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# Exploring the potentials of polyploidization, cytology, and histology of soursop (*Annona muricata* L.) genotypes

**Nwaoguala, C. N. C [1]*; Omere, A. E.[1]; Emede, T.O.[1]; Nwafor, C.C.[2]; Eze, G.I[3]; Law-Ogbomo, K.E.[1]**

1. Department of Crop Science, University of Benin, P.M.B. 1154, Ugbowo, Benin City, Edo State, Nigeria.
2. Rubber Research Institute of Nigeria, Iyanomor Edo State Nigeria
3. Department of Anatomy, University of Benin, P.M.B. 1154, Ugbowo, Benin City, Edo State, Nigeria.

## ABSTRACT

**This study explored the potential applications of polyploidization, cytology, and histology in different soursop (*Annona muricata* L.), by means of colchine treatments and characterized resultant genotypes via flow cytometric DNA content analysis, chromosomal counts and leaf histology. Polyploidization was initiated ex vitro, soursop seeds were imbibed in four different colchicine levels (0, 0.5, 1, and 1.5%) and exposed to six exposure durations (0, 6, 12, 18, 24, and 30 hours). Fresh soursop leaf samples collected from the treated seedlings were used for flow cytometric analysis, direct chromosome count, and histology. Direct somatic chromosome count revealed that soursop genotypes had a consistent diploid chromosome number of 2n=13 and 2n=14 across treatments, with no unequivocal polyploidy cells. Additionally, Flow cytometry studies confirmed the ploidy status of colchicine-induced soursop genotypes as diploids and triploids. Histology assay showed activities of cell proliferation with an increased stratification of epithelial cells in the epidermis, and hyperplasia of the palisade and spongy mesophyll layers and increased chlorophyll pigment signals at 12, 18, 24, and 30 hours of exposure. For ex vitro polyploidization of soursop, higher percentage of colchicine concentration (2% to 4%) and longer exposure durations (24, 48 and 72 hours) are recommended, as well as the use of primordial tissues or newly sprouted seedlings. Based on these findings, optimization of colchicine concentration, exposure time and selection of regenerants is recommended to achieve stable polyploidy lines in soursop, and historical markers may provide early screening tools.**

## ARTICLE'S INFO

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***Corresponding Author***

*Nwaoguala, C. N. C*

***E-mail:*** *chibuzo.nwaoguala@uniben.edu*

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## INTRODUCTION

Soursop (*Annona muricata* L.), belonging to the genus *Annonaceae*, is a tropical fruit tree native to the American tropics and Caribbean that has since been successfully introduced and cultivated in other tropical and subtropical regions worldwide, including Africa, Asia, and Australia. It is popularly known in Africa as "sawasop" (Osaigbovo *et al*., 2023). As an important and widespread perennial fruit tree, soursop has received burgeoning attention in recent years due to its pharmaceutical and nutraceuticals potential, particularly for the presence of secondary metabolites reported to have anticancer/antitumoral properties (Montejo-Mendez *et al*., 2025). The secondary metabolite isolated from different tissues of various species of the genus *Annonaceae,* called annonaceous acetogenins, is a large family of naturally occurring polyketides. These compounds feature a long fatty acid chain with tetrahydrofuran (THF) rings and a terminal gamma-lactone ring (Agu *et al*., 2017). The recorded ethnomedicinal properties of soursop include, analgesic, antibacterial, anticancer, antifungal, antioxidant, antitumor, antiulcer, antiviral, anti-arthritic, anti-diabetic, anti-hypertensive, anti-inflammatory, anti-insomnia, anti-rheumatic, anti-stress, immune-enhancing. (Agu *et al*., 2017; Moghadamtousi *et al*., 2015; Nsor *et al*., 2024; Kazaure *et al*., 2025).

Soursop is an exotic fruit that biologically and ecologically shares similar morphological and edaphic similarities with some indigenous Nigerian fruit trees, including *Adansonia digitata*, *Parkia biglobosa*, and *Vitellaria. Paradoxa.* The, soursop genetic improvement encompass the selection of desirable progenies and integration of desirable traits in the fruits (Oni *et al*., 2022). Osaigbovo *et al* (2023) posited that conservation strategies should be developed in order to forestall the threat of extinction of soursop. Successful nursery production of good quality seedlings is critical for the conservation of *A. muricata* (Osaigbovo and Nwaoguala, 2011; Osaigbovo *et al*., 2023).

Polyploidy involves an increase in the number of chromosomes beyond the typical diploid sets and is believed to play a major role in speciation and diversity (Neenu *et al*., 2024; Raebild *et al*., 2024). Similarly, karyotyping is a technique for confirming ploidy status at the chromosomal level, and it has been reported that polyploid organisms exhibit increased vigour and often perform better than their diploid relatives in various aspects. Historically and more recently, the innate capacity of polyploids has been the focus of many plant breeders (Omere *et al*., 2022). Artificial induction of polyploidy may be a promising approach to enhance the production of pharmaceutically important secondary metabolites, improve tolerance to both biotic and abiotic stresses, and achieve higher yield levels (Salma *et al*., 2017; Omere *et al*., 2022; Neenu *et al*., 2024; Zhang *et al*., 2024).

Histology, the microscopic study of cells and tissues, is closely linked to anatomy, cell biology, and physiology (Karabiyik and Sen, 2022). During development, it helps reveal structural changes (Moreno-Sanz *et al*., 2020). Chromosome counting, flow cytometry, and histological assays are useful for assessing polyploidy.

