Polyploid Induction in *Centella asiatica* (L.) Urban by Colchicine Treatment

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Diploid Asiatic pennywort, *Cantella asiatica* (L.) Urban, underwent a polyploid induction procedure using 1 and 2% colchicine. Two methods of application were used, one by placing seedlings on colchicine-saturated cotton balls, the other by placing colchicine-containing agar on seedling shoot tips. Results showed that the cotton ball method with 2% colchicine gave the highest rate of polyploid induction, while no significant difference was obtained by the agar method. Polyploid plants differed from the diploid ones in the observable phenotypes and containing a higher number of guard cell chloroplasts.

Key words: Asiatic pennywort, *Centella asiatica,* colchicine, polyploid.

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การชักนำให้เกิดพอลีพลอยด์ในต้นบัวบก โดยใช้สารโคลชิซีน

วรวุฒิ จุฬาลักษณานุกูล และ วิสา ฉิมน้อย (2542)
วารสารวิจัยวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **24 (2)**

ได้ทำการชักนำต้นบัวบกชนิดดิพพลอยด์ให้เป็นต้นพอลิพลอยด์โดยใช้โคลชิซีน และทำการทดลองชักนำ 2 วิธี คือหนึ่งใช้สำลีชุบสารละลายโคลชิซีน 1-2% วางลงบนต้นอ่อน วิธีที่สองโดยผสมโคลชิซีน 1-2% กับวุ้นแล้ววางลงบนปลายยอดของต้นอ่อน พบว่าการใช้สำลีชุบสารละลายโคลชิซีน 2% วางบนต้นอ่อน สามารถชักนำให้เกิดพอลีพลอยด์ดีที่สุด ในขณะที่การใช้วุ้นผสมโคลชิซีนไม่ว่าจะเป็น 1 หรือ 2% ให้ผลการชักนำไม่ต่างกัน ต้นพอลิพลอยด์ที่เกิดขึ้นแสดงลักษณะแตกต่างจากต้นดิพพลอยด์ จำนวนคลอโรพลาสต์ในเซลล์คุมและจำนวนโครโมโซมในเซลล์ของต้นพอลิพลอยด์สูงกว่าต้นดิพพลอยด์

คำสำคัญ บัวบก, โคลชิซีน, พอลิพลอยด์

INTRODUCTION

The herbaceous Asiatic pennywort plant, *Centella asiatica* (L.) Urban, is classified as a vegetable and thought to have some herbal properties in the reduction of blood pressure, fever, bacterial and fungal infection, and inhibition of cancerous cells.[1] There also have been reports on the effect of this plant in healing stomach infection and stimulation of immune response in humans.[2]

Polyploidy is one of the successful methods in the improvement of economic crops such as wheat, oats, cotton, coffee, apples, roses, and bananas.[3] The process can occur naturally or through human manipulation. In general, polyploid plants exhibit superior phenotypes to those of diploids such as stronger stems, and thicker and larger leaves, flowers, fruits, and seeds. Polyploid induction can be used as a means to create and select new and better breeds for further use.[4] In order to produce polyploid plants, the chemical colchicine is widely used because of its effectiveness, abundance and being non-toxic to cells.[5] By producing polyploid *C. asiatica,* the production of better-quality herbal substances from its extract will be possible and more economically feasible.[6]

Specific objectives for this experiment were to determine the effective concentration and appropriate method of colchicine application in the polyploid induction of *C. asiatica*. Characterization of the obtained polyploid plants was also conducted. The overall objective of this research was to produce polyploid *C. asiatica* stock plants for future breeding and propagation programs.

MATERIALS AND METHODS

Colchicine Treatment

One hundred *C. asiatica* plants were divided into four treatmentsets and a control set, at 20 plants each. In the first treatment, a drop of agar containing 2% colchicine was placed on seedling shoot tips. The second treatment received a similar treatment to the first except the concentration of colchicine used was 1%. In the third treatment, cotton balls saturated with 1% colchicine were placed on seedling shoot tips. The last treatment was similar to the third one but with 2% colchicine. The cotton balls were then covered with aluminum foil. All treatments were left for 6 days.

