INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY: APPLIED BUSINESS AND EDUCATION RESEARCH
2026, Vol. 7, No. 6, 2696 – 2709
http://dx.doi.org/10.11594/ijmaber.07.06.25

Research Article

Stomatal and Morphological Characteristics of In-Vitro Grown Tomato (*Solanum lycopersicum* L.) Treated with Different Concentrations of Colchicine and Pendimethalin

Jessa R. Gines*, Catherine C. Arradaza[2], Marilyn M. Belarmino[3], Marilou M. Benitez[3], Rosemarie B. Gonzaga[3]

[1]Cebu Technological University-Barili Campus, Barili, Cebu, Philippines
[2]Plant Tissue Culture-Department of Horticulture, Visayas State University, Baybay City, Leyte, Philippines
[3]Department of Horticulture, Visayas State University, Baybay City, Leyte, Philippines

Article history:
Submission 21 May 2026
Revised 15 June 2026
Accepted 23 June 2026

**Corresponding author:*
E-mail:
jessa.gines@gws.ctu.edu.ph

ABSTRACT

This research investigated the stomatal and morphological response of in vitro grown tomato (*Solanum lycopersicum* L.) plantlets to different concentrations of colchicine and pendimethalin. The effects of treatments on stomatal parameters, chlorophyll content and morphological characteristics were studied. The study was conducted using a Completely Randomized Design (CRD) with seven treatments replicated three times with ten samples per replicate at the Plant Tissue Culture Laboratory, Department of Horticulture, Visayas State University. To evaluate the responses of various concentrations of colchicine (1.5, 3.0 and 5.0 mM) and pendimethalin (10, 20 and 30 µM), induced polyploid-associated traits and overall plant growth and development rate on tomato plantlets have also been compared with normal diploid plantlet used as control in subsequent measurement. The results indicated that the mutagen treatments have a substantial effect on explant viability, stomatal traits and chlorophyll content as well as vegetative growth of tomato plants. Moderate concentrations had more profound effect with higher survival percentages of explants found for 20 µM pendimethalin (91%) and 5.0 mM colchicine (84%), which produced regeneration and adaptation, as compared to other concentrations in this study. It was found out with an increased stomatal length, width and aperture on mutation treated plantlets but decreased stomatal density shows induction of polyploid trait. Colchicine resulted in the greatest stomatal size together with favourable impact on chlorophyll content. In contrast, at the highest concentrations of pendimethalin (30 µM), growth was inhibited and chlorophyll was decreased as a result of phytotoxicity. Colchicine and pendimethalin in moderate concentrations exerted a

How to cite:
Gines, J. R., Arradaza, C. C., Belarmino, M. M., Benitez, M. M., &Gonzaga, R. B. (2026). Stomatal and Morphological Characteristics of In-Vitro Grown Tomato (*Solanum lycopersicum* L.) Treated with Different Concentrations of Colchicine and Pendimethalin. *International Journal of Multidisciplinary: Applied Business and Education Research*. *7*(6), 2696 – 2709. doi: 10.11594/ijmaber.07.06.25

favorable response on stomatal and morphological arrangements, traits associated with polyploidy can enhance the vegetative vigor.

Keywords: *Chemical mutagens, Colchicine, In vitro culture, Pendimethalin, Physiological response, Polyploidy, Stomatal traits, Tomato*

Introduction

Among the various important vegetables of world tomato (*Solanum lycopersicum* L.) is ranked as the first most economically viable and nutritionally rich cash crop. It is a rich source of vitamins, minerals, antioxidants and thus lycopene for human absorption and use. However, tomato has been considerably compromised by drought stresses, high temperature and climate variability leading to reduction of plant vigor, growth photosynthesis and yield. Thus, with climate change challenges intensifying, enhancing the trait of resistance responses to market and stresses in respect to tomatoes is needed.

Polyploidization is an increase of whole sets of chromosomes in cell has been succeffully used to enhance plants' yield through crop improvement. Its event frequently leads to increased cell appendage size, leaf thickness, stomatal density, chloroplast content as well as drought tolerance and vegetative vigor which are of enormous value for other plant breeding program (Tossi et al., 2022). Polyploidization also influences stomatal traits, which affect physiological functions such as gas exchange and water-use efficiency by determining the size and density of stomata. Colchicine and pendimethalin are frequently used antimitotic agents for artificially inducing polyploidy. They are primarily known to interact with the extremely dynamic spindle fiber formation during mitosis and therefore prevent chromosome separation which ultimately triggers doubling of chromosomes (Dhooghe et al., 2011). Colchicine acts by binding to tubulin and inhibiting microtubule polymerization, thereby disrupting spindle formation during mitosis and inducing chromosome doubling (Singh et al., 2025). It has been noted polyploid plants applied with colchicine tend to have a larger stoma alongside with fewer stomatal density in contrast with the diploid counterparts, therefore making stomatal attributes a common sign of polyploidization (Dhooghe et al., 2011). Moreover, polyploid plants have thicker leaf, more vegetative growth and biomass in comparison to diploids (Talukdar, 2013). Similarly, another agent for polyploidization is Pendimethalin, which is also a dinitroaniline herbicide. Pendimethalin is a tubulin-disrupting agent that inhibit assembly of microtubules and formation of mitotic spindle just like colchicine. Pendimethalin is defined as an effective chromosome doubling agent with less phytotoxicity effect than colchicine (Kermani et al., 2003). Plants exposed to pendimethalin have been reported with enhanced vegetative growth, enlarged stomata and different polyploid-related characteristics, confirming its utility as an alternative antimitotic agent for chromosome doubling and polyploid induction (Eng et al., 2021).

It is regarded that some of the recognized stomatal features can serve as authentic markers for successful induction for polyploid. In general, it has been observed that polyploid plants have larger stomata and they have fewer stomata per unit area than diploid plants (Beaulieu et al., 2008). These anatomical modifications may functionally help improve the efficient use of water loss while concurrently preventing excessive transpiration when under stress. Polyploidization also may influence chlorophyll accumulation, transpiration rate, stomatal conductance and photosynthetic efficiency. Nevertheless, mutagenic effects are dose-dependent. While moderate plant concentration has the key role for enhancing growth and adaptation physiological response, exposure to high concentrations would cause cytotoxicity, oxidative stress, and growth inhibition (Calabrese & Baldwin 2003).

While several studies have been performed using induced polyploidy, only few, reporting on the comparative study of colchicine and pendimethalin effects on anatomical and physiological responses in vitro grown tomato plants have emerged. Therefore, this research

was conducted to analyze the effect of different concentrations of colchicine and pendimethalin on polyploidy induction, explant survivability, stomatal characteristics, chlorophyll content and vegetative growth of tomato plants grown in vitro. These findings may contribute to breeding tomato with better performance and tolerating abiotic stresses.

