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Chemosensitivity analysis of tiger nuts (*Cyperus esculentus* L.) using ethyl methanesulfonate (EMS) and colchicine mutagens

Patrick Twumasi [a,*], Pual Agu Asare [a], Emmanuel Afutu [a], Godwin Amenorpe [b], Sylvester N.T.T. Addy [c]

[a] *Department of Crop Science, University of Cape Coast (UCC), Ghana*
[b] *Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC), Ghana*
[c] *Center for Scientific and Industrial Research – Crop Research Institute (CSIR-CRI), Fumesua, Ghana*

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ABSTRACT

Four hundred tubers of four genotypes, two brown and two black tiger nuts were subjected to Ethyl Methanesulfonate (EMS) and Colchicine treatments at concentrations of 0 %, 0.1 %, 0.25 %, 0.5 % and 1.0 % for 24 h. Each genotype had twenty tubers treated with each of the five different concentrations and were planted using Complete Randomized Design (CRD) in a greenhouse. Quantitative data was collected and LD_{50} and RD_{50} were analysed using Excell 2016 and Genstat 11.2. A general decreasing trend in percentage germination and plant height was observed with increasing concentrations of mutagens applied. An EMS treatment had LD_{50} and RD_{50} values of 0.97 % and 1.49 % for black and 0.63 % and 1.63 % for brown genotypes.

Similarly, the percentage colchicine treatment had LD_{50} and RD_{50} values of 1.65 % and 19.51 % concentrations for black and 0.91 % and 1.71 % concentrations for brown genotypes.

1. Introduction

Tiger nut (*Cyperus esculentus* L.), commonly called 'chufa', 'atadwe', nut grass, earth almond, water grass, rush nut, yellow nut sedge and northern nut grass [1,2], is a root tuber crop belonging to the sedge family. The origin of the crop to date is uncertain. While some believe that it is a native of Africa and tropical Asia [3–5] others are of the view that it originated from Europe and North America [6,7].

The tuber (nut as affectionately called) is a source of feed, food, medicine and perfumes [8,9]. It can be eaten raw, roasted, dried, baked or made into a refreshing beverage called Horchata De Chuf (in Spain), 'kunnu aya' (in northern Nigeria) and 'atadwe milk' (in Ghana).

The tuber is highly valued for its protein (7–8%) [10], fibre (8–10 %) [11], vitamins (C and E), and rich minerals (Sodium, Calcium, Potassium Magnesium, Zinc and traces of Copper) [12]. It contains almost all the functional nutrient components for a balanced diet. Approximately, the tuber contains 26–30 % starch and 21–25 % fat, providing about 400–450 kcal 100 g^{-1} energy [13].

Medically, by potency, the crop is an aphrodisiac, has carminative and diuretic effects, and is used as a stimulant and tonic. This moderates the incidence of colon cancer, coronary heart diseases, obesity, diabetes, excessive thirst, gastrointestinal disorders [13–15] constipation, high blood pressure, and diarrhoea [16]. Economically, the crop provides a source of foreign exchange for its high export

* Corresponding author.
E-mail address: ptrcktwumasi@yahoo.com (P. Twumasi).

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potential [17].

Though the crop is inundated with a lot of nutritional, medical and economic benefits, the inadequate attention invested in its breeding has resulted in a lower genetic base, limiting its genetic advancements. There is the risk of local germplasm in the hands of farmers becoming endangered with time as there is a lack of germplasm resources for the accessions coupled with no varietal release. Studies so far have been on morphological characterization and have revealed low genetic diversity within the populations studied [18,19].

Tiger nut is a vegetatively propagated crop and it scarcely undergoes natural hybridization and therefore has low genetic variability, unlike sexually propagated crops. There is no available documentation for the crop's improvement by any method. Improving any crop, first demands creating variation in a population for selection. A mutation is the ultimate source of variability and provides unique germplasm for plant breeders [20], especially in vegetatively propagated crops. This can occur by natural means, which takes several years or by artificial (induced) mutation, which gives results in a relatively shorter period. Induced mutation has been used successfully in the genetic improvements of many crop genotypes. This technique has been used by plant breeders since the 1920s to create genetic variation [20–23].

