SOME REMARKS ON THE CYTOLOGY OF NORMAL AND COLCHICINE-TREATED HEMP-PLANTS. (CANNABIS SATIVA L.)

by

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Several investigations have pointed out that in the roots of *Cannabis sativa* the plerome consists for the greater part of diploid cells, while the periblem is tetraploid in the majority of cases. Something like this is further only known for *Spinacia* and *Mercurialis*. The origin of these tetraploid cells may be understood in this way that the chromosomes in the periblem divide, but the halves do not separate, in other words, they do not pass the metaphase stage. Hence nuclei are formed, having now double the number of chromosomes. In accordance herewith LANGLET found nuclei with chromosomes forming exquisite pairs in the metaphase, which proves that they have just been produced by splitting. Further away from the apex the chromosomes are no longer arranged in this manner (1).

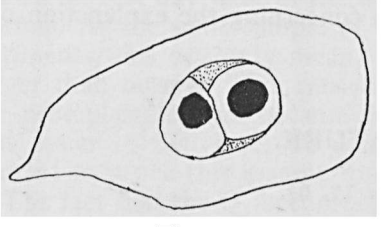

Fig. 1.
Resting nucleus from the periblem, showing two nucleoli with lucid zones, artificially produced after fixation.

BRESLAWETZ only is of another opinion (2). The tetraploid nuclei should proceed from a fusion of two resting nuclei. From this event, which would be unparelleled in cytology, he gives several figures. We also found similar figures in hemp-roots (Fig. 1). The explanation, however, is quite another one. Two nucleoli are clearly seen, each surrounded by a lucid zone. It is generally known that this figure is due to a fault of fixation, in other words is something artificial. The nucleolus possibly pushes the contracting nuclear substance to the periphery by its Brownian movement. Therefore, we are not allowed to identify such a zone with a nucleus, as BRESLAWETZ did. When two nucleoli are present, the zones may border

upon each other. It would be wrong to conclude that we have to do with a fusion.

We too frequently saw the chromosomes in tetraploid nuclei lying in pairs, so that the explanation of LANGLET seems to us to be the right one.

In spite of repeated cytological investigations of *Cannabis sativa* one peculiarity still remained undetected, namely the occurrence of satellites. In diploid cells we generally find an equal number of satellites. In this respect we found the hemp-plant to behave in a different manner. The diploid nuclei in our preparations only showed one chromosome carrying a satellite. Tetraploid nuclei never contained more than two satellite chromosomes. Since the work of HEITZ (3) we know that possibly a relation exists between satellite chromosomes and nucleoli. In correspondence with this opinion the diploid nuclei in hemp-roots show one nucleolus, whereas the tetraploid nuclei at most contain two nucleoli. As a fusion of the nucleoli often occurs, many tetraploid nuclei only possess one nucleolus.

The occurrence of only one satellite chromosome in each diploid-nucleus may be connected with the following. In the male hemp-plant a pair of xy-chromosomes has been described by SINOTO (4). The most plausible inference of our findings therefore is that the observed satellite chromosome represents the y-chromosome of the male plant. In harmony with this a satellite chromosome has not been found in all of the roots studied, which might be due to the occurrence of female hemp-plants with 2 X-chromosomes.

We did not succeed, nor did LANGLET, in finding tetraploid tissue in the meristem of the stem. The nuclei as well as the metaphase plates differ somewhat in size and the number of the nucleoli varies from one to two, but well countable plates were not observed. Though the occurrence of scattered tetraploid cells remains possible, we may assume that the stem on the whole is diploid.

One chromosome was noticed to show an irregular behaviour with respect to the metaphase plate. This is, as will be known, a peculiarity of sex-chromosomes.

Since chromosome doubling is the rule in hemp, this plant seemed to be a favourable object for treatment with colchicine. KOSTOFF (5) already used hemp-roots for this same reason and observed the occurrence of tetraploid cells. But, as the latter are normal in the periblem of hemp-roots, his observation tells us nothing about the influence of colchicine.

Hemp-seeds were soaked in a 0.2 per cent colchicine-solution for two days. After that period they were carefully washed off and

allowed to germinate between filter-paper in Petri-dishes. When the rootlets had sufficiently grown, fixation followed in NAWASHIN's fluid. The sections were stained with gentianviolet.

