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KINETICS OF THE MITOTIC CYCLE OF ISOLATED BARLEY EMBRYOS

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The use of isolated embryos for cytogenetic research is superior to the use of intact seeds, especially in cereals. With the use of isolated embryos, endosperm does not interfere with the mutagenic action of chemical mutagens by storing or docomposing the mutagen or by supplying energy. Defined cultivation media allow to control all external conditions by a better way than do whole seeds. When rare chemicals are applied, the use of isolated embrya reduces the required volumes of solutions. Sterile work eliminates possible heterogeneity of results due by bacterial contamination which is very important for instance in experiments with application of exogenous DNA.

Material and methods.

Seeds of spring barley cv. Ametyst, with diameter range 2.5-2.8 mm were used throughout the experiments. Before dissection, seeds were sterilized by rinsing into 2 % chloramin for 16 hrs. Anaerobiotic conditions prevents the onset of the S-phase. After sterilization, seeds were washed in sterile distilled water and embryos were isolated with forcepts and scalpel. Extirped embryos were stored in refrigerator. Four month storage does not decrease the viability. Dissected embrya were cultivated by a modification of already described method [3]. Before cultivation, embryos were sterilized again in 2 % chloramine for 15 min. and washed five times in sterile distilled water. Miflin's complete medium [5] was used.

Embryos were cultivated in vials 9 cm high, with diameter 2.5 cm, in 4 ml of liquid medium. Ten embryos were put into each vial and cultivated on a shaking device RT 60 with the rotation diameter 4 cm and with speed 180 cycles per minute, in temperature 26 ^{o}C. Samples were fixed in Farmer's fixa - tive and Feulgen squashes were made.

In the first series of experiments, the overall dura - tion of the mitotic cycle was determined by labelling the naturally occuring synchronously dividing fraction of the cell population by tetraploidy with the use of the method of VAN'T HOF (11). After constant period of cultivation (20 hrs or 60 hrs) embrya were applied also on a shaking device for 30 min. Colchicine concentrations 0.1, 0.2 and 0.4 % or 0.1 % colchicine in 2 % dimethylsulfoxide were used. After colchicine treatment, embryos were re-sterilized and transferred into fresh media. Samples were fixed in 2 hrs intervals. Before fixation, embrya were pre-treated by 0.15 % colchicine for 2 hrs to facilitate the chromosome count - ing. The interval of between colchicine application and the incidence of tetraploid cells corresponds to the duration of the mitotic cycle. Five embryos were used in each expe - rimental variant and at least 200, but usually over 500 metaphases were scored. Roots were divided into three gro - ups: a) central roots, b) longest lateral roots, c) shortest lateral roots.

For the estimation of mitotic index values, embryos were fixed without colchicinepretreatment. Interphase nuclei and individual phases of mitosis were calculated. Ten fields were counted on each slide, with approximately 100 cells in each field and four slides were made in each experimental variant. In the experiments with synchronization of mitosis, 5-aminouracil p., trade mark Lachema was used. Embryos were put into vials which contained solution of 5-aminouracil

750 mg/l, dissolved in distilled water. Embrya were culti - vated on the shaking device for 36 hrs, washed and transfer -red into Miflin's medium. Mitotic index was determined and samples were fixed in two hours intervals.

Results

Labelling of the naturally occuring synchronously dividing cell fraction by tetraploidy.

The time sequence of the occurence of tetraploid meta- phases after short-time colchicine treatment was followed. The effect of concentration on the incidence of tetraploid cells in the root meristems of central roots, after treat- ment of 20 hrs cultivated embrya is seen in Fig. 1. The 0.4 concentration of colchicine yielded the maximum of tetraplo- id cells 16 hrs after treatment, similarily as 0.1 % concen -tration, It demostrates that colchicine does not prolonge the mitotic cycle by its toxic action. The 0.4 % concentra- tion induced more tetraploid cells than the 0.1 % concen- tration, but the difference was not too large. The degree of heterogenity of central and lateral roots is shown in the Fig. 2. There are slight differences in the possition of maxima and the course of the curves and therefore only cen- tral roots were used for further experiments with labelling the naturaly occuring synchronously dividing fraction of the cell population by tetraploidy. Fig. 3 demonstrates the eff- ect of dimethylsulfoxide on the induction of tetraploid cells by colchicine action. Dimethylsulfoxide is known to enhance the polyploidogenic action of colchicine (4). We used 0.1 % colchicine in 2 % dimethylsulfoxide and we found no considerable effect of dimethylsulfoxide on the frequency of polyploid cells. The concentration of dimethyl- sulfoxide used decreased the mitotic index and slowed down

the mitotic cycle. The position of the first maximum was in 18 hrs instead of in 16 hrs. From these reasons we cannot recomend the use of dimethylsulfoxide for this kind of ex - periments. When colchicine is applied 20 hrs after the on - set of cultivation, cells of the first prometaphase are affected and the second mitotic cycle is timed. Question arises, whether the average time of the mitotic cycle re - mains constant for longer period of cultivation. This que- stion was solved by comparison of the response of colchi - cine treatment on 20 hrs cultivated and 60 hr cultivated embrya. Fig. 4 shows that the first induction of the te- traploid cells in embrya treated after 60 hrs of cultiva - tion is also 16 hrs after colchicine treatment like in most other cases but the peak is much less pronounced which de- monstrates high degree of heterogeneity of the mitotic cycle duration in older roots.