Materials and Methods

Plant Materials and Sterilizations

The tomato seeds variety Diamante Max F1 were used in this study. For five minutes, the seeds were washed and sterilized with 0.01% sodium hypochlorite (bleach) and 70% (v/v) ethanol for an additional five minutes. Then, the seeds were rinsed with sterilized distilled water three times (Kumar et al., 2022).

The washed and sterilized seeds were sown and pre-germinated in a container with MS basal medium (Murashige and Skoog 1962) for five (5) days until radicle emergence of 5-7 mm, indicating a high level of meristematic activity (Lehrer et al., 2008).

Culture Media Preparation

The medium used was Murashige and Skoog (1962) formulation supplemented with vitamins, Fe-EDTA, 30 g L^{-1}sucrose, and 5 g L^{-1} agar for solidification. The pH of the medium was adjusted to 5.8±

0.1 with either 1.0N HCl OR 1.0N NaOH before dispensing. Culture vessels containing 15 ml of the medium were sterilized by autoclaving at 121°C and 15 psi for 20 minutes (Murashige and Skoog, 1962).

Experimental Design and Treatments

The study was conducted at Plant Tissue Culture Laboratory (PTCL) of the Department of Horticulture, Visayas State University, Baybay City, Leyte from March to May 2025. The experiment was laid out in a Complete Randomized Design (CRD) with seven (7) treatments replicated three (3) times having ten (10) samples per treatment per replication. The treatments were designated as follows:

- **T_1** – No application of chemical mutagens
- **T_2** – 1.5 mM of Colchicine
- **T_3** - 3.0 mM of Colchicine
- **T_4** - 5.0 mM of Colchicine
- **T_5** – 10 µM of Pendimethalin
- **T_6** – 20 µM of Pendimethalin
- **T_7** – 30 µM of Pendimethalin

Application of Chemical Mutagens

The five-day-old pre-emerged tomato seeds were used with radicle emergence of 5-7 mm. The dosage employed by Ramulu et al (1991) and Mulyana et al (2023) was modified to 0, 1.5, 3.0, and 5.0 mM for colchicine and 0, 10, 20, and 30 µM for pendimethalin.

These antimitotic agents were added to the Murashige and Skoog (MS) medium. The radicle-emerged tomato seeds were treated in the treatment medium under light conditions with a temperature of ±25ºC for four (4) days. After the four-day treatment exposure, the seedlings were removed from the treated culture media. These were rinsed for 10 minutes using three 10 ml changes of deionized water (Lehrer et al., 2008). Modifying the method employed by Mulyana et al (2023), at the end of the four-day treatment, the seedling was transferred to MS medium (Murashige and Skoog 1962) + 2 mg of BAP and 0.1 mg of IAA + 2% sucrose, pH of 5.8 to stimulate new growth for thirty (30) days.

Explants Survival (%)

This was done by counting the number of surviving explants divided by the number of explants applied with chemical mutagens multiplied by 100 (Mulyana et al., 2023).

Morphological Characteristics of Tomato

Morphological characteristics of tomato plants were evaluated based on leaf length, leaf width, leaf area and plant height. The method of measurements was following the procedure employed in the study of Zhang et al (2024). These data were quantified using ImageJ 1.53 k software.

Stomatal Morphology

For stomatal characteristics, samples were gathered from the third fully expanded leaves from the top and imprinted using transparent nail polish. A compound microscope was used to magnify imprints at 4x and 10x (Zakariyya et.al., 2017). The area of the field of view was determined using a calibrated micrometer slide. Stomatal length, stomatal width, stomatal

aperture and stomatal density were analyzed using Image J software (Mathias et.al., 2024).

Chlorophyll Content

The Hiscox and Israelstam (1979) method were used to measure the amount of total chlorophyll. Samples were thinly sliced, bleached and were incubated using a water bath that contains 80% ethanol for 24 hours. Then, the absorbance of the solution was determined using a spectrophotometer (Epoch microplate spectrophotometer, Biotek ™, Epoch ™) set at 645 and 663 nm, and the rate was recorded and calculated using the equation of Arnon (1949).

Results and Discussions

Plantlet Survival (%)

The survival response of the tomato plantlets was significantly different depending on the concentration of colchicine and pendimethalin (Figure 1). Comparing with the explant viability without exposure to mutagens, ranging 62% high survival percentage of non-mutagen applied plantlets for colchicine and pendimethalin. Notably, explants cultured in 1.5 mM (69%), 3.0 mM (75%) and 5.0 mM (84%) colchicine showed slightly improved survivals. While that of pendimethalin, at 20µM (91%) showed the highest survival percentage indicating significantly greater at $p<0.005$. This was followed by 10µM (82%) and the least was at 30µM (62%). This indicates that the doses were not sufficiently toxic to influence the tissue variation. This is in line with the reports of colchicine microtubule inhibition and chromosome doubling without drastic reduction of regenerative capacity within tolerable doses (Dhooghe et al., 2011). In addition, this agreed with demonstration that colchicine-induced polyploidization increases cellular metabolic efficiency and structural strength leading to improved to plantlet survival (Dhooghe et al., 2011).

The highest number of surviving plantlets (91%) was observed in 20µM pendimethalin treatment amongst all the other applied chemical mutagens, higher than without mutagens and other concentrations of colchicine and pendimethalin. This increased survival could be due to hormesis, a phenomenon in which multiple cellular stress responses are activated at low doses of chemicals, resulting in improved regenerative ability (Calabrese & Baldwin, 2003). Moreover, this is in conformity with the previous reports for the low-dose pendimethalin mediated polyploidization without considerable reduction in explant viability (Sajid and Afab, 2013). The low doses of dinitroaniline herbicides can promote cell division and/or lead to polyploidy with little cytotoxicity (Belz and Duke, 2014; Kumar and Rai, 2021). This is dose-dependent and parallel known responses of antimitotic mutagens that stimulate cellular adaptation and growth at low-to-intermediate doses, but inhibit mitoses and disrupt tissue integrity at higher levels (Sattler et. al., 2016).

