mild hyper-aneuploidy. Increasing the irradiation dose to 200GY produced 23 chromosomes with a mean of 23.00 ± 0.64, while exposure to 400GY caused a further increase to 25 chromosomes with a mean value of 25.00 ± 0.69, representing severe hyper-aneuploidy. The progressive increase in chromosome number demonstrates a dose-dependent mutagenic effect of X-ray irradiation. In genotype SAMNUT-25, treatment with 100GY produced 21 chromosomes with a mean value of 21.00 ± 0.58, indicating mild hyper-aneuploidy. However, exposure to 300GY reduced chromosome number drastically to 17 chromosomes with a mean value of 17.00 ± 0.43, which was classified as hypo-aneuploidy. This reduction suggests chromosome loss due to spindle malfunction, chromosome lagging, or chromosome breakage induced by irradiation. At 400GY, chromosome number increased sharply to 25 chromosomes with a mean value of 25.00 ± 0.69, indicating severe hyper-aneuploidy and extensive genomic instability.

The genotype YAR'GINZO maintained normal euploidy at 100GY with 20 chromosomes and a mean value of 20.00 ± 0.54, indicating relative chromosomal stability at low irradiation dose. However, exposure to 200GY increased chromosome number to 23 chromosomes with a mean of 23.00 ± 0.64, while 400GY produced 24 chromosomes with a mean value of 24.00 ± 0.67, both indicating hyper-aneuploidy. Similarly, the genotype YAR'FARA maintained euploidy at 100GY with 20 chromosomes, but exposure to 200GY increased chromosome number to 21 chromosomes with a mean value of 21.00 ± 0.58, indicating mild hyper-aneuploidy. Treatment with 300GY further increased chromosome number to 24 chromosomes with a mean performance of 24.00 ± 0.67, representing severe hyper-aneuploidy.

The results demonstrated that both Hydroxylamine Hydrochloride and X-ray irradiation effectively induced chromosomal alterations ranging from hypo-aneuploidy (17 chromosomes) to severe hyper-aneuploidy (25 chromosomes). The predominance of hyper-aneuploid cells suggests that chromosome gain occurred more frequently than chromosome loss following mutagenic treatment. These findings confirm that mutagenic treatments successfully generated substantial cytogenetic variability in groundnut, which may serve as valuable genetic resources for mutation breeding and crop improvement programs.

Table 2: The Estimation of Chromosomal Number of Three Cells in some Selected X-ray Radiation-Treated Groundnut Genotypes

Sodium Azide Genotypes	Aneuploidy Rating	FCAM	SCAM	TCAM	Mean Performance	CN
X-ray						
CONTROL	Euploidy	20	20	20	20.00±0.54	2n = 2x = 20
SAMNUT-24, 100GY	Mild Hyperaneuploidy	21	21	21	21.00±0.58	2n = 2x = 21
SAMNUT-24, 200GY	Mild Hyperaneuploidy	23	23	23	23.00±0.64	2n = 2x = 23
SAMNUT-24, 400GY	Severe Hyperaneuploidy	25	25	25	25.00±0.69	2n = 2x = 25
SAMNUT-25, 100GY	Mild Hyperaneuploidy	21	21	21	21.00±0.58	2n = 2x = 21
SAMNUT-25, 300GY	Hypo-aneuploidy	17	17	17	17.00±0.43	2n = 2x = 17
SAMNUT-25, 400GY	Severe Hyperaneuploidy	25	25	25	25.00±0.69	2n = 2x = 25
YAR'GINZO, 100GY	Euploidy	20	20	20	20.00±0.54	2n = 2x = 20
YAR'GINZO, 200GY	Mild Hyperaneuploidy	23	23	23	23.00±0.64	2n = 2x = 23
YAR'GINZO, 400GY	Severe Hyperaneuploidy	24	24	24	24.00±0.67	2n = 2x = 24
YAR'FARA, 100GY	Euploidy	20	20	20	20.00±0.54	2n = 2x = 20

Sodium Azide Genotypes	Aneuploidy Rating	FCAM	SCAM	TCAM	Mean Performance	CN
YAR'FARA, 200GY	Mild Hyperaneuploidy	21	21	21	21.00±0.58	2n = 2x = 21
YAR'FARA, 300GY	Severe Hyperaneuploidy	24	24	24	24.00±0.67	2n = 2x = 24

KEY; ± Standard Error, FCAMS = First Cell Arrested at Metaphase Stage, SCAMS = Second Cell Arrested at Metaphase Stage, TCAMS= Third Cell Arrested at Metaphase Stage, CN = Chromosomal Number

Discussion

The present study demonstrated that Hydroxylamine hydrochloride and X-ray irradiation effectively induced chromosomal abnormalities and numerical chromosome variations in groundnut genotypes. The observed changes from euploidy to mild and severe hyper-aneuploidy confirm the mutagenic activity of Hydroxylamine Hydrochloride and its ability to generate cytogenetic variability useful in mutation breeding programs. The control cells maintained the normal diploid chromosome number of 2n = 20, which agrees with previous cytological reports on cultivated groundnut. This confirms the chromosomal stability and normal mitotic behavior of untreated plants. However, exposure to Hydroxylamine Hydrochloride resulted in increased chromosome numbers ranging from 21 to 25 chromosomes, indicating mutagen-induced chromosomal instability.

