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Research Article

Harnessing Induced Mutations to Develop Superior Tiger Nut Varieties Using Ethyl Methanesulfonate (EMS) and Colchicine

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Abstract

The limited breeding advancements in tiger nuts (Cyperus esculentus L.) have left African farmers reliant on unimproved gene pools with no released varieties. The vegetative nature of the crop restricts natural hybridization, necessitating induced mutation breeding to improve productivity, nutritional value, early maturity, and resilience to environmental stresses. Four high-yielding genotypes (two brown and two black) were selected for improvement and treated with Ethyl Methanesulfonate (EMS) and Colchicine at varying concentrations to determine lethal dose (LD50) and reduction dose (RD50) values. Following these, 600 tubers were treated and planted in successive generations (M1-M4). Results showed that tuber coat colour was unaffected by mutagen treatments, while significant effects were observed in growth traits such as plant height, girth expansion, and leaf length. The mutant Cb5 achieved the highest mean plant height (115.75 cm), and colchicine treatments induced early maturity, with four brown mutants maturing within 75-77 days. Yield analysis revealed ten mutants surpassing controls in hundred tuber weight, with Eb2 recording the highest weight. Nutritional and mineral analysis across 13 selected genotypes highlighted significant variability in 12 nutrient elements. Hierarchical cluster analysis of 25g enotypes demonstrated high genetic diversity, reflecting the potential for breeding improvement and varietal release. This study highlights the utility of induced mutation breeding in developing tiger nut cultivars with enhanced agronomic and nutritional traits. These findings contribute to food security, economic development, and nutrition-sensitive agriculture, offering a pathway for sustainable tiger nut cultivation and improved livelihoods for farmers.

Keywords: Tiger Nut Breeding; Induced Mutation; Genetic Diversity; Ethyl Methanesulfonate (EMS); Colchicine Treatment; Nutritional Analysis

Introduction

Currently, the world's human population is a little over 8 billion and still experiencing a gradual increase every day. It is envisaged to be 9 billion by 2050 [1]. This surge in population presents a massive concern for a reliable and efficient food supply system to meet the nutritional demands of the rising population for assured food security. According to [2], 8.9% of the global human population suffers from hunger, 3 billion people face malnutrition problems, and 2 billion are food insecure [3,4]. This underscores the need for innovative crop improvement techniques by plant breeders to forestall the food and nutritional need challenges ahead of time.

As an underutilized and neglected food crop, tiger nut tubers are rich in all the food nutrients [5] providing quick access to a balanced and nutritious diet for healthy body growth and development. Once harvested, the tubers are ready for consumption without any tedious and sophisticated cooking process. They can be wash-dried, eaten raw, roasted, or milled into powder for consumption. Tiger nut tubers account for a great deal of fiber for digestive health, fats (monounsaturated and polyunsaturated) for a healthy heart, vitamins such as vitamin E providing antioxidants to protect cells from damage, and minerals such as calcium, magnesium, potassium, and iron [6,7]. These minerals help in the mineralization of bones, cofactors to enzyme systems, the sustenance of muscles and nerve excitation, and the maintenance of the oxygen-carrying capacity of the blood (Clarkson, 2013; Ghosh., *et al*., 2016) [8,9]. Tiger nut is not only a source of food in the form of chewable tubers, processed oil, and milk but also beneficial as feed, medicine, and perfumes [10,11], and provides great returns of foreign exchange.

Over decades, agricultural practices for crop improvement had been on the approach of conventional breeding which takes several years to create a diversity of gene pool for genetic advancement to meet breeder's interests. To improve crop plants, plant scientists including plant breeders have to rely on three methods: cross-breeding, mutation, and transformation [12]. For a fast and reliable approach to creating genetic variation for trait improvement in crop plants, plant breeders since 1920 have exploited the use of induced mutation [13-15]. Mutation breeding for crop improvement utilizes the deliberate action of generating genetic variations among existing genotypes [16]. Mutation can spontaneously occur naturally taking several years or be artificially induced within a shorter space of time using mutagens. Induced mutation has been frequently applied in widening the genetic diversity of vegetatively propagated crops of which tiger nut is no exception. It has been successfully used in bananas, potatoes, cassava, and other crops [17,18]. It makes use of either physical or chemical mutagens. Physical mutagens employ the use of ionizing radiations such as alpha rays, beta rays, X-rays, gamma rays, cosmic rays, and others, or non-ionizing radiations, for example; ultraviolet rays, infrared rays, microwaves, and laser among others. Ionising radiations have been extensively employed in plant mutagenesis due to their greater convenience compared to chemical mutagens. They cause biological injuries in plants resulting in point mutation, deletion, single and double chromosomal strand break, and chromosomal rearrangement [12]. However, they are costly compared to chemical mutagenesis.

Commonly used chemical mutagens in plants include alkylating agents, for example; ethyl methane sulfonate (EMS), mustard gas, ethyleneimine (EI), dimethyl sulfide (DMS), sodium azide (SA) [19] and Colchicine [20]. Chemical mutagens such as EMS causes point mutation and has also relatively silent (missense) mutational effect (50%) with about 5% nonesense mutations [21]. Colchicine on the other hand induces polyploidy by doubling or multiplying the chromosome sets of plants resulting in changes in plant characteristics. Some polyploids blatantly produce bigger organs via flowers, seeds, leaves, stems, and roots [22]. Induced mutations are crucial for crops enhancement by creating genetic diversity, which if properly managed and screened would result in the creation of novel and superior crop varieties with promising traits fulfilling the breeder's objectives. Breeding by this technique proffers a substantial scope for promoting sustainable agricultural progress and food security [23,24]. It increases crop yield while addressing the problems of pests and diseases, nutrition, and climate shocks [25-27].

The cultivation of tiger nuts has over the years witnessed little or no significant breeding improvements. Hence, the tiger nut accessions presently in the hands of African farmers constitute a warehouse of an unimproved natural gene pool coupled with no va-

rietal release. Additionally, the vegetative nature of this crop does not promote natural hybridization over years of cultivation. Improving on such natural germplasm therefore calls for immediate implementation of induced mutation breeding techniques in order to create cultivars that exhibit increased productivity, superior nutritional value, early maturity, and resilience to environmental stressors.

Materials and Methods

Experimental site and land preparation for putative mutants

The trials were conducted at the CSIR-Crops Research Institute, situated in Fumesua within the Ashanti Region (010 360W; 060 430N). This locale is characterized by an elevation of 186 m above sea level, positioned within the semi-deciduous forest zone. The average temperature range recorded falls between 21°C as the minimum and 31°C as the maximum. The annual cumulative rainfall averages at 1727.2mm, exhibiting a bimodal distribution pattern. The primary rainy season extends from March to July, while the secondary rainfall phase spans from August to November. The mean relative humidity stands at 95% during mornings and decreases to 61% by noon.

The experimental site was ploughed and harrowed for M_1 and M_2. Row ridges were subsequently made. For M_3, sacks filled with soil (sandy loam) were used. The soil was mixed with groundnut husk and packed into the sacks to enrich the soil fertility and also enhance soil porosity for good soil-water infiltration, plant root penetration, and tuber development. Prepared sacks filled with soil were sprayed with a solution of Dusban insecticide at a dose of 200 ml per 15 litres of water to control termites and other insects' threat to the crop.

Planting materials and treatment

A total of six hundred tubers were planted for the M_{1g} eneration. These tubers comprised 50 BUO-B control, 100 BUO-B EMS-treated, 50 OFF-b control, 100 OFF-b EMS-treated, 50 ENK-B control, 100 ENK-B colchicine-treated, 50 APR-b control, and 100 APR-b colchicine-treated specimens. The EMS treatment for the black and brown genotypes involved solution concentrations of 0.97% and 0.63%, respectively. Meanwhile, the colchicine-treated black and brown genotypes were exposed to solution concentrations of 1.65% and 0.83%, respectively, as determined by their LD_{50} and RD_{50} computations in an earlier work [5].

For the subsequent M_{2g} eneration, a total of one thousand one hundred and sixty-eight tubers were screened from the M_1 harvest and used for bulk planting. Among these, there were 73 BUO-B control tubers, 219 BUO-B EMS-treated tubers, 73 OFF-b control tubers, 219 OFF-b EMS-treated tubers, 73 ENK-B control tubers, 219 ENK-B colchicine-treated tubers, 73 APR-b control tubers, and 219 APR-b colchicine-treated tubers.

Proceeding to the M_{3g} eneration, one hundred tubers were allocated for planting. These included 3 BUO-B control tubers, 22 BUO-B EMS-treated tubers, 3 OFF-b control tubers, 22 OFF-b EMS-treated tubers, 3 ENK-B control tubers, 22 ENK-B colchicine-treated tubers, 3 APR-b control tubers, and 22 APR-b colchicine-treated tubers.

