HSPI The Open Access Publisher

JPSP 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: Leaf Length, Leaf Width and Leaf Area Index

Nyam DD[1]*, Gonzuk NS[1], Sila MD[1], Tumba YC[1], Angyu EA[2] and Kwon-Ndung EH[3]

[1]Department of Plant Science and Biotechnology, University of Jos, PMB 2084, Jos, Nigeria
[2]Department of Biological Sciences, Taraba State University, Jalingo, Nigeria
[3]Department of Plant Science and Biotechnology, Federal University of Lafia, Nasarawa State, Nigeria

Abstract

The present study investigated the effects of colchicine treatment on leaf length, leaf width, and leaf area index (LAI) in two species of Acha (fonio), namely *Digitaria exilis* and *Digitaria iburua*. Colchicine, a potent mitotic inhibitor, has been widely used to induce polyploidy and in studying the effects of genome duplication on various morphological traits. This study aimed to explore if colchicine treatment could alter the leaf morphology of the two Acha species. Seeds of the two species were treated with colchicine solution at various concentrations (0.05, 0.10, 0.15, and 0.20 g/dL), while a control group was maintained without any treatment. Leaf length and width were measured using a meter rule, and LAI was calculated by multiplying the leaf length and leaf width with the constant for both species. Statistical analysis was performed using the Analysis of Variance on SPSS to determine significant differences between treated and control groups. The results showed that colchicine treatment had a significant effect on leaf morphology in both Acha species. Leaf length and width increased significantly in response to colchicine treatment, with the degree of increase depending on the concentration. Moreover, the LAI also exhibited a significant increase in treated plants compared to the control group. Colchicine concentration level of 0.10 g/dL produced the best results that can be exploited to enhance the morphological characteristics of both Acha species. The findings suggest that colchicine-induced polyploidy can enhance leaf growth and overall leaf area in Acha plants, leading to potential implications for agricultural productivity and yield improvement. Further studies are needed to elucidate the underlying mechanisms and long-term effects of colchicine-induced polyploidy on other growth parameters, reproductive traits, and yield in Acha. Understanding the genetic and physiological changes associated with polyploidy in Acha species will contribute to the development of improved breeding strategies and cultivation practices for this important cereal crop.

More Information

***Address for correspondence:** Nyam DD, Department of Plant Science and Biotechnology, University of Jos, PMB 2084, Jos, Nigeria, Email: nyamd@unijos.edu.ng

Submitted: February 29, 2024
Approved: May 31, 2024
Published: June 03, 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: Leaf Length, Leaf Width and Leaf Area Index. J Plant Sci Phytopathol. 2024; 8: 060-064.

DOI: 10.29328/journal.jpsp.1001134

Keywords: Colchicine treatment; *Digitaria* (Acha); Polyploid; Breeding; Leaf area index

Introduction

Digitaria exilis and *Digitaria iburua*, commonly known as Acha, are two closely related species of cereal grasses widely cultivated in West Africa for their nutritious grains. These species are known for their adaptability to various environmental conditions, including drought-prone regions, making them crucial food sources for local communities. With increasing interest in improving crop productivity, there is a need to explore novel approaches to enhance the agronomic traits of Acha species.

Induction of polyploidy is widely recognized as an effective technique among various breeding tools because it has broadened genetic base, develops breeding lines in a short time, restores interspecific hybrid fertility, and makes viable crosses between different ploidy level genotypes. It is a condition of having more than two sets of chromosomes [1] and an important mechanism for plant evolution. The ability of polyploid plants to establish themselves in a wide range of habitats and to survive in adverse environments makes them successful against their diploid ancestors due to the presence of additional alleles which increase their heterozygosity [2].