Integrating genetic tools into agriculture can support the creation of pharmaceuticals from bioactive compounds, benefiting stakeholders (Montejo-Mendez *et al*., 2025). Currently, there's no data on artificially induced polyploidy in soursop and limited cytological information—critical for future research and utilization. Hence, this study sought to explore the potential of colchicine-induced polyploidization in soursop genotypes.

## MATERIALS AND METHODS

### Experimental site and sample collection

Fruits of soursop (*Annona muricata* L.) were randomly purchased from different markets in Edo State. Purchased fruits were transported to the Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria, which lies between latitude 6° 14' N and 7° 34' N and longitude 5° 40' E and 6° 43' E in a high-humidity (80%) region (Falodun and Bakare, 2023).

### *Ex vitro* initiation of polyploidization

### Colchicine treatment

Fruits were depulped, and the seeds extracted from the pulp. Before sowing, seeds were treated with colchicine (a mutagenic and antimitotic agent for chromosome doubling) at four colchicine levels of 0, 0.5, 1.0, and 1.5 % for six exposure durations (0, 6, 12, 18, 24 and 30 hours). After treatment, seeds were sown in a 3 kg polythene bags in the screen house of the Department of Crop Science, Faculty of Agriculture, University of Benin,

### Flow cytometry for ploidy analysis

### Sample collection and preparation

Soursop leaf tissue were sampled from colchicine treated seedlings in the screen house for flow cytometric analysis of relative DNA content (fluorescence) following standard protocol in the Bioscience Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State.

A piece of freshly harvested young plant tissue was cut out and put into a clean dry Petri dish. 500ul of OTTO 1 solution (0.1 M citric acid + 0.5% (v/v) tween 20) was pipetted into the Petri dish containing the plant tissue and with the use of a sterile razor blade, the plant tissue was chopped into pieces to release the nuclei from the cells. Another 500ul of the OTTO 1 solution was added and the chopped leaf was slowly washed downwards in slanted position, filtered through a suitable cell trics disposable filter (0.22um) into a clean dry sample test tube and incubated at room temperature for about 2 minutes. One (1 ml) of the

OTTO 2 working solution (0.4 M $Na_2HPO_4.12H_2O$) was added and mixed thoroughly. The filtered solution was set into the flow cytometer, and the ploidy value was measured.

**Determination of chromosome number**

**Sample collection and preparation**

Fixative solution (96 % ethanol mixed with acetic acid in a ratio of 3:1) was prepared in order to preserve the root cutting for onward chromosome number determination. Young root meristem deriving from treated seedlings were harvested and pre-treated (8-hydroxyquinoline), fixed, hydrolyzed and stained for metaphase chromosome observation.

**Histological assay**

**Sample collection and preparation**

Leaf samples from each treatment (control and selected colchicine exposure times; 6, 12, 18, 24 and 30 hours) were fixed, embedded, sectioned and stained with haematoxylin and cosin (H and E) (Ewere *et al*., 2022). The anatomical features evaluated included: epidermal stratification, thickness of palisade and spongy mesophyll layers, chlorophyll pigmentation distribution (CP), presence of hyperplasia (HP), vascular structure condition (VS) and count of epidermal and palisade layer cells.

**Data analysis**

Data was subjected to analysis of variance (ANOVA) at 0.05 probability level of significance for differences between groups using Statistical Package for the Social Sciences (SPSS - 24th edition).

## RESULTS

The mean nuclear DNA ((Deoxyribonucleic acid) content, ploidy level and status based on flow cytometric analysis is presented on Table 1. Samples analysed were either remarked as diploids or triploids, with a mixoploid recorded. The control (no colchicine) had a mean DNA content of 12681.51 units and was assigned as diploid (2×). Exposure duration of 0 hour for 0.5, 1 and 1.5 % of colchicine treatment for 0-hour duration had mean DNA contents of 15938.55, 19254.45 and 19218.26, with ploidy levels of 260, 320 and 320, respectively. The exposure duration of 6 hours for 0.5, 1 and 1.5 % of colchicine treatment had mean DNA contents of 18710.85, 18819.47 and 17933.47, with ploidy levels of 300, 300 and 280, respectively.

Colchicine treatments of 0.5, 1, and 1.5 % at 12 hours resulted in DNA contents of 17924.58, 18263.99, and 17661.1, with ploidy levels of 280, 290, and 270, respectively. At 18 hours, DNA contents were 18064.58, 17307.51, and 17844.65, with ploidy levels of 280, 270, and 280, respectively. At 24 hours, DNA contents were 16436.84, 16436.84, and 8015.35/13400, with ploidy levels of 260, 260, and 160/220, respectively. At 30 hours, DNA contents were 12977.06, 14183.89, and 12659.34, with ploidy levels of 210, 240, and 200, respectively.

Some of the flow cytometric histograms of soursop treated with 0.5, 1 and 1.5 % colchicine at 0, 6, 12, 18, 24 and 30 hours are presented on figs. 1 – 7. The control (no colchicine nor exposure duration) had a count of 78 (y-axis) and gave a sharp peak at 200 based on the fluorescent intensity (x-axis) which is a function of the ploidy level (fig. 1). fig. 2 displays the histogram of 0.5 % colchicine treatment at 0 hour, with a count of 340 and a peak of 260. Presented on fig. 3 displays 1 % colchicine treatment at 6 hours peaked at 300 with a count of 650. fig. 4 presents the histogram of 1.5 % colchicine treatment at 12 hours with a count of 700, and peak of 270. The histogram of colchicine treatment of 0.5 % at 18 hours of exposure is presented on fig. 5 showing peak of 280 counts of 310. fig. 6 displays the histogram of colchicine treatment of 1 % at 24 hours with a peak of 260 and a count of 900. The histogram of colchicine treatment of 1.5 % at 30 hours of exposure is presented on fig. 7 revealing peak of 200 with count of 125.