Induced mutation has been functionally performed either physically or chemically. Physical mutagens are mostly electromagnetic radiations such as gamma rays, X-rays, UV light and particle radiation (beta and alpha particles). Chemical mutagens are usually alkylating agents and include ethyl methanesulfonate (EMS), ethidium bromide, and base analogues such as bromouracil [20,24,25]. Colchicine is another chemical mutagen, an alkaloid for mutation induction and more purposely for polyploidy evocation in plant breeding. EMS is also noted to be a powerful chemical mutagen producing random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation. EMS generally produces only point mutations in a genome. The higher doses of mutagen completely arrest seed germination [26]. Hence knowing the effective dose of a mutagen on genotypes is a prerequisite to successful mutation breeding. Therefore, there is a need to determine LD_{50} (lethal dose that will kill 50 % of the population) and RD_{50} (reduction dose that will reduce 50 % plant height or traits) of EMS and Colchicine for actual mutagenesis.

2. Materials and methods

The research work was carried out at the Center for Scientific and Industrial Research, Crop Research Institute (CSIR- CRI), Fumesua in the Ashanti region of Ghana in late March 2022 in a greenhouse at the onset of the major rainy season. Four genotypes made up of two different brown genotypes (OFF-b and APR-b) and two other different black genotypes (BUO–B and ENK-B) of tiger nut tubers were used. These genotypes were landraces made up of accessions drawn from a pool of earlier work on the morphological diversity of tiger nuts from major growing areas in Ghana. BUO-B was collected from Buoyam in the Bono East region of Ghana. ENK-B and APR-b were picked from Enkroful and Assin Praso respectively, all in the Central region of Ghana. While OFF-b was sourced from Offinso in the Eastern region of Ghana. The genotypes were selected based on yield performance as the highest recorded yielding genotypes.

One category of brown type and black type genotypes (BUO–B and OFF-b) were subjected to EMS treatment at five different concentrations respectively. The other category of brown and black type genotypes (ENK-B and APR-b) were treated with Colchicine also at five different concentrations respectively. The concentrations for each mutagen were; 0 %, 0.1 %, 0.25 %, 0.5 % and 1.0 %. In

Fig. 1. Plants at 16 days of growth after germination (sprout).

all, four hundred tubers made up of twenty tubers for each concentration were used for mutagenesis for the four genotypes.

The solutions were prepared with distilled water and the chemical mutagens. The mutagen Colchicine, which was powdered and bottled was manufactured by KEM Light Spechem Laboratory Private Limited located in Mumbai, India. The EMS was a liquid and bottled chemical product from CDH Private Limited located in New Delhi, India. First, 1 % stock solution (1 g chemical mutagen: 100 ml distilled water) was made and later diluted to the required concentration levels. However, the control (0 %) was made up of only distilled water.

The dried tubers were soaked in each of the concentrations of EMS and Colchicine for 24 h as observed by Ye and others [27] to soften tubers and open up tuber pores for efficient inhibition of chemicals. Soaked tubers were later removed and washed with distilled water for immediate planting. Treated tubers and the controls were planted in polybags filled with steamed sterilized sandy-loam soil in a greenhouse in three replications using Completely Randomized Design (CRD). Irrigation was continuously done according to the demand needs of the soil and plants. No fertilization and insecticide treatments were performed as insects were not a problem as well as soil for the trial was intact with nutrients without any deficiency symptoms. Germination was assessed on the seventh day after planting. Germination started with some of the controls and later from the eighth day onwards the treated ones also began sprouting.

Data collected were germination percentage, plant height, number of tillers, the diameter of the main plant and the number of leaves per plant. Germination count started on the same day as the appearance of germination till the tenth day, and percentage germination was determined by the number germinated to total planted expressed in percentage. Plant architectural traits such as plant height were calculated from the averages of all three replications. Plant height was taken from the surface of the soil to the tip of the primary (terminal) leaf when the plants were a month old (3 weeks after germination) using a measuring ruler. Fig. 1 shows plant growth stage in the third week after germination.

During this same period, the number of tillers was recorded and the diameter of the mother plant was determined using a digital vernier calliper taking readings at the base of the plant just above the soil and expressed in millimetres. The number of leaves borne on the mother plant was counted and recorded. The number of tillers produced by the mother plant was also documented.

Chemosensitivity analysis for LD_{50} and RD_{50} was performed with Excel 2016, and Genstat statistical software version 11.2. The LD_{50} and RD_{50} for the genotypes were estimated using a simple linear regression model by fitting the straight-line equation $y = mx + c$; where y is the response variable (percentage germination per plant height), x is the independent variable (concentration of mutagenesis), while m and c represent the slope and constant, respectively.

