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Variability of Agro Morphological Traits in *Vignea subterranean* Seedling Stage through Induced Mutation using Colchicine

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Abstract

Bambara groundnut (*Vigna subterranea*) a drought-tolerant legume with significant nutritional value, remains underutilized in genetic improvement programs. This study evaluated the mutagenic effects of colchicine on two Bambara groundnut landraces (MOK-01 and PTG-01) to enhance agronomic traits. Seeds were treated with colchicine at 0.025%, 0.05%, and 0.075% concentrations for 24 hours, while control seeds were soaked in distilled water. A Randomized Complete Block Design (RCBD) was employed, with seeds sown in sandy loam soil and evaluated four weeks after planting. Germination rates, seedling height, root length, lateral root formation, and leaf production were recorded. Data were analyzed using one-way ANOVA and Duncan's Multiple Range Test at $P \leq 0.05$. The results showed a reduction in germination rates with increasing colchicine concentrations. MOK-01 exhibited enhanced lateral root formation and stable seedling height at higher concentrations, while PTG-01 demonstrated improved root length and leaf production at moderate colchicine levels. Colchicine at 0.05% produced the most consistent improvements across traits. This study highlights colchicine's potential for genetic enhancement of Bambara groundnut. It is recommended that future studies focus on large-scale field trials and assess the long-term genetic stability of colchicine-induced traits to optimize its application in crop improvement programs.

Keywords: *Vigna Subterranean*, Colchicine-Induced Mutation, Agromorphological Traits, Phenotypic Variability, Mutation Breeding.

Introduction

Bambara groundnut (*Vigna subterranea* (L.)) is a valuable but underutilized leguminous crop belonging to the Fabaceae family. Originating from the tropical African region, it is widely cultivated in Central and West Africa due to its nutritional importance and remarkable drought resistance, a trait that distinguishes it from other legumes (Khan *et al.*, 2021; Mabhaudhi *et al.*, 2018). Despite its resilience and potential to contribute to food security, Bambara groundnut has been largely neglected in genetic improvement programs. This neglect is often attributed to its perceived limited economic significance compared to major crops such as wheat, rice, maize, and potato (Cullis & Kunert, 2017). Consequently, farmers continue to rely on genetically diverse landraces, which exhibit significant variability in morphological traits (Ntundu *et al.,* 2006) and nutritional content (Azman *et al.*, 2019).

Genetic variability within Bambara landraces is a key advantage, providing adaptability to local conditions, stress tolerance, and yield stability. These attributes make the crop particularly suitable for sustainable seed production in challenging environments, such as drought-prone regions (Dwivedi *et al.*, 2016). Historically, landraces have been selected for improved traits like seed yield, nutritional quality, and drought tolerance, enabling their cultivation by small-scale farmers for household consumption (Karikari, 2000; Hillocks *et al.,* 2012). The genetic diversity preserved in these landraces suggests their potential for breeding high-protein and high-oil cultivars, which could play a critical role in enhancing food security across the region (Azman *et al.*, 2019; Tan *et al.,* 2020).

However, conventional breeding methods for Bambara groundnut improvement face significant challenges. The crop's development has been constrained by a limited understanding of the genetic mechanisms governing its agronomic traits and unsuccessful attempts at hybridization (Lacroix *et al.,* 2003; Chimdi *et al.,* 2022). Although landraces represent valuable sources of genetic variation, they often lack optimal trait combinations required for consistent yields across diverse environments, highlighting the need for further genetic enhancement (Massawe *et al.*, 2005; Muhammad *et al.*, 2020).

The use of colchicine, a chemical mutagen, offers a promising alternative to conventional breeding approaches. Colchicine has been shown to induce desirable genetic mutations and create new phenotypes, providing an innovative pathway for genetic improvement (Anbarasan *et al.,* 2013). This study aims to investigate the mutagenic effects of colchicine on Bambara groundnut at the seedling stage, with the goal of addressing genetic constraints and improving its agronomic characteristics.

