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THE ELECTRON MICROSCOPIC STUDY OF MICROTUBULES AND THE EFFECT OF COLCHICINE DURING MITOSIS IN *Glycine max* (L.) Merr. (Soybean)

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ABSTRACT

This study was carried to investigate the formation and inhibition of microtubules in ultrastructural level by analysing all the stages of cell division in the control and colchicine treated cells of root tip meristematic cells of *Glycine max* (L.) Merr. (Soybean). In control cells, circular sections of microtubules were observed near the cell walls in interphase. At the end of the metaphase, it was observed that the nuclear envelope at the poles ruptured and microtubules entered the karyoplasm from there. The number of microtubules reached its maximum level during metaphase. Three different types of microtubules were distinguished namely kinetochoral, free and continuous microtubules. The number of microrubules were observed to decrease starting from the anaphase. There observed some phragmoplast microtubules in the telophase. The maximum effect of colchicine was observed at metaphase and there was no microtubules found in those root tip cells treated with colchicine. It was also determined that the chromosomes could not seperate from each other and stayed constantly at metaphase.

INTRODUCTION

The studies on the formation of microtubules goes back many years. They started in 1900's after the presence of microtubules had been observed and in 1960's it became possible to comprahensively investigate their formation after the invention of electron microscope. Remarkable developments were made in those studies and protein subunits of microtubules were examined by biochemical analysis.

A part from that the inhibiting effect of colchicine upon microtubules has been known for a long time. The thought that investiga-

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tion of these effects would be a great contribution to cancer research made many scientist to concentrate upon this subject. In 1960's the effect of colchicine in cell division were investigated but they remained at the level of light microscope. There were also some researchs upon this subject after the invention of electron microscope.

Ledbetter and Porter (1963) were the first scientists who ultrastructurally investigated the structure of microtubules in telophase of meristematic cells of *Triticum vulgare.* Then, there were studies investigating the structure of microtubules by Pickett Heaps and Northcote (1966) during the metaphase and cytokinesis of the same plant; Burgess and Northcote (1968) during the mitosis of high plants; Bajer (1968) during the formation of phragmoplast and cell plate of high plants; Sakai (1969) during the cell division of high plants such as *Hyacinthus orientalis* and *Tradescantia reflexa*; Wilson (1970) during the cell division of various high plants; Burgess (1970) during fern mitosis and Lambert and Bajer (1972) during the formation of anaphase and phragmoplast in the endosperm of *Haemanthus* upon the organisation of endoplasmic reticulum and microtubules, but they were not able to fully enlighten the distribution and unification of endoplasmic reticulum and microtubules. Burgess and Northcote (1967) tried to examine the function of the pre-prophase band of microtubules in *Phleum pratense.*

Bajer (1973) tried to examine the interaction of microtubules and the mechanism of chromosome movement. Lambert (1980) investigated the relation of spindle with nuclear membrane and chromosomes during the formation of anaphase in *Haemanthus albiflos, H. katharinae* and *Mnium hornum.* Tippit, Pillus and Pickett-Heaps (1980) examined the organisation of microtubules and classified them in *Ochromonas danica.* Hawes (1981) examined the mitosis and cytokinesis in maize roots by using a low and high voltage microscopes.

Mazia (1961), Inoue and Sato (1967) were the first workers who studied the effect of varying concentrations of colchicine upon the microtubules by using the electron microscope and pioneered the new era during which many other workers concentrated upon the same subject. Burgess and Northcote (1969) studied the effect of heavy water and colchicine on the polymerization of microtubules in wheat meristems. Braselton and Bennett (1980) examined lumicolchicine effects on plant development in *Triticum* roots.

This study was carried out to clarify the situation of microtubules and the effect of colchicine, used in polyploidy investigation upon the microtubules during the mitosis in Soybean *(Glycine max* L. Merr).

MATERIAL AND METHODS

Plant Material

Chippewa type Soybean *(Glycine max* L. Merr.) seed used as a research material was obtain from the University of Ankara, Faculty of Agriculture, Department of Field Crops. The experimental seeds which were left to germinate were classified into two groups when their roots reached to a length of 0,5 mm. The first group was left to germinate at 25–27 °C while the second were put in a 0,1 % colchicine solution for 3h at room temparature. Then the seeds were washed with distilled water and left germinate for 24 h before starting the fixation process.