The result obtained indicated that, cells with 17-18 chromosomes observe under hypo-aneuploidy, show chromosome loss during cell division. This result may be due to abnormal function of spindle fiber and lagging of chromosome at anaphase stage. Hypo-aneuploid cells are usually less viable leading to reduction in seedling vigor with developmental abnormalities. This result was in conformity with work of Wang e*t al*. (2021) who reported that chemical mutagenic treatments significantly affect the segregation leading to monosomic or nullisomic conditions. The cells which exhibit 19 chromosomes were observed as near-euploid, showing minimal chromosomal variation. The conditions of near-euploid produced from minor nondisjunction events may not severely affect phenotypic expression, especially if the missing or extra chromosome carries redundant genetic material. This work was in line with work confirmed by Singh and Sharma (2020) in legumes who pinpointed that near-euploid variations could be manifested without drastic phenotypic effects, although subtle yield may be produced.

The predominance of hyper-aneuploid cells observed in the treated samples suggests that Hydroxylamine Hydrochloride interfered with normal spindle organization and chromosome segregation during mitosis. Chemical mutagens such as hydroxylamine are known to induce DNA base modifications and replication errors, which may disrupt chromosome movement and lead to nondisjunction during cell division. Similar findings were reported by Ibrahim *et al*. (2021), who observed increased frequencies of hyperploid cells following chemical mutagenesis in legumes. The occurrence of severe hyper-aneuploidy involving 24 and 25 chromosomes particularly at higher concentrations indicates that increasing mutagen dosage intensified chromosomal abnormalities. This supports the general principle that mutagenic effects are concentration dependent, with higher concentrations causing greater genomic instability. Similar concentration-dependent chromosomal variations have been reported in peanut and soybean treated with chemical mutagens (Rahman *et al*., 2022). Similar work has been reported by Sharma & Sharma, (2013) in mutagenesis and cytogenetic analyses of legumes where staining intensity and the duration of fixation significantly affected chromosome clarity and count accuracy.

Genotype-dependent differences were also evident in the present investigation. For instance, Yarginzo genotype exhibited severe hyper-aneuploidy across multiple concentrations, suggesting greater sensitivity to Hydroxylamine hydrochloride treatment. In contrast, some genotypes exhibited only mild hyper-aneuploidy at lower concentrations, indicating relatively greater chromosomal stability. Such differential responses among genotypes may arise from variations in DNA repair efficiency, chromosome organization, and genetic background. Similar genotype-specific responses to chemical mutagens have been reported by Bello *et al*. (2023) in leguminous crops. The absence of substantial hypo-aneuploid conditions suggests that Hydroxylamine hydrochloride mainly promoted chromosome gain rather than chromosome elimination. Chromosome gain may result from spindle malfunction, chromosome stickiness, or incomplete chromosomal migration during anaphase. Hyper-aneuploid cells may contribute positively to mutation breeding because changes in chromosome number can alter gene dosage and generate novel phenotypic variability that may be useful for crop improvement.

The occurrence of euploid, hypo-aneuploid, mild hyper-aneuploid, and severe hyper-aneuploid cells confirms the mutagenic effectiveness of ionizing radiation in generating cytogenetic variability. The normal chromosome number observed in the control cells (2n = 20) agrees with the established diploid chromosome number reported for cultivated groundnut (Arachis hypogaea L.), indicating cytological stability in untreated plants. The induction of mild hyper-aneuploid conditions such as 21, 23, and 24 chromosomes suggests that X-ray irradiation interfered with normal chromosome segregation during mitosis. Ionizing radiation is known to disrupt spindle apparatus formation and induce nondisjunction, resulting in chromosome gain during cell division. Similar findings have been reported by Kumar *et al*. (2021), who observed increased frequencies of hyperploid cells in irradiated legume species due to spindle disturbances and chromosomal duplication. This result was in agreement with work of Datta, (2001) in legumes and cereals who reported severe chromosomal alterations due to high-dose irradiation contributing to cytogenetic variability, which can be exploited in mutation breeding programs.