Research Problem

Despite its resilience and nutritional value, Bambara groundnut (Vigna subterranea) remains underutilized in genetic improvement programs, limiting its potential to address food security challenges. Conventional breeding methods have been constrained by insufficient genetic understanding and unsuccessful hybridization attempts. This underscores the need for alternative approaches, such as induced mutagenesis, to enhance agronomic traits. Colchicine, a chemical mutagen, offers a promising avenue for generating beneficial genetic variations, yet its effects on Bambara groundnut remain underexplored. This study aims to address these gaps by evaluating colchicine's impact on the germination, growth, and morphological traits of Bambara groundnut landraces.

Aims and Objectives

This study investigates the mutagenic impact of colchicine on Bambara groundnut at the seedling stage

Objectives:

- To determine the effect of varying colchicine concentrations on the germination rate of Bambara groundnut landraces.
- To evaluate the influence of colchicine on the Agro morphological traits of Bambara groundnut seedlings.
- To assess genotype-dependent responses to colchicine treatments.

Research Questions

- How does colchicine concentration affect the germination rate of Bambara groundnut seeds?
- What is the impact of colchicine on the growth and morphological traits of Bambara groundnut seedlings?
- Are there significant genotype-specific responses to colchicine treatment?

Materials and Method

Study Area

The field trial investigations were executed at the experimental garden of the Department of Biological Sciences, Ahman Pategi University, Patigi, Kwara State, Nigeria. Patigi is situated in the north-central region of Nigeria. Patigi is located at a latitude of 8° 43' 42.64" N and a longitude of 5° 45' 20.20" E. United Nations - World Population Prospects, 2022.

Source of Research Materials

Two Bambara groundnut landraces were sourced from the local farmers in Kwara and Niger states, Nigeria.

Preparation of Colchicine Solution

Colchicine solutions of different concentrations were prepared following the methods of Tammu *et al*. (2021). The stock solution of 0.1% colchicine were generated by immersing 0.1 g of colchicine in 100 mL of distilled water. The stock solution was diluted to achieve concentrations of 0.025%, 0.050%, and 0.075%, respectively. Subsequently, the Bambara groundnut seeds were submerged in 15 ml vials containing the respective colchicine solution treatments for 24 hours.

Viability Test

Viability test was done following the method of Udofia *et al*. (2023). The seeds were submerged in solutions containing 0.025 %, 0.050 %, and 0.075 %, colchicine. Simultaneously, the non-treated control seeds (0%) undergo soaking for the similar duration using distilled water. This was subjected to this treatment for 24 hours. The germination test was applied to evaluate the impact of colchicine on the treated seeds.

$$\text{Germination percentage (\%)} = \frac{number\ of\ emerged\ seedlings}{total\ number\ of\ seeds} \times 100$$

Experimental Design and Sowing of Seeds

Two treated landraces of Bambara groundnut were cultivated in a Randomized Complete Block Design (RCBD) with three replications. The seeds were sown in 20-liter plastic rubber buckets, 50 cm in height, filled with sandy loamy soil in a 2:1 ratio, leaving 10 cm of space from the top. Five seeds were initially planted and subsequently thinned to an average of 3 seeds per buckets. Each of the two landraces were subjected to four treatments with three replications for colchicine treatment. Crop performance were evaluated at 4 weeks after planting.

Data Analysis

The data derived from the agro morphological traits underwent one-way ANOVA at P = 0.05, utilizing Plant Breeding Tools (PB Tools) developed by the International Rice Research Institute (IRRI) from 2013 to 2020. The Duncan Multiple Range Test was employed to separate the means when variances are detected.

