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Research Article

The Effects of Colchicine on the Mitotic Behaviour of Selected Indigenous Tomato (*Solanum Lycopersicum* L.) Accession

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Abstract
Colchicine is among the chemical mutagens that are widely used for inducing mutations in plants. This study was carried out to observe the mutagenic effect of colchicine on the chromosomal characteristic of tomato cells. Seeds of tomato accession (NG/SA/07/10/002-NorthEast) were treated with five different colchicine concentrations (0.1mM, 0.3mM, 0.5mM, 1.0mM) and 0.0mM as control. Cytological examination revealed that colchicine was found to induce changes in the chromosomal characteristics of tomato cells in the mitotic stage of development. Different types of chromosomal anomalies such as chromosomal clumping, condensation, fragmentation and chromosomal stickiness were observed. Somatic chromosome number were found to be 2n=24 in control cells and 2n=48 in colchicine treated cells indicating polyploidy.

Keywords: *Colchicine, Mitotic, Mutation, Polyploidy, Tomato*.

Introduction

Solanum lycopersicum (2*n*=24) is the edible, often red berry-type fruit of the nightshade or *Solanaceae* family which includes more than 3,000 species, occupying a wide variety of habitats (Bhala and Verma, 2018). It arises from the ovary of the plant after fertilization and its flesh is made up of pericarp walls. It has hollow spaces full of seeds and moisture called locular cavities (Krishna, 2007). It is a usually sprawling plant, herbaceous, as its close cousins are tobacco, eggplant and chilli peppers.

Ketchups, stew, sauces, salads, and drinks are diverse ways tomato is being consumed as well as used as ingredients in many dishes and also made into paste for canned tomato, (Babalola *et al*., 2010). Mutation is the basic source of heritable variation among species and induced mutation has been used universally and quickly to produce new varieties of crop. Induce mutation has led to exceptional advances in crop improvement by being able to change specific qualities in varieties in a limited amount of time by introducing some relevant variant (Aggarwal, *et al*., 2022). The development of crop plants rely to a large extent on genetic variability within the species and these changes in the sequence and gene structure can be arbitrarily caused by rupturing the DNA through chemical and physical mutagens (Viana *et al*., 2019).

Induced mutagenesis is a system where mutagenic chemical are used to create mutation in

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plants for the improvement of their agronomic traits. It is one of the most important tools used to study the nature and function of genes, which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic valued crops (Girma *et al*, 2014). Chemical mutagenesis causes the formation of polyploidy in plants (crops) when seeds are exposed to chemicals or radiation thereby generating mutants with desirable traits to be bred with other cultivars. The technology of induced mutagenesis plays an important role in creating genetic variations among crop species where hybridization is very challenging by improving one or two quantitative characters without affecting the rest of the genotypes (Khark and Shu, 2010). This technology has been used to improve major crops such as rice, wheat, sesame, pepper, peanut which are cultivated through seeds.

Increasing favorable mutations in plants is accomplished using various chemical mutagenic agents such as colchicine, paradichlorobenzene, dinitroanilines and hydroxylamine hydrochloride (Girma *et al*., 2014). The utilization of these physical and chemical mutagenic agents has resulted in significant progress in enhancing crops, including improvements in height, disease resistance, yields (vegetative/seed), and nutritional qualities (Ntuenibok *et al*., 1995, and Iwo *et al.*, 2015). This method allows for the introduction of desired traits that may not naturally occur or have been lost during evolution. Colchicine, a powdery mutagenic agent, is specifically used for inducing polyploidization and inhibiting the mitotic process to enhance crops (Manzour *et al.*, 2020). It appears as a white to yellow powder and is soluble in water at a concentration of 10mg/ml. With a molecular formula of $C_{22}H_{25}NO_6$ and a weight of 399.44g, it has been observed to influence plant physiology, leading to polyploidy, which is essential for crop improvement.