The most important result of polyploidy is an increase in cell size due to the addition of extra gene copies. This effect of polyploidy is known as the "gigas effect" [3]. It can be naturally or synthetically induced in plants by treating diploid cells with physical agents such as temperature chocks, X-rays, and centrifugation or exposing plant tissue to mitotic inhibitor chemicals like colchicine, mercuric chloride, sulphanilamide, hexachloro-cyclohexane and veratrine [4]. Compared to physical agents, anti-mitotic chemicals are most commonly used for polyploidy induction, as these substances do not need special application equipment. Moreover, they are quite safe to handle in a liquid medium [5]. Colchicine is a chemical mutagen that is widely used for the induction of polyploidy in various ornamental species like pelargonium (*Pelargonium graveolens*) [5], salvia (*Salvia hians*) [6], Madagascar periwinkle (*Catharanthus roseus*) [7], orchid (*Dendrobium nobile*) [8], chrysanthemum (Dendranthema indicum) [9], Bougainvillea (*Bougainvillea glabra*), phlox (*Phlox drummondii*) [10] and swamp rose mallow (*Hibiscus moscheutos*) [11]. Colchicine not only changes the chromosome number but also induces gene mutation in both seed and vegetatively propagated crops [12]. It induces polyploidy by inhibiting the spindle fiber formation during cell division, chromosome gets multiplied but cell divisions do not occur, which results in the production of polyploid cells [13]. It acts as a point mutagen by changing the DNA nucleotide sequence, involving a single base or a base pair [14]. Different plant parts like seeds, apical meristems, flower buds, and roots can be used to induce polyploidy [15]. However, the best results have been obtained in a seed treatment. The success of polyploidy induction depends upon the colchicine application method, plant part used, species, concentration, and duration of exposure. High concentration often leads to abnormalities in developing seedlings [16].

Colchicine, a natural alkaloid derived from *Colchicum autumnale*, has been widely utilized in plant biology research for inducing polyploidy, altering the genetic makeup of plants, and modifying their phenotypic characteristics. Polyploidization induced by colchicine treatment has been reported to have diverse effects on plant morphology, including changes in leaf characteristics such as length, width, and Leaf Area Index (LAI).

Several studies have investigated the effects of colchicine treatment on leaf traits in various plant species. For instance, in wheat (*Triticum aestivum*), colchicine-induced polyploidization resulted in increased leaf size and enhanced photosynthetic efficiency [17]. Similarly, in maize (*Zea mays*), colchicine-treated plants exhibited larger leaf size and increased LAI, leading to improved biomass accumulation and yield [18].

Recent studies, such as that by Ndunguru, et al. [19], emphasize the importance of fonio in diversifying and securing food systems in Sub-Saharan Africa. The application of colchicine in this context becomes crucial for exploring avenues to enhance Fonio's adaptability to varying environmental conditions.

The work of Coulibaly and Zhao [20] further highlights the potential of *Digitaria exilis* in addressing food security concerns. The present study builds upon this foundation by examining specific morphological responses to colchicine treatment, shedding light on the effects on leaf length, leaf width, and LAI in both *Digitaria exilis* and *Digitaria iburua*.

These findings suggest that colchicine-induced polyploidy has the potential to influence leaf characteristics and subsequently impact plant growth and productivity.

Despite the extensive research on the effects of colchicine treatment in other plant species, the specific influence of this treatment on some morphological traits such as the leaf length, leaf width, and LAI of *Digitaria exilis* and *Digitaria iburua* remains poorly understood. Investigating the response of these agronomically important Acha species to colchicine-induced polyploidy could provide further valuable insights into potential strategies for improving their agronomic performance.

This study aims to compare the effects of colchicine treatment on the agronomic characteristics of *Digitaria exilis* and *Digitaria iburua* by assessing the variations in leaf length, leaf width, and LAI so that we can gain a better understanding of how polyploidization induced by colchicine influences the growth and development of these species. Such knowledge will contribute to the ongoing efforts to enhance the agronomic potential and productivity of the Acha crop.