Electron Microscopy.

Root tips were fixed in 1 % gluteraldehyde + 0.5 % paraformaldehyde (Karnovsky, 1965) in 0,1 M phosphate buffer at pH 6.8 for an hour at room temparature; washed in phoshate buffer twice for 15 mm intervals; postfixed 1 % OsO_4 in 0,1 M phosphate buffer at room temparature for 2h; rewashed with buffer solution and twice with distilled water for 10 min. Samples were postfixed and prestained with 2 % aqueous uranyl acetate; dehydrated in an ethanolic series; rinsed in propylene oxide and embedded in Epon 812. Sections were double stained in a saturated solution of uranyl acetate in 50 % ethanol for 15 min, followed by a treatment in Sato's (1967) lead citrate for 10 min and examined using a Hitachi HS-9 transmission electron microscope at 75 kV.

RESULTS

In interphase stage, circular microtubules sections were found in the regions near to cell walls (Figure 1).

Prophase:

During the early stage of prophase, it was observed that the nuclear envelope was continuous and the chromatin material started to deposit at the edge of the membrane (Figure 2).

Figure 1: Detail picture of pre-prophase band microtubules (arrow). ER, Endoplasmic reticulum; r, ribosome; Pr, polyribosomes; CW, cell wall; D, dictyosome. x23.000

At mid prophase, nuclear envelope at the pole regions was observed to make an indentation towards the cell before breaking up.

In late prophase, the microtubules were observed to enter the karyoplasm with a few ER and cytoplasmic material after the puncture of the nuclear envelope, from the regions corresponding the spindle pole (Figure 3). Also in the same stage as seen in Figure 3 some of the microtubules were determined to bound to kinetochore of chromosome before rearranging at the equatorial plate. The number of microtubules were seen to increase towards to metaphase.

Metaphase:

During the early stage of metaphase, it was observed that the chromosomes were completely shaped and there were pieces of nuclear

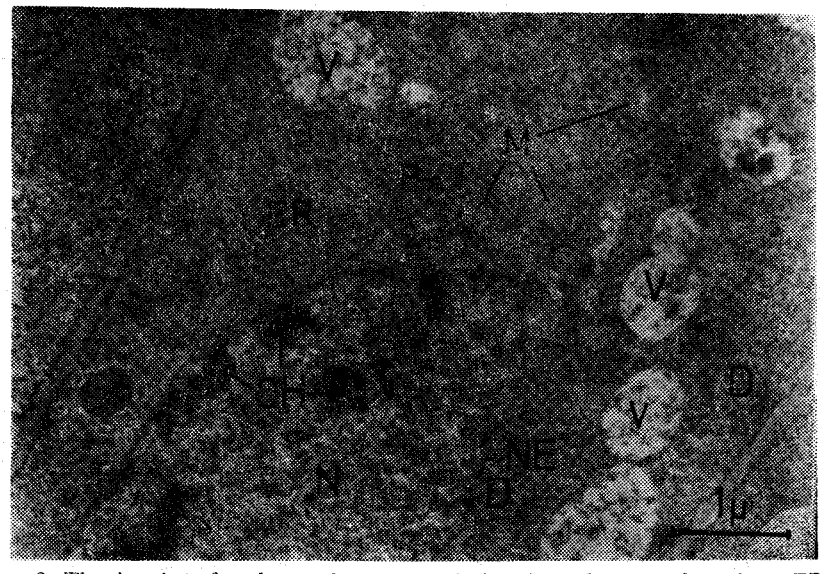

Figure 2. The situation of nucleus and cytoplasm during the early stage of prophase. ER, granulated endoplasmic reticulum; D, dictyosomes; M, mitochondria; V, vacuols; CH, chromosome; N, nucleus; NE, nuclear envelope; P, plastid. x 23.000.