Experimental design, planting, Selection and cultural practices

The M_1, M_2, and M_3 accessions were cultivated using a Randomized Complete Block Design (RCBD) without replications, as each distinct putative mutant line was unique and still undergoing segregation to attain homozygosity. Selection of useful mutants was based on the evaluation of yield parameters (overall tuber weight, hundred tuber count weight, tuber size), earliness to maturity, flowering capability, and attachment of tubers to plants during harvest.

Regular hoe weeding was conducted to manage weeds when necessary. Throughout each planting season of M_1-M_3, manual weed control was implemented three times. The initial weeding occurred 3 weeks after planting, followed by the second and third weeding at two and three months after planting, respectively. Irrigation was carried out according to the water requirements of both the soil and the plants, with no application of fertilizers. In the case of M_3 plants, weeds in sacks were manually removed.

Evaluation of selected mutant lines (M_4)

The chosen mutants underwent experimental assessment openly at the Legumes Department site of the Crop Research Institute, Fumesua, located in the Ashanti Region (010 360W; 060 430N). In alignment with breeding objectives, twenty-five tubers were designated from the M_3 as planting materials for the M_4 evaluation trial. These tubers comprised of 1 BUO-B control, 5 BUO-B EMS

treated, 1 OFF-b control, 6 OFF-b EMS treated, 1 ENK-B control, 5 ENK-B colchicine treated, 1 APR-b control, and 5 APR-b colchicine treated. These genetic types were sown using a Randomised Complete Block Design with two replications. Two tubers per hole were seeded for each genotype within a block and three in replications, resulting in a total of 150 tubers.

Prior to planting, the soil-filled sacks, each with a volume of 21,606.67 cm^3 were treated with Dusban solution to protect against termites. The sacks subsequently, were arranged on a cleared open field but covered with a black polythene sheet to control weed growth from the ground (Figure 1).

Figure 1: Sacks filled with soil containing one week old plants arranged on black polythene sheet.

Data collection and analysis of mutant lines

Agro-morphological data

A total of 20 agro-morphological traits were meticulously gathered in this study. These traits encompassed Days to Germination (DG), Number of Tillers per Stand (NT), Distance from the Last Tiller to the Mother Plant (DTP), Plant Height (PH), Girth of mother plant (GP), Number of Leaves (NL), Leaf Length (LL), Leaf Width (LW), Days to Flower/Inflorescence Set (DF), Number of Inflorescence (NI), Days to Physiological Maturity (DPM), Number of Tubers Attached (NTA), Number of Tubers Detached (NTD), Number of Tubers per Stand (NTS), Number of Rings per Tuber (NRT), Length per Tuber (LT), Width per Tuber (WP), Tuber Shape (TS), Hundred Tuber Weight per Genotype (HWT), and Overall Tuber Weight (OTW).

Data were cautiously collected from each plant within all three replications. Specifically, the Number of Tillers per Stand (NT), Distance from the Last Tiller to the Mother Plant (DTP), Plant Height (PH), and Percentage Inflorescence (PI) were assessed at the time of flowering (inflorescence). The Number of Tubers per Stand (NTS) and Attachment of Tubers to the Plant were recorded at harvest. The Number of Rings per Tuber (NRT), Length per Tuber (LT), Width per Tuber (WP), Tuber Shape (TS), Hundred Tuber Weight per Genotype (HWT), and Overall Tuber Weight (OTW) were measured in four days after harvest and subsequent drying.

For specific measurements, the Number of Tillers (NT), Number of Tubers (NTS), and Number of Rings (NRT) were quantified. The Distance from the Last Tiller to the Mother Plant (DTP) and Plant Height (PH) were determined using a meter rule. The Percentage Germination (PG) and Percentage Inflorescence (PI) were observed and expressed as percentages. Days to Germination (DG) and Days to Flower/Inflorescence Set (DF) were counted from the day of planting. Days to Harvest/Maturity (DM) was noted at the period corresponding to the physiological maturity of the crop retrospective to planting. Number of Tubers Attached (NTA), Number of Tubers Detached (NTD), and Number of Tubers per Stand (NTS) were counted and recorded at harvest. Length per Tuber (LT) and Width per Tuber (WP) were precisely measured using a vernier caliper. Tuber Shape (TS) was computed using the length-to-width (L/W) ratio, categorized as oval (<1.3), ovoid (1.3-1.8), or oblong (>1.8) based on previous research (Pascual., *et al*., 2000). Overall Tuber Weight (OTW) and Hundred Tuber Weight per Genotype (HWT) were determined using an electronic balance. The latter was randomly selected from the composite of all three replications per genotype.

Proximate and mineral data

After two weeks of mild sun-air drying, the tubers were subjected to proximate and mineral analysis. The proximate analysis was carried out at the Department of Biological Science, Kwame Nkrumah University of Science and Technology (KNUST) following the protocol of the Association of Official Analytical Chemists (AOAC) to determine the protein, fat, ash, carbohydrate, moisture,

and energy contents in triplicates of the thirteen tiger nut genotypes. Thirteen (13) out of the 25g enotypes were selected by screening following the objective of the research for the nutrients analyses due to resources at our disposal at the course of the experiment.

Moisture content was determined using AOAC 930.15; crude fat (AOAC 2003.05); ash (AOAC 942.05); crude fibre (AOAC 978.10); and crude protein (AOAC 2001.11). The percentage of carbohydrates was calculated using the formula

Carbohydrate (%) = 100 - (% moisture + % fat + % protein + % ash + % fibre).

On the part of mineral analysis, six (6) mineral elements made up of phosphorous, potassium, calcium, magnesium, sodium, and iron were determined for the 13g enotypes in three (3) replications at the Department of Crops and Soil Sciences, KNUST using the protocol of [28] and [29].

Data analysis

Univariate analysis was conducted, which involved employing methods such as Analysis of Variance (ANOVA) and descriptive statistics encompassing mean, minimum, maximum, mean square, standard error, standard deviation, and coefficient of variation. Genstat version 11.1 and Minitab version 21.1.1.0 were used for statistical analyses.

Results and Discussion

Variation in plant architecture

Qualitatively, few phenotypic variations were observed among the putative mutants at M_1 to M_{3g} enerations for plant architectural traits. This observation can be explained by several factors: Plant architectural traits, such as height, branching patterns, and leaf shape, are often controlled by multiple genes and can be highly stable. These traits might not exhibit significant changes immediately after mutation because the alterations may not impact the key regulatory pathways that control these traits. For example, plant height and branching are controlled by a network of genes, including those involved in hormone signalling pathways such as auxins, gibberellins, and cytokinins [30]. Also, the chemical mutagenesis may not have been efficient enough to cause visible changes in plant architecture. Many induced mutations are silent or affect non-coding regions of the DNA, leading to no visible phenotypic changes. Research has shown that the majority of mutations induced by chemical mutagens like EMS (ethyl methanesulfonate) are point mutations that often result in subtle or no phenotypic effects [31].

In addition, during the early generations (M_1 to M_3), there might be natural or artificial selection pressures that favour the survival of plants with typical architectural traits. Mutants with severe or noticeable deviations might be less viable or fertile, thus being less represented in subsequent generations. Studies on mutagenized plant populations have documented this phenomenon [32]. Again, some mutations might not express their phenotypic effects until later generations due to genetic background interactions, environmental conditions, or epigenetic factors. In the early generations, the phenotypic effects of mutations might be masked by dominant alleles or not yet fully manifest. Recessive mutations, for instance, do not express their effects until the mutant alleles are homozygous [33]. Above all, plant architectural traits are often complex and polygenic. Mutations in single genes might not produce noticeable changes because the overall architecture is controlled by the interaction of many genes. It may take several mutations in specific genes to produce a visible phenotypic change. Complex traits like plant architecture are often influenced by quantitative trait loci (QTLs), where multiple small-effect genes contribute to the overall phenotype [34]. Finally, the expression of architectural traits can be highly influenced by environmental conditions. If the environmental conditions remain consistent, there may be limited observable variation in these traits despite underlying genetic mutations. For example, the expression of branching patterns or plant height can vary with light, water, and nutrient availability, which can mask genetic mutations if the environment remains constant [35].

EMS (ethyl methanesulfonate) is a well-known mutagenic agent used in plant breeding and genetic studies to induce random mutations in the genome. It works by alkylating DNA bases, primarily guanine, leading to base-pair substitutions during DNA replication [31]. These substitutions can result in point mutations, which may alter gene function and consequently affect phenotypic traits.