Number of Guard Cell Chloroplasts

Sample tissues of leaf epidermis were placed on microscope slides and the size and density of guard cells were measured. To confirm that the obtained plants were indeed polyploid, 1% $AgNO_3$ solution was added to the peeled surfaces of the samples in order to count the number of guard cell chloroplasts.

Cytological Observation

Root tips of the *C. asiatica* plants were dissected and stained for chromosomal studies using hematoxylin staining.[7] Tip sections of 1-1.5 cm in length were fixed in Carnoy's solution (ethanol: acetic acid = 3:1). The samples were left in the fixative at room temperature for 2 hours and then preserved at 4°C in 70% ethanol solution. In order to conduct cytological studies, the preserved tips were washed twice with distilled water for 5 minutes each and transferred onto microscope slides and several drops of 45% acetic acid were added. A coverslip was placed on each slide over the root tip sections which were then gently squashed. The slides were immediately immersed in liquid nitrogen in order to release the coverslip, and were left to air dry for 30 minutes at room temperature. The slides were subsequently dipped in 5 M HCl at room temperature for 20 minutes, and were again air dried. The samples were later stained with acetohematoxylin (1g of iron alum dissolved in 100 ml of 45% acetic acid). The sections were left to stain for 5 minutes and were again gently squashed under a coverslip for further chromosomal analyses.

Comparison of Morphology between Diploid and Polyploid *C. asiatica*

Morphological data on the plant height, stem diameter, leaf number, width and length of leaf were collected from 20 each of the diploid and polyploid *C. asiatica* plants and were statistically evaluated using the paired t-test at a significance level of 0.05.

RESULTS AND DISCUSSION

Polyploid Induction

Table 1 shows that using colchicine-saturated cotton balls and agar-containing colchicine and applying both to the seedlings could successfully induce polyploidy in *C. asiatica*. However, the application method using cotton balls saturated with 2% colchicine solution was the most effective means for the induction. Although colchicine is generally known to arrest mitosis at metaphase by disrupting spindle fiber formation, its mode of action in organogenesis is still unknown.

Method	Colchicine Concentration (%)	% of survived plants	% of obtained polyploids
Control	-	100	-
1. Agar solution drop method	1	60	20
	2	50	20
2. Cotton plug method	1	60	20
	2	80	60