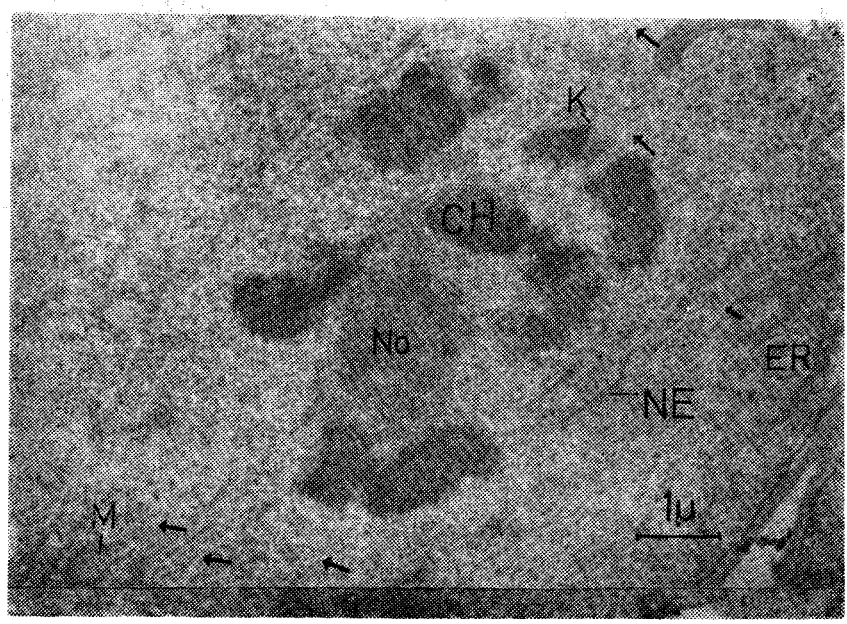

Figure 3. The microtubules which entered to karyoplasm after the breakage of nuclear envelope from the poles regions were shown by arrows. ER, granulated endoplasmic reticulum; M, mitochondria; CH, chromosome; K, kinetochore; No, distributed nucleolus; NE, nuclear envelope. x 16.100.

envelope partly dispersed around them. Then the microtubules reached to their maximum number by the rearrangement of chromosomes along the equatorial plate (Figure 4). Different types of microtubules were found at that stage. The big portion of microtubules found in cytoplasm were seen to be bound to kinetochore regions of chromosomes from one end, and to the pole from the other (Figure 4).

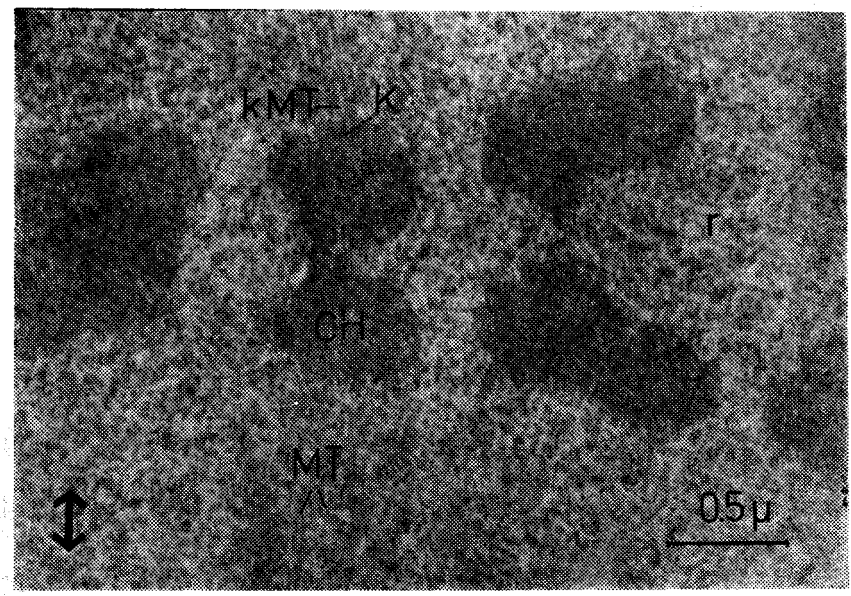

Figure 4. The electron micrograph which shows the kinetochore region of chromosomes (K) and the sister chromosomes which started to reproduce by dividings from top to bottom. CH, chromosome; kMT, the microtubule bound to kinetochore; MT, the microtubule not bound to kinetochore; r, ribosome; ↔ the direction of cell poles. x 46.000.