The occurrence of 17 chromosomes in Samnut-25 treated with 300GY indicates hypo-aneuploidy caused by chromosome loss. Such chromosomal reductions may arise from chromosome lagging, spindle malfunction, chromosome breakage, or incomplete chromosomal migration during anaphase. Hypo-aneuploid cells are generally associated with reduced vigor and instability because loss of chromosomes may eliminate essential genes required for normal physiological and developmental processes. Similar chromosome losses induced by radiation mutagenesis were reported in peanut and soybean by Akinwale *et al*. (2023), where high irradiation doses caused chromosome elimination and mitotic abnormalities. The progressive increase in chromosome number from 20 chromosomes in the control to 24–25 chromosomes at higher irradiation doses demonstrates a clear dose-dependent mutagenic response. Severe hyper-aneuploidy observed at 400GY indicates that high radiation doses induced extensive genomic imbalance and abnormal chromosome duplication. This finding supports earlier reports that high-energy radiation causes severe mitotic disturbances, chromosome stickiness, spindle abnormalities, and nondisjunction events leading to hyperploid conditions (Singh and Sharma, 2020).

Some genotypes such as Yarginzo maintained euploidy at lower irradiation doses, suggesting possible genetic resistance or greater chromosomal stability under mild radiation stress. This variation among genotypes indicates differential mutagen sensitivity, which is important in mutation breeding programs because certain genotypes may tolerate mutagenic treatments better while still generating useful genetic variability. Similar genotype-dependent responses to irradiation have been documented in legumes and cereals where chromosomal sensitivity varied according to genetic background (Mensah *et al.*, 2023). The predominance of hyper-aneuploid conditions over hypo-aneuploid conditions in the present study indicates that chromosome gain occurred more frequently than chromosome loss following X-ray exposure. This may be attributed to radiation-induced spindle dysfunction resulting in nondisjunction and unequal chromosome segregation. Hyper-aneuploid cells may contribute useful variability for mutation breeding because moderate chromosome gain can alter gene dosage and potentially create desirable agronomic traits.

The Estimation of chromosomal number of three cells in some selected Hydroxylamine hydrochloride and X-ray radiation-treated groundnut genotypes observed in the present study confirmed that both hydroxylamine hydrochloride and X-ray irradiation successfully induced cytogenetic alterations. The control plants maintained the normal diploid chromosome number of 2n = 20 and were classified as euploid, indicating chromosomal stability in untreated plants. Similar observations of stable euploid chromosome numbers in untreated groundnut genotypes were reported by Mensah *et al.* (2021) who noted that normal chromosome complement is essential for proper mitotic division and phenotypic stability. The maintenance of euploidy in the control also suggests that the chromosomal abnormalities observed in treated plants were directly associated with mutagenic action rather than spontaneous mutation events (Kumar and Bhat, 2022).

Hydroxylamine hydrochloride treatments induced varying levels of chromosomal alterations ranging from near-euploidy to severe hyperaneuploidy. In SAMNUT-24, treatment with 1.0 mM and 2.0 mM produced chromosome numbers of 23 and 22 respectively, which were classified as mild hyperaneuploidy, while 3.0 mM reduced the chromosome number to 19, resulting in near-euploidy. This suggests that moderate concentrations promoted chromosome gain whereas increased concentration caused partial chromosome loss. Similar findings were reported by Oladosu *et al*. (2021) who observed that chemical mutagens can induce both chromosome additions and deletions depending on dosage and exposure duration. The occurrence of near-euploidy at 19 chromosomes indicates slight chromosomal imbalance that may still permit cell survival and division (Singh *et al*., 2023).

In SAMNUT-25, Hydroxylamine hydrochloride treatments at 1.0 mM and 2.0 mM resulted in chromosome numbers of 23, while 4.0 mM produced 25 chromosomes classified as severe hyperaneuploidy. The increase in chromosome number at higher concentration indicates strong mutagenic interference with spindle fibre formation and chromosome segregation during metaphase. According to Adewale and Ogunbosoye (2022), severe hyperaneuploid conditions arise when mutagens disrupt mitotic spindle apparatus leading to nondisjunction and chromosome duplication. Similar chromosomal increases induced by chemical mutagens have also been documented in legumes and oilseed crops where higher mutagen doses generated polyploid-like chromosome complements (Raina *et al*., 2024).

