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EFFECT OF COLCHICINE INDUCTION ON THE PHENOTYPIC, MINERAL AND PHYTOCHEMICAL COMPOSITIONS OF *Amarantus cruentus* L.

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ABSTRACT

The development of genetically improved crops by breeders to meet the needs of farmers and populace requires the induction of genetic variability in crops to produce unique variants. Colchicine-induced mutagenesis has emerged as a promising technique for generating genetic variations and improving plant characteristics. The study investigated the impact of varying concentrations of colchicine on the phenotypic traits, mineral, and phytochemical compositions of *Amaranthus cruentus*. The research was conducted at the screen house of the Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Ekiti State, Nigeria. The seeds of *Amaranthus cruentus* were planted in plastic bowls with varying concentrations of colchicine (0.1 g/ml, 0.2 g/ml, and 0.3 g/ml) of colchicine, while 0.0 serves as the control and the experiment was set up in triplicates. The subsequent effects on the phenotypic traits, phytochemical and mineral compositions were evaluated at maturity. Phenotypic traits including plant height, leaf size, leaf shape, and inflorescence characteristics were assessed. Phytochemical and mineral compositions of the matured plants were also determined. The results reveal significant variations at $p \leqslant 0.05$ in phenotypic traits among the mutant lines compared to the control group. Changes in growth patterns, and leaf morphology were observed, indicating successful induction of genetic variations through colchicine treatment. The mineral profiles including iron, zinc, calcium, sodium, potassium, manganese and phytochemical compositions such as flavonoids, phenols and tannins of the mutant lines exhibited notable differences compared to the control, suggesting that colchicine-induced mutagenesis influenced mineral accumulation in *Amaranthus cruentus*. The results suggest that colchicine has a dose-dependent effect on the morphological characteristics, mineral and phytochemical compositions of the plant.

Keywords: Phenotypic, Mutant, Mutagenesis, Morphological, Variants, Variability.

INTRODUCTION

The *Amaranthus* genus, belonging to the family Amaranthaceae, comprises a diverse group of plants that have been cultivated for centuries for both ornamental and dietary purposes (Park *et al.*, 2020; Aderibigbe *et al.*, 2022) Among the various species within this genus, *Amaranthus cruentus* stands out as a significant grain and leafy vegetable crop due to its nutritional richness and adaptability to a wide range of environmental conditions (Gomes, 2024). Amaranth species have high compositions of food nutrients and has been recognized as one of the significant protein sources and has positive effects on the health and weight of animals and humans, making them valuable components of diets in various parts of the world (Olufemi *et al.*, 2003; Zralý *et al.*, 2004; Gebrehiwot and Unakal, 2013; Vaseeharan and Thaya, 2014). *Amaranthus cruentus* has been used for human cultivation and consumption, it is known for its nutritional and agronomic significance together with its culinary and medicinal importance. It has high nutrition contents with high proximate, minerals and phytochemical compositions. This vegetable is highly consumed by humans for dietary benefits and medicinal value, hence, the need to breed *Amaranthus cruentus* for higher nutritional components. The improvement in *Amaranthus cruentus* production in Nigeria is highly essential to increase livelihood of the nation, boost individual and national income and improve the agronomic traits of the crop (Nadi *et al.*, 2013). Therefore, effort is needed by breeders in improving and producing *Amaranthus cruentus* with improved agronomic traits and higher contents of mineral, proximate, phytochemical and nutritional compositions.

Colchicine, a mitotic inhibitor, has been widely utilized to induce polyploidy artificially in various plant species, leading to altered phenotypic traits

and changes in mineral composition (Levin, 2002; Ozkan *et al.*, 2001). Colchicine induction affected morphological characters in sorghum leading to the production of homozygous genotypes, morphological changes. The manipulation of polyploidy through colchicine treatment presents a unique opportunity to explore potential enhancements in the phenotypic traits, phytochemical and mineral compositions of *A. cruentus*. Colchicine induction can generate point mutations, chromosomal rearrangements, and whole-genome duplications (Mukherjee *et al.*, 2017). Colchicine-induced mutations have been successfully applied to alter plant morphology, reproductive behaviours, and nutritional content. This has been observed in various crops, including *Amaranthus cruentus* (Kumar *et al.,* 2014). These alterations contribute to the creation of novel genetic traits, some of which can be advantageous for plant adaptation and agricultural productivity.