Polyploidy is the possession of three or more complete sets of chromosomes in eukaryotic organisms such as yeast, plants, fishes, and even the mammalian genome (Ramsey and Schemske, 1998). Cytology, which is the study of cells of living organisms, can reveal the effects of mutagens in crops. Cells that come into contact with mutagens often exhibit evidence of polyploidy, as observed in forage sorghum cultivars (Maria and Sakunkan, 2018). Polyploidy refers to the condition where a typically diploid cell or organism gains one or more additional sets of chromosomes. Generally, polyploids have larger cells compared to their diploid counterparts, sluggish metabolism and growth, altered genomic interactions between the nucleus and cell wall, and a reduced number of cell divisions during development (Sander *et al.*, 2019). Chromosome doubling can occur either in the zygote, resulting in a completely polyploid individual, or locally in some apical meristems to give polyploid chimeras. Somatic polyploidy is seen in some non-meristematic plant tissues as well (Ramsey and Schemske, 2002). In somatic doubling, the main cause is mitotic non-disjunction which may occur in purely vegetative tissues (as in root nodules of some leguminous plants) or at times in a branch that may produce flowers or in early embryos (and may therefore be carried further) (Grant, 1981).

Spontaneous somatic chromosome doubling is a rare event and the only well documented instance of the same was in case of tetraploid *Primula kewensis* which arose by somatic doubling in certain flowering branches of a diploid hybrid (Lewis, 1980) Therefore, this study becomes necessary to assess the mutagenic effect of Colchicine on mitotic behaviours of selected indigenous tomato accession.

Materials and Methods

Source of Materials: The tomato accession (NG/SA/07/10/002-North East) was used for this study due to its farmers and consumers preference. It was collected from National Centre for Genetics Resources and Biotechnology (NAGRAB), Ibadan and the chemical mutagen, (Colchicine) was obtained from Sigma Company, India.

Certification of Seed Viability: The seed viability test was carried out before the application of colchicine using floating method (Falusi *et al.*, 2012) and germination test was carried out using the method of Stephen (2009). The collected matured air-dried seeds of tomato were presoaked in distilled water for one hour to collect settled seeds which were later treated with different

concentrations of colchicine. Ten seeds (for each replicate) were spread on three separate petri-dishes containing moist filter paper and partially covered in the Tissue Culture Laboratory, NBRDA. The experiment was monitored on daily basis for ten days to ensure seed viability.

Mutagenic Treatment of Seeds: Treatment of seeds with colchicine was done under the fume cupboard in the tissue culture laboratory, Agricultural Biotechnology Department, National Biotechnology Research Development Agency (NBRDA), Abuja. One molar stock solution of colchicine was prepared in g/ml (that is, 1.0g of colchicine dissolved in 2.50ml of distilled water). Four different concentrations, 0.1mM, 0.3mM, 0.5mM and 1.0mM were prepared in 50ml distilled water from the stock solution using serial dilution method. The seeds were soaked in each prepared concentration (Falusi *et al*., 2014) as well as distilled water to serve as control for 24 hours at room temperature (Girma *et al.*, 2014). The treated seeds were rinsed thoroughly with distilled water and partly dried.

Germination Test: Fifty seeds each of three replicates for control and treated tomato were placed on moist filter paper in petri-dishes for ten days to germinate and the numbers of germinated seeds were counted. The percentage germination was then calculated using the formula below;

$$\% \text{ germination} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds used}} \times 100$$ (Udofia *et al*.,2022)

Statistical Analysis: The collected data were analyzed statistically following the analysis of variance (ANOVA) at a 5% probability level. Duncan multiple range tests were employed to distinguished between means in cases where difference were observed.

Cytological Study: This study was carried out in the Biological science laboratory, federal University of Technology, Minna, Niger state. About 1.0cm to 1.5cm growing root tips were harvested before 11am and fixed in Paradichlorobenzene for some minutes to condense the chromosome. It was further fixed (or preserved) in freshly prepared 1:3 glacial acetic alcohol for 24 hours. The fixative was decanted and the root tips were subsequently rinsed with distilled water and then stored in 70% ethanol for further examinations. Root tips were hydrolyzed with warm 1 normal HCL for 15minutes and rinsed with distilled water (Abubakar *et al*., 2015). The root tips were further macerated. This was done by placing two drops of 2% aceto orcein stain on a glass slide and 2mm end of the root tips were placed on the stain. The absorption of stain enables the visibility of the chromosomes when viewed under an Olympus trinocular microscope. Cover slips were placed on the stained root tips follow by filter paper to enable squashing while the filter paper mopped up excess stain leaving a clean clear slide. Squashing was done by applying pressure gently on the glass slide using the thumb to flatten the cells, revealing the chromosome structure, number etc. The slides were then viewed under a trinocular microscope starting from the low power objective to the high power objective. Stages of mitosis and chromosomal aberrations were observed and then micro-photographs were taken (Abubakar *et al*., 2015).