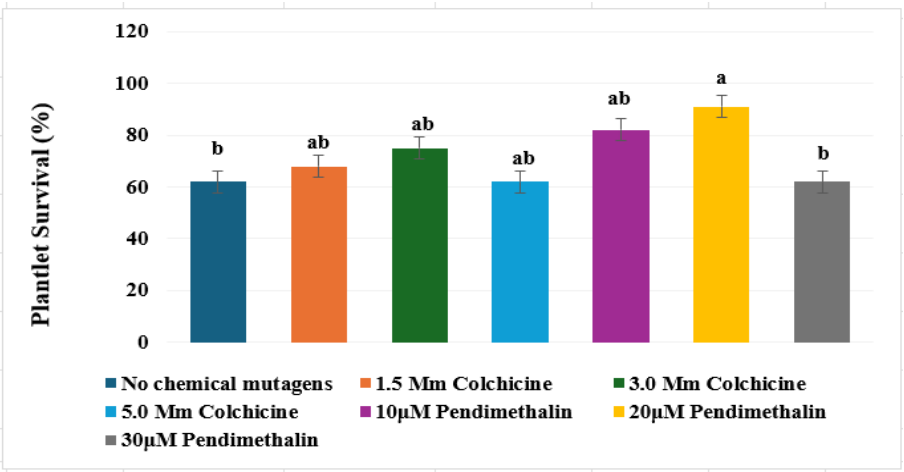

Figure 1. Plantlet survival (%) of tomato plantlets as treated with different concentrations of chemical mutagens, the colchicine and pendimethalin.

Leaf length, leaf width and leaf area

At $p<0.005$, a higher mean leaf length (1.6cm and 1.7cm, respectively) was observed in when 5.0mM colchicine and 10µM pendimethalin were applied. This indicates that at this concentration colchicine has effectively stimulated both cell division and expansion, leading to stronger vegetative growth. However, moderate dosages of the two mutagens at 1.5mM colchicine (1.3cm), 3.0mM colchicine (1.5cm) and 20µM and 30µM pendimethalin (1.5cm and 1.2cm, respectively) which signified partial increase in growth compared with non-mutagenized plants. On the contrary, leaves from plants treated with low (1.5mM colchicine) and high (30µM pendimethalin) concentrations were significantly shorter (1.2cm and 1.3cm respectively,) compared to non-mutagenized plants. Each reduction may be due to small chromosomal modifications at a low dose or cytotoxic effects and mitotic spindle damage at high doses. The observed increase in leaf length may be attributed to successful polyploidization induced by colchicine and pendimethalin. These findings were supported by previous studies where colchicine induced polyploid plants which have a larger leaf size, thickness and vigor than the diploid due to an increase into cell volume and the expression of genes (Thao et al., 2003). Similarly, pendimethalin (a microtubule polymerization interfering herbicide) has been reported to induce polyploidy at sublethal concentrations causing enhanced vegetative characters (Kumar et al., 2017). Polyploid plants often exhibit the gigas effect, characterized by increased cell size, enlarge nuclei, and enhance organ development such as larger leaves and stems. Artificially induced plants commonly develop larger vegetative structures due to increased cellular volume and enhanced metabolic activity and the gigas effect associated with chromosome doubling (Sattler et al., 2016). Moreover, colchicine induced polyploids exhibited improved morphological traits including leaf size, resulting from chromosome duplication and enhanced cell expansion (Mangena & Mushado, 2023).

It was revealed that the leaf width of tomato plants treated with 1.50mM colchicine, 5.0mM colchicine and 10µM pendimethalin (1.0cm, 1.2cm, and 1.1cm, respectively) were comparable to the length of tomato plants not treated with chemical mutagens ($p<0.05$). There may be an occurrence of mixoploidy (cytochimerism) where both diploid and polyploid cells coexist within the same plant in which mixoploid plants often exhibit intermediate growth responses because the enlarged polyploid cells are diluted by normal diploid cells. According to Sakhanokko et al. (2009), incomplete chromosome doubling frequently produces plants with morphological traits that overlap with the ones that are not exposed or treated with chemical mutagens. In contrast, the high pendimethalin dose (30µM) had a profound effect on leaf width (0.5cm), suggesting growth repression and potential phytotoxicity. Inhibition of mitotic activity can generate defects in cell division, or far-grain diversity reduce meristematic activity and thereby curtail organ growth (Hassan et al., 2020). The selective dose-dependent inhibitory effects of antimitotic agents on leaf development have been found in tomato and other solanaceous crops (Eng & Ho, 2019). It represents a dose-dependent response, in which moderate concentrations of mutagens promote leaf expansion and excess concentrations inhibit growth. A width increases at the two optimum concentrations of colchicine and pendimethalin is also in accordance with other traits associated to polyploidy such as larger mesophyll cells sizes or anatomical features related to photosynthetic capacity and stress resistance (Sattler et al., 2016). However, the reduction observed at higher doses indicates that mutagen dose should be optimized to provide an adequate balance between polyploid induction efficiency and plant viability.

The maximum average leaf area was observed in plantlets treated with 5.0 mM of colchicine and 10µM pendimethalin ($2.0cm^2$). Plantlets without the application of mutagen 1.5mM and 3.0 mM colchicine and 20µM pendimethalin ($1.2\ cm^2$, $1.3\ cm^2$, $1.3\ cm^2$, and $1.3\ cm^2$, respectively) had comparable leaf width while 30µM pendimethalin ($0.6cm^2$) caused the lowest size of the leaf area indicating a phytotoxic or growth inhibitory effect at high doses ($p<0.05$). The increase in leaf area encountered in plants treated with moderate colchicine and pendimethalin concentrations (5.0 mM and

10μM, specifically) is most likely due to the induction of polyploidy by colchicine that inhibits the formation of spindle fibers during mitosis resulting in chromosome doubling. It has been frequently reported that polyploid plants have larger cells, more cell volume and greater organ size such as leaf (Dhooghe et al., 2011; Talukdar, 2013). Increased leaf area is frequently accompanied by an increase in mesophyll cell size and in photosynthetic capacity, and a resulting better vegetative growth. Pendimethalin, is also a dinitroaniline herbicide disrupts the microtubule polymerization and mitotic spindle assembly similar to colchicine (Vaughn & Lehnen, 1991). The amount of increased leaf area detected at 10 μM pendimethalin may indicate that lower concentrations potentially can be used to induce chromosomal alterations without causing severe cytotoxicity. Similar results have been demonstrated by Kermani et al. (2003) that reported a better inclusion of vegetative characters at the adequate mutagen concentration. However, decrease of leaf area at 30μM pendimethalin shows phytotoxicity including enhanced mitosis disruption beyond a certain level leading to decreased cell division and metabolic functions. Risk has high dose causes chromosomal aberrations, low meristematic activity and growth reduction (Datta, 2009). The generally lower leaf area of non-mutated plantlets indicates that the mutagenic treatments applied at optimum can lead to a morphological improvement beyond the normal growth. It underpins the idea that induced polyploidy can be a useful element in crop improvement programs which target enhancement of vegetative vigor. The results indicate that levels of colchicine such as 5.0 mM and 10μM pendimethalin are optimum in improving leaf area with higher concentrations causing growth retardation. These observations are consistent with the literature and current established on chemical mutagenesis and induction of polyploidy, emphasizing the need for dose optimization to achieve beneficial morphological characteristics.