**Table 1: Summary of the mean DNA content of flow cytometric analysis**

| Treatment | DNA Content | Ploidy | Remarks |
|---|---|---|---|
| Control | 12681.51 | 200 | Diploid |
| 0.5 % 0 hour | 15938.55 | 260 | Diploid |
| 1 % 0 hour | 19254.45 | 320 | Triploid |
| 1.5 % 0 hour | 19218.26 | 320 | Triploid |
| 0.5 % 6 hours | 18710.85 | 300 | Triploid |
| 1 % 6 hours | 18819.47 | 300 | Triploid |
| 1.5 % 6 hours | 17933.47 | 280 | Diploid |
| 0.5 % 12 hours | 17924.58 | 280 | Diploid |
| 1 % 12 hours | 18263.99 | 290 | Diploid |
| 1.5 % 12 hours | 17661.1 | 270 | Diploid |
| 0.5 % 18 hours | 18064.58 | 280 | Diploid |
| 1 % 18 hours | 17307.51 | 270 | Diploid |
| 1.5 % 18 hours | 17844.65 | 280 | Diploid |
| 0.5 % 24 hours | 16436.84 | 260 | Diploid |
| 1 % 24 hours | 16436.84 | 260 | Diploid |
| 1.5 % 24 hours | 8015.35/13400 | 160/220 | Mixoploid |
| 0.5 % 30 hours | 12977.06 | 210 | Diploid |
| 1 % 30 hours | 14183.89 | 240 | Diploid |
| 1.5 % 30 hours | 12659.34 | 200 | Diploid |

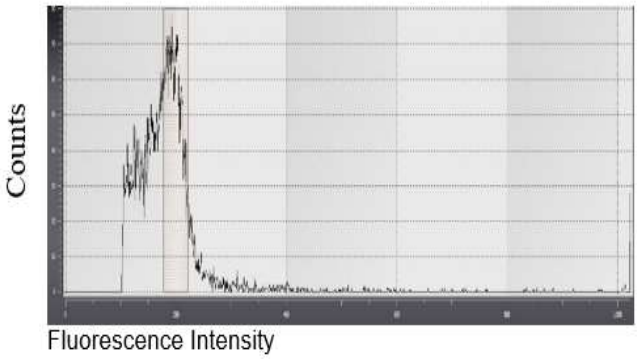


**Fig. 1: Histogram of relative DNA content for control**

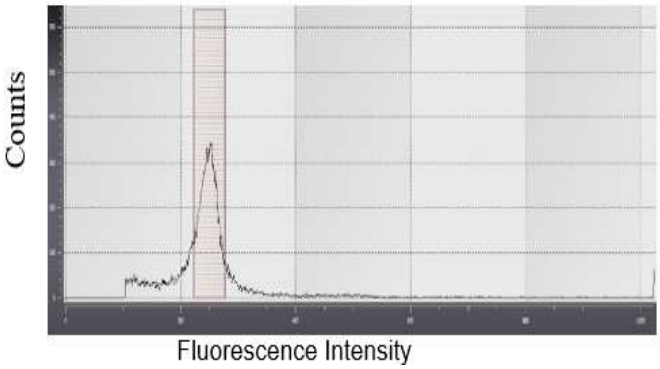


**Fig. 2: Histogram of relative DNA content for 0.5% colchicine at 0 hour of exposure**

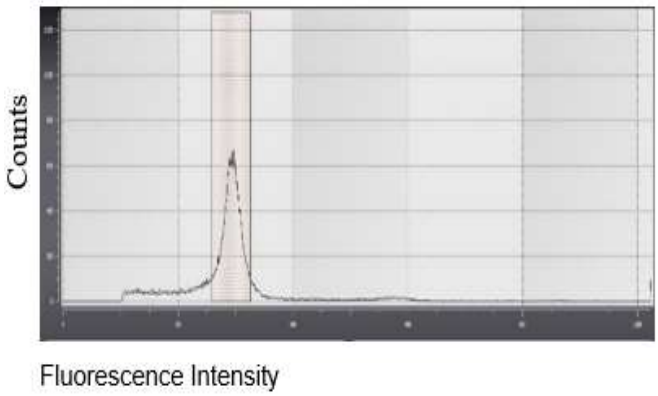


Figure 3: Histogram of relative DNA content for 1% colchicine at 6 hours of exposure

