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JOURNAL OF PLANT SCIENCE AND PHYTOPATHOLOGY
ISSN 2575-0135

Research Article

Agro-morphological Growth Response of Acha (FONIO) (*Digitaria exilis* and *Digitaria iburua* [Kippist] Stapf.) Exposed to Colchicine: Germination, Plant Height and Leaf Number

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Submitted: February 29, 2024
Approved: May 29, 2024
Published: May 30, 2024

How to cite this article: Nyam DD, Gonzuk NS, Sila MD, Tumba YC, Angyu EA, et al. Agro-morphological Growth Response of Acha (FONIO) (*Digitaria exilis* and *Digitaria iburua* [Kippist Stapf.) Exposed to Colchicine: Germination, Plant Height and Leaf Number. J Plant Sci Phytopathol. 2024; 8: 055-059.

DOI: 10.29328/journal.jpsp.1001133

Keywords: Colchicine; *Digitaria* spp (Acha); Germination; Polyploidy; Variability; Yield

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Abstract

Acha (Fonio) *Digitaria* sp. is a valuable cereal crop widely cultivated in several African countries. The genetic improvement of Acha through induced mutagenesis has gained attention for enhancing desirable traits. Colchicine, a mitotic inhibitor, is commonly used to induce polyploidy, which can lead to alterations in plant characteristics. This study aimed to investigate the effects of colchicine treatment on germination, plant height, and leaf count of two Acha species. Seeds from two *Digitaria* species, *Digitaria exilis,* and *Digitaria iburua*, were subjected to colchicine treatment using different concentrations (0.05, 0.10, 0.15, and 0.20g/dL). The control group was treated with distilled water. Germination rates were assessed by measuring the percentage of seeds that successfully sprouted. Plant height and leaf number were measured at maturity and analyzed using ANOVA on SPSS Version 26. The results indicated that colchicine treatment significantly affected germination, plant height, and leaf number in both *Digitaria* species. Germination rates varied depending on the concentration, with lower germination observed at higher concentrations (70.70% and 74.3% for 0.20g/dL treatment in both species). In terms of plant height, colchicine-treated Acha plants exhibited significant differences compared to the control group. The majority of treated plants showed increased growth (57.00 cm to 60.70 cm in *D. exilis* and 114.10 to 122.40 cm in *D. iburua*) compared to the control. Variability in plant height was observed among the treated plants, suggesting that colchicine-induced polyploidy had varying effects on height across different genotypes. Similarly, the number of leaves was affected by colchicine treatment. Treated plants exhibited an increase in leaf number (56.70 to 60.7 for *D exilis* and 32.60 to 36.30 for *D. iburua*), compared to the control group (49.7 and 29.7 respectively). This variability suggests that colchicine-induced polyploidy may have influenced leaf development and growth patterns in Acha. Colchicine treatment had significant effects on germination, plant height, and leaf count in both varieties of Acha. The results suggest that colchicine-induced polyploidy can alter these plant characteristics. These findings provide insights into the potential use of colchicine treatment for inducing desirable traits in Acha through polyploidization, which may have implications for crop improvement and breeding programs. Further research is warranted to explore the underlying mechanisms and assess the impact of these alterations on overall agronomic performance and yield potential.

Introduction

Colchicine, a natural alkaloid derived from the autumn crocus plant (*Colchicum autumnale*), has garnered significant attention in recent years due to its potential applications in plant breeding and genetic research. This compound has been widely used to induce chromosomal doubling and alter the ploidy level of plants, leading to the development of new and improved crop varieties. Acha (*Digitaria spp*) is a cereal grain of increasing agricultural importance in many African regions, known for its nutritional value and adaptability to diverse environmental conditions.

This research study aimed to investigate the effects of colchicine treatment on the germination process, plant height, and leaf number of two species of Acha. Understanding the impact of colchicine on these agro-morphologic growth parameters will provide valuable insights into its potential use for enhancing the agronomic traits of Acha and potentially other cereal crops.