As a result, some of the EMS-treated genotypes showed chimeric features in the form of chlorination of the leaves (Figure 2B), and multiple-shoots for the main plant (Figure 2C) among others at the M_1 to M_3 compared to their control (Figure 2A). These were much visible in the EMS brown-treated genotypes, suggestive of the occurrence of mutation. This agreed with studies that have shown that EMS treatment can induce a wide range of mutations in plants, including single nucleotide substitutions, insertions, deletions, and rearrangements [32]. These mutations can lead to various phenotypic changes, such as alterations in leaf morphology, pigmentation patterns, flowering time, growth habits, and reproductive structures [30].

Chimeric features, such as chlorination of leaves and the development of multiple shoots, can arise as a result of somatic mutations induced by EMS [36]. Somatic mutations occur in somatic cells and can give rise to localized changes in phenotype within the plant, leading to chimeric or mosaic patterns. For example, chlorination of leaves may indicate altered chlorophyll production due to mutations affecting chloroplast function or pigment biosynthesis pathways.

Multiple shoots on the main plant can result from mutations affecting the regulation of shoot development pathways, such as the balance between apical dominance and branching. Mutations in genes controlling shoot meristem activity or hormone signaling pathways, such as auxin and cytokinin, can disrupt normal shoot growth patterns, leading to the formation of multiple shoots.

Comparing EMS-treated genotypes to untreated controls allows us to assess the phenotypic effects of mutagenesis and identify mutants with desirable traits for further breeding or genetic studies [33]. The frequency of chlorophyll mutation is said to be frequent in populations treated with EMS [37] and the higher the frequency of observing chimeras, the higher the chances of selecting true useful mutants [38,39].

Plant growth characteristics

Plant growth parameters such as plant height, stem girth, number of leaves, leaf length, and leaf width are essential morpho-physiological traits that contribute to increased biomass. A significant difference existed for the 25g enotypes for plant height of the current study at $p < 0.001$. It ranged from 92.00 cm for CBC (colchicine black control) to 115.75 cm for Cb5 (colchicine brown mutant). The majority of the colchicine-treated genotypes appreciated in plant height than their control and also than their EMS-treated genotypes (Table 1).

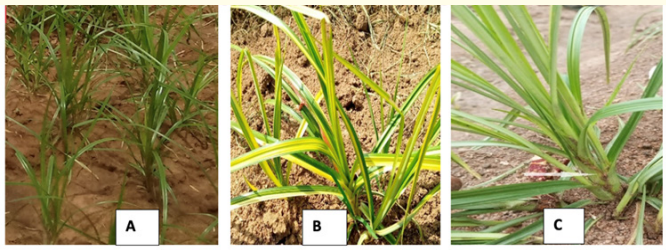

Figure 2: Phenotypic expressions of control and putative mutant morphotypes – A: Control, B: Chlorination of leaves, and C: Multiple shoot emergence.

According to recent research by [40], the application of colchicine in plant breeding has been associated with significant increases in plant height due to induced polyploidy. Colchicine disrupts microtubule formation during cell division, leading to the doubling or multiplication of chromosomes, a phenomenon known as polyploidy [41]. This increase in chromosome sets can result in larger cell sizes and enhanced growth characteristics, including taller plants. Additionally, the study found that colchicine-treated genotypes generally surpassed their counterparts treated with EMS in terms of plant height. This finding suggests that while EMS-induced mutations may lead to phenotypic changes, the impact of colchicine-induced polyploidy on plant height is more pronounced, highlighting the significant role of polyploidy in influencing this trait [41,40]. This is not surprising as colchicine is known to stimulate growth in plants due to the modification of the signalling pathway [42].

Similarly, stem girth ranged from 10.8 mm (Eb22) to 13.5 mm (EB2). Four colchicine mutants, Cb5 (13.3 mm), CB5 (13.0 mm), Cb19 (12.7 mm), and CB3 (12.6 mm) ranked second, third, fourth, and fifth to the highest observed control (EbC) which ranked sixth with 12.59 mm. However, a non-significant difference ($P > 0.05$)

was recorded among the genotype means for stem girth suggesting that both EMS and Colchicine mutants and their controls do not differ much from each other in terms of plant stem girth. Though, the mutagen colchicine had some level of influence on the stem girth expansion for most of the colchicine mutants relative to their controls (CbC and CBC) (Table 1).

The number of leaves (NL) and leaf width (LW) both recorded non-significant differences ($P > 0.05$). NL ranged from a mean of 14.7 for EB7 to 16.2 each for five genotypes; CB5, Eb8, Cb11, Eb14, and the control, EBC respectively. Also, LW ranged from an average of 1.4 (EB8) to 1.5 (EbC, Cb11, Eb12, CB5, Cb19, Eb2) (Table 1) without wide variation.

A notable variation was detected in leaf length (LL) among the genotypes, with a p-value of 0.000. The range of LL spanned from 48.8 cm (CB7) to 69.2 cm (Cb9). The majority of colchicine brown mutants (Cb9, Cb2, Cb19, Cb11) outperformed their control (CbC) and many of the genotypes including their black colchicine counterparts (Table 1). This was in expectance as colchicine multiples the chromosome number of organisms leading to increased plant growth characteristics and larger size development [43; 39; 44]. The results indicated that the influence of colchicine had a significantly greater effect on the growth characteristics of brown tiger nuts compared to the black genotypes. This aligns with previous research indicating that brown tiger nut accessions are more susceptible to the impacts of mutagens in plant growth traits than the black types [5].

Earliness to maturity

Ordinarily, tiger nuts take 90 to 110 days after planting to maturity if properly managed [45]. Physiological maturity involving signs of yellowing of leaves and the cessation of new inflorescence occurs when the plant is 75 to 90 days old after planting [46]. Overall, all the 25g enotypes of the current study physiologically matured in a space range of 75 to 86 days indicating general earliness to maturity for the mutants and their controls. This is not surprising as earliness was an important trait considered in the selection process of the genotypes for the successive generations from M_1 to M_4. The genotypes therefore present a choice of candidates for breeding for earliness. However, six mutants were extra early maturing than other mutants and the control (EbC) which physiologically matured at 78 days (Figure 3). The extra early maturing mutant, Eb8 which initiated flower set as early as 17 days (Table 1) had a mean of 75.7 days to reach physiological maturity. This was followed by the mutants; Eb1, Cb9, Cb2, Cb5, and Cb11 which respectively took 76.3, 76.3, 76.7, 76.7, and 77.0 days to mature physiologically (Figure 3).

All six extra-early mutants had a brown colour and this suggests that mutagens may have a greater influence on early maturity in brown tiger nuts than black ones. Previous research has highlighted the notably sensitive nature of brown tiger nuts to chemicals and other environmental stresses [47,48]. The findings of the current study extend beyond earliness, encompassing variables such as days to germination (DG), number of tillers (NL), plant height (PH), and number of inflorescences per plant (NIP), among others (Table 1).

The flower set started early with a mean of 17.2 days for the mutant Cb19 and late with a mean of 27.5 days for the mutant CB16. Flowering was one hundred percent as all genotypes flowered with an average of 1.00 or 2.3 inflorescences in number per plant (Table 1). This is very promising for breeding advancement for the crop as tiger nuts scarcely flower. Poor flowering was observed in earlier work on the phenotypic characterization of tiger nut accessions in Ghana [49] as opposed to the current study. The revelation of the present study for flowering can be attributed to the mutagens and the careful selection in successive generations.

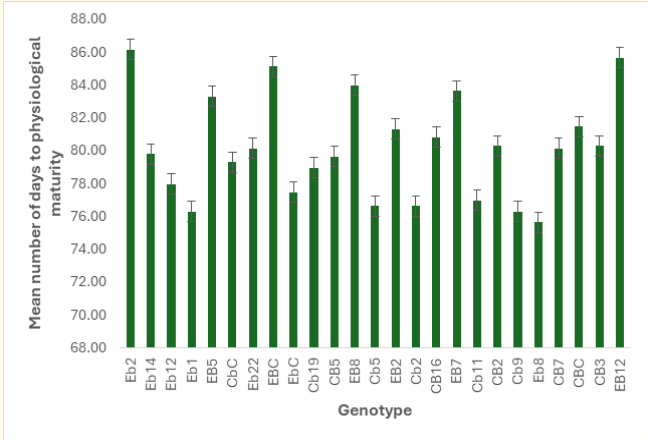

Figure 3: Effects of mutagens on days to physiological maturity among the 25 genotypes.