3. Results and discussion

In general, a gradual reduction in the percentage of germination or sprouts of tuber was observed as per corresponding increasing levels of mutagen concentrations for both EMS and Colchicine (Fig. 2). This is in agreement with the findings of Horn and others in cowpea [28] and Rangaswamy in sesame [29]. However, each genotype responded differently to the different levels of mutagen

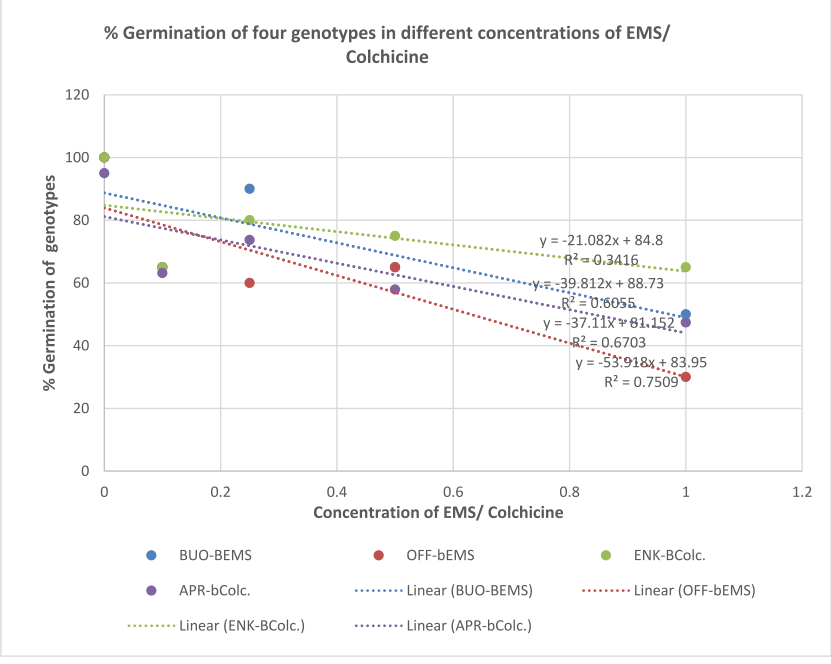

Fig. 2. Regression graph showing the percentage germination of the four tiger nut genotypes in the different concentrations of EMS and Colchicine treatments. BUO-B_{EMS}= Black EMS-treated tiger nut genotype from Buoyam; OFF-b = Brown EMS-treated tiger nut genotype from Offinso; ENK-$B_{Colc.}$ = Black Colchicine-treated tiger nut genotype from Enkroful and APR-$b_{Colc.}$ = Brown Colchicine-treated tiger nut genotype from Assin Praso.

concentrations in germination (Fig. 2) and plant architectural traits (Table 2 and Table 3). Nonetheless, the percentage rate of germination was higher with the black tiger nut genotypes than observed in the brown genotypes in both EMS and Colchicine (Fig. 2). Evidence of this nature shows that the black genotypes in their original state are hardier than their brown counterparts as the latter were more sensitive to the effects of the mutagens.

The black genotype's reaction to EMS (BUO-B_{EMS}) revealed 100 % germination under control conditions and a steady decline in germination with rising mutagen concentrations (Fig. 2). Ninety per cent of seeds germinated at a concentration of 0.25 %, whereas only 50 % did at a concentration of 1.0 %. The population-wide EMS concentration dose to produce LD_{50} was calculated to be 0.97 % (Table 1) for black tiger nuts.

The regression graph of Fig. 2 establishes an LD_{50} of 0.63 % with the equation, $y = -53.918x + 83.95$ (Table 1) for the corresponding brown tiger nuts treated with EMS. The black genotypes are confirmed to be less sensitive to the mutagen than the brown type because of the lower LD_{50} value observed for the brown compared with the black (0.97 %) (Table 1). Hence, the black genotype is more resilient to environmental changes than the brown genotype due to the hardier character of the nuts. Except for 0.1 % concentration which was drastically reduced by 65 %, all concentrations of Colchicine used to treat ENK-B_{Colc} resulted in a proportional decrease in germination. Fig. 2's regression equation, which showed an LD_{50} value of 1.65 %, was $y = -21.082x + 84.8$. (Table 1). The brown genotypes (APR-$b_{Colc.}$) (Fig. 2), on the other hand, consistently had a severe reduction in germination as Colchicine concentration rose, except 0.25 % Colchicine concentration, which had a 70 % germination rate. The lethal dose effect of 0.91 % (Table 1) could wipe out fifty per cent of the population, according to the regression equation $y = -37.11x + 81.152$ (Fig. 2).