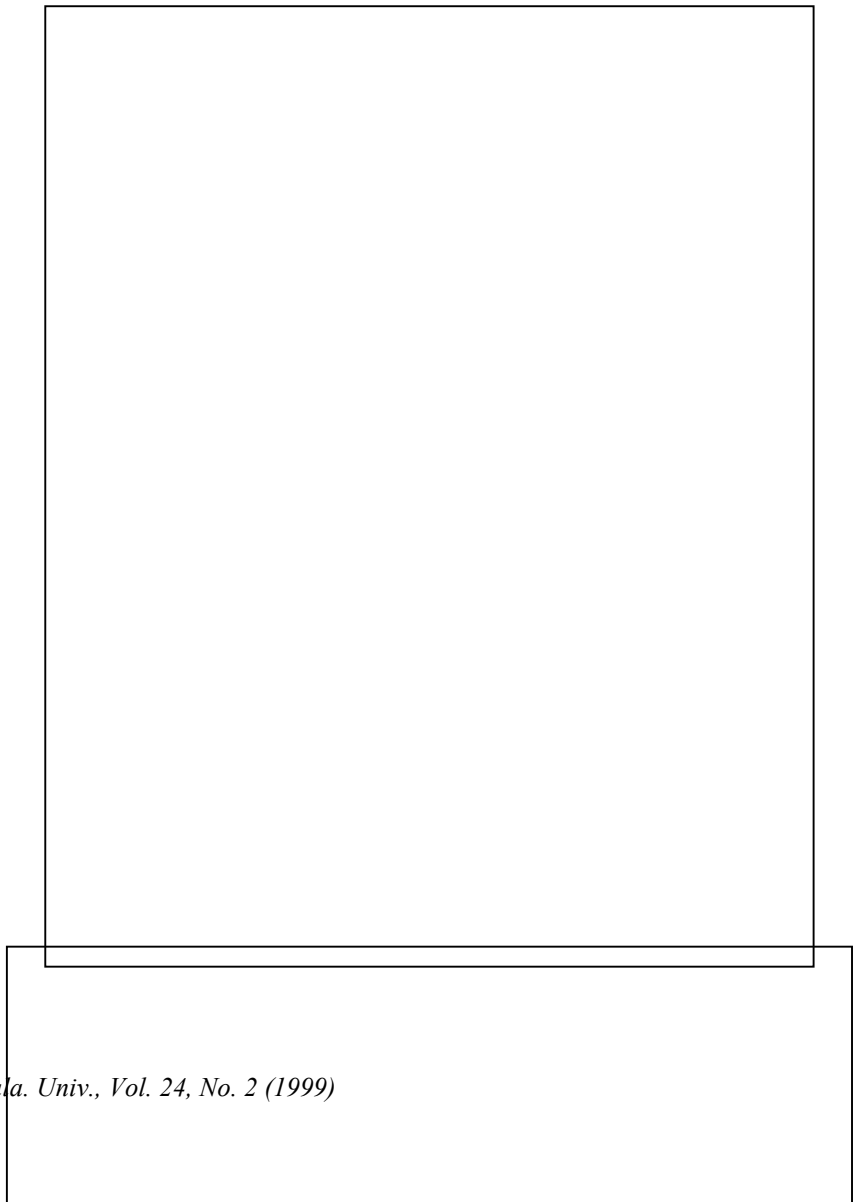
Table 1. Polyploid induction in *C. asiatica* by colchicine treatment

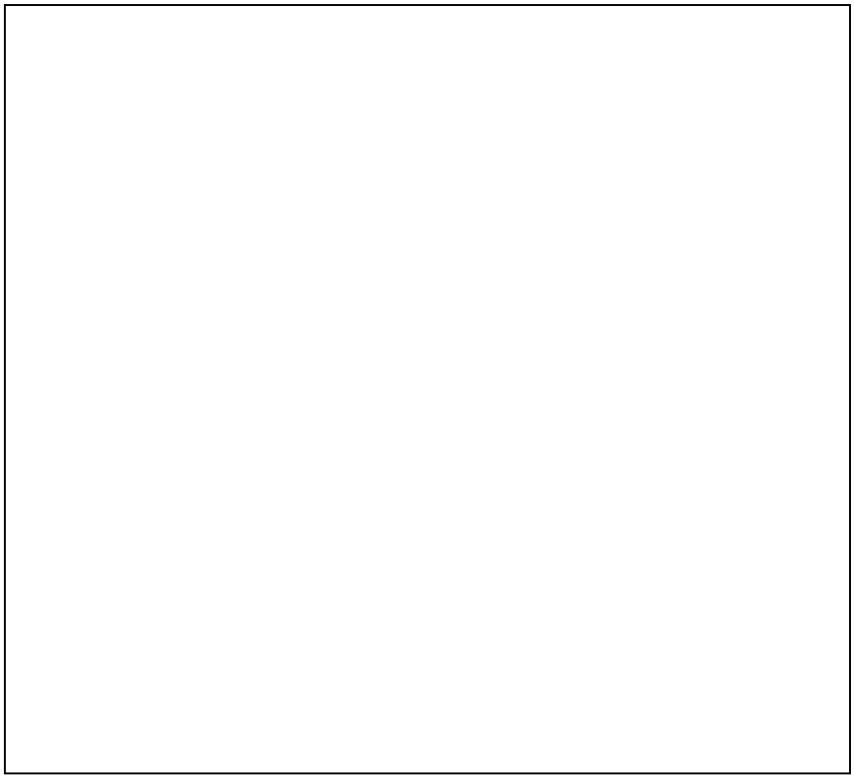
Comparison of Morphology between Diploid and Polyploid *C. asiatica*