But some of the microtubules were determined to be not bound to chromosomes, had spaces between them and were shorter than usual. A part from those, a very rarely seen microtubules type which continuous from one pole to another though the chromosomes was also determined. Especially during this stage the kinetochore regions of chromosomes were seen to be easily distinguishable from chromatine by their low constrast and the most common microtubules type was found to be those which had one end bound to kinetochore and the other to the pole (Figure 4). The separation from late metaphase from early metaphase is related to organisation of microtubules. The

microtubules at late metaphase were observed to be more organised and in the form regular line and therefore more easily followed than those at early metaphase. It was not possible to determine termination points of microtubules. But they were observed to approach towards the ER concentrated at the pole region.

Anaphase:

The microtubules bound to the kinetochores of chromosomes were observed to shorten and pull the chromosomes towards the poles during the anaphase. Therefore, the microtubules shortened and decreased in number after the start of the anaphase.

During the anaphase the microtubules types are those which had one end bound to kinetochore of chromosome and the other to the pole, continued from one pole to another in the interzonal region and no ends bound to any poles respectively (Figure 5). The number of microtubules bound to chromosomes was observed to be higher than the number of other microtubules. The microtubules were also obser-

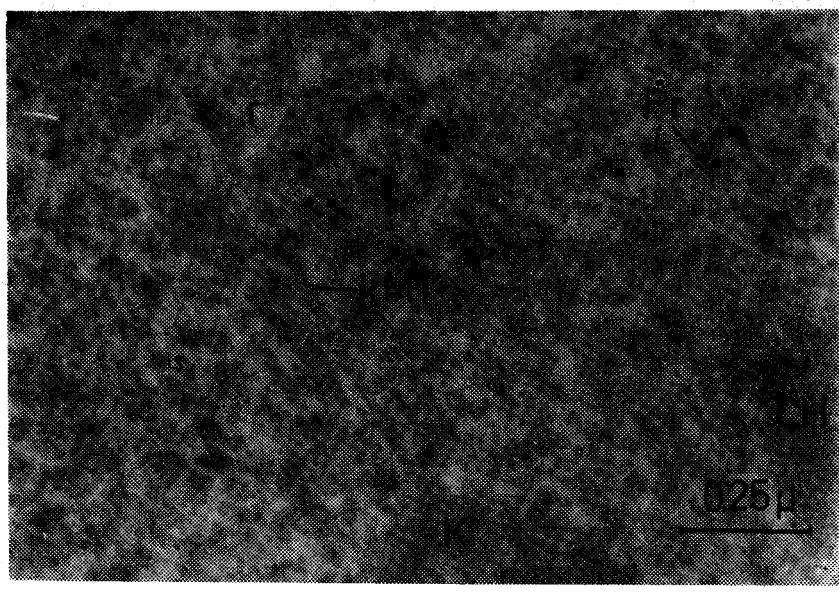

Figure 5. The electron micrograph showing chromosome kinetochore and the microtubules bound and not bound to kinetochore. CH, chromosome; kMT, the microtubule bound to kinetochore; K, kinetochore; r, ribosome; Pr, polyribosome. x 100.000.

ved to continue towards the ER lamels system and terminated there.
Telophase:

The phragmoplast vesicles were observed to be arranged in the interzonal region of the cell. The phragmoplast formation was understood to be consisted of the microtubules clusters in the interzonal region which make a right angle to the cell plate to be formed in the future and the number of the microtubules was seen to increase in the interzonal region during the mid-anaphase and phragmoplast formation (Figure 6). The phragmoplast microtubules were observed

Figure 6. The phragmoplast microtubules (fMT) which will consist the phragmoplast structure during the cell plate formation at the telophase stage are shown with arrows. CP, cell plate; P, plastid; V, vacuol. x 46.000.

to overlap each other and buried into the electron-dense granular material, between the phragmoplast vesicles (As shown in Figure 7A, 7B). These can be considered as the remains of continuous microtubules in the interzonal region. The vesicles located among the phragmoplast microtubules were determined to gather in order to form cell plate and during this process the number of microtubules were observed to decrease as the number of vesicles increased and consequently there remained a few microtubules near the edge of the phragmoplast (Figure 7A, 7B).