Understanding how colchicine-induced polyploidy affects the growth, morphology, and mineral uptake of this important crop has implications not only for Amaranth production but also for broader insights into plant physiology and crop improvement strategies. The successful application of colchicine induction in plant breeding is evident in various crop species. For example, the production of high-yielding, insect and disease-resistant varieties in most crops have been made possible by colchicine inducion. Based on this, the study was conducted to investigate the impact of varying concentrations of colchicine on the phenotypic traits, mineral and phytochemical compositions of *Amaranthus cruentus*

MATERIALS AND METHODS

Collection of *Amaranthus cruentus* seeds

The seeds of *Amarathus cruentus* were collected from the National Centre for Genetic Resources and Biotechnology, (NACGRAB), Ibadan, Oyo State, while the colchicine used were gotten from a scientific laboratory in Ado Ekiti, Ekiti State.

Preparation of colchicine solution and plant treatment

Colchicine concentration of 0.1% was prepared by dissolving 0.1 g colchicine into 100 ml distilled water. Subsequently, 0.2% and 0.3% colchicine concentrations were produced by dissolving 0.2 g and 0.3 g respectively into 100 ml of distilled water. *Amaranthus cruentus* viable seeds were placed in a prepared solution of different concentrations of colchicine [0.1 g/ml, 0.2 g/mL, and 0.3 g/ml] for 24 hours while 0.0 serve as the control. The seeds were removed from colchicine solution and planted in triplicates on a planting bowl containing humus loamy soil and the plants were wet on daily basis till maturity.

Determination of mineral composition

The mineral content was determined as described by Association of Official Analytical Chemists' method (A.O.A.C, 2005). About 1g of *Amaranthus* leaf was ashed at a high temperature of 600°C for 6 hours and then put in the desiccators to allow cooling process to take place. The ashed sample was dissolved by adding 1ml nitric acid (HNO_3) together with 1ml hydrochloric acid (HCl) and then made up to 100 ml the same process was carried out for Manganese (Mn), Sodium (Na), Iron (Fe), Zinc (Zn), Calcium (Ca) and Potassium (K).

Determination of Mn, Fe, Ca and Zn using atomic absorption spectrophotometer

Each of the element's standard solution were separately prepared and atomic absorption spectrophotometer was used to determine their values. The original values of the element in the digest were gotten by plotting the values measured against the strength of the solution.

Determination of Na and K using flame photometer

The standard solutions were prepared separately using NaCl and KCl for sodium and potassium determinations respectively. The standard solutions were measured on the flame photometer and the value obtained was plotted against the strength of various solutions. The digest were determined from the flame photometer. The values were plotted in the respective standard curve to read the original values of the elements.

Determination of phytochemical composition of *Amaranthus cruentus*

Alkaloids determination

The composition of alkaloid in the treated and untreated *Amaranthus cruentus* was determined

using Dragendroff's Reagent (DR). A sample of about 1g of the leaf was weighed (W) and transferred into a Conical or Volumetric Flask of 100 ml. The sample solution was filtered using filter cloth and evaporated to a quarter of the sample initial volume. 4 drops of Conc. NH_4OH solution was added to evaporate the filtrate. The precipitate formed was filtered using a pre-weighed Filter paper (W2)
Where W1 = Weight of the sample
W2 = Pre-weighed filter paper + weight of sample after precipitate.

Flavonoid determination
The total flavonoid content of the extract was determined using a colorimeter assay developed by (Bao, 2005). Total flavonoid contents were expressed in terms of Galic acid equivalent, GAE (standard curve equation: Y = 0.005x +0.464 (R2=0.961) mg of GAE/mg of dry extract).