Results and Discussion

The Effects of Colchicine on germination: The results of the study indicated significant difference between the lower concentrations and the highest concentration. Significant variations ($P>0.05$) were observed among the treatments (Table 1), with the 0.1mM and 1.0mM, 0.3mM and 1.0mM as well as 0.5mM and 1.0mM treatments recording the highest mean value of 93.33%, 86.7% and 93.33% respectively. However, the lowest germination percentage of 53.33% was observed in the 1.0mM (highest) treatment, which differed significantly from the other treatments. This result highlights that the 1.0 mM concentration has a notably different effect on germination percentage compared to several other concentrations, which may warrant further investigation.

The observed response demonstrated a decrease in germination percentage with an increase in Colchicine treatment (Table 1). This finding aligns with the research conducted by Adelanwa and Habeeb (2011), who observed a highly significance difference for germination percentage in the Roma VF tomato variety when subjected to

4.0mM colchicine treatment. Also, the research on the effect of sodium azide on groundnut by Mensah and Obadoni (2007) reveals a decrease in survival percentage with increase concentration of the mutagen. The reduction in the germination percentage mean performance of mutagenic treatments has been attributed to the delayed or inhibited physiological and biological processes necessary for seed germination, including enzyme activity, hormonal imbalance and inhibition of mitotic process (Salim Khan, 2009).

The Effects of Colchicine on the Mitotic Behaviour of Tomato Accessions: Mitotic observations in cells of the treated seeds and the control are presented in plates I and II. Plate I shows the different phases of cell division with 2n=24 number of chromosomes in a diploid cell (control). The chromosomes were generally small in size. On the other hand, plate II shows some abnormalities as a result of mutation. The observed abnormalities induced by colchicine were: chromosomal clumping at metaphase, chromosomal stickiness and the doubling of chromosome (2n=48) at prophase.

The result of the cytological analysis confirm the presence of diploid number of chromosome 2n=24 as reported by earlier authors (Charles and Butler, 2008). Abnormalities and irregular mitotic cell division such as doubling of the chromosome number as observed in this study is similar to the findings of Obute *et al.* (2005). They observed an exact addition of two somatic genomes in *Solanum aethiopicum* giving 2n = 2x = 48 and an abnormal increase in number of chromosomes 2n= 2x = 69 in *S. indicum* treated with colchicine where their diploid cells have 2n=24 and 2n=26 respectively. The chromosome doubling resulted to increase in stomata density, size and chloroplast count in guard cells (Tammu *et al*., 2021). This phenomenon is collectively referred to as the gigas effect (Acquaah, 2002).

Clumping of chromosome observed at metaphase in this study is in accordance to the results of Al-Ahmadi, (2013) and Alam Qamre *et al*., (2022) who observed clumping and stickiness of chromosomes of mutant *Allium cepa* and *Triticum aestivum* L. respectively. Appearance of the clumping and stickiness might be due to the inactivation of the spindle followed by a random scattering of the chromosomes over the cell (Auti *et al.*, 2010). Similarly, mitotic investigations of *Lablab purpureus* (L.) Sweet recorded stickiness as one of the chromosomal aberrations as studied by Jagtap and More (2019). This aberration is characterized by sticky clumps of chromatin which brings about sterility. It was suggested that the chromosomal aberrations developed due to physiological and chromosomal damage caused by the mutagen.

However , the clumping and stickiness of chromosomes have been associated with the impact of chemical compounds on the physical-chemical properties of DNA, protein, or both, as well as on the formation of complexes with phosphate groups in DNA, DNA condensation, or the creation of inter and intra chromatid cross-links (Turkoglu, 2007). The cause of clumping and stickiness of chromosomes could also be a disturbance in nucleic acid metabolism in the cell, as indicated by Chidambaram *et al.* (2009). Additionally, according to Obute *et al.* (2005), colchicine leads to the destruction of the spindle, resulting in the interruption of anaphases and the accumulation of metaphases where chromosomes shorten, thicken, and split, ultimately causing clumping and stickiness of chromosomes observed in this study.

Conclusion

In conclusion, cytological analysis is recognized as a reliable method to evaluate the mutagenic potential of a substance by examining the frequency of cellular changes it induces in plants and provides the better prospects of selecting the appropriate mutagenic dosage that could induce desired characters in order to improve plant breeding

The present study indicated that tomato cells at mitotic stage were evidently influenced by the doses of colchicine used in this study. The cytological examination also confirmed the mutagenic effect of colchicine on tomato plant cell although fewer clearer photomicrographs were obtained; there were clear abnormal behavior of the cells at mitosis. These chromosomal aberrations include clumping and stickiness of chromosomes at metaphase and chromosome doubling at prophase which shows

the importance of colchicine in mutation breeding for crop improvement. This study affirms that colchicine induced plants produced effective and beneficial traits that can enhance crop improvement in plant breeding.