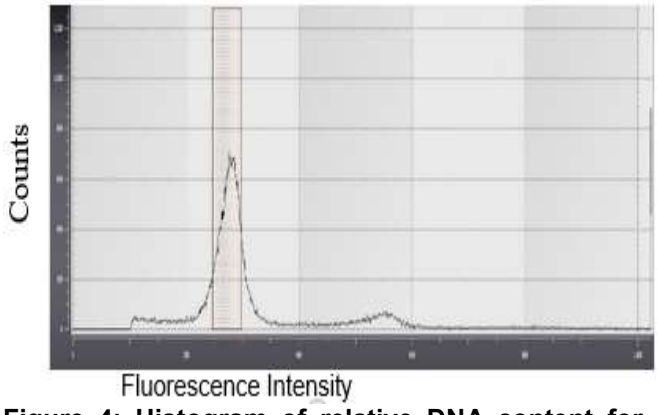


**Figure 4: Histogram of relative DNA content for 1.5% colchicine at 12 hours of exposure**

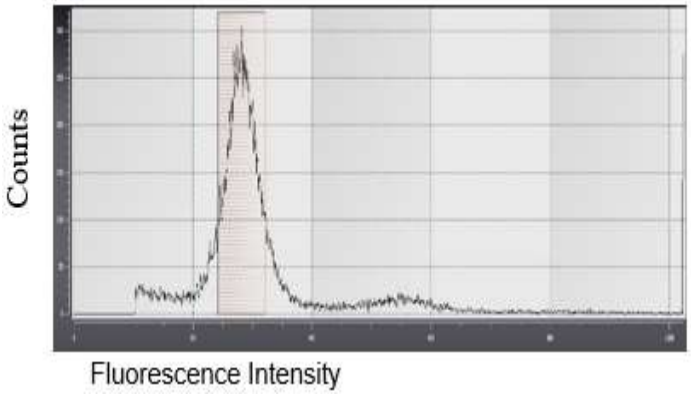


**Fig. 5: Histogram of relative DNA content for 0.5% colchicine at 18 hours of exposure**

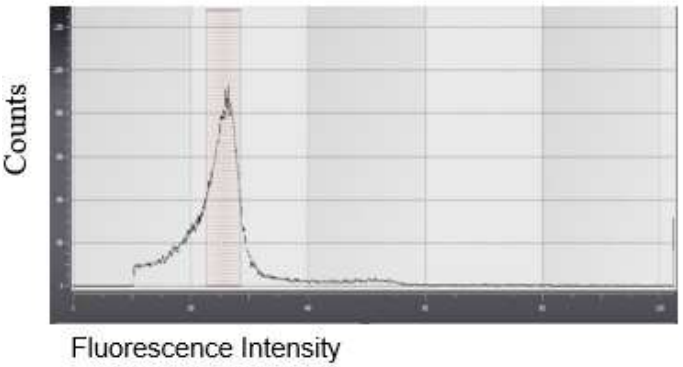


**Fig. 6: Histogram of relative DNA content for 1% colchicine at 24 hours of exposure**

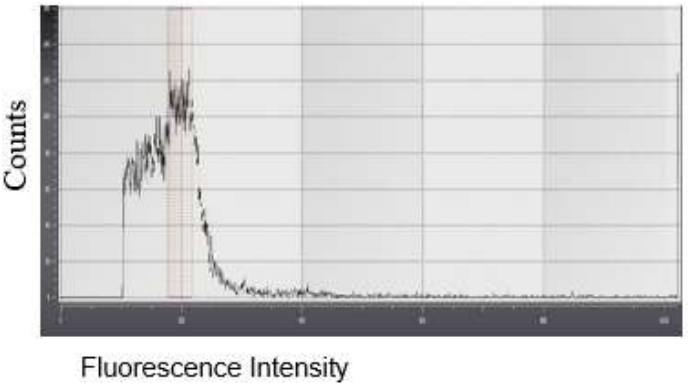


**Figure 7: Histogram of relative DNA content for 1.5% colchicine at 30 hours of exposure**

The average of the 7 cells, chromosome number and the ploidy status for the varying colchicine treatment levels (%) and exposure durations are presented on Table 2. The control (no colchicine) had number of chromosomes counted ranging from 10 – 14 with an average of 13, giving chromosome number of 2n = 2x = 13. All samples analysed were diploids.

Colchicine treatment of 0.5 % and exposure duration of 0 hour and 18 hours, 1% at 24 hours, and 1.5% at 12 hours and 30 hours had 14 chromosomes counted across the 7 cells with an average of 14, chromosome number of 2n = 2x = 14. Exposure duration of 6 hours and colchicine treatment of 1 % had 13 chromosomes counted across the 7 cells with an average of 13, chromosome number of 2n = 2x = 13.

**Table 2: Chromosome analysis from 7 cells counted**

| Treatment | Number of chromosomes counted in individual metaphase cell | | | | | | | Average | Chromosome number | Ploidy status |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | | |
| Control | 10 | 13 | 12 | 12 | 14 | 14 | 14 | 13 | 2n=2x=13 | Diploid |
| 0.5 % 0 hour | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 1 % 0 hour | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 2n=2x=13 | Diploid |
| 1.5 % 0 hour | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 0.5 % 6 hours | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 2n=2x=13 | Diploid |
| 1 % 6 hours | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 2n=2x=13 | Diploid |
| 1.5 % 6 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 0.5 % 12 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 1 % 12 hours | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 2n=2x=13 | Diploid |
| 1.5 % 12 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 0.5 % 18 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 1 % 18 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 1.5 % 18 hours | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 2n=2x=13 | Diploid |
| 0.5 % 24 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 1 % 24 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 1.5 % 24 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 0.5 % 30 hours | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 2n=2x=13 | Diploid |
| 1 % 30 hours | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 2n=2x=14 | Diploid |
| 1.5 % 30 hours | 14 | 14 | 14 | 14 | 14 | 13 | 13 | 14 | 2n=2x=14 | Diploid |

Plates 1 – 7 show micrographs of cut section of samples at metaphase for estimation of somatic chromosome number of soursop. Thirteen (13) chromosomes were recorded for control (no colchicine) and 1% colchicine level at 6 hours of exposure. Fourteen (14) chromosomes were counted for 0.5% at 0 hour, 1.5% at 12 hours, 0.5% at 18 hours, 1 % at 24 hours, and 1.5% at 30 hours.