Results and Discussion

Germination Percentage

The results (fig 1) indicate that as colchicine concentration increases, there is a noticeable reduction in the germination percentage of both Bambara groundnut landraces (MOK 01 and PTG 01). The control group shows a high germination rate of 100% for both landraces, which sharply decreases when exposed to colchicine treatments. Specifically, MOK 01 maintains a germination rate of 70% at all colchicine concentrations (0.025%, 0.050%, and 0.075%). PTG 01 shows a slight resilience at the 0.025% concentration with an 80% germination rate, but drops to 70% at the higher concentrations (0.050% and 0.075%).

This trend aligns with findings from similar studies on mutagenic agents. In particular, Olawuyi *et al.* (2021) reported depletion in germination percentage when Bambara groundnut was treated with sodium azide, a chemical mutagen similar in some effects to colchicine. Similarly, Mangena (2020**)** reported a comparable decline in germination in soybeans when exposed to increasing concentrations of colchicine, highlighting the negative impact of colchicine on seed viability and initial germination success.

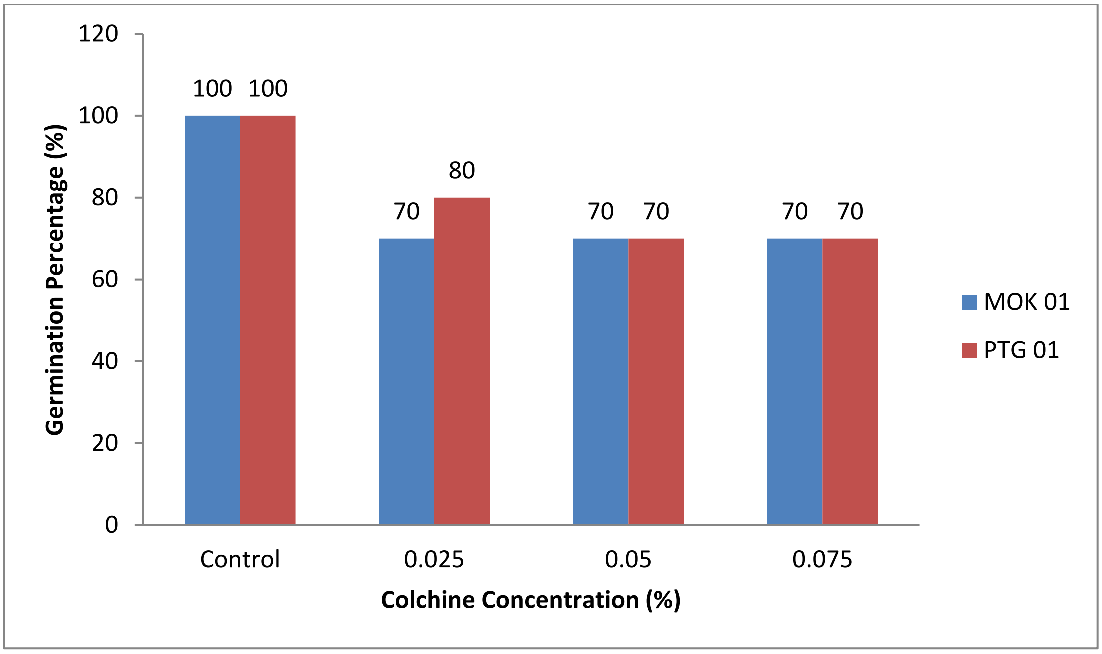

Figure 1: Effect of Colchicine Concentration on Germination Percentage

Growth Characteristics (Tables 1 and 2)

Across both landraces (MOK-01 and PTG-01), no significant differences in seedling height between the control and colchicine treatments (0.025%, 0.050%, and 0.075%) as indicated in table 1 & 2 across all treatments ($P > 0.05$). This suggests that varying colchicine concentrations did not significantly impact the overall height of the Bambara groundnut seedlings in either landrace.

MOK-01 showed a decrease in root length at lower colchicine concentrations (7.00 ± 1.00 cm for 0.024%) compared to the control (10.67 ± 1.20 cm). However, the root length increased with higher colchicine concentrations (9.33 ± 0.67 cm at 0.075%), though differences were not statistically significant in most cases.