Yield

A wide variability, in general, was identified in yield among the twenty-five tiger nut genotypes in terms of hundred tuber weight (HTW) and overall tuber weight (OTW). For instance, HTW demonstrated a large variability in means with highly significant ($P < 0.01$) (Figure 4). In all, eleven mutant genotypes made up of both EMS and Colchicine-treated brown and black tiger nuts performed better than the best control in HTW ranking. The highly performed mutant was Eb2 and recorded a remarkable HTW of 166.0g than the control, CbC (132.0g). The others included EB8 (163.0g), EB12 (151.7g), Cb11 (140.3g), Eb14 (138.7g), Cb19 (138.0g), CB2 (137.7g), Eb22 (136.0g), CB16 (135.7g), CB7 (135.0g), and Cb9 (133.7g) (Figure 4).

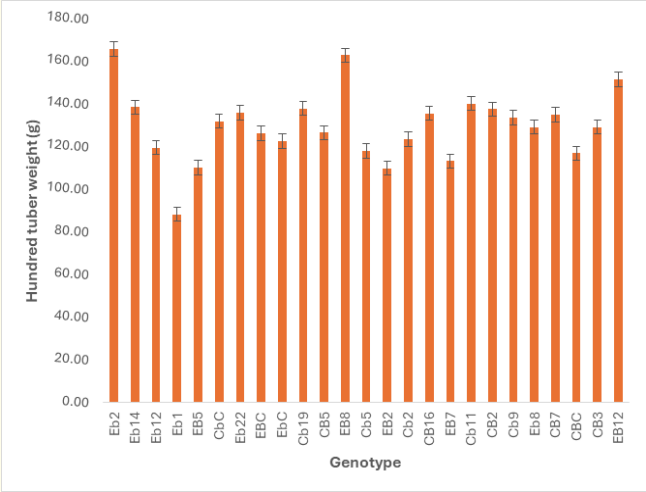

Figure 4: Effects of mutagens on hundred tuber weight among the genotypes.

The results of HTW in the current study are higher than the recorded mean weights of 99.28g in 2019 and 102.85g in 2020 [50]. Again, they outweighed the majority of the accessions evaluated in Ghana. In a morphological characterization of local tiger nut accessions in Ghana, Donkor and her colleagues recorded 17.8- 132.3g for HTW among 23 out of 24 accessions. It was only one out of the 24 accessions recorded a higher figure of 295.3g more than that observed in the current study [51]. Nonetheless, the mutants generated by the recent study are commendable in HTW and present added lines to tiger nut accessions in Ghana. Seven of these mutants were the highest-performing genotypes (Figure 4). They outperformed the best control genotype EBC, which recorded 119.8g. The mutants included EB8 (148.8g), Eb2 (147.5g), CB16 (136.8g), Cb11(134.5g), EB12 (131.8g), Eb22 (128.5g), and Cb19 (127.0g) (Figure 4).

On an individual plant basis, overall tuber weight (OTW) ranged from 67.5g for the mutant Eb1 to 148.8g for the mutant EB8. Indication of significant variability among the accessions means at $p < 0.05$. Among all the treatment genotypes, only the colchicine black mutants outperformed their control. The rest however had some mutants outperformed their control and the control also outperformed other mutants. For example, two of the EMS black mutants, EB8 (148.8g) and EB12 (131.8g) outperformed their control EBC (119.8g). The control on the other hand performed better in overall weight than the mutants EB7 (112.2g), EB5 (110.2g), and EB2 (105.3g). Overall tuber weight is influenced by several factors involving genetics, environmental conditions, soil quality, water availability, and cultivation practices [52]. The differences in overall weight are attributed to genetics as the genotypes were given the same environment apart from the mutagen applied. According to [53], the impact of a mutagen is species-specific and also affected by the type and dose of the mutagen. Hence given the optimum or recommended dose of mutagen as per the current study, it can be concluded that colchicine influenced the genotype of the black tiger nuts translating into an overall increased in tuber weight.

Tuber characteristics

In general, tuber coat colour among the genotypes for putative mutant and mutant lines remained unchanged to the effects of the mutagens (both EMS and Colchicine) from M_1 to M_4. EMS black and Colchicine black treated tubers were black respectively upon successive generations. Also, EMS brown and Colchicine brown treated genotypes showed no colour change (Figure 6) with the mutagens.

This is suggestive that coat colour is sparsely affected in vegetatively propagated crops when exposed to mutagens, unlike other sexually propagated crops. Affirming this assertion is the observation made when out of 600gamma irradiated genotypes, only 5 root flesh colour variants were detected in sweet potatoes. The root tuber colour remained the same for all [54]. This was also reported