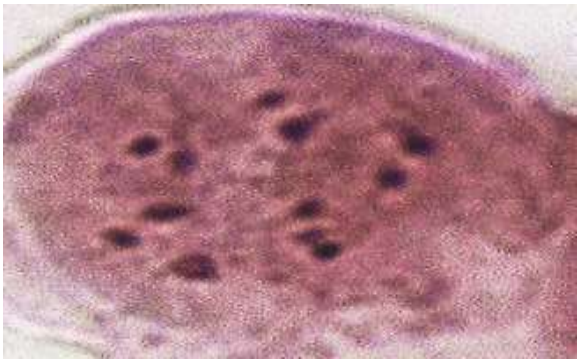

**Plate 1: Photomicrographs of chromosomes for control**

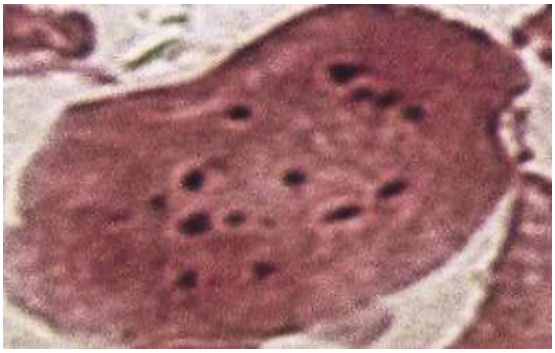

**Plate 2: Photomicrographs of chromosomes for 0.5 % colchicine at 0 hour of exposure**

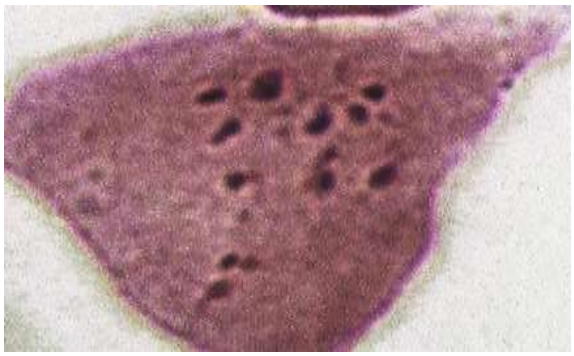

**Plate 3: Photomicrographs of chromosomes for 1 % colchicine at 6 hours of exposure**

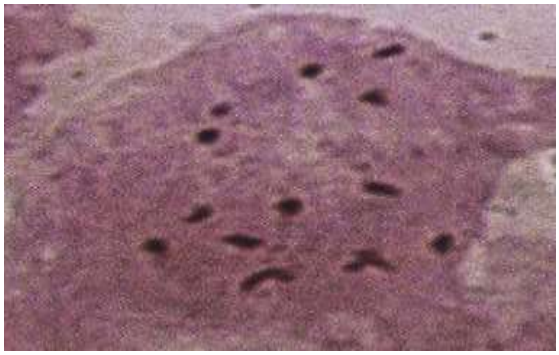

**Plate 4: Photomicrographs of chromosomes for 1.5 % colchicine at 12 hours of exposure**

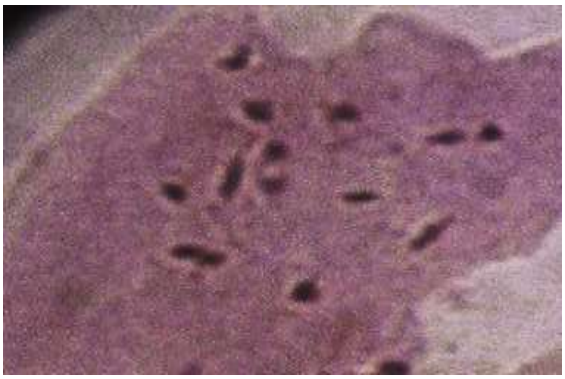

**Plate 5: Photomicrographs of chromosomes for 0.5 % colchicine at 18 hours of exposure**

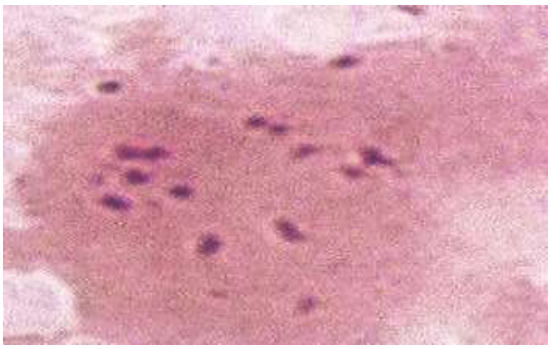

**Plate 6: Photomicrographs of chromosomes for 1 % colchicine at 24 hours of exposure**

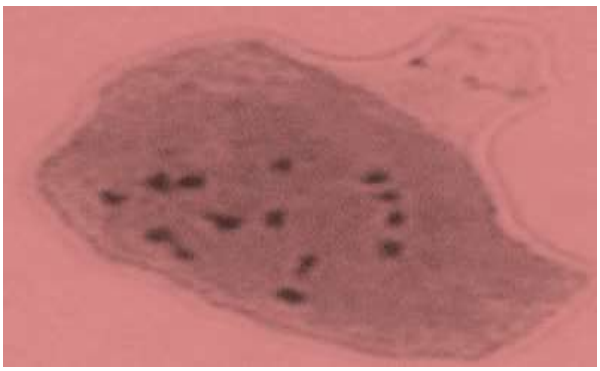

**Plate 7: Photomicrographs of chromosomes for 1.5 % colchicine at 30 hours of exposure**

Histological assessment of soursop treated with colchicine at 6, 12, 18, 24 and 30 hours is presented on Plates 8 – 13. At 30 hours of exposure, the epidermal layer had a mean cell count of 531.30, significantly higher than 410.00 at 18 hours, 378.30 at 6 hours, 349.00 at 24 hours, and 288.30 at 12 hours. The control (no colchicine) recorded a mean cell count of 205.5. The palisade layer had 285.00 mean number of cells counted for 30 hours of exposure which was the higher than 247.80 for the control, 199.00 for 24 hours, 95.00 for 6 hours, 78.00 for 18 hours, and 54.00 for 12 hours (Figure 8).