In PTG-01, root length increased significantly with colchicine concentration, peaking at 14.33 ± 0.33 cm at 0.075% colchicine ($P > 0.05$), suggesting PTG-01's roots may be more responsive to colchicine treatments than MOK-01.

MOK-01 displayed a significant increase in leaf production under 0.050% colchicine (48.67 ± 3.93 leaves) compared to the control, indicating that moderate colchicine levels could stimulate leaf proliferation.

For PTG-01, however, the number of leaves significantly increased at 0.050% colchicine but decreased substantially at 0.075% (26.67 ± 0.33 leaves). This suggests a concentration-dependent response, where moderate colchicine may promote leaf production, but higher concentrations (0.075%) could hinder growth.

In MOK-01, a significant rise in lateral root formation were noticed at 0.075% colchicine (58.00 ± 2.52 roots) compared to lower treatments, suggesting that higher colchicine concentrations may encourage lateral root proliferation.

PTG-01 did not exhibit significant changes in lateral root numbers across treatments, implying a genotype-dependent response to colchicine regarding lateral root development. These findings partially align with Eng *et al.* (2021), who documented varied colchicine effects on root length across concentrations in *Neolamarckia cadamba*, underscoring genotype-dependent variability in legume root response to treatment.

Table 1: Growth Characteristics of Bambara Groundnut (MOK-01)

Treatments	Seedling height(cm)	Root length(cm)	No. of leaves	Number of lateral roots
Control	23.67 ± 3.48^{a}	10.67 ± 1.20^{b}	35.00 ± 1.00^{a}	28.67 ± 1.86^{b}
0.024	22.00 ± 1.15^{a}	7.00 ± 1.00^{a}	41.00 ± 1.00^{ab}	17.00 ± 2.08^{a}
0.050	23.33 ± 2.33^{a}	9.67 ± 0.33^{ab}	48.67 ± 3.93^{c}	16.00 ± 2.00^{a}
0.075	23.00 ± 2.08^{a}	9.33 ± 0.67^{ab}	43.67 ± 1.33^{bc}	58.00 ± 2.52^{c}
Total	23.00 ± 1.04	9.17 ± 0.55	42.08 ± 1.76	29.92 ± 5.19

Values are expressed as means ± SE. Values sharing the identical letter(s) in the column are not significantly different at P > 0.05.

PTG 01

Table 2: Growth Characteristics of Bambara Groundnut (PTG-01)

Treatment	Seedling height(cm)	Root length(cm)	No. of leaves	Number of lateral roots
Control	23.00 ± 0.58^{a}	9.33 ± 0.67^{a}	37.00 ± 1.00^{b}	58.00 ± 1.73^{a}
0.024	22.33 ± 2.19^{a}	12.33 ± 0.33^{ab}	35.00 ± 1.00^{b}	62.67 ± 4.48^{a}
0.050	23.33 ± 1.76^{a}	13.67 ± 1.86^{b}	65.00 ± 1.00^{c}	64.67 ± 2.03^{a}
0.075	20.67 ± 1.45^{a}	14.33 ± 0.33^{b}	26.67 ± 0.33^{a}	58.67 ± 1.86^{a}
Total	22.33 ± 0.75	12.42 ± 0.72	40.92 ± 4.37	61.00 ± 1.45

Values are expressed as means ± SE. Values sharing the identical letter(s) in the column are not significantly different at P > 0.05.

Biomass and Survival Rate (Tables 3 and 4)

For MOK-01, fresh and dry weights were significantly higher at the highest colchicine concentration (0.075%) compared to the control and lower concentrations, indicating an enhanced biomass accumulation at higher colchicine levels. This suggests that 0.075% colchicine could enhance biomass production in MOK-01.