Averagely, a relatively low concentration of both mutagens resulted in an increase in plant architecture, including plant height, leaf count, main plant diameter, and tiller count (Table 2). For example, plant height growth was positively affected at lower concentration doses of mutagens (Fig. 3). This is not surprising as mutagens such as colchicine have both stimulatory and inhibitory effects on mutants at different concentrations [30–32], and hence at lower concentrations such as 0.1 %, an increase in plant architectural traits was observed (Table 2). Based on their means, these quantitative traits to some extent showed a steady increase with lower doses of mutagen concentrations up to a dose of 1.0 % before eventually declining (Tables 2 and 3). This is in line with the observed effects of RD_{50} for both mutagens, that to establish RD_{50}, the concentrations for both mutagens should be from 1.5 % and above for the black and brown tiger nut genotypes (Fig. 3). On the contrary, results from Fig. 3 show that at very low concentrations below the control where water loses its chemical properties, plant height is likely to be negatively affected.

Similar findings about a decline in plant heights, when chemical mutagen concentrations rose in onions were reported [33]. For instance, in Table 2, the mean plant heights for the control treatments for the black and brown genotypes were 34.84 cm and 34.50 cm, respectively. At a concentration of 0.1 % EMS, they were different with higher values of 38.26 cm and 36.89 cm, respectively. This pattern persisted for EMS concentrations of 0.25 %–0.5 %, and at an increase in EMS concentration of 1.00 %, it changed to a decrease in height levels of 23.66 cm and 22.28 cm in the black and brown genotypes, respectively.

On an individual plant basis, there was a wide range measurement of values recorded for most genotypes treated with mutagens than their control counterparts. For example; the minimum height for brown genotype treated with 0.5 % Colchicine in Table 3 was 21.63 cm and the maximum was 40.53 cm as compared with the control which recorded 35.37 cm for the minimum and 37.17 cm for the maximum. This confirms the event of mutation by chemical mutagens as being distributed randomly across the genome [34,35] and hence results in high mutation densities. To effectively induce about 50 % reduction in plant architectural traits for the population, an observation made for this study recommended the use of high doses of mutagen concentrations of both EMS and Colchicine for the mutagenesis of the genotypes.

In Fig. 3, the RD_{50} regression analysis for the genotypes based on plant height revealed varied regression coefficients. Additionally, RD_{50} values for various genotypes ranged from 1.49 % to 19.51 %. (Table 1). The RD_{50} for the black genotype treated with EMS was 1.49 %, as opposed to 1.63 % for the brown genotype (Table 1). The brown genotype (APR-b_{Col}) had an RD_{50} based on Colchicine treatment of 1.71 % as opposed to the enormous value of 19.51 % for black Colchicine treated. However, this was expected because the black Colchine-treated genotype outperformed its control in height. Overall, the genotypes treated with Colchicine had greater RD_{50} values than the genotypes treated with EMS (Table 1).

4. Conclusion

The results of the current investigation showed that EMS and Colchicine mutagens prolonged the time of germination in tiger nut tubers. The brown tiger nut is more sensitive to these chemical mutagens than the black genotypes. Additionally, it was seen that the percentage rate of germination gradually decreased when the concentration dose was increased. The effective dose (LD_{50} value) for the

Table 1
LD_{50} and RD_{50} of EMS and Colchicine among the treated genotypes with their corresponding regression equations.