Leaf cross section showed anatomical changes associated with colchicine treatments. Visual examination of the control in Plate 8 shows the normal leaf cell architecture, composed of the stratified (ST) layer of the epidermis (EP), the mesophyll (ME) composed of palisade mesophyll (PM) and spongy mesophyll (SP) with dense concentration of chlorophyll (CH). The plate was generated by viewing at 100 X magnitude with Hematoxylin and Eosin (H and E) staining. At 6 hours of exposure (Plate 9), there was marginal proliferation of the leaf cells as the epidermis had a single layer of flattened cells, palisade mesophyll layer already started undergoing herperplasia, spongy mesophyll had dense chlorophyll pigments crisscrossed by veins of vascular structures (VS). Plate 10 displayed the section of leaf exposed to colchicine for 12 hours (Plate 10) having doubled layered stratification of epithelial cells for the epidermis, there was hyperplastic palisade mesophyll layer, spongy mesophyll contained dense chlorophyll pigments crisscrossed by veins of vascular structures.

At 18 hours of exposure (Plate 11), the epidermis had multi-layered stratification of cells, there was hyperplastic palisade mesophyll layer, spongy mesophyll contained dense chlorophyll pigments in the upper and lower parts, and crisscrossed by veins of vascular structures. In the 24 hours of exposure (Plate 12), the epidermis had multi-layered stratification of epithelial cells, there was hyperplasia in the palisade mesophyll layer, spongy mesophyll contained dense chlorophyll pigments in the lower part, and crisscrossed by veins of vascular structures. Displayed on Plate 13 is leaf section of 30 hours of exposure to colchicine having multi-layered stratification of epithelial cells in the lower epidermis, hyperplastic palisade mesophyll, and spongy mesophyll with dense chlorophyll in the lower part. fig. 8 indicated variation in mean numbers of epidermal and palisade cells, among treatments.

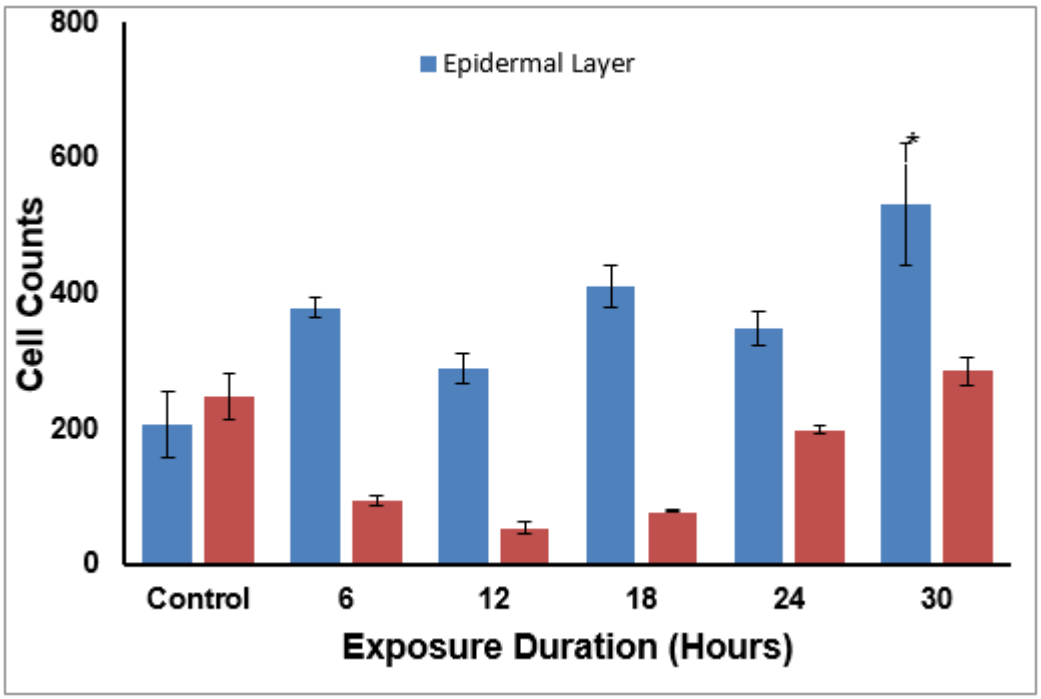


**Figure 8: Histogram showing the mean number of cells counted for epidermal and palisade layer**

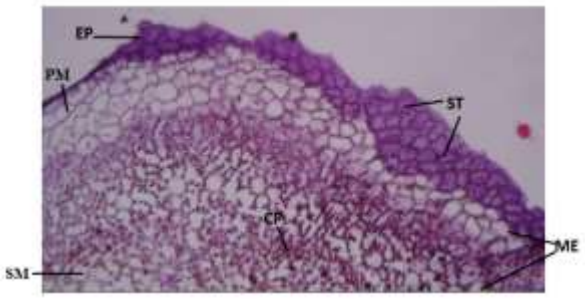


**Plate 8: Leaf specimen for control (not induced) H and E 100 X**
EP: Epidermis; PM: Palisade mesophyll; SM: Spongy mesophyll; ST: Stratification; CP: Chlorophyll pigments; ME: Mesophyll