Genotype	DG	NT	DTP (cm)	PH (cm)	GP (mm)	NL	LL (cm)	LW (cm)	DF	NIP	DPM	NTA	NTD	TNT	OTW (g)	HTW (g)	TL (mm)	TW (mm)	TS (mm)	NRT
EBC	8.5^{abc}	18.2 ^{ab}	9.9^{a}	92.8^{c}	11.0^{a}	16.2^{a}	51.3^{def}	1.4^{a}	24.7^{a}	1.8^{a}	85.2^{ab}	59.3^{abcd}	9.2^{cdefg}	68.5^{ab}	79.8^{ab}	126.3^{ab}	21.2 ^{cdefg}	17.4^{abc}	1.2^{de}	6.7^{ab}
EB2	9.5^{a}	17.2 ^{ab}	9.9^{a}	97.5^{abc}	13.5^{a}	15.8^{a}	52.3^{cdef}	1.5^{a}	17.5^{a}	0.7^{a}	81.3^{abcdef}	75.7^{abc}	10.2^{bcdefg}	85.8^{a}	71^{ab}	110.0 ^{ab}	15.7^{g}	15.7^{bcdef}	1.0^{e}	6.4^{abc}
EB5	8.3^{ab}	18.2 ^{ab}	10.0^{a}	96.5^{abc}	12.3^{a}	15.3^{a}	50.0^{ef}	1.4^{a}	24.5^{a}	1.3^{a}	83.3^{abcde}	80.3^{a}	3.8^{fg}	84.2^{a}	73.5^{ab}	110.3^{ab}	17.2^{fg}	16.8^{abcd}	1.0^{e}	6.2^{bc}
EB7	7.7^{abc}	16.2 ^{ab}	8.8^{a}	98.3^{abc}	11.5^{a}	14.5^{a}	55.3^{abcdef}	1.4^{a}	25.8^{a}	1.2^{a}	83.7^{abcd}	79.5^{ab}	6.7^{cdefg}	86.2^{a}	74.8^{ab}	113.3^{ab}	17.2^{fg}	16.0^{abcde}	1.1^{e}	6.2^{bc}
EB8	8.2^{ab}	17.5 ^{ab}	10.8^{a}	99.7^{abc}	12.1^{a}	14.8^{a}	56.3^{bcdef}	1.4^{a}	24.7^{a}	1.8^{a}	84.0^{abc}	73.8^{abcd}	4.0^{efg}	77.8$_{ab}$	99.2^{a}	163.0^{a}	22.6 ^{cdefg}	18.2^{a}	1.2^{de}	6.8^{ab}
EB12	9.3^{ab}	13.2 ^{b}	8.2^{a}	99.4^{abc}	12.0^{a}	14.8^{a}	51.4^{def}	1.4^{a}	26.7^{a}	1.3^{a}	85.2^{ab}	65.7^{abcd}	4.7efg	70.3^{ab}	87.8^{ab}	151.7^{ab}	20.0^{defg}	17.8^{ab}	1.1^{de}	6.8^{ab}
EbC	7.2^{abc}	18.0^{ab}	12.7^{a}	95.0^{bc}	13.0^{a}	14.7^{a}	66.7^{ab}	1.5^{a}	19.3^{a}	1.0^{a}	77.5^{cdef}	46.3^{bcd}	11.0^{bcdefg}	57.3^{ab}	67.7^{ab}	122.7^{ab}	22.9^{bcdef}	13.1^{gh}	1.8^{abc}	6.8^{ab}
Eb1	6.8^{abc}	18.7^{ab}	9.9^{a}	109.5^{abc}	11.0^{a}	14.8^{a}	59.5^{abcdef}	1.5^{a}	25.0^{a}	1.5^{a}	76.3^{ef}	44.0^{cd}	2.8^{g}	46.8^{b}	45.0^{b}	88.3^{b}	28.3^{ab}	12.7^{h}	2.2^{a}	6.7^{ab}
Eb2	6.2^{c}	23.0^{a}	13.0^{a}	113.2^{abc}	12.0^{a}	14.8^{a}	59.7^{abcdef}	1.5^{a}	26.2^{a}	1.3^{a}	86.2^{a}	72.7^{abcd}	7.8^{cdedfg}	80.5^{a}	98.3^{a}	166.0^{a}	31.9^{a}	15.5^{cdef}	2.1^{ab}	7.3^{a}
Eb8	6.2^{c}	15.0^{ab}	12.2^{a}	104.4^{abc}	12.2^{a}	16.2^{a}	63.4^{abc}	1.5^{a}	24.7^{a}	2.0^{a}	75.7^{f}	47.0^{bcd}	23.0^{abcd}	70.0^{ab}	76.0^{ab}	129.3^{ab}	22.6^{bcdef}	14.1^{efgh}	1.6^{bcd}	6.0^{bc}
Eb12	5.8^{c}	20.4^{ab}	10.8^{a}	107.1^{abc}	10.9^{a}	14.8^{a}	66.0^{ab}	1.5^{a}	25.8^{a}	1.5^{a}	78.0^{cdef}	59.8^{abcd}	5.8^{defg}	65.7^{ab}	66.5^{ab}	119.7^{ab}	26.5^{abcd}	13.6^{fgh}	2.0^{ab}	6.3^{bc}
Eb14	7.0^{abc}	20.5^{ab}	10.9^{a}	108.0^{abc}	11.9^{a}	16.2^{a}	66.2^{ab}	1.5^{a}	25.5^{a}	1.8^{a}	79.8^{abcdef}	60.0^{abcd}	11.5^{bcdefg}	71.5^{ab}	78.8^{ab}	138.7^{ab}	27.2^{abc}	13.5^{fgh}	2.0^{ab}	6.2^{bc}
Eb22	7.2^{abc}	18.2^{ab}	11.8^{a}	109.0^{abc}	10.8^{a}	15.0^{a}	62.3^{abcd}	1.4^{a}	24.2^{a}	2.3^{a}	80.2^{abcdef}	54.2^{abcd}	20.0^{abcdefg}	74.2^{ab}	85.7^{ab}	136.0^{ab}	20.6 ^{cdefg}	15.1^{defg}	1.4^{cde}	5.7^{c}
CBC	7.7 ^{abc}	14.3^{ab}	11.0^{a}	92.0^{c}	11.7^{a}	15.5^{a}	49.0^{f}	1.4^{a}	24.8^{a}	1.0^{a}	81.5^{abcdef}	50.3^{abcd}	11.5^{bcdefg}	61.8^{ab}	70.7^{ab}	117.0^{ab}	17.2^{fg}	16.9^{abcd}	1.0^{e}	6.4^{abc}
CB2	7.8 ^{abc}	15.3 ^{ab}	10.0^{a}	93.3 ^{c}	11.8^{a}	15.7^{a}	51.5^{def}	1.5^{a}	22.5^{a}	1.5^{a}	80.3^{abcdef}	55.8^{abcd}	5.7^{defg}	61.5^{ab}	77.8^{ab}	137.7^{ab}	18.4 ^{efg}	16.9^{abcd}	1.1^{de}	6.6^{abc}
CB3	6.5^{bc}	13.5^{b}	10.5^{a}	97.0^{abc}	12.6^{a}	15.2^{a}	50.7^{ef}	1.5^{a}	22.8^{a}	1.5a	80.3^{abcdef}	58.5^{abcd}	6.3^{defg}	64.8^{ab}	78.3^{ab}	129.3^{ab}	17.1^{fg}	16.9^{abcd}	1.0^{e}	6.4^{abc}
CB5	7.8 ^{abc}	17.8 ^{ab}	10.8^{a}	93.5^{bc}	13.0^{a}	16.2^{a}	52.9^{cdef}	1.5^{a}	25.5^{a}	1.5^{a}	79.7^{abcdef}	62.2^{abcd}	3.5^{fg}	65.6^{ab}	70.8^{ab}	126.7^{ab}	18.3^{efg}	16.6^{abcd}	1.1^{de}	6.5^{abc}
CB5	7.8 ^{abc}	17.8 ^{ab}	10.8^{a}	93.5^{bc}	13.0^{a}	16.2^{a}	52.9^{cdef}	1.5^{a}	25.5^{a}	1.5^{a}	79.7^{abcdef}	62.2^{abcd}	3.5^{fg}	65.6^{ab}	70.8^{ab}	126.7^{ab}	18.3^{efg}	16.6^{abcd}	1.1^{de}	6.5^{abc}
CB7	7.5^{abc}	14.5 ^{ab}	12.0^{a}	95.7^{bc}	12.0^{a}	15.0^{a}	48.8^{f}	1.5^{a}	25.0^{a}	1.5^{a}	80.2^{abcdef}	55.2^{abcd}	4.5^{efg}	59.7^{ab}	73.8^{ab}	135.0^{ab}	20.3^{defg}	17.0^{abcd}	1.2^{de}	6.4^{abc}
CB16	8.2 ^{abc}	16.3 ^{ab}	11.6^{a}	94.2 ^{bc}	12.0^{a}	16.0^{a}	50.5^{ef}	1.4^{a}	27.5^{a}	1.2^{a}	80.8^{abcdef}	73.0^{abcd}	7.2^{cdefg}	80.2^{a}	91.2^{a}	135.7^{ab}	16.1^{fg}	16.5^{abcd}	1.0^{e}	6.2^{bc}
CbC	6.2^{c}	18.2 ^{ab}	12.5^{a}	109.2^{abc}	12.3^{a}	16.0^{a}	64.5^{ab}	1.5^{a}	25.0^{a}	2.3^{a}	79.3^{abcdef}	45.2^{cd}	28.3^{ab}	73.5^{ab}	75.7^{ab}	132.0^{ab}	20.0^{defg}	14.1^{efgh}	1.4^{cde}	6.1^{bc}
Cb2	6.2^{c}	17.0 ^{ab}	11.8^{a}	109.2^{abc}	11.3^{a}	15.7^{a}	66.6^{ab}	1.5^{a}	25.5^{a}	2.0^{a}	76.7^{def}	47.0^{bcd}	18.0^{abcdefg}	65.0^{ab}	69.5^{ab}	123.7^{ab}	20.1 ^{defg}	14.7^{defgh}	1.4^{cde}	6.1^{bc}
Cb5	6.0^{c}	17.3 ^{ab}	10.8^{a}	115.8^{a}	13.3^{a}	15.2^{a}	61.5^{abcde}	1.4^{a}	26.0^{a}	2.2^{a}	76.7^{def}	44.3^{cd}	22.2^{abcde}	66.5^{ab}	66.8^{ab}	118.0^{ab}	24.8^{bcde}	14.0^{efgh}	1.8^{abc}	6.1^{bc}
Cb9	6.2^{c}	15.0 ^{ab}	13.6^{a}	105.2^{abc}	12.5^{a}	14.8^{a}	69.2^{a}	1.5^{a}	26.8^{a}	1.7^{a}	76.3^{ef}	41.3^{d}	21.7^{abcdef}	63.0^{ab}	75.3^{ab}	133.7^{ab}	21.7^{bcdefg}	15.4^{cdef}	1.4^{cde}	6.3^{bc}
Cb11	6.8^{abc}	15.8 ^{ab}	13.3^{a}	105.0^{abc}	12.0^{a}	16^{a}	64.7^{ab}	1.5^{a}	26.5^{a}	2.2^{a}	77.0^{cdef}	51.3^{abcd}	29.8^{a}	81.2^{a}	89.7^{ab}	140.3^{ab}	21.4^{cdefg}	15.5^{bcdef}	1.4^{cde}	6.1^{bc}
Cb19	6.8^{abc}	18.0 ^{ab}	14.9^{a}	101.7^{abc}	12.8^{a}	16.2^{a}	66.3^{ab}	1.5^{a}	17.2^{a}	1.3^{a}	79.0^{bcdef}	51.7^{abcd}	24.7^{abc}	76.3^{ab}	84.7^{ab}	138.0^{ab}	21.5 ^{cdefg}	15.4^{cdef}	1.4^{cde}	6.4^{abc}

Table 1: Means of tiger nut genotypes (21 mutants and 4 controls) across 20 morpho-descriptors.

Means having letter(s) in common do not significantly differ, using Tukey's mean separation test at p= 0.05 level of significance with adjusted individual confidence level of 99.97%.