Comparison of morphology between diploid and polyploid *C. asiatica* at the same age revealed that phenotype of the polyploids was different from that of the parental diploid plants. The polyploid plants exhibited slightly slower growth than the diploid plants. Figure 1 shows that the petiole length of the polyploid was taller, the sizes of the leaves and the guard cells were larger, and the leaf color was dark green. Table 2 shows that the petiole length, diameter, leaf number, width and length of leaves in diploid and polyploid *C. asiatica* were statistically different at $P<0.05$. Guard cells of the diploid and polyploid plants were also of different sizes. The averages of the width and length of guard cell pairs, along with the number of chloroplasts, of the polyploids are significantly larger than those of the diploids ($P<0.05$).

Cytological Study

The chromosomal characteristics of *C. asiatica* were not well defined, due to their small sizes and number and inadequate condensation. This makes them quite difficult to study using the Feulgen squash technique. Therefore, the acetohematoxylin method described by Fujii and Guerra[7] was applied instead. Figure 2 compares the metaphase, anaphase, and prophase stages between the diploid and polyploid *C.asiatica.* Although the number of chromosomes could not be accurately counted, it was clearly visualized that the obtained plants were indeed polyploid.





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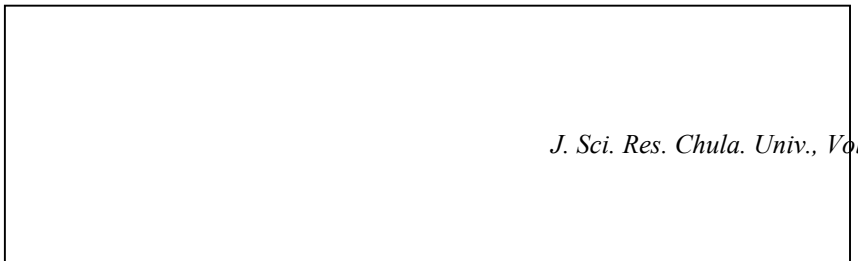