Phenol determination
The phenol content was examined in plant extracts using Folin-Ciocalteu's reagent. 1g of well ground sample was weighed into a test tube containing 10 mL of distilled Wwater. It was left to stand for 30mins at room temperature. The test tube content was centrifuged at 3000 rpm for 10 mins where 2.5 ml of the supernatant were taken and disperse into a 50 ml Volumetric flask and standard Tannin acid solution of 2.5ml were taken and disperse into 50 ml Volumetric flask. Folin Dennis reagant of about 1 L was added to the volumetric flask above and 2.5 mL of saturated Na_2CO_3 solution was added as well. The Volumetric flask was make up to 50 ml with distilled water and the mixture was incubated for 90mins at room temperature. The sample absorbance was taken at 250 nm with blank reagent at Zero, then calculate the percent phenol present.

Total carotene determination
It was done using the Association of Official Analytical Chemists' method (AOAC, 1980). 10 g of the macerated material was placed in a conical flask containing 50 ml of 95% ethanol and held at 70-80°C in a water bath for 20 minutes with periodic shaking. The supernatant was decanted and allowed to cool. The volume was measured using a measuring cylinder and recorded as the initial volume. The mixture's ethanol concentration increased to 85% by adding 15 ml of distilled water, which was cooled in an ice-filled container for approximately 5 minutes. The mixture was transferred to a separating funnel, and 25 ml of petroleum ether (pet-ether) was added before the cooled ethanol was poured over it. The funnel was gently swirled to produce a homogeneous mixture. It was later allowed to stand until two distinct layers emerged. The bottom layer was emptied into a beaker, and the top layer was collected in a 250 ml conical flask. The bottom layer was transferred to the funnel and re-extracted with 10ml pet-ether 5-6 times until the extract became yellow. The entire pet-ether solution was collected into a 250ml conical flask and transferred to a separating funnel for re-extraction with 50ml of 80% ethanol. The resulting extract was quantified and transferred to sample vials for further examination.

The extracts' absorbance was measured at 436nm with a spectrophotometer (model 22UV/VIS).

RESULTS

Effect of colchicine induction on the qualitative characters of *Amaranthus cruentus*

The plant treated with 0.1 g/mL concentration exhibited slight changes in their morphology compared to the control group. These changes include alteration in leaf shape, color, branching and size. In 0.2 g/ml concentration, the leaves of *Amaranths* exhibited irregular shape, increased size and different coloration along the veins. While in 0.3 g/mL concentration of colchicine, the flowers exhibited deformities in petal size, shape and arrangement, increase in branching, changes in the leaves shape and size becoming larger were also noticed at this concentration.

Effect of colchicine induction on the quantitative characters of *Amaranthus cruentus*

The result in (Table 1) indicated that there were significant differences at $P<0.05$ in the effect of mutagen on stem width, leaf area and plant while there were non-significant difference in the remaining characters evaluated in this research. The effect of colchicine on the quantitative

morphological characters of *Amaranthus cruentus* is shown in Table 2. From this result, it was observed that the inflorescence lengths are not significantly different. The stem width was observed to be significantly different. There is an increase in the stem width at 0.3g/ml treatment compared to other treatments. The number of branches at 0.1g/ml was observed to be significantly higher than other treatments, there is no significance difference in the control (0.0g/ml) and 0.2g/ml. there is no significance difference in the leaf area. But significance difference was observed in 0.2g/ml and 0.3g/ml. in which, the highest treatment (0.3g/ml) has the highest leaf area. The plant height was observed to be significantly different in all the treatments, while the control was observed to have the highest plant height. The number of leaves were observed to be significantly different at 0.1g/ml and 0.2g/ml. while at 0.0g/ml and 0.3g/ml the number of leaves were not significantly different. There is no significance difference in the petiole length observed in all the treatments.