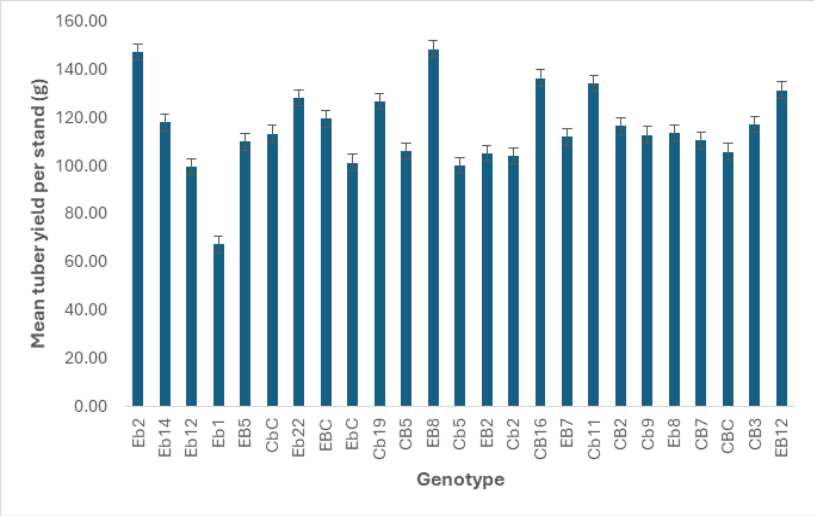

Figure 5: Effects of mutagens on overall tuber weight of tiger nut mutants and their controls.

again in sweet potato [55]. However, with sexually propagated crops, the effect is clear and domineering. Colour changes in the seeds of cowpea when exposed to mutagens have been frequently reported [56,57].

Another interesting observation made on tuber development was twined or multiple tuber formation among some of the mutants (Figure 6). Four of the EMS brown mutants had multiple tuber development, resulting in enhanced tuber size and shape. For example, the tuber lengths of Eb2 (47.9 mm), Eb1 (42.5 mm), Eb14 (40.8 mm), and Eb12 (39.8 mm) were longer than their control, EbC (33.9 mm) (Table 1). Also, by shape, they were classified as oblong by recording the tuber L/W ratio greater than or equal to 2.0 mm than their control, EbC which had 1.8 mm (Table 1). Larger tuber sizes are mostly the consumers' choice.

In all, the mutant EB5 recorded the highest mean tuber number attached of 80.3 out of the total mean of 84.2 tubers underground (Table 1), representing 95.4% of tuber attached to the plant during harvesting. This was followed by EB7 (79.5), EB2 (75.7), and EB8 (73.8), all of which were black tiger nuts treated with EMS (Figure 6). Colchicine-treated mutant, CB16, another black tiger nut ranked 5th next in all with a mean tuber count of 73.0 (Figure 6) out of a total mean per stand of 80.2 (Table 1), accounting for 91%. Among the EMS brown mutants, Eb2 observed the highest mean number of tuber attached with 72.7 out of 80.5 tubers per stand representing 90.3% to rank 6th.

Effects of mutagens on tuber attachment to plant on harvest

The mutants EB12, CB5, Eb14, and Eb12 ranked next in successive order until the control EBC occupied the 11th position with the mean number of 59.3 tubers attached out of 68.5 tubers harvested (86.6%). For colchicine brown, 3 mutants; Cb19 (51.7 tubers), Cb11 (51.3 tubers), and Cb2 (47.0 tubers) did perform better than their control CbC which documented 45.2 mean tubers attached (Figure 7).

In addressing the problem of difficulty in harvesting arising from detachment of the tubers in the soil at harvesting as lamented by farmers [46,58], producers and plant breeders stand the chance of selecting these identified mutants with such resilient and robust runners for tuber attachment on plants during harvesting.

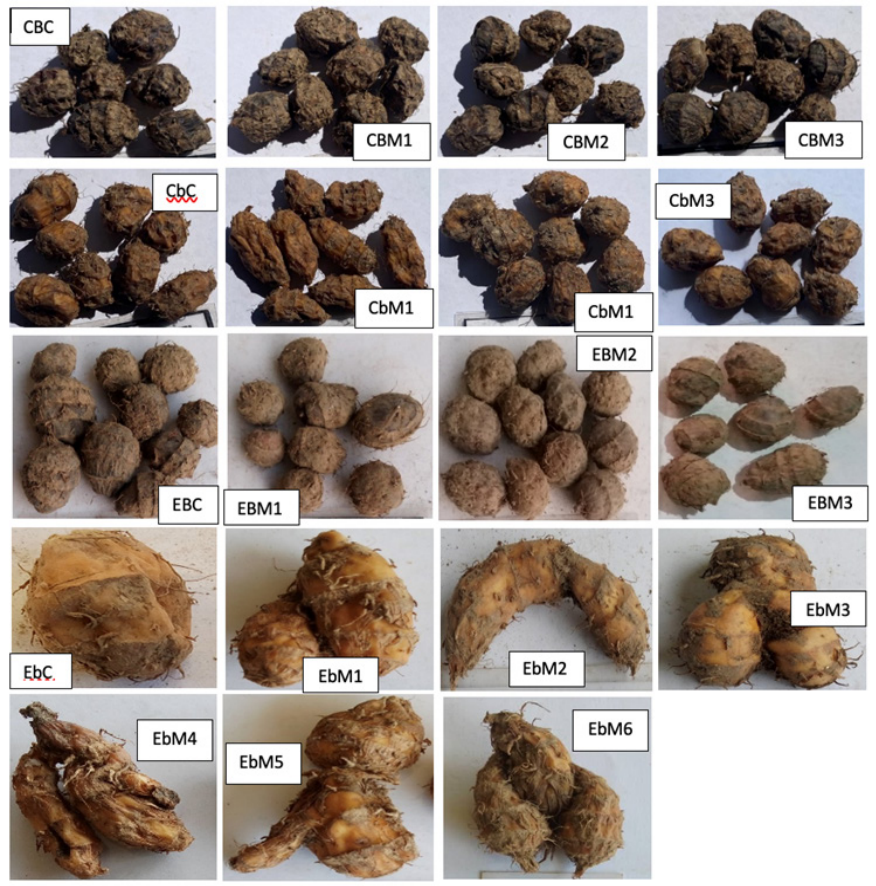

Figure 6: Tuber characteristics of mutants and control tiger nut genotypes.

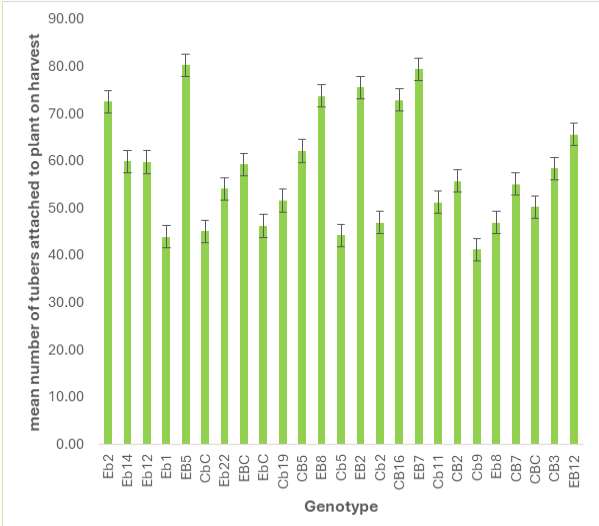

Figure 7: Effects of mutagens on mean number of tubers attached to genotypes at harvest.

Genetic relationship of genotypes

The genetic similarity among 21 mutants and their 4 controls was estimated utilizing standardized data for cluster analyses of 20 traits. A wide range of variations existed among the genotypes. The study revealed 9 cluster groupings at a Euclidean distance similarity measure of 48.76% (Figure 8).

Three clusters; cluster I, V, and IX were independently grouped from the rest. Cluster I and Cluster V were made up of only Eb2, and Eb1 mutants respectively. They were distantly apart from each other and their control (EbC) which clustered with Cb19 in cluster VIII (Figure 8) on the premise of having a broader leaf width of 1.5 mm. Cluster IX on the other hand consisted of only one black EMS genotype (EB2) which varied distantly from its control (EBC) in another cluster (VI). This indicates high genetic variability and substantial genetic diversity in existence. Cluster II was made up of two genotypes consisting of the mutants Eb12, and Eb14. These mutants were all brown EMS-treated genotypes.

The red colour indicates black genotypes treated with EMS; green colour shows black genotypes treated with Colchicine; blue colour signifies brown genotypes treated with EMS; and yellow colour denotes brown genotypes treated with Colchicine.

Cluster III comprised five genotypes, with four exhibiting a brown colouration induced by colchicine treatment, while the remaining genotype (Eb8) displayed a brown mutation induced by EMS. Among the colchicine brown genotypes, one was the control (CbC), and the other three were colchicine brown mutants (Cb2, Cb5, and Cb9) (Figure 8; Figure 9). These genotypes were notably characterized by tall plant height, the presence of two or more inflorescences, a significant number of tubers detached in the soil, and oval-shaped tubers, among other traits (Table 1).