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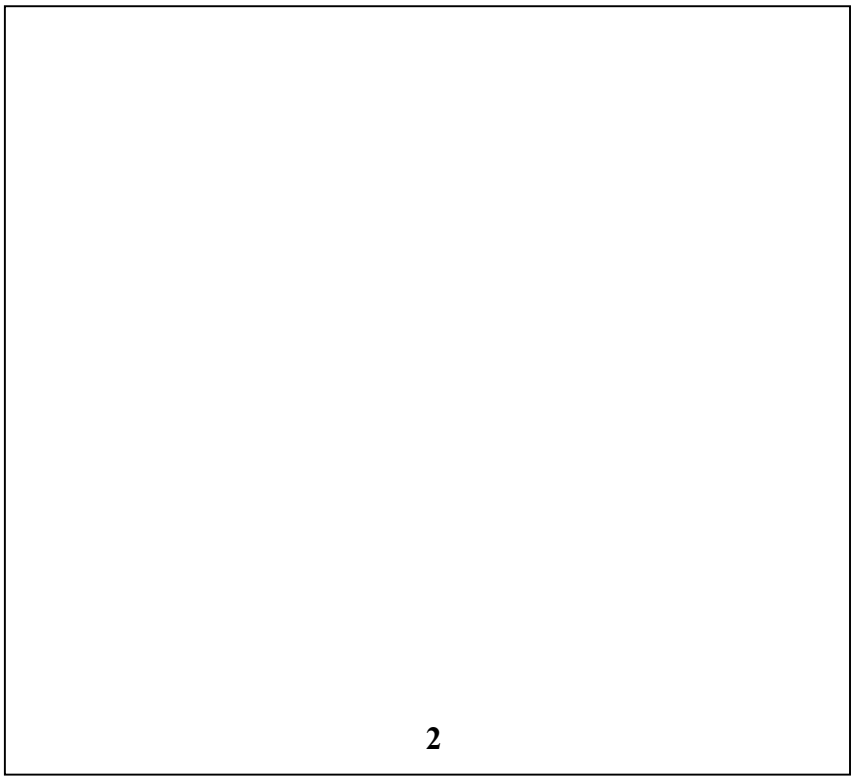
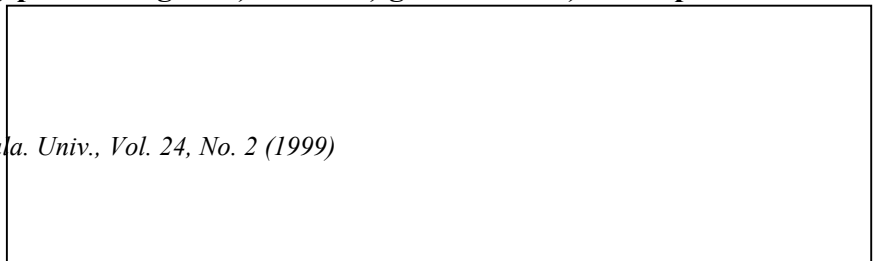
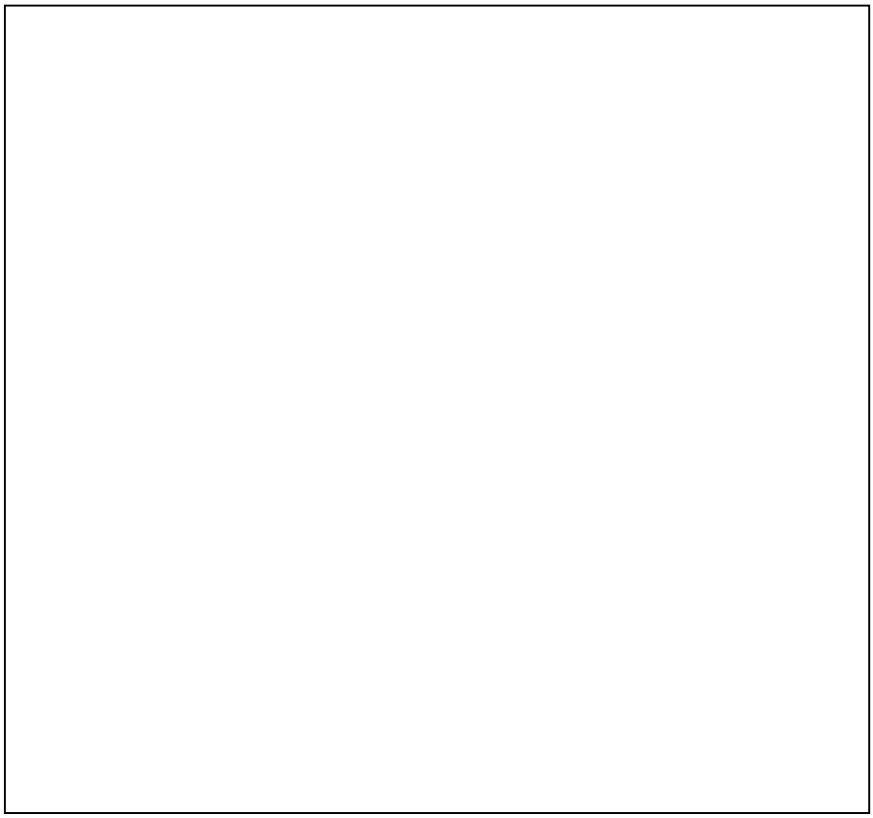