Genotype/Mutagen	LD_{50} Regression Equation	% LD_{50} Calculated	RD_{50} Regression Equation	% RD_{50} Calculated
BUO-B_{EMS}	$y = -39.812x + 88.73$	0.97	y = 38.468 x - 7.3965	1.49
OFF-b_{EMS}	$y = -53.918x + 83.95$	0.63	y = 37.013 x - 10.5	1.63
ENK-$B_{Colc.}$	$y = -21.082x + 84.8$	1.65	y = 2.6686 x - 2.0875	19.51
APR-$b_{Colc.}$	$y = -37.11x + 81.152$	0.91	y = 29.866 x - 1.1461	1.71

BUO-B_{EMS}= Black EMS-treated tiger nut genotype from Buoyam; OFF-b = Brown EMS-treated tiger nut genotype from Offinso; ENK-$B_{Colc.}$ = Black Colchicine-treated tiger nut genotype from Enkroful and APR-$b_{Colc.}$ = Brown Colchicine-treated tiger nut genotype from Assin Praso.

Table 2
The mean, minimum, maximum and standard deviation of genotypes treated with EMS.

Genotype	Treatment in Concentration (%)	PHT (cm)	NLP	NTP	DMP (mm)
Black	Control (0)	34.84^{a} (33.43–36.80) 1.75	7.56^{ab} (7.00–8.33) 0.69	1.67^{bc} (1.33–2.00) 0.33	3.78^{a} (3.59–4.10) 0.26
	0.1	38.26^{a} (35.27–42.43) 3.73	7.89^{a} (7.33–8.33) 0.51	2.00^{abc} (1.67–2.33) 0.33	4.02^{a} (3.30–4.41) 0.62
	0.25	35.02^{a} (31.63–37.83) 3.14	7.67^{ab} (7.00–8.00) 0.51	2.22^{ab} (2.00–2.67) 0.39	3.83^{a} (3.48–4.16) 0.35
	0.5	30.53^{ab} (29.70–31.97) 1.25	7.78^{a} (7.00–9.00) 1.07	2.89^{a} (2.33–4.00) 0.96	4.49^{a} (3.85–5.09) 0.62
	1.0	23.66^{b} (12.47–30.50) 9.77	6.56^{ab} (4.33–8.67) 2.17	1.89^{abc} (1.67–2.33) 0.39	3.29^{a} (2.27–3.85) 0.88
Brown	Control (0)	34.50^{a} (30.60–36.47) 3.32	7.33^{ab} (7.00–7.67) 0.33	1.67^{bc} (1.33–2.00) 0.33	3.86^{a} (4.05–4.17) 0.44
	0.1	36.89^{a} (35.07–38.53) 1.74	7.33^{ab} (7.00–7.67) 0.33	2.78^{ab} (2.33–3.00) 0.39	3.85^{a} (3.56–4.02) 0.26
	0.25	35.96^{a} (29.97–42.67) 6.38	8.00^{a} (7.33–8.00) 0.39	2.22^{ab} (1.33–3.67) 1.26	3.99^{a} (3.78–4.29) 0.27
	0.5	37.37^{a} (34.80–40.90) 3.16	7.67^{ab} (7.33–8.00) 0.33	2.33^{ab} (1.33–3.33) 1.00	3.87^{a} (3.37–4.34) 0.50
	1.0	22.28^{b} (12.67–32.50) 9.93	5.44^{b} (2.67–9.00) 3.24	1.00^{c} (0.00–1.67) 0.88	3.14^{a} (1.08–5.43) 2.18

PHT: plant height, NLP: number of leaves per plant, NTP: number of tillers per plant, DMP: Diameter of the main plant.
Means within the same column with the same letter(s) is/are not significantly different using one-way ANOVA with Fisher's pairwise grouping comparison at $P \leq 0.05$.

Table 3
The mean, minimum, maximum and standard deviation of genotypes of Colchicine.