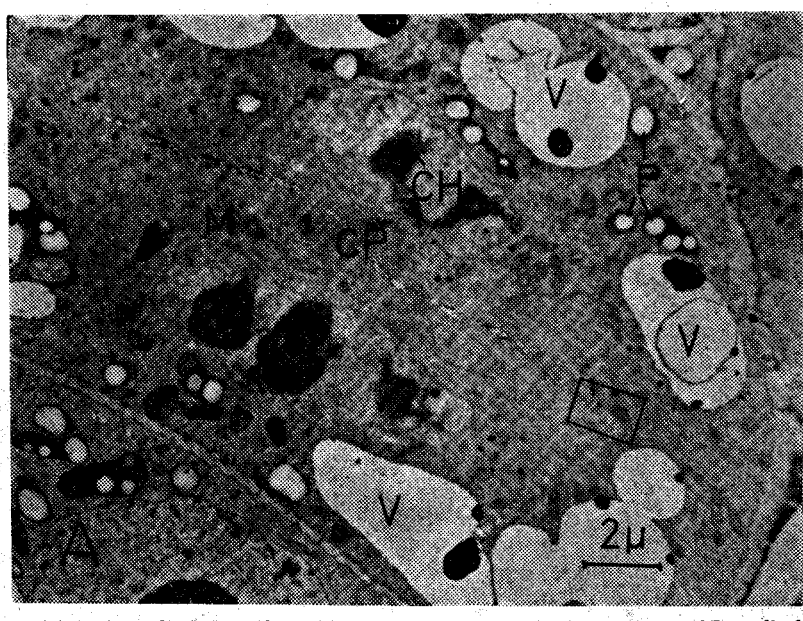

Figure 7A. The cell during the mid-telophase stage. CH, chromosome; CP, cell-plate; V, vacuols; P, plastids; M, mitochondria. x 7.500.

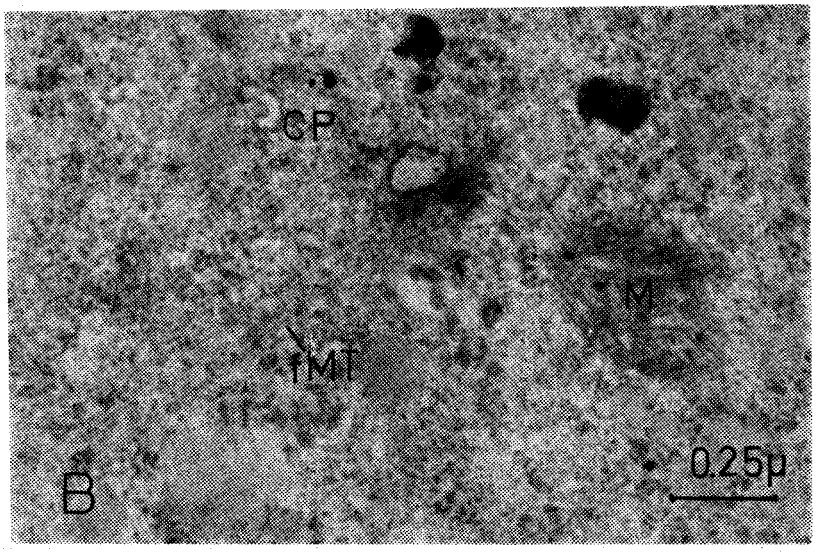

Figure 7B. The electron micrograph showing the phragmoplast microtubules buried into electron dense material near the edge of the cell plate framed in a square in figure 7A. fMT, phragmoplast microtubule; CP, cell plate; M, mitochondria. x 75.000.