Germination is a critical stage in the life cycle of plants, and its success greatly influences overall crop productivity. Colchicine treatment has been reported to influence germination rates by affecting various physiological processes, including seed coat permeability, water uptake, and hormonal regulation. By subjecting seeds of the two *Digitaria* Spp. to colchicine treatment at varying concentrations and exposure durations, we can evaluate its influence on germination efficiency and subsequent seedling development.

Furthermore, plant height is an important trait that contributes to biomass production and overall plant fitness. Colchicine treatment has been shown to affect cell division and elongation processes, resulting in altered plant heights. By measuring the height of colchicine-treated Acha plants compared to untreated control plants, we can assess the impact of this compound on plant growth.

Leaf number is another vital morphological trait directly associated with plant productivity. Colchicine has been documented to influence leaf development and morphology through its influence on cell division and differentiation. By quantifying the number of leaves produced by colchicine-treated Acha plants, we can determine whether this compound induces any noticeable changes in leaf production and potentially identify novel strategies for increasing foliage in agricultural crops.

Several studies have reported the successful induction of polyploidy in various plant species using colchicine treatment. For instance, Wang, et al. [1] demonstrated the efficient induction of polyploidy in *Trifolium repens* through colchicine treatment, resulting in improved forage quality and increased biomass production. Similarly, Zhang, et al. [2] employed colchicine treatment to induce polyploidy in *Arabidopsis thaliana*, resulting in altered leaf morphology and enhanced drought tolerance.

Moreover, investigations focusing on the effects of colchicine treatment on cereals have yielded promising results. Omondi, et al. [3] observed increased grain size and yield in colchicine-treated rice plants, highlighting the potential for improved crop productivity. However, limited research has been conducted on the impact of colchicine treatment specifically on Acha varieties.

Overall, this research study will provide valuable insights into the effects of colchicine treatment on the germination process, plant height, and leaf number of two varieties of Acha. The findings will contribute to our understanding of colchicine's potential applications in plant breeding and its implications for improving crop productivity and agronomic traits. By exploring the effects of colchicine on Acha, we aim to shed light on its broader applicability in crop improvement strategies, potentially benefiting food security and agricultural sustainability.

Materials and methods

A trial was conducted at The National Cereals Research Institute (NCRI), Acha Research Substation, Riyom. Seeds of two Acha species, *Digitaria exilis,* and *Digitaria iburua*, were also obtained from the Institute. The seeds were surface-sterilized by immersing them in a 0.5% sodium hypochlorite solution for 10 minutes, followed by rinsing with sterile distilled water. 1gram of colchicine powder was obtained from a chemical supplier and used for the study. Two doses each of 0.05g, 0.10g, 0.15g, and 0.20g colchicine powder were weighed and dissolved in 100ml of distilled water. The solution was stirred thoroughly until the colchicine was completely dissolved. Seeds of both varieties of Acha were soaked in distilled water for 24 hours. After soaking, the seeds were treated with colchicine solution for 24 hours. The control group seeds were soaked in distilled water for the same duration. The treated seeds were then rinsed thoroughly with distilled water and dried in shade for 24 hours before sowing. This study employed a Randomized Complete Block Design (RCBD) with two factors; Variety (V) and Colchicine Treatment (CT). There were two levels of Variety (V_1 and V_2) and five levels of Colchicine Treatment (0.00g/dL, 0.05g/dL, 0.10g/dL, 0. 15G/dL and 0.20g/dL) resulting in a total of ten treatments (0.00g/dLV1, 0.05g/dLV_1, 0.10g/dLV_1, 0.15G/dLV_1, 0.20g/dLV_1, 0.00g/dLV_2, 0.05g/dLV_2, 0.10g/dLV_2, 0. 15G/dLV_2, 0.20g/dLV_2). Each treatment was replicated three times, resulting in a total of 30 experimental units. Germination rates were recorded daily for three weeks after planting. Treated and untreated seeds were sown on the field. Percentage germination was determined by dividing the number of germinated seeds by the total number of sown seeds multiplied by 100. This was taken for three weeks. Plant height (cm) was taken by measuring the height of the plant from the soil surface to the tip of the tallest leaf using a ruler. Five plants were measured and the average was computed for each experimental unit. The number of leaves was determined by counting the number of leaves on each plant by visual inspection. The leaves of five plants were counted and the average was calculated for a more accurate estimate. Data obtained from the germination assay, plant height, and leaf numbers were subjected to statistical analysis. Two-way analysis of variance (ANOVA) was performed to determine significant differences among treatments. Post-hoc tests, Tukey's HSD (Honestly Significant Difference) test, were conducted to compare treatment means. The Turkey HSD test was used to compare the means of the groups to determine if there were significant differences between them. This test is used to identify which groups are significantly different from each other.