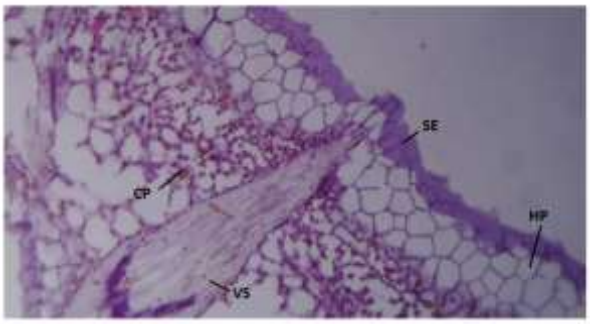


**Plate 10: Sections of leaf exposed to colchicine (12 hours) H and E 100X**
SE: Stratified epidermis; CP: Chlorophyll pigments; VS: Vascular structures; HP: Hyperplasia

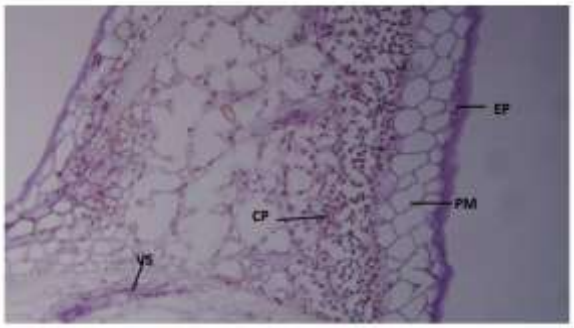


**Plate 9: Sections of leaf exposed to colchicine (6 hours) H and E 100X**
EP: Epidermis; PM: Palisade mesophyll;
CP: Chlorophyll pigment; VS: Vascular structures

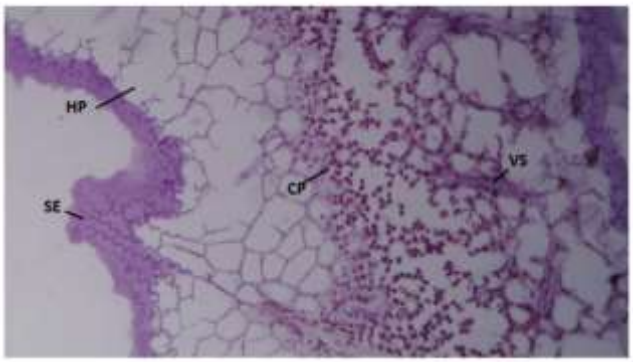


**Plate 11: Sections of leaf exposed to colchicine (24 hours) H and E 100 X**
SE: Stratified epidermis; CP: Chlorophyll pigments; VS: Vascular structures; HP: Hyperplasia

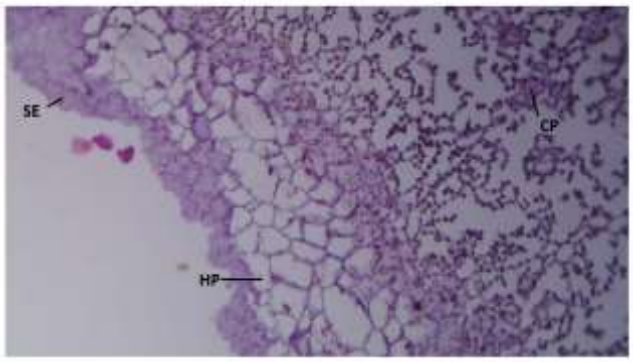


**Plate13: Sections of leaf exposed to colchicine (30 hours) H and E 100 X**
SE: Stratified epidermis; CP: Chlorophyll pigments; HP: Hyperplasia

## DISCUSSION

The induction of polyploidy is a common breeding strategy in horticultural crops, offering opportunities for improved traits such as larger organs, increased chloroplast content, altered stomatal anatomy and enhanced physiological performance (Eng and Ho, 2019).The flow cytometry data exhibited apparent increased in DNA content (up to ~1.52× relative to diploid control) in early treatments (1 and 5 % at 0 hour) and at 6 hour exposures, suggesting induction of genome duplication (i.e putative triploid status). This pattern aligned with Surson *et al*,. 2024 who reported that higher colchicine concentrations and shorter exposure times often yield higher ploidy induction rates., whereas prolonged exposure may reduce viability or lead to mixoploidy. However, chromosome counts did not confirm this putative polyploids: all treatments consistently produced 2n = 2× counts (13 or 14 chromosomes) indicating that stable whole-genome doubling did not occur or was not fixed in the cells analysed. This discrepancy in observed chromosome counts is likely attributable to challenges with micrograph clarity and chromosome clumping (Bridg, 2000; Dansi *et al*., 2001). The resulting diploid ploidy status and the somatic chromosome number of 2n=2x=14 aligned with previous chromosomal studies reported by Simmonds (1954), Darlington and Wylie (1958), and Bridg (2000). Notwithstanding, variation exists in published literature, with Bowden (1948) reporting a chromosome number of 2n=2x=16 and Bridg (2000) also reporting 2n=2x=18 for specific soursop genotypes.