There were a significant change during the prophase of the cells treated with colchicine (Figure 8). During the colchicine treatment,

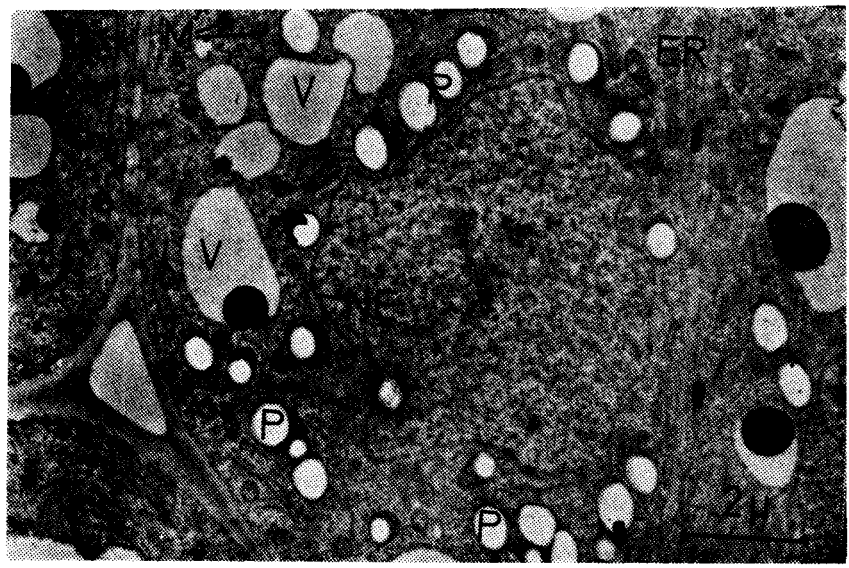

Figure 8. The cell nucleus and cytoplasm during the prophase of the roots treated with colchicine. M, mitochondria; P, plastids; V, vacuols; NE, nuclear envelope. x 11.500.

it was observed that the chromosome action came to halt, the cell division remained constantly at metaphase and consequently polyploidy formed (Figure 9, 10). During the analysis of the cell treated with colchicine the highest toxic effect was observed at the metaphase and therefore the chromosomes were not able to seperate from each other (Figure 9). The cytoplasm was also observed to be effected in the same way as seen in the nucleus.

DISCUSSION

Burgess and Northcote (1968), Wilson (1970), Burgess (1970) and other workers proposed that ER and partly the nuclear membrane played an important role in the polymerization of microtubules, by basing the fact that ER or membrane elements are generally found in spindles. Hawes (1981) investigated the mitotic cell division in *Zea mays* by using an electron microscope. He claimed that the ER

Figure 9. The micrograph showing the chromosomes which were not able to separate from each other during the metaphase in the roots treated with colchicine. The agranular endoplasmic reticulum (ER), forms colonies in the cytoplasm. CH, chromosome; M, mitochondria; D, dictyosome; V, vacuols. x 16.100.

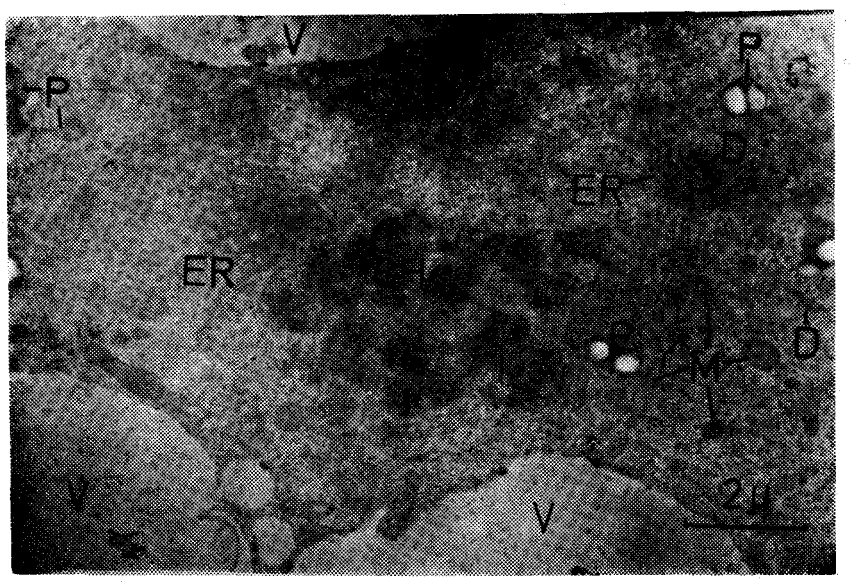

Figure 10. The electron micrograph showing the cell treated with colchicine and which also had polyploid situation. The mutated cytoplasmic organels are also appearent. CH, chromosome; ER, agranulated endoplasmic reticulum; D, dictyosome; M, mitochondria; P, plastids; V, vacuols. x 11.500.