Results and discussion

Germination

The germination rates of colchicine-treated seeds exhibited significant variations compared to the control group (Table 1). In *Digitaria exilis*, the highest germination rate (91%) was observed in the treatment group with a colchicine concentration of 0.05g/dL, while the lowest

Table 1: Effect of Colchicine treatment on Germination, Plant height and Number of leaves of two species of Acha.

Variety	Treatment (g/dL)	Germination (%)	Plant Height (cm)	Number of Leaves
D. exilis	0.00	88.00^{ab}	76.80^{c}	49.70^{e}
	0.05	91.00^{a}	81.70^{ab}	58.70^{b}
	0.10	85.00^{bc}	83.20^{a}	60.70^{a}
	0.15	81.70^{c}	81.00^{ab}	56.70^{d}
	0.20	70.70^{d}	80.30^{bc}	57.00^{c}
D. iburua	0.00	88.00^{ab}	109.30^{c}	29.70^{e}
	0.05	92.30^{a}	119.20^{ab}	34.70^{b}
	0.10	85.30^{bc}	122.40^{a}	36.30^{a}
	0.15	82.70^{c}	116.80^{ab}	33.70^{c}
	0.20	74.30^{d}	114.10^{bc}	32.60^{cd}

Tukey's HSD Test: Values with the same letters do not differ significantly at $p \leq 0.05$.

germination rate (70.70%) occurred in the treatment group with a concentration of 0.20g/dL. In *Digitaria iburua*, the highest germination rate (92.30%) was recorded in the treatment group with a colchicine concentration of 0.05g/dL, whereas the lowest germination rate (74.30%) was observed in the treatment group with a concentration of 0.20g/dL. No interaction existed between the effects of Colchicine treatment and Species ($F(4, 20) = [0.725]$, $p = [0.585]$).

Also, while Species did not have any significant effect on germination ($p = 0.110$) Colchicine treatment did have a significant effect on germination ($p = 0.00$).

Plant height

The application of colchicine resulted in alterations/variations in plant height. In *Digitaria exilis*, the tallest plants were observed in the treatment group with a colchicine concentration of 0.10g/dL (Table 1), reaching an average height of 83.20 cm. Conversely, the shortest plants were found in the control group, with an average height of 76.80 cm. In *Digitaria iburua*, the tallest plants were recorded in the treatment group with a colchicine concentration of 0.10g/dL, with an average height of 122.40 cm. The control group exhibited the shortest plants, measuring an average of 109.30 cm in height.

Whereas no interaction existed between the effects of Colchicine treatment and Species ($F(4, 20) = [1.48]$, $p = [0.25]$), both species and Colchicine treatment did have a significant effect on plant height ($p = 0.00$).

Leaf number

Colchicine treatment influenced leaf development in both varieties. *Digitaria exilis* plants treated with 0.10g/dL colchicine concentration exhibited the highest leaf count, with an average of 60.70 leaves per plant. The control group had the lowest leaf count, with an average of 49.70 leaves per plant. Similarly, in *Digitaria iburua*, the treatment group with a colchicine concentration of 0.10g/dL produced the highest leave number of 36.00. The control group recorded the least number of leaves (29.70). Interaction existed between the effects of Colchicine treatment and Species ($F(4, 20) = [10.21]$, $p = [0.00]$) and both Species and Colchicine had a significant effect on the Number of Leaves ($p = 0.00$).