Plant height

There were significant differences observed among treatments ($p<0.005$). The 5.0 mM colchicine-applied tomato plants showed approximately the same height when compared to the control (4.7cm), followed by those applied with 1.5 mM (4.4cm) and then with 3.0 mM (3.9cm). In the pendimethalin-applied tomato plantlets, 4.4 cm height was recorded at 10μM, followed by 3.5 cm at 20μM, with the lowest value observed at 30μM (1.3cm), indicating a pronounced inhibitory effect on the growth. The dose-dependent nature of tomato growth inhibition caused by both colchicine and pendimethalin (acting genetically on synthesis and breakdown depending upon exposure to transformation and chemical) was shown through the pattern revealed. Colchicine is one example of an alkaloid that disrupts spindle fiber formation in mitosis, and it is commonly used for polyploidy induction purposes (duplication of chromosomes). The application of 1.5-3.0 mM, however, induces abnormalities in mitosis, which biologically translates into transient shrinkage, mediated by deregulatory effects on cell cycle control resulting in abnormal division (Nishu et al., 2021). The recovery in growth at 5.0 mM, however, could be due to both as a result of successful polyploidization and drug dosage but also larger cell sizes or increased vigor also been reported previously (Dhooghe et al. (2011). The dinitroaniline herbicide pendimethalin acts differently by inhibiting microtubule polymerization and inhibiting normal cell division (Chen et al., 2021). The observed reduction in length from 10μM to 30μM indicates more phytotoxicity that inhibits the meristematic activity of cells and elongation growth at higher concentrations. Notably, the ability of pendimethalin to retard growth at 30μM indicates that too high concentrations induced phytotoxic responses in tomato plantlets. As a dinitroaniline, pendimethalin inhibits the polymerization of microtubules and ultimately spindle formation in mitosis, inhibiting cell division and limiting root and shoot development. Consequently, their high concentrations can inhibit meristematic activity, interfere with tissue formation and/or greatly reduce plant vigor. Similar observations have been made in previous studies where prolonged or excessive treatment with antimitotic agents resulted in the inhibition of plant growth, even though chromosome doubling was successfully attained (Ramulu et al., 1991).

Table 1. Leaf length (cm), leaf width (cm) and leaf area (cm²) of tomato (Solanum lycopersicum L.) as applied with different concentrations of colchicine and pendimethalin

Morphological Characteristics of Tomato				
Source	**Leaf length (cm)**	**Leaf width (cm)**	**Leaf Area (cm^2)**	**Plant height (cm)**
Control	1.0^{b}	1.2^{a}	1.2^{b}	4.9^{a}
1.5 mM of Colchicine	1.3^{ab}	1.0^{a}	1.3^{b}	4.4^{ab}
3.0 mM of Colchicine	1.5^{ab}	0.9^{ab}	1.3^{b}	3.9^{bc}
5.0 mM of Colchicine	1.6^{a}	1.2^{a}	2.0^{a}	4.7^{ab}
10 µM of Pendimethalin	1.7^{a}	1.1^{a}	2.0^{a}	4.4^{ab}
20 µM of Pendimethalin	1.5^{ab}	0.9^{ab}	1.3^{b}	3.5^{c}
30 µM of Pendimethalin	1.2^{ab}	0.5^{b}	0.6^{c}	1.3^{d}
Total	**9.8**	**11.3**	**9.7**	**27.1**
Mean	**1.4**	**1.6**	**1.3**	**3.8**
c.v. (%)	**15.22**	**19.29**	**6.23**	**8.04**

Groups sharing a letter are not significantly different (Tukey HSD, $p<0.05$)

Stomatal Morphology

As presented in Figure 2a, the tomato plantlets without colchicine and pendimethalin showed the lowest stomatal length (23.1µm). Among the colchicine applications, 5.0mM produced the highest increase in stomatal length (34µm), followed by 3.0mM (22.4µm) and 1.5mM (20.8µm). By contrast, the stomatal length of all pendimethalin-applied tomato plants (10, 20 and 30µM) were 48µm, 46µm and 47µm. Elongation of stomata assayed under mutagenic applications indicated that colchicine and pendimethalin caused over-elongation of cells, perhaps due not only to increase in cell dimension but also polyploidization. Each of the two mutagens also causes abnormalities in mitosis in which microtubules do not form properly, chromosomes are doubled-then-lost and cells grow to abnormal sizes. Colchicine is a tubulin-binding agent that disrupts spindle fiber formation and characterized by endoreduplication, thereby increasing the nuclear DNA (Dhooge et al., 2011). Polyploid cells are typically larger and carry over to produce bigger guard cells, stomata. This is consistent with Kumar et al. (2020) who found that stomatal sizes of colchicine-treated *Vigna radiata* were larger than in the untreated control, verifying that polyploid cells have increased length/size. Likewise, 3-5 mM colchicine treatments in *Allium cepa* increased both stomatal length and width relative to diploid parent plants (Dhooghe et al., 2011). Pendimethalin, a dinitroaniline herbicide, inhibits microtubule polymerization and induces mitotic arrest and chromosome doubling in the same way as colchicine (Battacharya, 2017). It is well established that pendimethalin application leads to bigger stomata and thicker leaves in *Brassica napus*, which are associated with increased ploidy levels (Doyle & Coate, 2019).

The interaction between the application of colchicine and pendimethalin had a significant effect on stomatal width in tomato plantlet ($p<0.05$). A key finding was that tomato plantlets exposed to colchicine opened their stomata directly in a dose-response way. The stomatal width was similar from 1.5mM (17µm) and 3.0mM (18.4µm) of colchicine compared with the highest value from the treatment with 5.0mM colchicine (27.7µm). Stomatal width was increased with the application of pendimethalin, 10µM being the highest (41.5µm) which not significantly exceeded to those not applied without mutagens and almost all colchicine applications. The stomatal width was slightly but significantly reduced with 20µM (38.1µm) and 30µM(37µm) pendimethalin, 33% and 12%, respectively as compared to control. This study found that the two mutagens were able to increase stomatal size, particularly at moderate concentrations. All the mutagens increased stigma width as compared to control and indicate that colchicine and pendimethalin are responsible for cytological (ploidy) & physiological changes in all described tomato plants