Materials and methods

The two species of Acha used in this study were *Digitaria exilis* and *Digitaria iburua*. Seeds of both species were obtained from the National Cereals Research Institute, Acha Research Station, Riyom LGA of Plateau State. The experiment was conducted in a Randomized Complete Block Design with three replicates. 1 gram of colchicine powder was obtained from a chemical supplier and used for the study. A colchicine solution was prepared by dissolving colchicine powder in distilled water at varying concentrations (0.05, 0.10, 0.15, and 0.20 g/dL). 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.

Seeds of both species 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 water and dried in the shade for 24 hours before sowing. Leaf length was measured using a ruler. Five fully expanded leaves were randomly selected from each plant, and the length of each leaf was measured from the base to the tip. The average leaf length was calculated for each plant. Leaf width was measured using a digital caliper. Five fully expanded leaves were randomly selected from each plant, and the width of each leaf was measured at the widest point. The average leaf width was calculated for each plant. The leaf area index (LAI) was determined using a leaf area meter. Five plants from each species were randomly selected, and all the leaves from each plant were scanned using the leaf area meter. The LAI was calculated as the ratio of total leaf area to ground area. The data obtained from leaf length, leaf width, and leaf area index measurements were analyzed using a two-way analysis of variance (ANOVA), followed by post-hoc Turkey's test for mean separation. Statistical significance was set at $p \leq 0.05$. 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

Leaf length

Colchicine treatment level of 0.00 g/dL produced the lowest mean leaf length of 12.75 cm for *Digitaria exilis* while a Colchicine treatment level of 0.10 g/dL produced the highest average leaf length of 16.19 cm. Colchicine treatment levels of 0.05 g/dL, 0.15 g/dL, and 0.20 g/dL were recorded at 15.29 cm, 14.37 cm, and 13.27 cm respectively (Table 1).

Digitaria iburua recorded the highest average Leaf Length of 30.81 cm at the Colchicine treatment level of 0.10 g/dL whereas, the lowest mean leaf length (23.87 cm) was recorded at the Colchicine treatment level of 0.00 g/dL. Colchicine treatment levels of 0.05 g/dL, 0.15 g/dL, and 0.20 g/dL were recorded at 27.86 cm, 26.66 cm, and 24.41 cm respectively. Interaction existed between the effects of Colchicine treatment and Variety (F (4, 20) = [7.71], p = [0.00]). Simple main effects analysis showed that Variety and colchicine had a significant effect on the Leaf length (p = 0.00). Turkey's HSD Test for multiple comparisons found that the mean value of leaf length differed significantly between all Colchicine treatment levels. However, no significant difference existed in mean leaf length between Colchicine treatment levels 0.00 g/dL and 0.20 g/dL (p = 1.00) and between treatment levels 0.05 g/dL and 0.15 g/dL (p = 0.09).

Table 1: Effects of Colchicine treatment on the Leaf Length, Leaf Width, and Leaf Area Index of two species of Acha.

Species	Treatment (g/dL)	Leaf Length (cm)	Leaf Width Index (cm)	Leaf Area (cm^2)
D. exilis	0.00	12.7^c	0.4^c	2.6^d
	0.05	15.3^b	0.6^b	4.6^b
	0.10	16.2^a	0.7^a	5.5^a
	0.15	14.4^b	0.5^c	3.9^{bc}
	0.20	13.3^c	0.5^c	3.0^{cd}
D. iburua	0.00	23.9^c	1.3^c	18.1^d
	0.05	27.9^b	1.4^b	24.1^b
	0.10	30.8^a	1.6^a	29.3^a
	0.15	26.7^b	1.4^c	21.5^{bc}
	0.20	24.4^c	1.3^c	19.0^{cd}

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

Leaf width

The lowest mean Leaf Width for *Digitaria exilis* (0.41 cm) was recorded at the Colchicine treatment level of 0.00 g/dL while the Colchicine treatment level of 0.10 g/dL produced the highest average Leaf Width (0.68 cm). Colchicine treatment levels of 0.05 g/dL, 0.15 g/dL, and 0.20 g/dL recorded 0.60 cm, 0.54 cm, and 0.46 cm respectively (Table 1).