2

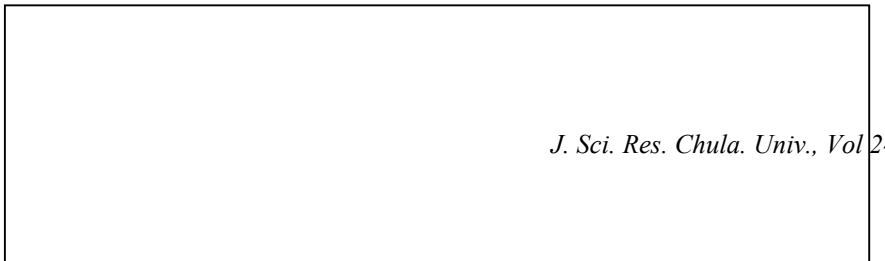
D

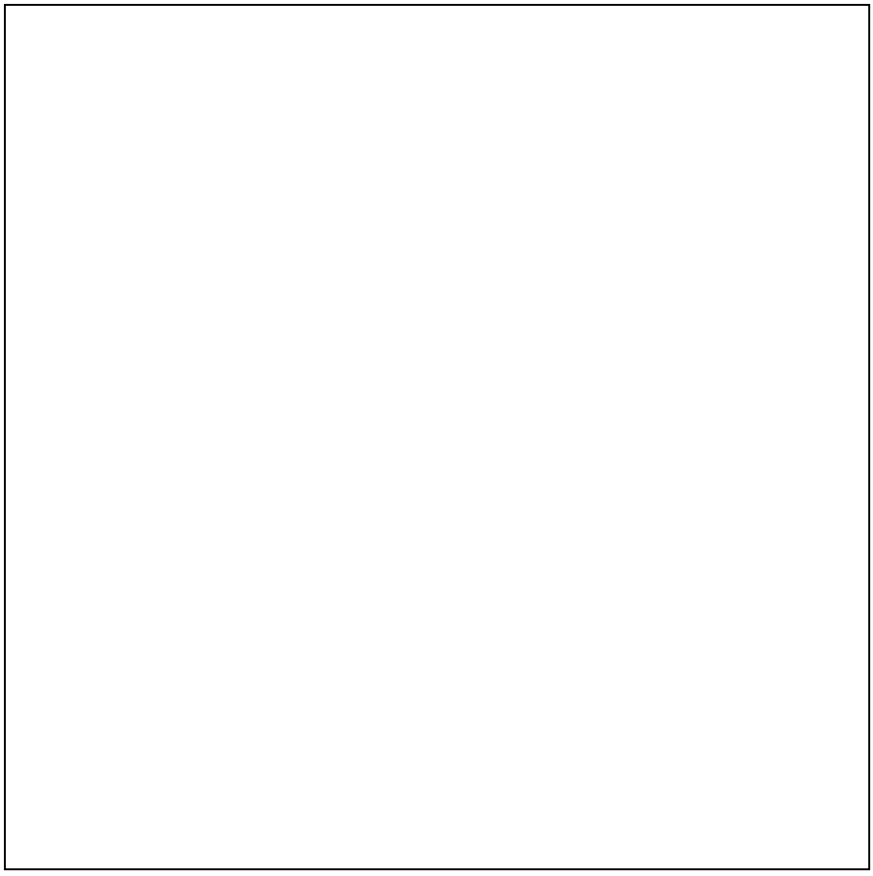
Figure 1. Morphological comparison between diploid (1) and polyploid (2) *C. asiatica* A) petiole length B) leaves C) guard cells D) chloroplast numbers.