considered. Colchicine is a mitotic inhibitor that binds to tubulin and inhibits spindle fiber formation thereby preventing segregation of the chromosomes, causing chromosome doubling and polyploidy (Dhooghe et al., 2011). In general, polyploid cells exhibit larger nuclear and cell size as seen in plants treated with colchicine for 24 h in the lemon grass (*Cymbopogon citratus*) leaves (Aina et al., 2012). Similarly, also dinitroaniline herbicide, pendimethalin disrupts microtubule polymerization and cell division causing chromosomal aberrations (Chen et.al, 2021). This disturbance at low levels may start chromosomal endoreduplication or partial polyploidization which create larger cells and stomata (Yao et al., 2023). But at higher doses, pendimethalin followed by stomatal aperture reductions may be due to cytogenetic toxicity and oxidative stress prevents the expansion and turgidity of cells (Gill, S.S., & Tuteja, N. 2010). This study provided evidence for the utility of colchicine and pendimethalin as inducers of stomatal traits as they were shown to induce chromosomal and cytological changes. However, as both mutagens are cytotoxic at high dose, derived by length of time and dosage required. The results are consistent with earlier reports demonstrating the biphasic, concentration-dependent effects of mutagens: beneficial at low concentrations and toxic above threshold levels (Datta, 2009). Pendimethalin was more effective than all these treatments at different concentrations, with maximum stomatal width recorded as 10µM (41.5 µm) when compared to the non-application of treatment and the colchicine treated plantlets. Moderate concentrations of both mutagens were found to increase size of stomata with respect to the control. The stomatal width of all the treated tomato plants was significantly increased and thus both colchicine & pendimethalin induced these cytological (ploidy) and physiological changes in tomato. It is a mitotic inhibitor, which works by binding to tubulin and inhibiting the formation of spindle fibers (Dhooghe et al., 2011), thereby preventing chromosome segregation and result in chromosome doubling or polyploidy as the outcome. Polyploid cells typically demonstrate an increased nuclear and cell size (e.g. in presence of colchicine on plant *Cymbopogon citratus* (Aina et al., 2012). This kind of disruption at the lower levels may lead to chromosomal endoreduplication or partial polyploidization, resulting in larger cell size and stomata (Yao et.al., 2023). In contrast, this could be due to cytogenetic toxicity and oxidative stress which limit the expansion and stability of cells at higher doses of pendimethalin (Chen et al., 2021). Results revealed that since they could induce chromosomal and cytological changes, thus colchicine and pendimethalin also confirmed as prior efficacy for augmenting stomatal features. Both types of mutagens become cytotoxic at high doses, necessitating that the concentration–time exposure be optimized. These findings support previous reports proposing that mutagens exert simultaneous or opposing concentration-dependent actions beneficial at lower and toxic at higher concentrations (Chen et al., 2021).

Table 2. Stomatal length (µm), width (µm) and aperture (mm²) of tomato (Solanum lycopersicum L.) as applied with different concentrations of colchicine and pendimethalin

Source	Stomatal Analysis			
	Stomatal length (µm)	**Stomatal width (µm)**	**Stomatal aperture (µm)**	**Stomatal density (mm²)**
No chemical mutagens	23.1^{c}	18.2^{c}	3.9^{c}	373^{a}
1.5 mM of Colchicine	20.8^{c}	17.0^{c}	4.0^{c}	289^{b}
3.0 mM of Colchicine	22.4^{c}	18.4^{c}	3.6^{c}	354^{a}
5.0 mM of Colchicine	34.0^{bc}	27.7^{bc}	3.8^{c}	293^{b}
10µM of Pendimethalin	48.9^{a}	41.5^{a}	12.6^{a}	88^{c}
20µM of Pendimethalin	46.6^{ab}	38.1^{ab}	10.3^{ab}	113^{c}
30µM of Pendimethalin	47.4^{ab}	37.0^{ab}	9.4^{b}	117^{c}
Total	**243.2**	**197.9**	**47.6**	**1627**

Source	Stomatal Analysis			
	Stomatal length (µm)	Stomatal width (µm)	Stomatal aperture (µm)	Stomatal density (mm²)
Mean	**34.7**	**28.2**	**6.8**	**232**
c.v. (%)	**14.28**	**15.07**	**13.61**	**7.77**

Groups sharing a letter are not significantly different (Tukey HSD, p<0.05)

The stomatal apertures in colchicine and pendimethalin applied plants were larger than that of not applied with chemical mutagens which were the smallest (3.9µm). An increased stomatal size was also observed in tomato when applied with 3.0mM and 5.0mM colchicine (4µm and 3.6µm, respectively) and 10µM pendimethalin (12.6µm), indicating that the mutagens had the potential to cause polyploid-associated morphological changes. During mitosis, colchicine disturbs the spindle microtubules between chromosomes and prevents chromosome separation causing a doubling of chromosomes (Dhooghe et al., 2011) and formation of polyploid cells. Nuclear DNA content is increased by polyploidy, and larger cell size (also enlarged guard cells) are frequently associated with wider stomatal apertures (Sattler et al., 2016). Pendimethalin, also a dinitroaniline herbicide sharing with trifluralin the microtubule-disruptive mechanism of action, was found to promote polyploidy and enlargement of stomata as well (Chen et al., 2021). The increase in aperture of the stomata observed in colchicine-applied plants are consistent with results from other reports on colchicine-induced polyploidy in wheat and ornamentals showing that larger stoma guard cells and wider stomatal pores occurred (Sattler et.al., 2016). Such results provide evidence that both colchicine and pendimethalin are capable to induce cytological cited by value morpho-structural changes in stomata characteristics in treated plants probably due to polyploidization mechanism which would influence physiological responses and adaptability of mutagen applied tomato plants.

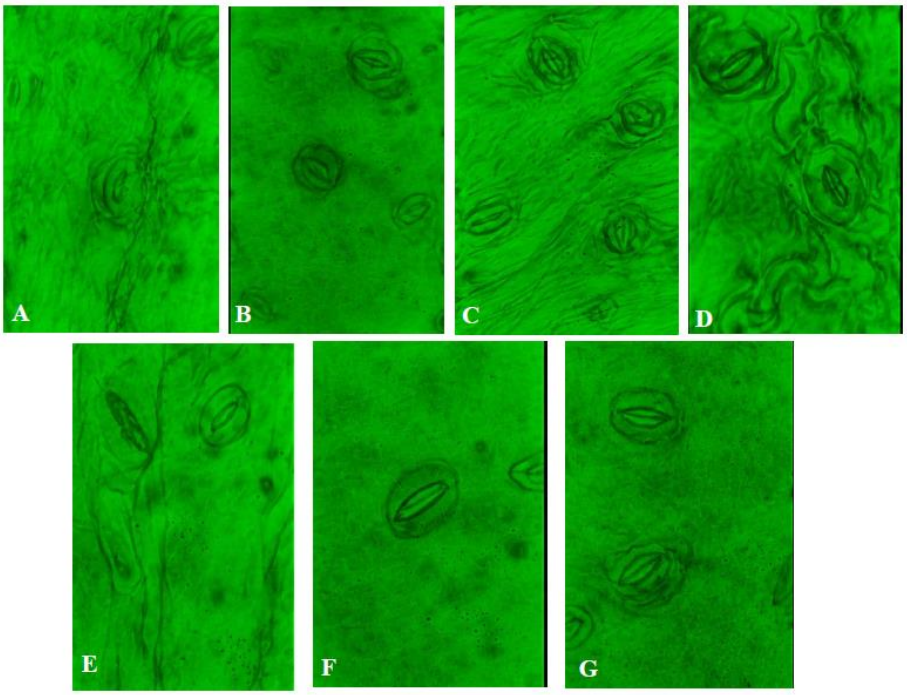

Figure 2a Stomatal morphology (length, width, and aperture) of tomato plants applied with chemical mutagens (colchicine and pendimethalin): A.) No application of chemical mutagens B.) 1.5mM Colchicine **C.)** 3.0mM Colchicine **D.)** 5.0mM Colchicine **E.)** 10µM Pendimethalin **F.)** 20µM Pendimethalin and **G.)** 30µM Pendimethalin