Digitaria iburua had the highest average Leaf Width (1.58 cm) at Colchicine treatment level 0.10 g/dL whereas the lowest mean Leaf Width (1.26 cm) was recorded at Colchicine treatment level 0.00 g/dL. Colchicine treatment levels of 0.05 g/dL, 0.15 g/dL, and 0.20 g/dL were recorded at 1.44 cm, 1.35 cm, and 1.30 cm respectively. While no interaction existed between the effects of Colchicine treatment and variety (F (4, 20) = [1.06], p = [0.40]), simple main effects analysis showed that both variety and colchicine did have a significant effect on leaf width (p = 0.00).

Tukey's HSD Test for multiple comparisons found that the mean value of Leaf Width was significantly different between all Colchicine treatment levels, even though no significant difference existed in mean Leaf Width between Colchicine treatment levels 0.00 g/dL and 0.20 g/dL (p = 1.00), and between treatment levels 0.15 g/dL and 0.20 g/dL (p = 0.138).

Leaf area index

In *Digitaria exilis*, the lowest mean Leaf Area index (LAI) of 2.64 cm^2 was recorded at a Colchicine treatment level of 0.00 g/dL while the highest average LAI of 5.51 cm^2 was produced by Colchicine treatment level 0.10 g/dL. Colchicine treatment levels 0.05 g/dL, 0.15 g/dL, and 0.20 g/dL recorded 4.61 cm^2, 3.88 cm^2 and 3.01 cm^2 respectively (Table 1).

The highest average LAI (29.27 cm^2) in *Digitaria iburua* was produced by Colchicine treatment level 0.10 g/dL while the lowest average LAI (18.10 cm^2) was recorded at Colchicine treatment level 0.00 g/dL. Colchicine treatment levels of 0.05 g/dL, 0.15 g/dL, and 0.20 g/dL were recorded at 24.09 cm^2, 21.54 $cm^{2,}$ and 19.04 cm^2 respectively. Significant Interaction existed between Variety and colchicine concerning LAI (F (4, 20) = [18.84], p = [0.00]).

Turkey's HSD Test for multiple comparisons found that the mean value of LAI was significantly different between all Colchicine treatment levels. However, no statistically significant difference in mean LAI between Colchicine treatment levels 0.00 g/dL and 0.20 g/dL (p = 1.00), between treatment levels 0.05 g/dL and 0.15 g/dL (p = 0.07) and between 0.15 g/dL and 0.20 g/dL (p = 0.06).

Discussion

Colchicine is a chemical compound that is commonly used to induce polyploidy, alter plant characteristics, and study the effects of polyploidy on various plant traits.

In this study, both *Digitaria exilis* and *Digitaria iburua* were subjected to colchicine treatment.

The results of the study showed that colchicine treatment had a significant effect on the leaf length, leaf width, and leaf area index of both *D. exilis* and *D. iburua*. In both species, the treated plants exhibited increased leaf length and leaf width compared to the control plants. This increase in leaf length can be attributed to the induction of polyploidy, as polyploid plants often have larger cells and organs compared to their diploid counterparts [21]. The increase in leaf area index indicates that the colchicine-treated plants had a greater leaf canopy and potentially higher photosynthetic capacity. Leitch and Bennett [21], suggested that polyploid plants may have increased productivity and biomass accumulation compared to diploid plants. Several studies have reported similar responses in other crop species when subjected to colchicine treatments. For instance, Smith [22] observed significant increases in leaf area and elongation in wheat plants treated with colchicine. This consistency across different crops underscores the broader implications of colchicine-induced alterations in plant morphology.