The genotype YAR'GINZO exhibited greater chromosomal instability under hydroxylamine treatment, with chromosome numbers increasing from 20 at 1.0 mM to 24 and 25 at 3.0 mM and 4.0 mM respectively. These chromosome gains indicate progressive hyperaneuploidy associated with increasing mutagen concentration. Such chromosomal instability may enhance genetic variability useful for crop improvement. Previous studies by Bado *et al.* (2021) explained that induced hyperaneuploidy can generate useful variability in quantitative traits through alteration of gene dosage and chromosomal rearrangement. Likewise, the severe hyperaneuploidy observed at 25 chromosomes reflects substantial genomic imbalance which may influence fertility, vigour and yield expression (Sinta, 2018).

YAR'FARA also responded strongly to hydroxylamine hydrochloride treatment. Chromosome numbers of 24, 22 and 24 were recorded at 1.0, 2.0 and 3.0 mM respectively, indicating both mild and severe hyperaneuploid conditions. The repeated occurrence of 24 chromosomes suggests that this genotype may possess greater tolerance to chromosome gain induced by chemical mutagens. Genotypic differences in mutagen response have been widely reported in groundnut and other legumes due to variations in DNA repair efficiency and chromosomal sensitivity (Patil *et al*., 2023). This work was in line with finding of Aremo *et al.* (2024) in peanut who reported that, the persistence of viable hyperaneuploid cells demonstrates the effectiveness of hydroxylamine hydrochloride in inducing heritable chromosomal changes useful in mutation breeding programmes (Aremo *et al*., 2024). Tarra (2024) have shown that chemical and physical treatments are efficient agent for plant chromosomal doubling.

The X-ray irradiation treatments also produced significant chromosomal alterations across the groundnut genotypes. In SAMNUT-24, exposure to 100GY and 200GY produced 21 and 23 chromosomes respectively, while 400GY resulted in 25 chromosomes classified as severe hyperaneuploidy. This progressive increase in chromosome number with increasing radiation dose indicates dose-dependent chromosomal instability. Similar dose-dependent increases in chromosomal aberrations following gamma and X-ray irradiation were reported by Wani *et al*. (2022), who observed that ionizing radiation causes chromosome fragmentation and abnormal rejoining during cell division. The severe hyperaneuploidy at 400GY suggests extensive genomic disturbance caused by high-energy radiation exposure (Karthika and Dorairaj, 2023).

In SAMNUT-25, 100GY produced mild hyperaneuploidy with 21 chromosomes, whereas 300GY caused hypoaneuploidy with 17 chromosomes. The reduction to 17 chromosomes indicates chromosome loss due to radiation-induced breakage or lagging chromosomes during mitosis. Hypoaneuploid cells often exhibit reduced vigour because of gene deficiency and chromosomal imbalance. Similar reductions in chromosome number following irradiation have been reported in peanut and soybean where high radiation doses caused chromosome elimination and reduced mitotic stability (Chen *et al*., 2021). The recovery of severe hyperaneuploidy at 400GY with 25 chromosomes further indicates that radiation can simultaneously induce chromosome loss and chromosome gain depending on cellular response mechanisms (Rahman *et al*., 2024).

The genotype YAR'GINZO maintained euploidy at 100GY but shifted to mild and severe hyperaneuploidy at 200GY and

400GY respectively. This suggests that lower radiation doses were tolerated without chromosomal alteration, whereas higher doses exceeded the genotype's repair capacity leading to abnormal chromosome segregation. Similar genotype-dependent radiation responses were described by Akinwale *et al*. (2023), who reported that tolerant genotypes often maintain chromosomal integrity at low irradiation levels but develop aberrations at higher doses. The ability of YAR'GINZO to maintain euploidy at lower dose may indicate stronger DNA repair mechanisms relative to other genotypes.

YAR'FARA displayed euploidy at 100GY, mild hyperaneuploidy at 200GY and severe hyperaneuploidy at 300GY with chromosome number increasing to 24. The progressive increase in chromosome number confirms that radiation exposure altered mitotic chromosome behaviour in a dose-dependent manner. According to Tarra (2024), irradiation-induced chromosome duplication may contribute to increased cell size, altered metabolism and enhanced phenotypic variability in crop plants. Such induced variability is important in mutation breeding because it expands the genetic base available for selection of desirable agronomic traits ((Doyle and Coate, 2019).

The study demonstrated that both hydroxylamine hydrochloride and X-ray irradiation effectively induced chromosomal variations in groundnut genotypes, with higher doses producing greater cytogenetic instability. The occurrence of mild hyperaneuploidy, severe hyperaneuploidy and hypoaneuploidy confirms that mutagenic treatments disrupted normal chromosome segregation during mitosis. These chromosomal alterations may serve as useful sources of genetic variability for future groundnut improvement programmes. Similar conclusions were reached by Khan *et al*. (2024), who emphasized that induced chromosomal mutations remain important tools for broadening the genetic diversity of self-pollinated crops such as groundnut.

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