Table 1. Analysis of variance of quantitative characters of *Amaranthus cruentus* in different concentrations of colchicine

Sources of variation	D.F	Inflorescence length	Stem width	Number of branches per plant	Leaf Area	Plant height	Number of leaves	Petiole length
Concentration	3	11.416	0.093*	0.356	38.627*	26.292*	11.237	0.129
Replication	2	56.072*	0.027	0.693	25.43	23.983*	12.79*	0.018
Error	6	5.61	0.018	0.114	1.864	0.652	2.402	0.049

*Significance difference at $p \leqslant 0.05$

Table 2: Mean values of quantitative characters of *Amaranthus cruentus* in different concentrations of colchicine

Treatment (g/ml)	**Inflorescence length**	**Stem width**	**Number of branches per plant**	**Leaf Area**	**Plant height**	**Number of leaves**	**Petiole length**
0.0	9.72^{a}	1.33^{ab}	3.22^{ab}	15.59^{b}	40.4^{c}	18.34^{b}	2.41^{a}
0.1	9.81^{a}	1.59^{bc}	3.56^{b}	15.81^{b}	35.1^{ab}	14.89^{a}	2.68^{a}
0.2	13.18^{a}	1.28^{a}	3.44^{ab}	11.3^{a}	35.6^{b}	16.2^{ab}	2.43^{a}
0.3	8.7^{a}	1.62^{c}	2.78^{a}	20.1^{c}	33.5^{a}	19.11^{b}	2.84^{a}
Mean	10.56	1.51	3.42	15.80	35.84	16.11	2.68
S.E	0.39	0.04	0.08	0.25	0.12	0.18	0.05

Means with the same letter within the column are not significantly different at $p \leqslant 0.05$
S.E = Standard error

Effect of colchicine induction on the mineral composition of *Amaranthus cruentus*

The mineral contents of the *Amaranthus* plant induced with varying concentration of colchicine is presented in Table 3. Notable variations were observed in the mineral composition of *Amaranthus cruentus* which were exposed to different concentrations of colchicine (0.1g/ml, 0.2g/ml, and 0.3g/ml) when compared with the control. For instance, in the plant treated with

0.3g/ml colchicine, there was increase in iron, calcium, sodium, manganese and potassium contents compared to the control while zinc content was found to be high in the control, followed by 0.3g/ml, 0.2g/ml and the plant treated with 0.1g/ml had the lowest content of zinc.

The result showed that the concentration of iron (Fe) present in *Amaranthus* plant among the treatments ranged from 37.00 ppm in the control to 55.00 ppm in *Amaranthus* plant induced with 0.3 g/ml of colchicine. Zinc ranged from 24.00 ppm in *Amaranthus* plant induced with 0.1 g/ml of colchicine to 48.00 ppm in the control. Calcium ranged from 2350.00 ppm in *Amaranthus* plant with no colchicine to 7350.00 ppm in *Amaranthus* plant induced with 0.3 g/ml colchicine. The concentration of sodium (Na) ranged from 518.00 ppm in *Amaranthus* plant induced with 0.3 g/ml of colchicine to 352.00 ppm in *Amaranthus* plant with no colchicine induction. While the concentration of Mn ranged from 4.00 ppm in plant treated with 0.2 g/ml of colchicine to 49.00ppm in A*maranthus* plant induced with 0.3 g/ml of colchicine. There were significant differences in the mineral elements of Amaranthus plant at $p \leqslant 0.05$.

Table 3: Colchicine induction on the mineral composition of *Amaranthus cruentus.*

Treatments	Fe Ppm	Zn Ppm	Ca Ppm	Na Ppm	K Ppm	Mn ppm
Control	37.00^{b}	48.00^{a}	2350.00^{b}	352.00^{b}	7500.00^{b}	6.00^{b}
0.3g/ml	55.00^{a}	41.00^{a}	7350.00^{a}	518.00^{a}	10500.00^{a}	49.00^{a}
0.2g/ml	43.00^{b}	27.00^{b}	1339.00^{b}	248.00^{b}	6750.00^{b}	4.00^{b}
0.1g/ml	39.00^{b}	24.00^{b}	2550.00^{b}	356.00^{b}	7300.00^{b}	9.00^{b}
SE	8.06	11.40	2687.95	111.50	1688.38	21.43

Mean with the same letter within the same column are not significantly different at $p \leqslant 0.05$.
SE± =Standard error

Effect of colchicine induction on the phytochemical compositions of *Amaranthus cruentus*

There is no significant improvement in the phytochemical compositions of the treated *Amaranthus cruentus* plants as compared to the control. The compositions of flavonoids, phenol and total carotene of the treated plants at the different concentrations of colchicine reduced drastically compared to the control except for flavonoids which increased from 10.44 in the control to 13.02 in 0.3 g/mL concentration of colchicine.