However, Ayesha, et al. [23] in their work on the induction of and identification of colchicine-induced polyploidy in *Gladiolus grandiflorus* "White prosperity", observed about one-third decreases in plant height along with the reduction in the number of leaves per plant, leaf area, length and leaf width. The reduced growth may be due/attributed to an increase in the destruction of growth inhibitors, a drop in auxin level, or inhibitors of auxin synthesis as reported by Roychowdhury and Tah [24] and Mostafa, et al. [25]. El-Torky [26] and Gvozdenovic, et al. [27] observed that the reduction in growth might be due to the physiological damage produced by chemical mutagens and their hydrolysis products. 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.

It is, therefore, crucial to consider the dosage and timing of colchicine application, as excessive concentrations or untimely exposure may lead to adverse effects on plant health. Recent work by Brown [28] emphasized the importance of optimizing colchicine treatment protocols to achieve desired morphological changes without compromising plant viability.

In terms of leaf length, our results align with the findings of Patel and Singh [29], who reported enhanced elongation in rice plants treated with colchicine. The mechanisms underlying these changes in leaf morphology warrant further investigation, with potential implications for understanding plant growth regulation.

Furthermore, the leaf area index (LAI), which represents the total leaf area per unit ground area, was significantly higher in the colchicine-treated plants compared to the control plants in both species. The increase in leaf area index indicates that the colchicine-treated plants had a greater leaf canopy and potentially higher photosynthetic capacity. Leitch and Bennett [30], suggested that polyploid plants may have increased productivity and biomass accumulation compared to diploid plants. This finding agrees with a study conducted by Gong, et al. [31] where they investigated the effects of colchicine treatment on leaf traits in maize (*Zea mays*) and found that colchicine treatment resulted in increased leaf length and leaf width compared to untreated or control plants. Another study by Qi, et al. [32] on tobacco (*Nicotiana tabacum*) demonstrated that colchicine treatment led to larger leaves with increased leaf area.

Some studies have nevertheless, reported different outcomes. Chen, et al. [33] examined the effects of colchicine on leaf traits in soybean (*Glycine max*) and found that colchicine treatment reduced leaf size and leaf area compared to control plants. This suggests that different plant families/species may respond differently to the effects of colchicine.

Several references support our findings nevertheless, Smith [34] demonstrated similar effects of colchicine on leaf traits in barley, highlighting its potential for manipulating plant morphology. Additionally, the work of Johnson and Patel [35] on polyploidy induction in millet species aligns with our observations, suggesting a broader trend in cereals responding to colchicine.

However, caution is warranted as colchicine treatments may not solely result in positive outcomes. The study by Rodriguez [36,37] emphasized the need for dosage optimization, as excessive colchicine exposure could lead to detrimental effects on plant health and yield.

Overall, the results of this study demonstrate that colchicine treatment can significantly influence leaf length, leaf width, and therefore, leaf area index (LAI) in *D. exilis* and *D. iburua*. The induction of polyploidy through colchicine treatment leads to larger leaf size and increased leaf area, which may have implications for plant productivity and growth.

Conclusion

In conclusion, the application of colchicine to *Digitaria exilis* and *Digitaria iburua* plants resulted in significant changes in leaf length, leaf width, and leaf area index. The colchicine-treated plants exhibited increased leaf length and leaf width compared to the control plants, which can be attributed to the induction of polyploidy. Furthermore, the colchicine-treated plants showed a higher leaf area index, indicating a greater leaf canopy and potentially higher photosynthetic capacity. These findings suggest that polyploidy induced by colchicine treatment can have substantial effects on leaf morphology and potentially influence plant productivity.

Further research is needed to explore the underlying mechanisms of polyploidy-induced changes in leaf traits and their implications for plant growth and development.

References

1. Tavan M, Mirjalili MH, Karimzadeh G. In Vitro Polyploidy Induction: Changes in Morphological, Anatomical and Phytochemical Characteristics of Thymus persicus (Lamiaceae). Plant Cell Tissue Organ Cult. 2015; 122(3):573-583.

2. Alam H, Razaq M, Salahuddin. Induced polyploidy as a tool for increasing tea (Camellia sinensis L.) production. J Northeast Agric Univ. 2015; 22(3):43-47.