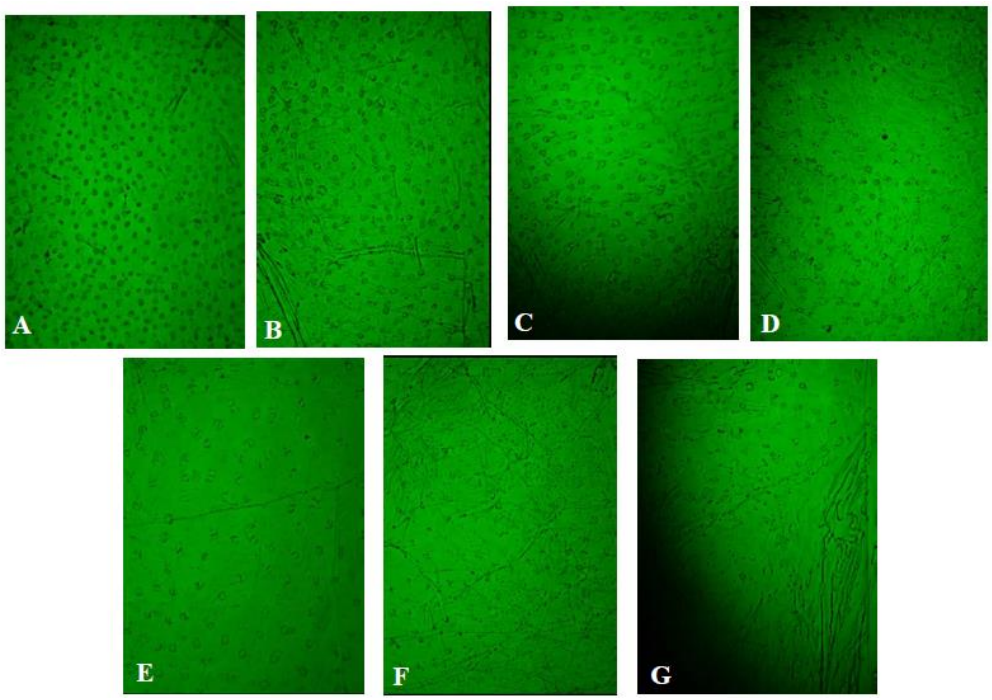

Figure 2b: Stomatal density of tomato plants applied with chemical mutagens (colchicine and pendimethalin): A.) No application of chemical mutagens B.) 1.5mM Colchicine C.) 3.0mM Colchicine D.) 5.0mM Colchicine E.) 10µM Pendimethalin F.) 20µM Pendimethalin and G.) 30µM Pendimethalin

As shown in Fig. 2b, both colchicine (5.0mM) and pendimethalin (10µM) applications affected the stomatal density which were 293mm^2 and 88mm^2, respectively, compared to without application of mutagens which showed highest stomatal density of 373 mm^2 ($p<0.05$). According to the normal anatomical features of diploid tomato leaves, their epidermal cells were relatively small and consequently maintained more stomata per unit area. Similar trends have been described in a number of diploid species in which high stomatal density is associated with smaller cell size and higher packing of epidermal cells (Beaulieu et al., 2008). The stomatal density of colchicine-treated tomato plantlets decreased significantly and predominantly under the higher concentrations. This response is commonly viewed as a characteristic of successful chromosome doubling and polyploidy induction. Colchicine interferes with microtubule formation at mitosis and causes an improper packing of metaphase chromosomes and thereby, results in polyploid cells (Dhawan & Lavania, 1996). Polyploid plants generally have larger epidermal and guard cells, which decreases the number of stomata per unit leaf area (Carputo et al., 2003).

Comparable decrease in the stomatal density and an increase in the size of stomata has been also noticed for colchicine-induced polyploids of tomato as well as other Solanaceae crops (Sattler et al., 2016). The results are in agreement with classical anatomical markers for polyploidization, such that good cytological changes caused by colchicine were induced in terms of stomatal development. The strongest reduction in the stomatal density of pendimethalin treatments was observed, with all concentrations (10-30µM) exhibiting similarly low values such as 88mm^2, 113mm^2, and 117mm^2, respectively. Pendimethalin adversely affects cell division through impaired microtubule polymerization resulting in mitotic arrest induction and modification of normal cellular organization (Vaughn & Lehnen, 1991). Despite being primarily employed as a pre-emergence herbicide, microtubule-disrupting herbicides have been shown to initially invoke polyploidy and large changes in plant tissue architecture (Morejohn et al., 1987). The consistent reduction in stomatal number resulting from

pendimethalin application suggests that the lowest pendimethalin concentration tested was at, or above, an androgenesis threshold level required to impair stomatal differentiation. This indicates that pendimethalin is more effective than colchicine in affecting development of epidermal cells and anatomy of leaf. The decrease in stomatal density caused by the two mutagens has significant physiological consequences. Reduced stomatal density is generally associated with high water-use efficiency, consistent with lower transpiration, which is typical of polyploids and stress-selected genotypes (Maherali et al., 2009). A decrease in stomatal density offers potential to limit maximum photosynthetic rates at high CO_2 concentrations under optimal growing conditions, since the number of available stomata are reduced, but a corresponding increase in size of the remaining stomata provides compensation (which is often observed in polyploid plants) to ensure that gas exchange is maintained (Sattler et al., 2016). In addition, polyploidy-associated structural changes are frequently associated with improved abiotic stress tolerance to drought and osmotic stresses (del Pozo et al., 2015).