Comparative studies have reported successful induction of tetraploid, for example, Zhang *et al*. (2018) achieved successful tetraploids (2n=4x=44) in Stevia *Rebaudiana bertoni* (an herbaceous perennial, 2n=2x=22) by imbibing germinating seeds in 0.5 and 1 % colchicine for 24 hours ex vitro. Mohammadi *et al* (2023) induced polyploidy *ex vitro* in thyme (*Thymus vulgaris*) using apical meristem (2n = 2x = 30) of 2 and 4-leaf seedlings, and recorded the highest number of tetraploids (2n = 4x = 60) for 3 % colchicine level at the 4-leaf stage. El-Shereif and Elyazid (2024) assayed the effectiveness of colchicine in inducing polyploidy in *Citrus reticulata* using bud tissues *ex vitro*. They reported tetraploid and 1 % colchicine concentration as most effective for producing tetraploid plants. Zhang *et al*. (2024) successfully doubled the chromosome number of *Lycium chinense* (an edible medicinal plant, 2n=2x=24) using higher concentrations of colchicine, specifically 4% to 6%, to yield tetraploids (2n=4x=48). However, the diploid state recorded for the treated plants in this study could be due to incomplete chromosome doubling as a result of low colchicine concentration, relatively short exposure durations, choice of tissue induced, environment of experiment (*ex vitro* vs *in vitro*), and nature of the plant. This aligns with the findings of Surson *et al*. (2024), who consider the type and concentration of the antimitotic agent, the duration of exposure, the plant organs targeted, and the treatment methods to be critical determinants for the efficiency and success of polyploidization.

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Flow cytometry results exhibited variation among the genotypes studied. The control (no exposure to colchicine) and 1.5 % colchicine concentration at 30 hours were complete diploids, whereas 1 % and 1.5 % of 0 hours, and 0.5 % and 1 % of 6 hours were triploids. The other concentrations displayed divergence from diploidy towards triploidy, which presents interesting genotypes that could be explored in future studies. Colchicine concentration of 1.5 % at 24 hours of exposure was a mixoploid, which indicates that there was some level of transformation at the genome level; however, chromosome doubling (tetraploid) was not recorded. Ho *et al* (2024) determined the ploidy level of colchicine induced polyploids of *Neolamarckia cadamba* (Burflower tree) *ex vitro* and *in vitro* using flow cytometry, and they recorded tetraploids, octoploids, and mixoploids. They stated that flow cytometry was a worthy tool in identifying polyploids (even mixoploids), and complementing conventional method like direct chromosome counting.

Histological assessment of soursop leaf tissues exposed to colchicine at varying durations (6, 12, 18, 24, and 30 hours) induced noticeable anatomical changes, consistent with either antimitotic stress or initial altered ploidy states (or endoreduplication). Polyploidy plants often have thicker leaves, increased mesophyll layers, larger cells, fewer but larger stomata, higher chloroplast counts and altered tissue architecture (Li *et al*., 2025). In this study, though stable ploidy change was not confirmed cytologically but, the anatomical modifications suggest that colchicine exposure elicited cellular and tissue-level responses, which may reflect either early stages of genome doubling, endoreduplication or stress induced compensatory morphological changes. These anatomical signatures may thus serve as early screening criteria for putative polyploidy plants before full verification. .

The epidermis is pivotal for plant growth, and is the protective outermost layer of plant cells that functions in the reduction of risk of dehydration, pathogenic invasion or protection of the photosynthetic tissue.

Epidermal cells produce and store different lipid molecules that play key roles in both defense and development (Javelle *et al*., 2010; Glover *et al*., 2016). Leaf mesophyll is the primary photosynthetic tissue in plants, and consists of the palisade mesophyll and spongy mesophyll involved in photosynthetic activities (Zhang and Ambrose, 2024).

Therefore, the proliferation of epidermal cells and mesophyll cells as the duration of exposure increased could suggests that polyploids may have more photosynthetic capabilities, defense, and development. This result is in alignment with Sabzehzari *et al* (2020), that recorded anatomical differences between diploid and colchicine-induced tetraploids of ispaghula (*Plantago ovata* Forsk) *ex vitro* using bud tissues with 3% colchicine concentration at 24 hours and 22.5% trifluralin at 72 hours performing better than other concentrations. Bhuvaneswari *et al* (2020) also recorded significant chloroplast and stomata differences between diploid and colchicine-induced tetraploid of *Citrus limon in vitro* with 0.25% colchicine at 24 hours outperforming.

## CONCLUSION

Direct somatic chromosome count did not exhibit any transformational activity or effect based on colchicine levels of induction and exposure durations on soursop genotypes studied. Flow cytometry delineated the genotypes into diploids and triploids, where as an outlier (mixoploid) between diploidy and triploidy was recorded. This is indicative that flow cytometry gives a more detailed ploidy results because it deals directly with DNA and genome, whereas chromosome counting functions at the cell division stage and may require complete reduplication of the genome DNA in order to accurately ascertain ploidy. The study could not foist clear polyploidization and this may have been due to the plant material used for the induction which was not yet mitotically active, thus polyploidization could not be successfully induced. Results of direct chromosome count were incongruous with findings from flow cytometry and histology, whereas, findings deduced from flow cytometry and histology were congruent.

Research on using sprouted seeds which could be mitotically active should be deployed in future efforts to induce *ex-vitro* polyploidization in soursop genotypes. For *ex vitro* polyploidization of soursop, higher percentage of colchicine concentration (2 to 4 %) and longer durations of exposure (24, 48 and 72 hours) are recommended. Plant tissues such as apical meristems, buds, and petioles should be used; perhaps where seeds are to be used for tree crops and other recalcitrant species, efficient scarification must be conducted in order to facilitate imbibition of the antimitotic agent. An *in vitro* protocol for soursop polyploidization through intense and meticulous effort is advised. For ploidy research, the combination of direct chromosome count, flow cytometry, and histological assay can be employed for holistic view of the state of the cell and genome of induced species.

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