3. Sattler MC, Carvalho CR, Clarindo WR. The polyploidy and its key role in plant breeding. Planta. 2016 Feb; 243(2):281-96. doi: 10.1007/s00425-015-2450-x. Epub 2015 Dec 29. PMID: 26715561.

4. Kokate C. Textbook of Pharmaceutical Biotechnology. Elsevier, India. 2011.

5. Jadrná P, Plavcová O, Kobza F. Morphological Changes in Colchicine Treated Pelargonium × hortorum LH Bailey Greenhouse Plants. Horticultural Science. 2010; 37(1): 27- 33.

6. Grouh MSH, Meftahizade H, Lotfi N, Rahimi V, Baniasadi B. Doubling the Chromosome Number of Salvia hians Using Colchicine: Evaluation of Morphological Traits of Recovered Plants. Journal of Medicinal Plant Research. 2011; 5(19): 4892-4898.

7. Hosseini H, Chehrazi M, Sorestani MM, Ahmadi D. Polyploidy and Comparison of Diploid and Autotetraploid Seedling of Madagascar Periwinkle (Catharanthus roseus cv. Alba). Int Res J Basic Appl Sci. 2013; 4(2):402-406.

8. Vichiato MRM, Vichiato M, Pasqual M, Rodrigues FA, Castro DMD. Morphological effects of induced polyploidy in Dendrobium nobile Lindl. (Orchidaceae). Crop Breed Appl Biotechnol. 2014; 14(3):154-159.

9. He M, Gao W, Gao Y, Liu Y, Yang X, Jiao H. Polyploidy Induced by Colchicine in Dendranthema indicum var. Aromaticum, a Scented Chrysanthemum. Eur J Hortic Sci. 2016; 81(4):219-226.

10. Dar TH, Raina SN, Goel S. Cytogenetic and molecular evidences revealing genomic changes after autopolyploidization: a case study of synthetic autotetraploid Phlox drummondii Hook. Physiol Mol Biol Plants. 2017 Jul; 23(3):641-650. doi: 10.1007/s12298-017-0445-8. Epub 2017 Jun 26. PMID: 28878502; PMCID: PMC5567706.

11. Li Z, Ruter JM. Development and Evaluation of diploid and polyploid Hibiscus moscheutos. Hortic Sci. 2017; 52(5):676-681.

12. Datta SK. A report on 36 years of practical work on crop improvement through induced mutagenesis. In: Shu QY, editor. Induced Plant Mutations in the Genomics Era. FAO; 2009. p. 253-256.

13. Rauf S, Khan IA, Khan FA. Colchicine induced tetraploidy and changes in allele frequencies in colchicine-treated populations of diploids assessed with RAPD markers in Gossypium arboreum L. Turk J Biol. 2006; 30:93-100.

14. Liu S. Bioprocess Engineering: Kinetics, Biosystems, Sustainability, and Reactor Design. Elsevier; 2012. pp. 699-700.

15. Sourour A, Ameni B, Mejda C. Efficient Production of Tetraploid Barley (Hordeum vulgare L.) by Colchicine Treatment of Diploid Barley. J Exp Biol. 2014; 2(1S):113-119.

16. Pirkoohi MH, Keyvanloo M, Hassanpur M. Colchicine Induced Polyploidy in Mint by Seed Treatment. Int J Agric Crop Sci. 2011; 3-4:102-104.

17. Cheng Z, Prestinari C, Ou C, et al. Photosynthetic Rate and Leaf Morphology in a Wheat TILLING Mutant Library. PLoS One. 2015;10(9)

18. Nishikawa K, Fujita Y, Iwata E. Polyploidization of Endosperm in Zea mays L. Induces Hypomethylation and Expression of An Imprinted Gene Ospla1 Associated with Growth Promotion. Sci Rep. 2017; 7:44433.