Genotype	Treatment in Concentration (%)	PHT (cm)	NLP	NTP	DMP (mm)
Black	Control (0)	33.73^{ab} (31.97–36.60) 2.50	7.56^{ab} (7.33–7.67) 0.19	2.44^{ab} (2.00–3.00) 0.51	4.26^{a} (3.47–5.19) 0.87
	0.1	34.24^{ab} (34.10–34.47) 0.20	7.89^{a} (7.67–8.33) 0.39	2.56^{a} (2.33–3.00) 0.39	4.01^{ab} (3.38–4.39) 0.55
	0.25	35.11^{ab} (35.07–35.17) 0.05	7.89^{a} (7.33–8.33) 0.51	1.89^{abc} (1.67–2.00) 0.19	4.39^{a} (3.99–4.83) 0.42
	0.5	34.23^{ab} (33.47–34.90) 0.72	8.11^{a} (7.67–9.00) 0.77	2.67^{a} (1.67–3.33) 0.88	4.50^{a} (4.33–4.58) 0.15
	1.0	33.20^{ab} (30.33–35.27) 2.56	8.00^{a} (7.67–8.33) 0.33	2.33^{ab} (2.33–2.33) 0.00	4.37^{a} (4.15–4.49) 0.19
Brown	Control (0)	35.97^{ab} (35.37–37.17) 1.04	7.78^{a} (7.67–8.00) 0.19	2.44^{ab} (2.00–2.67) 0.39	3.91^{ab} (3.80–4.13) 0.19
	0.1	30.42^{ab} (13.00–40.57) 15.16	5.56^{bc} (2.67–7.67) 2.59	1.56^{bc} (0.67–2.33) 0.84	3.55^{ab} (1.52–5.11) 1.84
	0.25	38.52^{a} (35.30–41.17) 2.98	7.00^{abc} (6.67–7.33) 0.33	1.56^{bc} (1.33–1.67) 0.19	3.96^{ab} (3.85–4.08) 0.12
	0.5	33.38^{ab} (21.63–40.53) 10.25	6.78^{abc} (5.00–8.33) 1.68	2.11^{ab} (1.67–2.33) 0.39	3.84^{ab} (2.56–5.11) 1.28
	1.0	23.73^{b} (9.93–38.63) 14.38	4.89^{c} (2.67–7.00) 2.17	1.11^{c} (0.33–1.67) 0.69	2.65^{b} (1.22–4.31) 1.56

PHT: plant height, NLP: number of leaves per plant, NTP: number of tillers per plant, DMP: Diameter of the main plant.
Means within the same column with the same letter(s) is/are not significantly different using one-way ANOVA with Fisher's pairwise grouping comparison at $P \leq 0.05$.

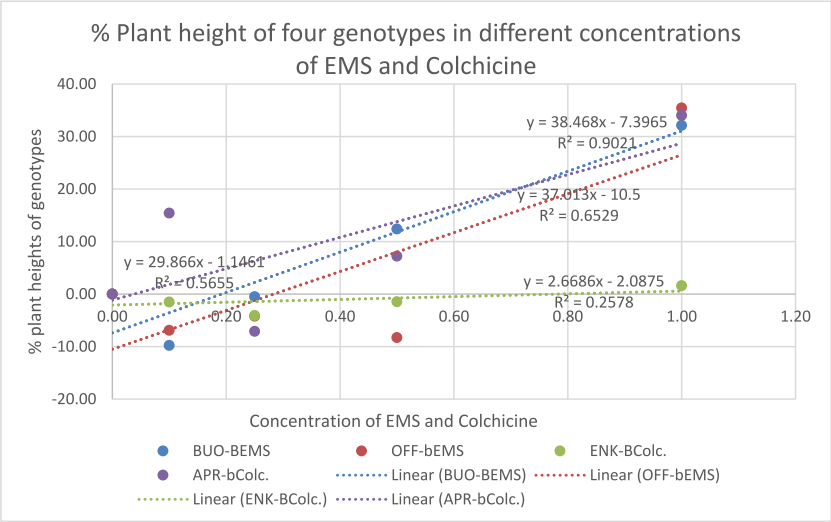

Fig. 3. Regression graph showing the RD_{50} for plant heights for the four different tiger nut genotypes in the different concentrations of EMS and Colchicine treatments. BUO-B_{EMS}= Black EMS-treated tiger nut genotype from Buoyam; OFF-b = Brown EMS-treated tiger nut genotype from Offinso; ENK-$B_{Colc.}$ = Black Colchicine-treated tiger nut genotype from Enkroful and APR-$b_{Colc.}$ = Brown Colchicine-treated tiger nut genotype from Assin Praso.

black genotypes was determined to be a high dose of 0.97 % EMS concentration as opposed to 0.63 % EMS concentration in the brown genotypes.

Genotypes responded to Colchicine treatment less sensitively compared to EMS. In comparison to a considerably lower concentration of EMS that could do the same task, a higher concentration of Colchicine—as high as 1.65 % and 0.91%—was required to kill half the population of the black and brown genotypes, respectively.