Chlorophyll Content

The results showed that application of 5.0mM colchicine (44.04 mg/g^{-1}) resulted in the maximum content of chlorophyll followed by those treated with 20µM pendimethalin (41.56mg/g^{-1}), and 3.0 mM colchicine as well there after minimum content appeared in plantlets applied with 30µM pendimethalin was recorded (11.65 mg/g^{-1}). The increased chlorophyll at the lower dose of colchicine and pendimethalin may indicate that the compound promoted the construction of chloroplasts and induced synthesis of photosynthetic pigments. Colchicine, however, is known to induce polyploidy in several plant species by blocking microtubule assembly during mitosis leading to chromosome doubling and increased cell size (Dhooghe et al., 2011). Polyploid plants usually have higher chlorophyll content, more numbers of chloroplasts per cell and better photosynthetic efficiency than diploids (Eng et al., 2021). This explains the much higher chlorophyll content of plants when exposed to 3.0-5.0mM colchicine in our experiment. Similarly, the application of less to moderate level of pendimethalin 10-20µM (34mg/g^{-1} and 41mg/g^{-1}) also indicated induction in chlorophyll accumulation probably due to beneficial chromosomal and metabolic changes. Despite its usage as herbicide, pendimethalin has been shown to exhibit a mutagenic effect at sublethal levels on different kinds of organisms through changing the expression of genes concerned with pigment biosynthesis and photosynthetic activities (Dhooghe et al., 2021). In contrast, in higher concentrations (30µM) of pendimethalin, there was a reduction in chlorophyll contents significantly and which suggests a phytotoxicity effects. Such reduction might be due to oxidative stress, suppression in pigment synthesis or structural injury caused by excessive mutagenic stress on chloroplast membrane (Gill & Tuteja, 2010).

These findings suggest that optimal doses of colchicine and pendimethalin can positively influence chlorophyll biosynthesis and photosynthetic capacity in tomato plants. However, higher concentrations may exert inhibitory effects, highlighting the importance of optimizing mutagen dosage to achieve beneficial genetic variation without compromising plant physiological performance. Nevertheless, higher concentrations are inhibitory, emphasizing the need to determine a suitable mutagen dose that will induce favorable genetic variation without affecting plant physiology.

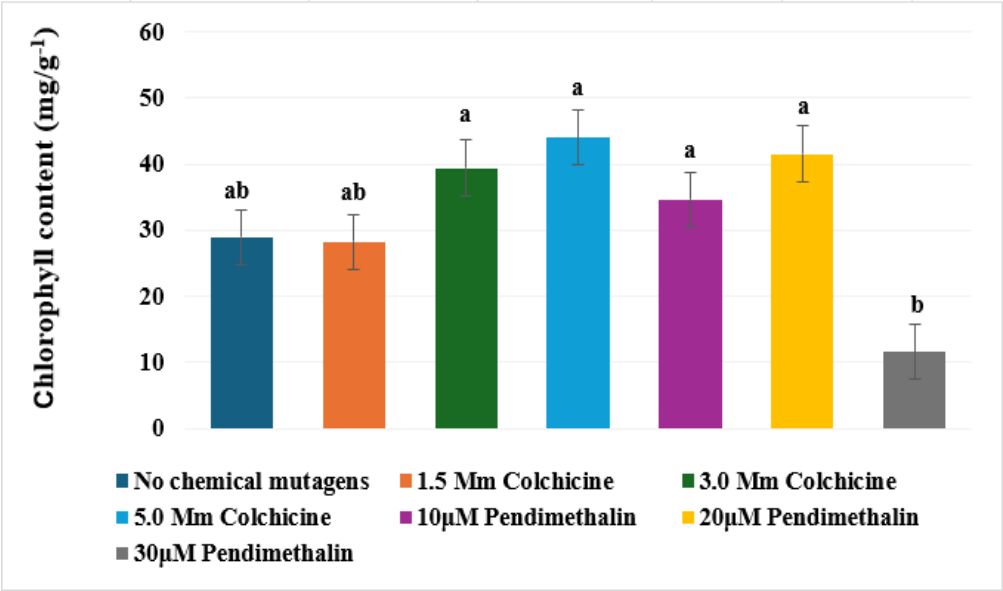

Figure 3. Chlorophyll content (mg/g-1) of tomato plant as treated with different concentrations of chemical mutagens, the colchicine and pendimethalin.

Conclusion

Significant effect on the survival, morphological and stomatal characteristics of tomato plantlets grown in vitro with the application of colchicine and pendimethalin. The plantlet survival, leaf development, stomatal size and chlorophyll which indicates a successful enhancement of polyploidy and improved vegetative vigor. However, with the higher concentrations of pendimethalin particularly 30µM caused growth inhibition and phytotoxic effects. Furthermore, both chemical mutagens reduced stomatal density while increasing stomatal dimensions indicating chromosome doubling. Generally, optimized mutagen concentrations can be effectively utilized to improve tomato growth traits. The study affirmed that colchicine and pendimethalin had a significant effect on the survival, morphological and stomatal characteristics of tomato plants grown in vitro. Moderate concentrations of colchicine and pendimethalin enhanced explant survival, leaf development, stomatal size and chlorophyll content which indicates a successful enhancement of polyploidy and improved vegetative vigor. However, with the higher concentrations of pendimethalin particularly 30µM caused growth inhibition and phytotoxic effects. Furthermore, both chemical mutagens reduced stomatal density while increasing stomatal dimensions indicating chromosome doubling. Generally, optimized mutagen concentrations can be effectively utilized for polyploidy induction thus improving tomato growth traits.

References

Aina, O.O., Quensberry, K,H., & Gallo, M. (2012). Colchicine-induced morphological and cytological changes in lemon grass (Cymbopogon citratus (DC.) (Stapf.). African Journal of Biotechnology, 11(3), 618-625. https://doi.org/10.5897/AJB10.2394.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in Beta vulgaris. Plant Physiology, 24(1), 1-15. https://doi.org//10.1104/pp.24.1.1. https://doi.org/10.1104/pp.24.1.1.

Beaulieu, J.M., Leitch, I.J., Patel S, Pendharkar A & Knight, C.A. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* 179(4): 975-986. https://doi.org/10.1111/j.1469-8137.2008.02528.

Calabrese EJ & Baldwin LA. (2003). Hormesis: The dose-response revolution. *Annual Review of Pharmacology and Toxicology*.

https://doi.org/10.1146/annurev.pharmtox.43.100901.140223.

Carputo D, Barone A, Frusciante L & Ricciardi L. (2003). Morphological and cytological characterization of colchicine-induced polyploidy in Solanum species. *Euphytica, 130*, 259–265. https://10.1002/9780470650202.ch2.

Eng, W. H., Ho, W. S., & Tan, S. G. (2021). Pendimethalin as an alternative antimitotic agent for polyploid induction in plants. *Plants, 10*(7), 1431. https://doi.org/10.3390/plants10071431.

Eng, W., & Ho, W. (2019). Polyploidization using colchicine in horticultural plants: A review. Sci. Hortic.-Amst., 246, 604–617. https://doi.org/10.1016/j.scienta.2018.11.010.