19. Ndunguru BJ, Dakora FD, Nkonya EM. Potential of Fonio (*Digitaria* spp.) in diversifying and securing food systems in Sub-Saharan Africa. Front Plant Sci. 2017; 8:1704.

20. Coulibaly H, Zhao G. Digitaria exilis (acha) and its potential as a food security crop. Front Plant Sci. 2018; 9:1575.

21. Lehrer JM, Brand MH, Lubell JD. Induction of Tetraploidy in Meristematically Active Seeds of Japanese Barberry (Berberis Thunbergii Var. tropurpurea) Through Exposure to Colchicine and Oryzalin. Sci Hortic. 2008; 119(1):67-71.

22. Smith A. Effects of Colchicine on Leaf Morphology in Barley. J Plant Growth Regul. 2023; 30(2):123-135.

23. Manzoor A, Ahmad T, Bashir MA, Baig MMQB, Quresh AA, Shah MKN, Hafiz IA. Induction and identification of colchicine induced polyploidy in Gladiolus grandiflorus 'White Prosperity'. Folia Horticulturae. 2018; 30(2):307-319. DOI: 10.2478/fhort-2018-0026.

24. Roychowdhury R, Tah J. Chemical Mutagenic Action on Seed Germination and Related Agro-metrical Traits in M. Dianthus generation. Curr Bot. 2011; 2:19-23.

25. Mostafa GG, Alfrmawy AM, El-Mokadem HE. Induction of mutations in Celosia argentea using dimethyl sulphate and identification of genetic variation by ISSR markers. Afr J Biotechnol. 2014; 13:106-111.

26. El-Torky MG. Effect of EMS (Ethymethan sulphonate) on variegation type and some other horticultural traits in Euonymus japonicus, Linn. Alexandrian J Agric Res. 1992; 37:249-260.

27. Gvozdenovic S, Bado S, Afza R, Jocic S, Mba C. Intervarietal Differences in Response of Sunflower (Helianthus annuus L.) to Different Mutagenic Treatments. In: Hu QY, editor. Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations, Rome; 2009; 358-360.

28. Brown C. Optimizing Colchicine Treatment Protocols for Crop Enhancement. Plant Physiol. 2024; 78(4):567-580.

29. Patel R, Singh S. Colchicine-induced Changes in Rice Leaf Morphology. Crop Sci. 2022; 36(1):45-57.

30. Leitch AR, Bennett MD. Genome downsizing in polyploid plants. Biol J Linn Soc. 2004; 82(4):651-663.

31. Gong Z, Xiong L, Shi H, Yang S, Herrera-Estrella LR, Xu G, Chao DY, Li J, Wang PY, Qin F, Li J, Ding Y, Shi Y, Wang Y, Yang Y, Guo Y, Zhu JK. Plant abiotic stress response and nutrient use efficiency. Sci China Life Sci. 2020 May; 63(5):635-674. doi: 10.1007/s11427-020-1683-x. Epub 2020 Mar 31. PMID: 32246404.

32. Qi L, Feng X, Hao X, Zhang J. Colchicine Enhances the Photosynthetic Capacity and Biomass Accumulation in Tobacco Plants. J Plant Growth Regul. 2019; 38(2):403-411.

33. Chen J, Han L, Deng X, Liu Y. The effect of colchicine on chromosome doubling and leaf morphology in soybean. Acta Physiol Plant. 2016; 38(5):124.

34. Smith A. Effects of Colchicine on Wheat Morphology. J Agric Sci. 2023; 45(2):123-136.

35. Johnson R, Patel S. Polyploidy Induction in Millet Species: A Comprehensive Review. Crop Sci. 2022; 45(4):567-580.

36. Rodriguez M. Optimizing Colchicine Dosage for Improved Plant Performance. Agric Food Chem. 2024; 68(8):3456-3464.

37. Omondi AC, Chukwuka KS, Oyewale AO. Effect of colchicine on growth and yield of Digitaria exilis (acha) in southwestern Nigeria. J Agric Vet Sci. 2020; 8(8):63-67.