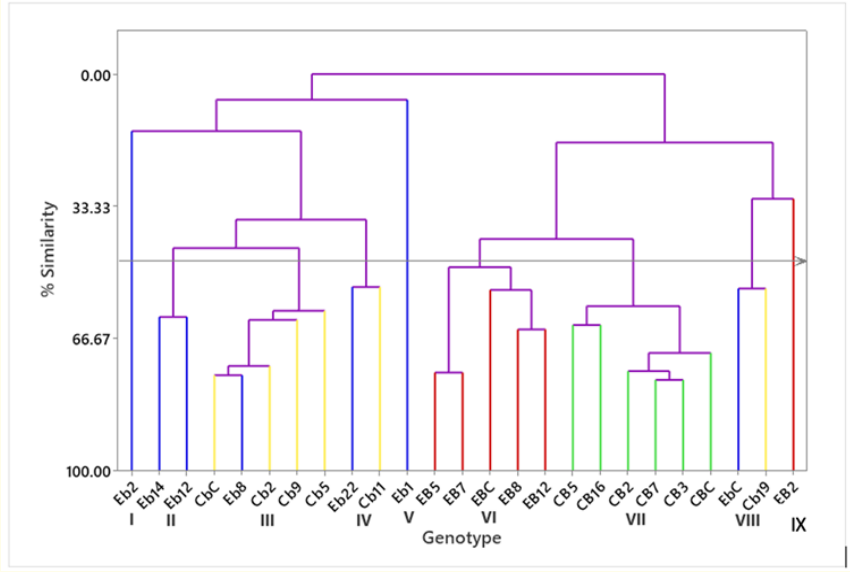

Figure 8: Dendrogram showing the diversity of mutants and control across twenty morpho descriptors using Euclidean measure similarity index.

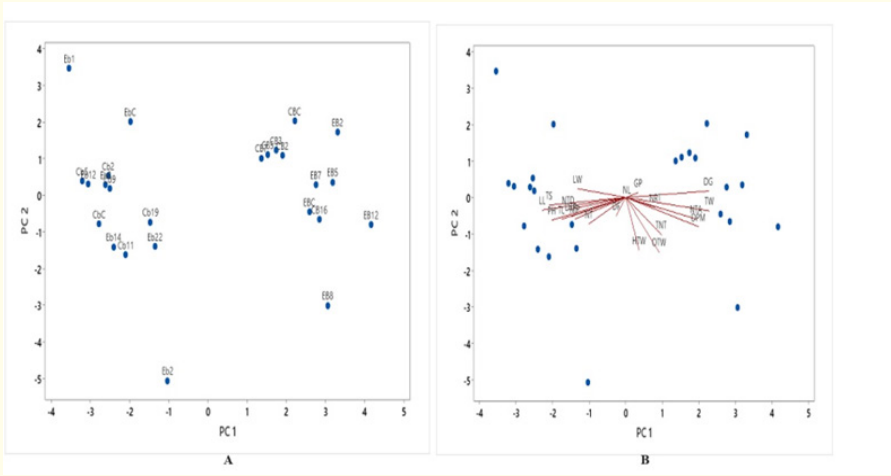

Figure 9: Principal components analysis on the correlation matrix of 25 genotypes (mutants and control) tiger nuts: A- PC score plot showing the distribution of genotypes. B- PC biplot showing the distribution of genotypes and morpho descriptors.

Members of cluster IV were made up of only two mutants (Cb11 and Eb22). Though they were different in their mutagens, they were put together by having more than 2 inflorescences (Figure 8b; Table 1).

Cluster VI, comprising five genotypes with four were identified as EMS-induced black mutants (EB5, EB7, EB8, EB12), clustering closely with their control genotype (EBC). An interesting observation is that these genotypes exhibited longer durations to reach physiological maturity, ranging from 83 to 86 days. Additionally, they displayed a higher number of tubers attached to the plant upon harvesting. The presence of a greater number of tubers attached to the plant at harvest is a valuable trait sought after by both breeders and farmers [59]. This trait addresses a common challenge in tuber crops, such as potatoes, where tubers left underground can be difficult to recover during harvesting. By having more tubers attached to the plant, farmers can potentially recover a higher yield, thus increasing productivity and profitability. Moreover, it can lead to savings in time and labor costs associated with harvesting, as fewer tubers are left behind.

Despite clustering with their control genotype, some mutants within Cluster VI exhibited significantly higher tuber numbers compared to their control counterparts. This finding suggests the presence of genetic variations within the mutants that contribute to enhanced tuber production. Identifying and understanding these genetic factors could hold significant implications for breeding programs aimed at improving tuber yield and quality [40].

Similarly, cluster VII constituted all black 6-member colchicine genotypes. It included the control (CBC) and 5 mutants (CB2, CB3, CB5, CB7, CB16) (Figure 9). They recorded 7-8 days for germination. They were not so early, but average germinators (Table 1).

Nutritional value of mutants

Increased in consumption and utilization of tiger nuts such as into flour, milk, or oil is influenced by their nutritional composition. The tubers are rich in almost all functional food nutrients. However, the nutrient composition of the tuber is dependent on the type or variety of the tuber (yellow, brown, or black), the area of cultivation, planting seasons, and the postharvest method used in the processing of the tubers [60,61]. The findings of the present study showed a high mean carbohydrate content of 52.6% for the mutant Cb19 among all the 13 selected genotypes with the least being EB8 registering 43.9% (Table 2). EB12 ranked second highest with 50.6%, and it was followed by Eb22, Cb11, and CB2 with 49.3%,

48.7%, and 48.1% respectively, all performing better in carbohydrates than the best-performing control, EBC. So interestingly the four controls; EBC, CbC, CBC, and EbC ranked in succession with each other in carbohydrate content. They recorded 46.9%, 46.7%, 46.6%, and 45.0% respectively (Table 2), indicating that the percentage carbohydrate of tiger nuts ranges from 45- 47%. This is supportive of 45.73% [7], and 46.99% of yellow-type tiger nut tuber [62]. However, the current finding is contrary in agreement to 43.3% [63], 41.22% of brown type, and 65.66% of black type [62], suggesting that the percentage nutrient content varies with the type of tiger nut tuber [64].

On the part of crude protein content, the control, EbC recorded the highest value of 4.60% with the mutant Eb14 observing the least of 1.89%. However, eight mutants outperformed the other 3 controls (EBC-2.51%, CbC-2.46%, CBC-2.12%). They included Cb11 (4.08%), Cb19 (3.74%), EB12 (3.74%), EB8 (3.6%), CB16 (3.51%), CB2 (3.47%), Eb2 (3.30%), and Eb22 (3.30%) (Table 2). Many of these mutants performed well also for the carbohydrates, hence, stand tall in using them for food. The crude protein content of genotypes of the current study is comparatively below 5.04% [63], 7.15-9.20% [65], and 7.15-12.00% [62]. However, they are supported by the findings of [66] (3.30-4.33 %), and also partly in agreement with the score range of 4.16- 5.56% of 24g hanaian accessions evaluated for proximate and mineral composition [67].

Genotype	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Fe (%)
EBC	0.59 ± 0.00^{b}	1.40 ± 0.00^{e}	0.34 ± 0.01^{c}	0.11 ± 0.00^{cd}	0.03 ± 0.00^{cd}	0.94 ± 0.03^{a}
EB8	0.49 ± 0.00^{h}	1.53 ± 0.01^{d}	0.33 ± 0.00^{cd}	0.09 ± 0.00^{f}	0.03 ± 0.00^{cd}	0.66 ± 0.03^{e}
EB12	0.52 ± 0.00^{e}	1.42 ± 0.01^{e}	0.33 ± 0.00^{d}	0.10 ± 0.00^{de}	0.03 ± 0.00^{ab}	0.83 ± 0.02^{b}
EbC	0.50 ± 0.00^{fg}	1.65 ± 0.00^{e}	0.33 ± 0.00^{cd}	0.11 ± 0.00^{bc}	0.03 ± 0.00^{ef}	0.68 ± 0.01^{de}
Eb2	0.64 ± 0.00^{a}	1.64 ± 0.01^{b}	0.34 ± 0.01^{c}	0.08 ± 0.00^{f}	0.03 ± 0.00^{de}	0.75 ± 0.03^{cd}
Eb14	0.49 ± 0.00^{gh}	1.75 ± 0.01^{a}	0.34 ± 0.00^{cd}	0.09 ± 0.00^{ef}	0.03 ± 0.00^{abc}	0.85 ± 0.03^{b}
Eb22	0.59 ± 0.01^{b}	1.64 ± 0.01^{b}	0.29 ± 0.00^{e}	0.09 ± 0.00^{f}	0.03 ± 0.00^{bc}	0.58 ± 0.01^{f}
CBC	0.57 ± 0.01^{c}	1.55 ± 0.02^{cd}	0.34 ± 0.00^{cd}	0.09 ± 0.00^{ef}	0.03 ± 0.00^{f}	0.78 ± 0.01^{bc}
CB2	0.59 ± 0.00^{b}	1.56 ± 0.01^{c}	0.29 ± 0.00^{e}	0.12 ± 0.00^{b}	0.03 ± 0.00^{de}	0.84 ± 0.03^{b}
CB16	0.50 ± 0.01^{ef}	1.41 ± 0.01^{e}	0.37 ± 0.00^{b}	0.14 ± 0.00^{a}	0.03 ± 0.00^{f}	0.67 ± 0.02^{e}
CbC	0.49 ± 0.01^{h}	1.28 ± 0.01^{f}	0.41 ± 0.01^{a}	0.14 ± 0.00^{a}	0.02 ± 0.00^{g}	0.74 ± 0.04^{cd}
Cb11	0.58 ± 0.00^{bc}	1.40 ± 0.00^{e}	0.34 ± 0.01^{cd}	0.09 ± 0.00^{f}	0.03 ± 0.00^{de}	0.81 ± 0.03^{bc}
Cb19	0.54 ± 0.01^{d}	1.55 ± 0.01^{cd}	0.30 ± 0.01^{e}	0.12 ± 0.01^{bc}	0.03 ± 0.00^{a}	0.76 ± 0.03^{c}