As hormesis (a growth vigour simulative effect of mutagen at lower concentrations) progressed, a rise in the plant's architecture, including its height, diameter, number of leaves, and tillers, was seen. The RD_{50} is established in addition to hormesis. For the black and brown genotypes treated with EMS, the ideal doses to reduce plant height were found to be 1.49 % and 1.63 %, respectively. Those that received Colchicine recorded high RD_{50} values of 1.71 % and 19.51 % for the brown and black genotypes respectively.

Data availability statement

Data is available as supplementary document attached.

CRediT authorship contribution statement

Patrick Twumasi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pual Agu Asare:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Emmanuel Afutu:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Godwin Amenorpe:** Writing – review & editing, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Sylvester N.T.T. Addy:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22619.

References

[1] M.P. Shilenko, G.S. Kalacheva, G.M. Lisovskiĭ, I.N. Trubachev, Chufa (Cyperus esculentus) as a source of vegetable fats in a sealed life-support system, Kosmicheskaia Biol. i Aviakosmicheskaia Meditsina 13 (5) (1979) 70–74.
[2] T. Bazine, Ş.F. Arslanoğlu, Tiger nut (Cyperus esculentus); morphology, products, uses and health benefits, Black Sea Journal of Agriculture 3 (4) (2020) 324–328.
[3] DAISIE, Delivery of Alien Invasive Species Inventories for Europe. European Invasive Alien Species Gateway, 2014.
[4] P. Acevedo-Rodríguez, M.T. Strong, Catalogue of Seed Plants of the West Indies, 2012.
[5] R. Govaerts, World Checklist of Lamiaceae, Royal Botanic Gardens, Kew, Richmond, London, UK, 2014.
[6] USDA-ARS, Germplasm Resources Information Network (GRIN). Online Database, National Germplasm Resources Laboratory, Beltsville, Maryland, USA, 2014. http://npgsweb.ars-grin.gov/gringlobal/taxonomysearch.aspx.
[7] L.G. Holm, D.L. Plucknett, J.V. Pancho, J.P. Herberger, The World's Worst Weeds, University Press of Hawaii, *Honolulu.* Hawaii, USA, 1977.
[8] F.T. De Vries, Chufa (Cyperus esculentus, Cyperaceae): a weedy cultivar or a cultivated weed? Econ. Bot. 45 (1) (1991) 27–37.
[9] A. Gambo, A. Da'u, Tiger nut (Cyperus esculentus): composition, products, uses and health benefits-a review, Bayero Journal of Pure and Applied Sciences 7 (1) (2014) 56–61.
[10] R.A. Oderinde, O.A. Tairu, Evaluation of the properties of yellow nutsedge (Cyperus esculentus) tuber oil, Food Chem. 28 (3) (1988) 233–237.
[11] S.C. Umerie, J.N. Enebeli, Malt caramel from tubers of Cyperus esculentus, Bioresour. Technol. 57 (2) (1996) 215–216.
[12] R.E. Sanful, The use of tiger-nut (Cyperus esculentus), cow milk and their composite as substrates for yoghurt production, Pakistan J. Nutr. 8 (6) (2009) 755–758.
[13] E. Sánchez-Zapata, J. Fernández-López, J. Angel Pérez-Alvarez, Tiger nut (Cyperus esculentus) commercialization: health aspects, composition, properties, and food applications, Compr. Rev. Food Sci. Food Saf. 11 (4) (2012) 366–377.
[14] E.R. Chukwuma, N. Obioma, O.I. Christopher, The phytochemical composition and some biochemical effects of Nigerian tigernut (Cyperus esculentus L.) tuber, Pakistan J. Nutr. 9 (7) (2010) 709–715.
[15] A.O. Adebayo-Oyetoro, O.O. Ogundipe, F.K. Lofinmakin, F.F. Akinwande, D.O. Aina, S.A.O. Adeyeye, Production and acceptability of chinchin snack made from wheat and tigernut (Cyperus esculentus) flour, Cogent Food Agric. 3 (1) (2017), 1282185.
[16] A.K. Oladele, J.O. Aina, Chemical composition and functional properties of flour produced from two varieties of tigernut (Cyperus esculentus), Afr. J. Biotechnol. 6 (21) (2007).
[17] J.P. Tetteh, E. Ofori, A baseline survey of tiger nut (Cyperus esculentus) production in the Kwahu South District of Ghana, Ghana J. Agric. Sci. 31 (2) (1998) 211–216.
[18] P.A. Asare, R. Kpankpari, M.O. Adu, E. Afutu, A.S. Adewumi, Phenotypic characterization of tiger nuts (Cyperus esculentus L.) from major growing areas in Ghana, Sci. World J. (2020), https://doi.org/10.1155/2020/7232591, 2020.
[19] E. Donkor, D. Nyadanu, H. Dapaah, Germplasm collection and morphological characterization of local accessions of tigernut (Cyperus esculentus L.) in Ghana for conservation and utilization, J. Plant Breed Crop Sci. 11 (8) (2019) 196–205.