PTG-01, however, showed less pronounced increases in fresh and dry weights, with a slight increase at 0.075% colchicine. This difference suggests genotype-specific responses, with MOK-01 exhibiting a more robust growth response to higher colchicine concentrations than PTG-01.

In both genotypes, survival rates decreased as colchicine concentration increased. Notably, MOK-01's survival rate dropped from 2.67 ± 0.33% in the control to 0.67 ± 0.33% at 0.075% colchicine, indicating potential toxicity at higher colchicine levels.

PTG-01 showed a similar trend, with a significant drop in survival rate to 1.00 ± 0.58% at 0.075%. This reduction suggests that higher colchicine concentrations can negatively impact plant viability in both genotypes.

No visual abnormalities were recorded across treatments in both genotypes. This lack of visible morphological deformities could imply that, despite reduced survival rates, surviving plants maintained relatively normal physical appearances. Compared to Aisyah et al. (2024), this study's results reflect similar findings of colchicine-induced variability in quantitative traits across genotypes, although they did not observe changes in stem or leaf color as reported in *Portulaca grandiflora*. This distinction suggests that colchicine might induce more pronounced phenotypic diversity in ornamental species, while its effects on Bambara groundnut may be limited to agronomic traits such as leaf count and root length.

Table 3: Biomass and Survival Rate of Bambara Groundnut (MOK-01)

Treatment	Fresh weight(g)	Dry weight(g)	Survival rate%	Visual abnormalities
Control	8.33 ± 1.20^{b}	3.67 ± 0.88^{b}	2.67 ± 0.33^{b}	-
0.024	4.00 ± 1.15^{a}	1.00 ± 0.00^{a}	1.33 ± 0.33^{ab}	-
0.050	9.33 ± 1.20^{bc}	3.67 ± 0.33^{b}	2.33 ± 0.67^{b}	-
0.075	13.00 ± 1.53^{c}	4.33 ± 0.33^{b}	0.67 ± 0.33^{a}	-
Total	8.67 ± 1.11	3.17 ± 0.44	1.75 ± 0.30	-

Values are expressed as means ± SE. Values sharing the identical letter(s) in the column are not significantly different at P > 0.05.

PTG 01

Table 4: Biomass and Survival Rate of Bambara Groundnut (PTG-01)

Treatment	Fresh weight(g)	Dry weight(g)	Survival rate%	Visual abnormalities
Control	6.67 ± 0.67^{ab}	2.00 ± 0.58^{ab}	3.00 ± 0.00^{b}	-
0.024	6.00 ± 1.00^{ab}	1.33 ± 0.33^{a}	2.67 ± 0.33^{b}	-
0.050	5.33 ± 0.33^{a}	3.33 ± 0.33^{b}	2.67 ± 0.33^{b}	-
0.075	7.67 ± 0.33^{b}	2.33 ± 0.33^{ab}	1.00 ± 0.58^{a}	-
Total	6.42 ± 0.38	2.25 ± 0.28	2.33 ± 0.28	-

Values are expressed as means ± SE. Values sharing the identical letter(s) in the column are not significantly different at P > 0.05.

Conclusion

This study demonstrates the mutagenic potential of colchicine in modifying certain agronomic traits of Bambara groundnut, particularly germination rate, root length, and leaf production. The responses of the two landraces to colchicine treatments varied, underscoring the function of genotype in deciding the extent of mutagenic influence. MOK-01 showed increased lateral root formation at the highest concentration, whereas PTG-01 displayed enhanced root length with moderate colchicine levels. These results suggest that controlled colchicine application could be a viable strategy for improving Bambara groundnut, potentially aiding its resilience and productivity in low-input farming systems.

Recommendations

- Optimize colchicine concentrations for large-scale field trials.
- Conduct long-term studies to assess genetic stability and potential yield improvements.
- Investigate the environmental impact of colchicine use in agricultural systems.

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Disclosure of conflict of interest

The authors of this article declared that there is no conflict of interest.

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