Discussion

The exposure of Acha (Fonio) to colchicine resulted in notable effects on key growth parameters. Germination patterns exhibited variations compared to control groups, suggesting potential alterations in the genetic makeup induced by colchicine. The germination percentage increased at lower concentrations and decreased significantly with increasing concentrations of colchicine, suggesting a negative effect on seed viability. Essel, et al. [4], also observed that the percentage of germination was high in the control (89.3) and 0.05g/dl (90.2) of first generation of colchicine treatment. The results showed that *D. brandisii* seeds were non-dormant, and seed lots achieved their highest germination rates on the 4th day and finished the whole germination period after 21 days. Colchicine inhibited seed germination and seedling growth but did not change its germination pattern. Seed germination and seedling growth decreased constantly with an increase in colchicine concentration. Colchicine showed more negative effects on seedling growth than on seed germination and root growth. High concentrations of colchicine retarded the development of plumules and even caused their aberrant development. This aligns with findings from previous studies where colchicine has been recognized for inducing genetic variations in crop plants. Lora [5], observed that the germination of *Petunia axillaris* seeds was stimulated by colchicine. According to her, there was an increase in the number of seeds that germinated as well as a reduction in germination time. The optimum concentration for germination (0.04 per cent) was high enough to induce polyploidy. Growth of seedlings was seriously retarded only by colchicine in concentrations of 0.1 per cent or stronger, the concentrations that have been most widely used in seed treatment. In view of these results, it would appear that seed treatment using weak solutions of colchicine rather than strong, the optimum concentration to be determined for each kind of plant, should yield satisfactory results in the practical production of large numbers of polyploid plants. Smith, et al. [6] demonstrated that colchicine treatment can alter the germination process by affecting cell division and influencing the hormonal balance within seeds. Zhuo, et al. [7] showed that *D. brandisii* seeds were non-dormant, and seed lots achieved their highest germination rates on the 4th day and finished the whole germination period after 21 days. According to them, Colchicine inhibited seed germination and seedling growth but did not change its germination pattern. They further maintained that seed germination and seedling growth decreased constantly with an increase in colchicine concentration. Reduced germination percentages were observed with increasing concentration of the mutagen especially in the C1 plants which were from the initially treated seeds, whereas in the C2 generation, the germination rate ranged from 63.0% in Treatment 0.15g/dl to 86.5% in the control. Colchicine showed more negative effects on seedling growth than on seed germination and root growth. High concentrations of colchicine retarded the development of plumules and even caused their aberrant development. In this work, our findings align with these studies, indicating that colchicine exposure may have a significant influence on germination rates.

In terms of plant height, the colchicine-treated plants of both *Digitaria exilis* and *Digitaria iburua* showed a significant increase in height compared to the control group. This increase in plant height can be attributed to the induction of polyploidy by colchicine treatment. The results indicate that colchicine treatment may have a stimulatory effect on the growth of Acha, potentially resulting in taller plants and higher biomass production. Polyploid plants often exhibit increased cell size and cell number, leading to larger overall plant size [8]. The polyploid cells induced by colchicine treatment likely contributed to the enhanced growth and elongation of the treated plants. These observations coincide with the research by Doku [9], emphasizing the importance of exploring unconventional methods for crop improvement in West Africa. The impact of colchicine on plant height has been investigated in various crops, revealing its potential to induce changes in growth patterns [10]. Our results show a notable difference in the height of fonio plants treated with colchicine compared to the control group. This suggests that colchicine may play a crucial role in modifying the elongation and development of fonio plants, which can have implications for overall yield and harvest management.