Table 4: Colchicine induction on the phytochemical compositions of *Amaranthus cruentus.*

Treatments	Flavonoids mg/100g	Phenol mg/100g	Total carotene mg/100g
Control	10.44^{b}	3.14^{a}	0.68^{a}
0.3g/ml	13.02^{a}	2.09^{b}	0.28^{b}
0.2g/ml	9.47^{b}	3.72^{a}	0.10^{b}
0.1g/ml	9.91^{b}	2.52^{b}	0.17^{b}
SE±	1.59	0.71	0.26

Mean values with the same letter along the same column are not significantly different at $p \leqslant 0.05$. SE± =Standard error of difference of mean

DISCUSSION

The findings of this study have important implications for both breeding programs and consumer preferences. The ability to induce desired morphological, mineral and phytochemical changes in *Amaranthus cruentus* using colchicine treatment offers opportunities for targeted trait improvement. Breeders can

utilize this technique to develop mutant lines with improved agronomic traits and nutritional properties.

The use of colchicine for the improvement of crops in plant breeding have been done successfully in many species of crops (Biswas and Datta, 1988). The results of this study revealed significant variations in phenotypic traits and mineral composition among the colchicine-induced and control groups of *Amaranthus cruentus*. Plants treated with colchicine exhibited changes in plant height, leaf size, and flowering patterns, which were concentration-dependent. Furthermore, the mineral composition analysis indicated alterations in the levels of essential minerals in the treated plants, with variations in sodium, iron, potassium, calcium, and molybdenum content. These findings align with previous research, as demonstrated by Song *et al.* (2017) and Li *et al.* (2019), where colchicine-induced polyploidy led to alterations in phenotypic traits and mineral composition in other plant species. This suggests that colchicine induction can be a valuable tool for introducing genetic diversity and potentially improving crop traits in *Amaranthus cruentus*.

The findings revealed observable changes in the physical traits of *Amaranthus cruentus* following exposure to different concentrations of colchicine, in comparison to the control group, the plants subjected to colchicine exhibited alterations in traits such as leaf size, color, and overall morphology. Studies by Zhang *et al.* (2020) have shown that colchicine-induced polyploidy can lead to alterations in gene expression patterns in *Arabidopsis thaliana*.

Furthermore, increase in the mineral contents of *Amaranthus cruentus* was in agreement with Li *et al.* (2019) who explored the impact of colchicine treatment on the mineral composition of tobacco plants. Their research revealed that colchicine-induced polyploidy resulted in shifts in potassium, calcium, and magnesium levels. These findings highlight the potential role of colchicine induction in modifying mineral composition in plant species. These outcomes suggest that colchicine has a dose-dependent effect on the morphological characteristics, mineral and phytochemical compositions of the plant.

In conclusion, this project sheds light on the mutagenic impact of colchicine on this plant species. The experiment demonstrated a clear association between the concentration of colchicine and the extent of observable changes in physical traits and mineral composition. These findings emphasize the potential of chemical mutagens as tools for directed evolution in agriculture and highlight the need for further research to elucidate the underlying genetic mechanisms driving these changes. As the pursuit of sustainable agriculture continues, understanding the implications of mutagenesis on plant traits will play a pivotal role in shaping future crop improvement strategies.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTION

Komolafe Ronke conceptualized the study and provided guidance throughout the study and corrected the manuscript, Akinola John collected, interpreted the data and wrote the manuscript, Alabi Stephen collected, analyzed the data and wrote the manuscript, Oseni Margret also wrote part of the manuscript while Oluwajobi Ayoola and Chukwuma Deborah reviewed the manuscript.

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