[20] A.M. van Harten, Mutation Breeding: Theory and Practical Applications, Cambridge University Press, 1998.
[21] B.S. Ahloowalia, M. Maluszynski, K. Nichterlein, Global impact of mutation-derived varieties, Euphytica 135 (2) (2004) 187–204.
[22] L.J. Stadler, Genetic effects of X-rays in maize, Proceedings of the National Academy of Sciences of the United States of America 14 (1) (1928) 69.
[23] A.B. Tambe, B.J. Apparao, Gamma Ray Induced Mutations in Soybean [Glycine Max (L.) Merill] for Yield Contributing Traits. Induced Plant Mutations in the Genomics Era, Joint FAO/IAEA Programme, Vienna, Austria, 2009, pp. 95–96.
[24] M. Girija, D. Dhanavel, Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatments in cowpea (Vigna unguiculata L. Walp), Global J. Mol. Sci. 4 (2) (2009) 68–75.
[25] C. Mba, R. Afza, S. Bado, S.M. Jain, Induced mutagenesis in plants using physical and chemical agents, Plant Cell Culture: Essential Methods 20 (2010) 111–130.
[26] M.M. Spencer-Lopes, B.P. Forster, L. Jankuloski, Manual on Mutation Breeding, Issue third ed., Food and Agriculture Organization of the United Nations (FAO), 2018.
[27] Y. Ye, X. Li, H. Qian, S. Yang, X. Han, J. Zhang, Effects of Different EMS Solution Concentration and Time Treatment on Morphological Traits of Cyperus Esculentus L, vol. 203, E3S Web of Conferences, 2020, https://doi.org/10.1051/e3sconf/202020302006.
[28] L.N. Horn, H.M. Ghebrehiwot, H.A. Shimelis, Selection of novel cowpea genotypes derived through gamma irradiation, Front. Plant Sci. 7 (2016) 262.
[29] M. Rangaswamy, Induced Mutagenesis in Gingella (Sesamum indicum L.) Treated with Gamma Rays and EMS, Tamil Nadu Agricultural University, Coimbatore, 1973. M. Sc. (Agri.) Thesis.
[30] Y.I. El-Nashar, M.H. Ammar, Mutagenic influences of colchicine on phenological and molecular diversity of Calendula officinalis L, Genet. Mol. Res. 15 (2) (2016) 1–15.
[31] S. Nura, A.K. Adamu, S. Mu'Azu, D.B. Dangora, Chemical mutagenesis for improved quality traits in jute (Corchorus olitorious L.), Continent. J. Biol. Sci. 4 (2) (2011) 22–27. //www.cabdirect.org/cabdirect/abstract/20123299339.
[32] K. Singh, O.P. Awasthi, A. Singh, V.K. Sharma, A.K. Dubey, L.S. Sisodia, Effect of colchicine on plant growth and leaf nutrient acquisition of sweet orange (Citrus sinensis (L .) Osbeck) cv. Mosambi 8 (3) (2020) 211–215.
[33] N. Joshi, A. Ravindran, V. Mahajan, Investigations on chemical mutagen sensitivity in onion (Allium cepa L.), Int. J. Bot. 7 (3) (2011) 243–248.
[34] E.A. Greene, C.A. Codomo, N.E. Taylor, J.G. Henikoff, B.J. Till, S.H. Reynolds, L.C. Enns, C. Burtner, J.E. Johnson, A.R. Odden, Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis, Genetics 164 (2) (2003) 731–740.
[35] B.J. Till, S.H. Reynolds, E.A. Greene, C.A. Codomo, L.C. Enns, J.E. Johnson, C. Burtner, A.R. Odden, K. Young, N.E. Taylor, Large-scale discovery of induced point mutations with high-throughput TILLING, Genome Res. 13 (3) (2003) 524–530.