However, Ravindra, et al. [11] observed that it causes depolymerisation and disappearance of the fibrillar microtubules in granulocytes and other motile cells, inhibiting their migration as well as metabolic and phagocytic activity. This according to them, caused the slow rate of growth and development due to physiological disturbance and reduced rate of cell division [12]. Amiri, et al. [13] also reported the characteristics of colchicine-treated plants to include slow growth, altered morphology, and prolonged flowering of polyploids which may, in part, result from slowed mitotic divisions and cell divisions of larger cells with more chromosomes. According to them, total plant height was lower in tetraploid lines compared to the diploids and the reduced stature was partly due to shorter internodal distances. Several other researchers Gu, et al. [14] in *Zizyphus jujuba*, He, et al. [15] in *Dendranthema indicum*, Kazi [16] in Solanaceae crops, Kushwaha, et al. [17] in *Chrysanthemum carinatum* L., Manawadu [18] in radish and Manzoor, et al. [19] in *Gladiolus grandiflorus* reported that colchicine treatment had decreased the plant height. Reduced growth may be attributed to the increase in destruction of growth inhibitors, drop in the auxin level, or inhibition of auxin synthesis as reported by Roychowdhury and Tah [20] and Mostafa, et al. [21]. These observations could perhaps be a result of higher levels of concentrations of the colchicine or the species of plant they worked on, expressing its norm of reaction.

The number of leaves is a key determinant of a plant's photosynthetic capacity and, consequently, its overall productivity. Colchicine-induced variations in leaf number have been documented in several plant species [22]. Our study reveals a significant correlation between colchicine exposure and alterations in leaf number in both *Digitaria exilis* and *Digitaria iburua*. This increase in leaf number is likely due to the stimulation of cell division and expansion resulting from polyploidy induction [23]. El-Torkey [24] also reported that the increase in leaf number in *Euonymus japonicus* may be due to the possibility of the physiological stimulation of the chemical mutagen. The highest of 90.2% was recorded in treatment at 0.05g/dl. Mean number of leaves, primary branches per plant and mean height and length of longest branch per plant were all significant ($p < 0.05$) within treatments but not between treatments in comparison to the control in C1.

This finding suggests that colchicine treatment may induce increased branching or proliferation of leaf primordia, leading to a greater number of leaves per plant. Enhanced leaf production can have important implications for photosynthesis and overall plant growth, ultimately influencing the yield of Acha crops. These findings agree with previous studies on colchicine-induced changes in various crops, such as rice [25]. This observation underscores the potential of colchicine to influence foliar development in fonio, with implications for biomass accumulation and nutrient assimilation.

This research sheds light on the agro-morphological growth response of Acha (Fonio) exposed to colchicine, emphasizing its impact on germination, plant height, and leaf number. These findings contribute to the understanding of colchicine-induced variations in fonio, providing valuable insights for future research on crop improvement strategies.

Conclusion

In conclusion, the findings of this study demonstrate that colchicine treatment has significant effects on the germination, plant height, and number of leaves in two species of Acha, *Digitaria exilis* and *Digitaria iburua*. Colchicine-treated seeds exhibited improved germination rates at lower concentrations but decreased with increasing concentration. Additionally, Acha plants treated with colchicine showed increased plant height, while both species displayed a greater number of leaves compared to the control group. These results suggest the potential use of colchicine treatment as a means to enhance the agronomic traits of Acha crops. Part of the limitations of this study is the avalanche of data presented and the period of the study. This work should be conducted for three years or more, depending on the phenotypic expression (s) of the polyploids, to properly establish the status of the mutants so generated.

Future studies should aim to elucidate the underlying mechanisms responsible for the observed effects of colchicine treatment on Acha crops. Understanding the molecular and physiological changes induced by colchicine can provide valuable insights into the pathways involved in germination, plant growth, and leaf development. In addition, the induction of polyploidy by colchicine treatment may lead to genetic instability in plants. Therefore, it is recommended to conduct molecular analyses, such as flow cytometry or chromosome counting, to verify the stability of the induced polyploid plants and assess the potential risks that could be associated with colchicine treatment.

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