Datta, S.K. (2009). Role of induced mutagenesis for mdvelopment of new varieties in floriculture. Acta Horticulturae, 836, 151-166. https://doi.org/10.17660/ActaHortic.2009.836.18.

del Pozo, J.C., & Ramirez-Parra, E. (2015). Whole genome duplications in plants: An overview from Arabidopsis. Journal of Experimental Botany, 66(22), 6991-7003. https://doi.org./10.1093/jxberv432.

Dhawan, V., & Lavania, U.C. (1996). Enhancement of plant secondary metabolites by mutagenic treatments. *Plant Cell, Tissue and Organ Culture, 44*, 45–55. https://doi.org./10.1007/BF00021884.

Dhooghe, E., Van Laere, K., Eeckhaut, T., Leus, L., & Van Huylenbroeck, J. (2011). Mitotic chromosome doubling of plant tissues in vitro. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 104(3), 359–373. https://doi.org/10.1007/s11240-010-9786-5.

Doyle, J.J., & Coate, J.E. (2019). Polyploidy, the nucleotype, and novelty: The impact of genome doubling on plant growth and physiology. Current Opinion in PlantBiology, 49, 1-7. https://doi.org/10.1016/j.pbi.2019.02.001.

Gill, S.S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48 (12), 909-930. https://doi.org/10.1016/j.plaphy.2010.08.016.

Hassan, J., Miyajima, I., Ozaki, Y., Mizune, Y., Sakai, K., & Wasimullah, Z. (2020). Tetraploid Induction by Colchicine Treatment and Crossing with a Diploid Reveals Less-Seeded Fruit Production in Pointed Gourd (*Trichosanthes dioica* Roxb.). https://doi.org/10.3390/plants9030370.

Kermani, M.J., Sarasan, V., Roberts, A.V., Yokoya, K. & Wentworth, J. (2003). *Oryzalin-induced chromosome doubling in Rosa and its effect on plant morphology and pollen viability. Theoretical and Applied Genetics*, 107(7), 1195–1200. https://doi.org/10.1007/s00122-003-1374-1.

Kumar, V., Singh, G., & Sharma, S. (2022). Surface sterilization for isolation of endophytes: Ensuring sterility while maintaining microbial culturability. *Journal of Basic Microbiology, 62*(3), 263–287. https://doi.org/10.1002/jobm.202100462.

Lehrer, J.M., Brand, M.H., & Lubell, J.D. (2008). Induction of tetraploidy in meristematically active seeds of Japanese barberry (*Berberis unbergii* var. Atropurpurea) through exposure to colchicine and oryzalin. *Scientia Horticulturae,* 119:67–71. https://doi.org/10.1016/j.scienta.2008.07.020.

Maherali, H., Walden, A.E., & Husband, B.C. (2009). Genome duplication and the physiology of polyploid plants. *New Phytologist, 183*(3), 751–760. https://doi.org./10.1111/j.1469-8137.2009.02997.x.

Mangena, P., & Mushadu, N. (2023). Colchicine-induced polyploidy in leguminous crops enhances morpho-physiological characteristics for drought stress tolerance. Life, 13 (10), 1966. https://doi.org./10.3390/life13101966.

Matias, K.M.L., Sotto, R.C., Bautista, N.S., Protacio,C.M., & Magdalita P.M. (2024). Influence of Drought Stress and Foliar Application of Salicylic Acid on Early Vegetative Stage of

Cacao. Philippine Journal of Science, 153 (2).

Morejohn, L.C., Bureau, T.E., Mole-Bajer, J., Bajer, A.S., & Fosket, D.E. (1987). Oryzalin, a dinitroaniline herbicide, binds to plant tubulin and disrupts microtubules. https://doi.org./10.1126/science.3576224.

Mulyana, A., Purwoko, B.S., Dewi, I.S., & Maharijaya, A. (2023). In vitro diploidization of haploid plantlet from anther culture of eggplant (Solanum melongena L.) using pendimethalin. *SABRAO Journal of Breeding and Genetics*, 55 (1) 115-122. https://doi.org/10.54910/sabrao2023.55.1.11.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. 15(3): 473- 497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Ramulu, K.S., Verhoeven, H.A., & Dijkhuis, P. (1991). Mitotic blocking, micronucleation, and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato. *Protoplasma* 160, 65-71. https://doi.org/10.1007/BF01322611.

Sajid M., & Afab, S. (2013). Low dose pendimethalin mediated polyploidy induction in plant tissue cultures. *Journal of Agricultural Science*, 5(2), 27-35.

Sakhanokho, H.F., Rajasekaran, K., Kelley, R.Y., & Islam-Faridi, M.N. (2009). Induced polyploidy in diploid ornamental ginger (Hedychium muluense R.M. Smith) using colchicine and oryzalin. HortScience, 44(7), 1809-1814. https://doi.org./10.21273/HORTSCI.44.7.1809.

Sattler, M.C., Carvalho, C.R., & Clarindo, W.R. (2016). The polyploidy and its key role in plant breeding. *Planta*, 243, 281–296. https://doi.org/10.1007/s00425-015-2450-x.

Singh, B., Yun, S., & Park, M.H. (2025). The role of colchicine in plant breeding. *International Journal of Molecular Sciences, 26*(14), 6743. https://doi.org/10.3390/ijms26146743

Talukdar, D. (2013). Cytogenetic characterization of induced autopolyploidy in plants. *Plant Systematics and Evolution*, 299, 631–646. https://doi.org/10.5772/55481.

Thao, N.P., Nakashima, A., & Tanaka, K. (2003). Colchicine-induced polyploidy in ornamental plants: Physiological and morphological outcomes. *Plant Cell Reports, 21*(12), 1151–1156. https://doi.org/10.1023/A:1021280611527.

Tossi, Valeria E.., Nichols, L. A., & other authors. (2022). *Impact of polyploidy on plant tolerance to abiotic and biotic stresses*. Frontiers in Plant Science, 13, 869423. https://doi.org/10.3389/fpls.2022.869423.

Vaughn, K.C., & Lehnen, L.P. Jr. (1991). Mitotic disrupter herbicides. Weed Science, 39(3), 450-457.

Yao, P.Q., Chen, J.H., Ma, P.F., Xie, L.H., & Cheng, S.P. (2023). Stomata variation in the process of polyploidization in Chinese chive (Allium tuberosum). BMC Plant Biology, 23, 595. https://doi.org/101186/s12870-023-04615-y.

Zakariyya, F.M., Setyawan, B., & Wahyo, S. (2017). Stomatal, Proline and Leaf Water Status Characters of Some Cocoa Clones (Theobroma cacao L.) on Prolonged Dry Season. Pelita Perkebunan: a Coffee and Cocoa Research Journal 33(1): 109-117. https://doi.org/10.3390/foods12118351 9.