Table 3: Mineral composition of tiger nut mutants and their controls.

Means and standard deviations for n =3. Means having the same letter(s) do not significantly differ by Tukey's test at ($p > 0.05$).

Percentage fat content ranged from 14.86% for EbC (control) to 20.61% for EB8. The mutant, EB8 outperformed the EMS black control (EBC) which recorded 20.08% (Table 2). A general overview of the results showed that black tiger nut tubers have a lot of fat deposits than brown tubers. Five black genotypes including one (1) EMS black mutant, two (2) colchicine black mutants, one (1) EMS black control, and two (1) colchicine black control ranked higher than the rest of the brown genotypes (Table 2). Strong evidence that the black tiger nut accessions in Ghana have tubers containing higher levels of crude fat (ether extract) than their brown and yellow counterparts, and are therefore in good standing to be used for tiger nut oil production. This is in support of the higher percentages of crude fat observed for the black tiger nut tubers than their yellow and brown counterparts evaluated in Ghana for their proximate and mineral composition [67]. However, these observations are different from other researchers recording higher crude fat percentages in the yellow and brown tiger nut tubers than the black types [62,63,65,66]. According to the proximate/nutritional composition of the brown and black type tiger nut tubers is significantly affected by the site and planting periods.

Besides the chemical constituents of proximate composition, tiger nut tubers are also valued for their rich mineral deposits. Table 3 revealed high levels of phosphorous, potassium, and iron in the tubers with calcium, magnesium, and sodium being in low amounts.

In general, the mutagens increased the phosphorous, and potassium contents among the accessions as in each case some mutants outperformed the controls by recording the highest values. Contrarily, the calcium and Iron levels were reduced by the effects of the mutagens as the accessions recorded reduced values than some of the controls. However, there was little or no effect pronounced by both EMS and Colchicine mutagens on the values of magnesium, and sodium contents to their controls (Table 3).

Genotype	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Fe (%)
EBC	0.59 ± 0.00^{b}	1.40 ± 0.00^{e}	0.34 ± 0.01^{c}	0.11 ± 0.00^{cd}	0.03 ± 0.00^{cd}	0.94 ± 0.03^{a}
EB8	0.49 ± 0.00^{h}	1.53 ± 0.01^{d}	0.33 ± 0.00^{cd}	0.09 ± 0.00^{f}	0.03 ± 0.00^{cd}	0.66 ± 0.03^{e}
EB12	0.52 ± 0.00^{e}	1.42 ± 0.01^{e}	0.33 ± 0.00^{d}	0.10 ± 0.00^{de}	0.03 ± 0.00^{ab}	0.83 ± 0.02^{b}
EbC	0.50 ± 0.00^{fg}	1.65 ± 0.00^{e}	0.33 ± 0.00^{cd}	0.11 ± 0.00^{bc}	0.03 ± 0.00^{ef}	0.68 ± 0.01^{de}
Eb2	0.64 ± 0.00^{a}	1.64 ± 0.01^{b}	0.34 ± 0.01^{c}	0.08 ± 0.00^{f}	0.03 ± 0.00^{de}	0.75 ± 0.03^{cd}
Eb14	0.49 ± 0.00^{gh}	1.75 ± 0.01^{a}	0.34 ± 0.00^{cd}	0.09 ± 0.00^{ef}	0.03 ± 0.00^{abc}	0.85 ± 0.03^{b}
Eb22	0.59 ± 0.01^{b}	1.64 ± 0.01^{b}	0.29 ± 0.00^{e}	0.09 ± 0.00^{f}	0.03 ± 0.00^{bc}	0.58 ± 0.01^{f}
CBC	0.57 ± 0.01^{c}	1.55 ± 0.02^{cd}	0.34 ± 0.00^{cd}	0.09 ± 0.00^{ef}	0.03 ± 0.00^{f}	0.78 ± 0.01^{bc}
CB2	0.59 ± 0.00^{b}	1.56 ± 0.01^{c}	0.29 ± 0.00^{e}	0.12 ± 0.00^{b}	0.03 ± 0.00^{de}	0.84 ± 0.03^{b}
CB16	0.50 ± 0.01^{ef}	1.41 ± 0.01^{e}	0.37 ± 0.00^{b}	0.14 ± 0.00^{a}	0.03 ± 0.00^{f}	0.67 ± 0.02^{e}
CbC	0.49 ± 0.01^{h}	1.28 ± 0.01^{f}	0.41 ± 0.01^{a}	0.14 ± 0.00^{a}	0.02 ± 0.00^{g}	0.74 ± 0.04^{cd}
Cb11	0.58 ± 0.00^{bc}	1.40 ± 0.00^{e}	0.34 ± 0.01^{cd}	0.09 ± 0.00^{f}	0.03 ± 0.00^{de}	0.81 ± 0.03^{bc}
Cb19	0.54 ± 0.01^{d}	1.55 ± 0.01^{cd}	0.30 ± 0.01^{e}	0.12 ± 0.01^{bc}	0.03 ± 0.00^{a}	0.76 ± 0.03^{c}

Table 3: Mineral composition of tiger nut mutants and their controls.

Means and standard deviations for n =3. Means having the same letter(s) do not significantly differ by Tukey's test at ($p > 0.05$).

Percentage phosphorous ranged from 0.49% (EB8) to 0.64% (Eb2). The mutant Eb2 outperformed all, including the four controls (EBC, CBC, EbC, CbC) which recorded 0.59%, 0.57%, 0.50%, and 0.49% respectively (Table 3). The value of 0.64% far exceeds that of 0.141% [7], 0.121% [65], 0.229- 0.283% [66], and the majority of accessions evaluated in Ghana [67]. Eb2 with its bigger size could be used for breeding improvement and also a ready source of phosphorus. This is phenomenal as consumers usually prefer bigger tiger nuts, tasting good and rich in nutrients.

Potassium content ranged from 1.28% for CbC (control) to 1.75% for Eb14 (mutant). Eb14 ranked the highest overall and was followed by EbC (control) with 1.65%. The high percentage of potassium in Eb14 exceeds that of 0.556-0.845% [66], and 0.216% [65]. Notwithstanding, the high potassium values including that of the controls for the current study as also identified by [67], are indicative that Ghanaian tiger nut tubers are endowed with a good amount of potassium for the normal functioning of the heart, regulation of muscular contraction, and the synthesis of protein and carbohydrates metabolism.

Conclusion

This study demonstrated the potential of EMS and colchicine mutagens to induce genetic variability and improve key agronomic traits in tiger nut (*Cyperus esculentus* L.). Screening reduced 1,168 M2 plants to 21 putative mutants and 4 controls in the M4g en-

eration. While tuber color remained unaffected, colchicine significantly enhanced plant height, stem girth, and leaf length, with mutant Cb5 exhibiting the tallest growth (115.75 cm). Additionally, colchicine and EMS treatments promoted early maturity in select mutants (75-77 days). Yield analysis identified 11 high-performing mutants, with EMS and colchicine treatments enhancing tuber weight, size, and shape. Mutants also exhibited strong tuber attachment during harvest, with EB5 and Eb2 recording the highest attachment rates among black and brown genotypes, respectively. Proximate and mineral analyses revealed extensive genetic diversity, supported by morphological clustering across 20 descriptors. These findings highlight the efficacy of mutagenesis in generating variability and improving agronomic traits, offering valuable insights for tiger nut breeding programmes.

Conflict of Interest

The authors declare that they have no conflict of interest.

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