The treatment appeared to be suitable for doubling the number of the chromosomes. In the periblem metaphase plates with 80 chromosomes were found. (Fig. 2). The colchicine, however, reduced the staining power of the chromosomes, so that the observation of satellites became very difficult. Nevertheless two satellite chromosomes were seen in one metaphase plate of the plerome, in which, peculiarly enough, only half the number of chromosomes had been doubled and which therefore contained 30 chromosomes. Judging from their position, they had originated by the splitting of the one satellite chromosome present in diploid cells (Fig. 3).

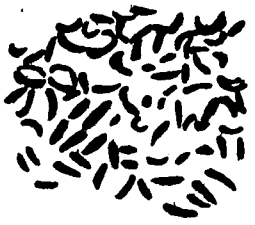

Fig. 2.
Octoploid nucleus, produced by the action of colchicine.

The result of the influence of colchicine is not an increase of the number of nuclear divisions, as was first supposed, but a failing development of the spindle. Consequently the daughter chromosomes do not separate after the division. They are seen lying side by side like pairs of skis (6). Our figure of an octoploid nucleus does

Fig. 3.
Cell from the periblem, containing 30 chromosomes, only half of the diploid number, the satellite-chromosome included, being doubled after treatment with colchicine.

Fig. 4.
Metaphase chromosomes which by action of colchicine are prevented from forming a „plate".

not show this phenomenon. We must assume that this nucleus had already passed some time in a doubled condition and now has entered into a division anew.

Fig. 4 represents metaphase chromosomes, which display a beginning of splitting. The influence of the colchicine has prevented

their arrangement into an equatorial plate. Hence they lie scattered in the nucleus in an irregular manner.

In fig 5. we clearly see the result of the influence of the colchicine on the plantules. As usual a thickening of the roots and the hypocotyls has been effected. After bedding out the growth is checked for a long time to become normal again only after two or three weeks. Moreover the growing stem gradually is reduced to the normal dimensions. Generally, the explanation for the thickening is seen in the fact that the tetraploid cells are much larger than diploid ones. In our opinion, however, this is not the only cause. Whilst in a normal

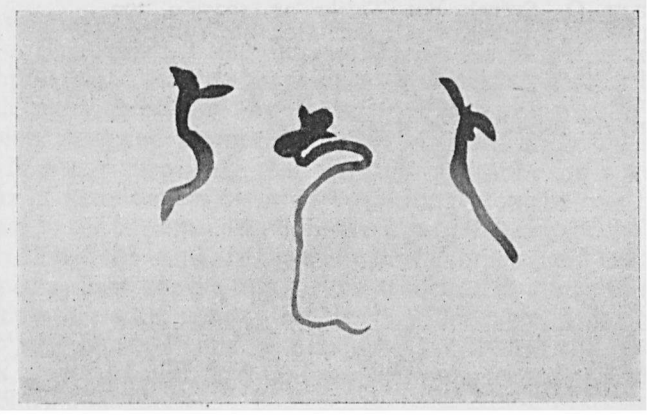

Fig. 5.
Photo, showing in the middle a normal Hemp plant and on the right and left a colchicine treated plant.

rootlet the direction of the divisions runs mainly parallel to the principal axis, many deviations from this rule are found after treatment with colchicine. The divisions then occur to a large extent in a direction perpendicular to the axis of the rootlet. In this direction also new cells are formed, and this is the main cause of the thickening of the roots and hypocotyls.

After treatment of hemp-roots with colchicine at most 4 nucleoli can be observed in the resting nuclei. From the above discussion it follows that these nuclei are octoploid, one nucleolus belonging to each diploid set of 20 chromosomes. Our observations therefore confirm the conceptions of HEITZ.

The colchicine not only prevents the development of the spindle fibers, it also checks the dividing rate of the nuclei. Hence in each

cross section much more prophase stages are found than normally. Earlier investigators therefore thought that the number of nuclear divisions had been increased. After treatment with colchicine hemp also displays a great number of prophase stages. The twenty chromosomes of such diploid nuclei can be easily counted.

Till now no doubling of the number of the chromosomes has been found after treatment with acenaphtene. The experiments, however, are continued.

The author wishes to thank especially Dr. N. P. BADENHUIZEN for his valuable advice.

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