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Table 1: Effect of Colchicine on Germination % of Tomato

Concentration (mM)	Germination Percentage			
	Rep 1	Rep 2	Rep 3	Mean %
0	80	80	80	80.0[a]
0.1	80	100	100	93.33[a]
0.3	100	80	80	86.67[a]
0.5	100	100	80	93.33[a]
1.0	80	20	60	53.33[b]

Values followed by the different superscript down in a column are significantly different $p<0.05$.

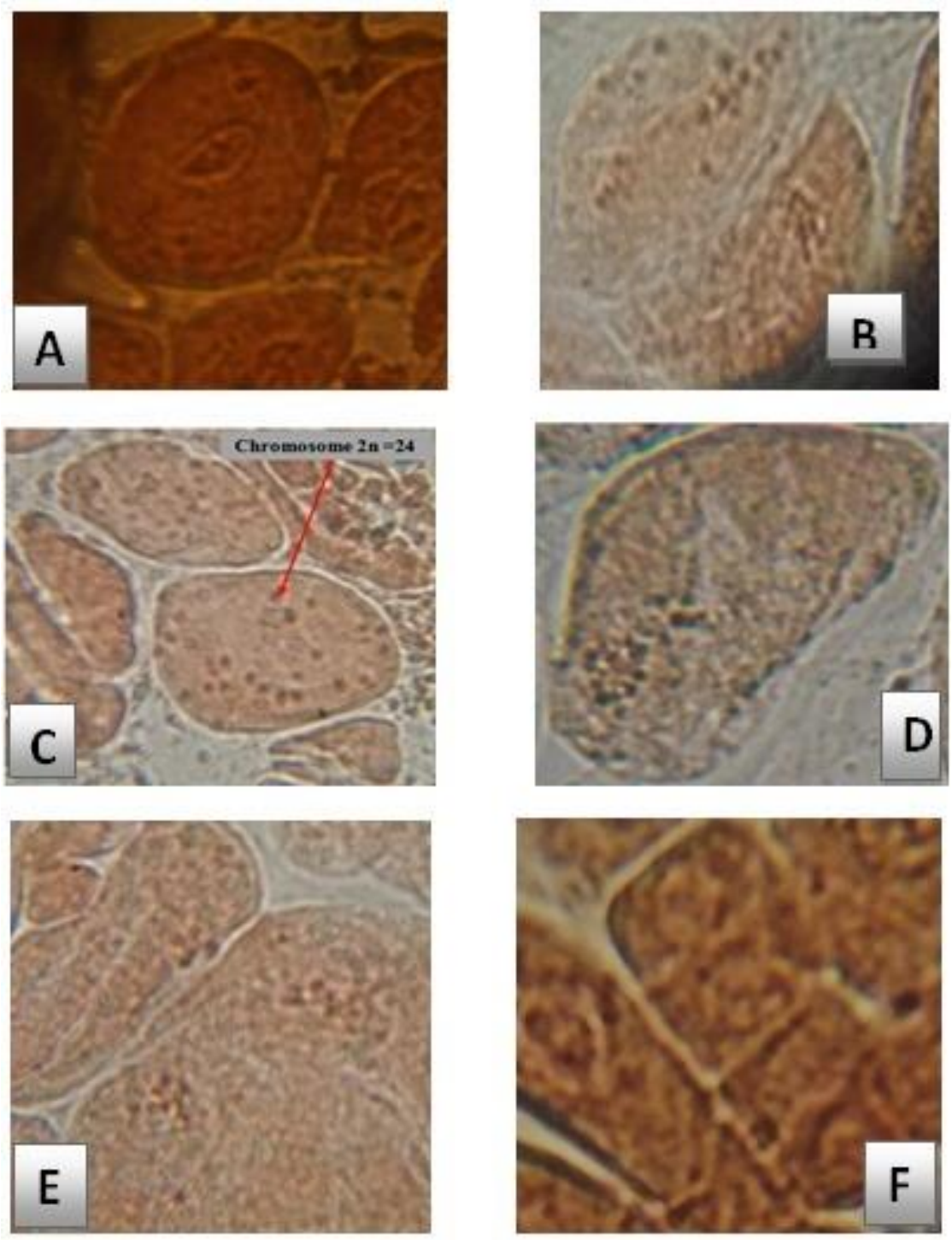

Plate I: Mitotic Behaviours of Untreated Tomato (control) Accession: A– Interphase; B– Late Interphase and Early Prophase; C– Prophase chromosome 2n = 24; D – Metaphase; E – Anaphase; F – Early telophase.