elements were shaped at the poles and the mitotic apparatus was consisted of the nuclear envelope of the whole membrane system and this ER.

The indistinguishable similarity between the pieces of nuclear membrane and ER, in our observation during mid-prophase verifies these proposals as well.

Pickett-Heaps and Northcote (1966) determined that the mitotic microtubules were originated from the microtubules of pre-prophase band. According to those workers the microtubules begin to disappear during the prophase and the mitosis begin to appear and as a result of this the microtubules migrate from band to poles. Burgess and Northcote (1967) made the same observation upon the pre-prophase band microtubules of *Phleum pratense* and claimed that the pre-prophase band microtubules plays an important role in the formation of mitotic stage microtubules. Fowke (1974) said that there were no pre-prophase microtubules after his studies on Soybean tissue cultures. But in our experiments, there observed circular microtubules sections similar to those pre-prophase microtubules but they were observed in very low numbers at the start of the prophase. But there were no such structures observed in the following stages. It is highly possible that these play an important role in the formation of other microtubules (Figure 1).

Pickett-Heaps and Northcote (1966), observed that the number of microtubules reached to maximum and the kinetochores were completely formed at the metaphase in their studies upon wheat meristems. Bajer and Mole-Bajer (1969) pointed out that the number of microtubules which were 10–20 during pro-metaphase reached to 75–100 during metaphase.

In our studies the number of microtubules was pictorialy determined to increase gradually after prophase and reached to its maximum at metaphase (Figure 4). A part from that the kinetochore region to which the chromosomes are attached at metaphase is clearly distinguishable (Figure 4).

Tippit and et. al (1980) investigated the metaphase, early anaphase, late anaphase and telophase stages of the cell division in their study they carried out on the microtubules of *Ochromonas danica* and categorised and named the microtubules as follows:

1– Free microtubules,

2– Interdigitated microtubules,

3– Polar microtubules,

4– Kinetochoral (Chromosomal) microtubules.

According to this study the microtubules laying one pole to another is very seldomly observed. The workers stated that the kinetochoral, polar and free microtubules were observed for a very short time before they disappeared. We classified the locations and the arrangements of the mitotic apparatus microtubules after the analysis of all the stages of soybean mitosis as follows:

1– Kinetochoral or chromosomal microtubules,

2– Free microtubules,

3– Continuous or interpolar microtubules.

The classifications of the afore mentioned workers are also in compliance with our classification.

The fact that colchicine has an inhibiting effect on the polymerization of microtubules has been emphasised by many workers for a long time.

Burgess and Northcote (1969) investigated the effect of % 0.1 colchicine concentration on the polymerization of microtubules in the meristematic cells of wheat. They reported that colchicine continuously kept the cells in metaphase and the microtubules were either not present or those formed had very small lengths.

In our studies the microtubules were not observed and the chromosomes could not separate from each other and the cell stays continuously at metaphase (Figure 9).

Lambert (1980) observed the inhibition of microtubules formation in cells treated with colchicine for a long time and called this situation "C-metaphase" in his study on the endosperm of *Haemanthus* treated with % 0.4 colchicine. A part form that he also observed the seperation of sister chromatids, called this "C-Anaphase" in the cells which he treated with % 0.5 colchicine but he definitely stated that there was no migration of chromosomes to the poles.

In our results, there observed the phenomenon of C-metaphase, but there was a polyploid situation in chromosomes (Figure 10). But

the stage of C-Anaphase was not observed. This situation supports the theory that the cell reproduction continues no matter whatever happens after the formation of microtubules and therefore colchicine has an inhibiting effect on the formation of microtubules rather than destroying them.

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