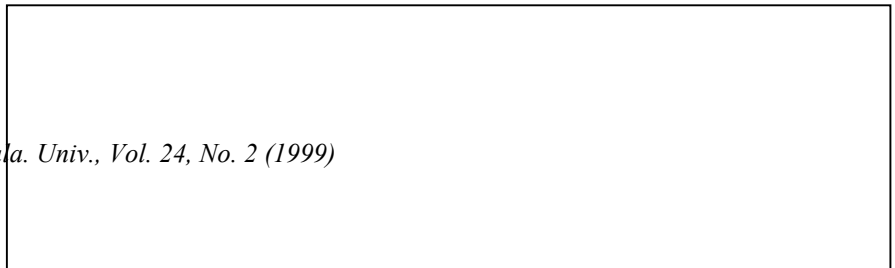


A





B



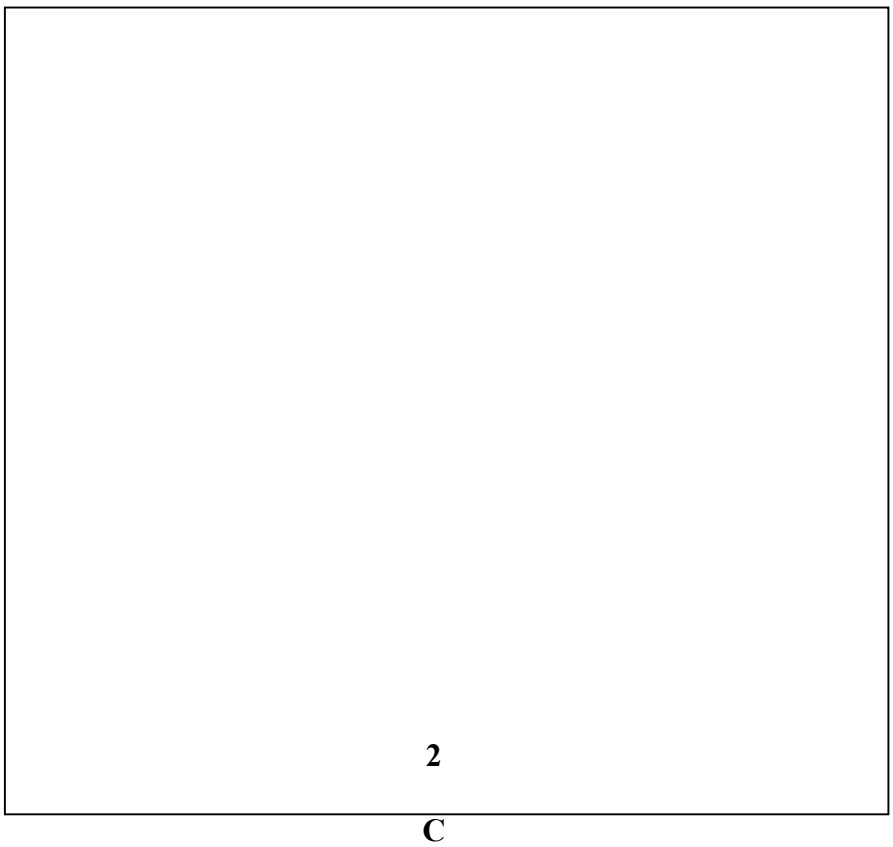


Figure 2. **Cytological comparison between diploid (1) and polyploid (2) *C. asiatica* A) metaphase B) anaphase C) prophase**

Chloroplast Counting

The number of chloroplasts could be used to estimate the number of chromosome sets from leaf epidermal cells.[8] By counting the chloroplast number in guard cells (Figure

1D), it could be confirmed that the plants obtained from the experiment were polyploid, possessing twice the number of diploid chloroplasts (Table 2).

In conclusion, the application of 2% colchicine-saturated cotton balls was the most effective method of polyploid induction of *C. asiatica*. The resulting polyploid plants can then be used in future plant breeding programs where the polyploid stability can be followed in many successive generations. In addition, the chemical compositions of the leaves from the diploid and the polyploid *C. asiatica* can be further analyzed.

Characteristic	Diploid	Polyploid	Statistical Significance At P<0.05
Petiole length (cm)	3.66	5.37	ns
Stem diameter (mm)	1.51	2.15	s
Leaf number	4	12	s
Width & Length of Leaf (cm)	2.78,1.51	3.57,1.92	s
Width & Length of Stoma (μ)	17.48, 22.43	24.37, 29.03	s
Chloroplast number	12	20	s

s = significant
ns = not significant

Table 2. Morphological characteristics (size and density of guard cells) of diploid and polyploid *C. asiatica.*

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