Source: Field Photograph

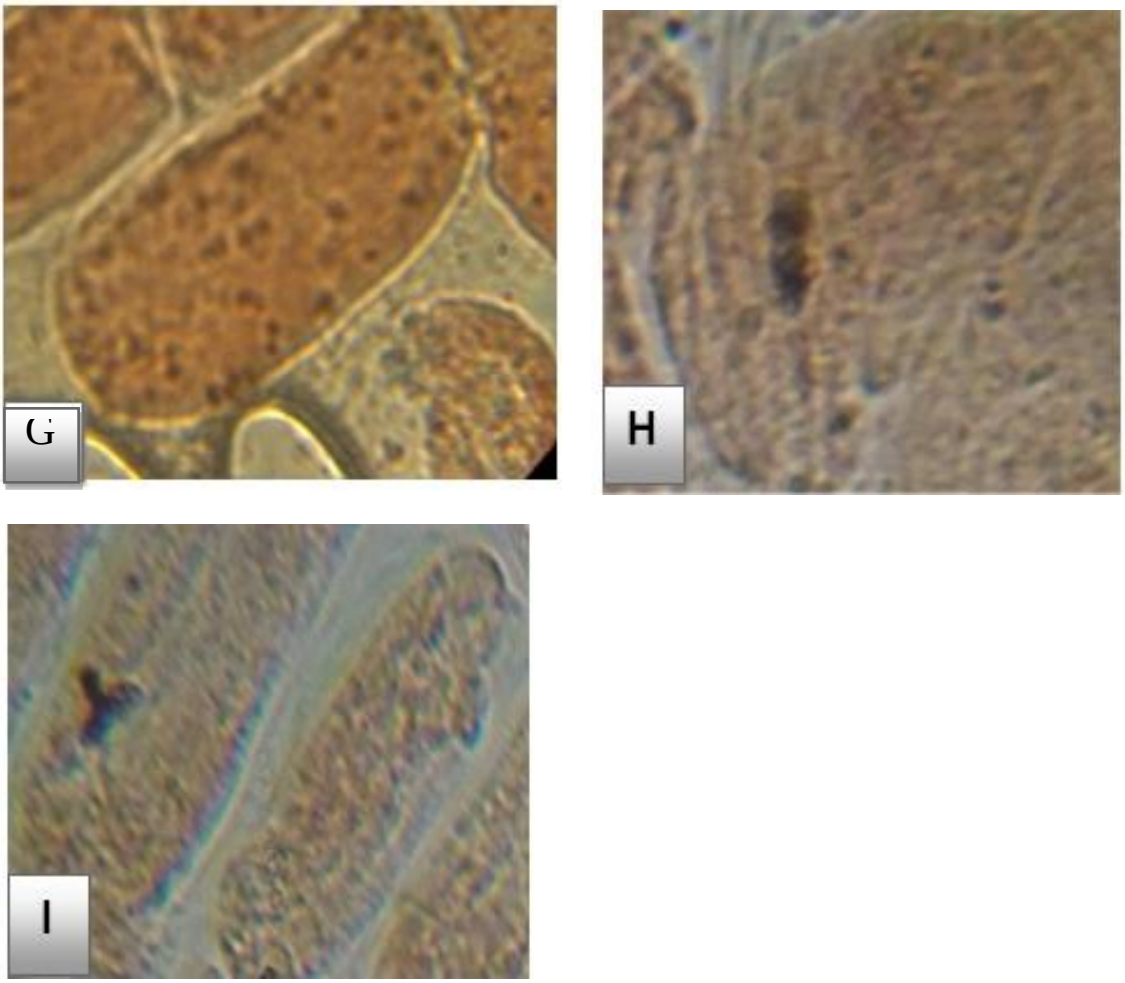

Plate II: Mitotic Behaviour of Colchicine Treated Tomato. G- Chromosome 2n=48 at Prophase; H and I–Clumping and stickiness of chromosomes at metaphase indicating abnormalities caused by mutation

Source: Field Photograph