Experiments on the timing of the duration of the mi - totic cycle by labelling the naturaly synchronously divi - ding fraction of the cell population by tetraploidy were replicated several times Fig. 5 shows the overall picture in all replications. Only the positions of the first maxi- ma are shown. In most cases the first maximum was 16 hrs after the treatment, in four cases 18 hrs after treatment, which determines the average lenght of the mitotic cycle slightly over 16 hrs.

The dynamics of the mitotic index

The value of the mitotic cycle time, as determined by labelling of the naturally occuring fraction synchronous- ly dividing fraction of the cell population by tetraploidy cannot be considered as the real time of the mitotic cycle unless it is confirmed by other method. There is a possibi- lity that the duration of the mitotic cycle in tetraploid

cells might be longer than that in diploid cells. For this method reason we used also another method of the estimation of duration of the mitotic cycle. Root meristem cells in dormant embryos are usually in G_1 phase. After soaking, they pass through S, G_2 and enter mitosis with partial synchrony. This natural partial synchrony lasts also in the second mitosis. On the basis of the time difference of the peaks of the mitotic index, corresponding the first and the second mitosis the mitotic cycle duration can be estimated (Fig.6). The first mitosis appears 14 hrs after the beggining of cultivation is 18 hrs after the soaking, for lateral roots and 20 hrs for central roots. The second maximum appears after next 16 hrs, which is in good agreement with the results of labelling mitosis by tetraploidy. In this case, there are again slight differences in the position of peaks in central and lateral roots, but the difference between peaks is 16 hrs in both cases. When the mitotic index of central roots was fractionated into individual phases of mitosis (Fig.7), it became evident that the dynamic of the mitotic index is determined by the dynamic of the prophase stage, as pro - phase is the longest phase of mitosis. The other phases of mitosis followed approximately the same pattern with ex - ception of some time shift, which was most strikingly expressed in anaphase and telophase stages.

In order to get more pronounced maxima, partial synchronization of the mitotic cycle was induced by 5-aminouracil (Fig. 8.). First mitoses, in central roots, were observed after 10 hrs of cultivation in nutrient solution and maximum over 20 % was reached in central roots after 12 hrs. In synchronized roots the second peak was mani - fested 16 hrs after the first one. The results with synchronization thus confirm the average duration of the mitotic cycle to be 16 hrs. It is interesting to compare the effect of 5-AU on the central roots and lateral ones.

While there was a marked synchronization in central roots, lateral roots showed only general depression of mitosis. The toxic action was obviously higher than the synchronizing one. This differential action of 5-AU, which can be suppo - sed also for other chemicals, is additional reason why it is advisable to use only central roots. Under our experi - mental conditions, 5- AU induced a low frequency of aberrations in anaphases (Tab. I.). On the basis of the knowledge of the duration of the mitotic cycle (16 hrs) and the ave - rage mitotic index (9.21) we estimated the duration of mi - tosis and of the individual phases of mitosis. (Tab. II).

Discussion

In our experiments we have found that the duration of the mitotic cycle in root meristems of isolated barley embryos cultivated in vitro was, under our experimental conditions, 16 hrs. It differs from the data on intact seeds (1, 8), where 12 hrs were repeatedly found. In our experiments results with three different approaches were simillar and therefore the difference should be attributed to the cultivation of embryos in vitro.

The method of the estimation of the duration of the mitotic cycle by labelling the naturaly synchronously dividing fraction of the cell population by tetraploidy did not shift the durstion of mitotic cycle in tetraploid cells, which is in good agreement with the data in literature (2, 10).

The method of synchronization of the cell population by 5-aminouracil is the most frequently used way of syn - chronization of mitosis in plant meristems. 5-aminouracil causes the depression of the rate of DNA synthesis, which brings about the accumulation of cells in the S-phase and

delay in the exit from G_2 period (6). In barley, possitive results with synchronization were already achieved in roots (9) as well as in shoots (7). The induction of chromosome aberrations by 5 - aminouracil in our experiments was not find in other experiments, dealing with synchronization by 5-aminouracil. Our concentration and period of treatment were higher than those used by other authors. Even if we succeeded, in future experiments, to modify the method in order to avoid the induction of aberrations, it must be kept in mind that 5- aminouracil is a chromosome breaking agent and one should be very careful in the interpretation of cytogenetic results of induction of aberrations by different mutagens after synchronization by 5 - aminouracil, because there might be some interaction of mutagen with DNA . On the other hand, synchronization is a very perspective method, which can be applied in studying many cytogenetic problems like the treatment of defined stage of the mitotic cycle, induction of polyploidy and others.

References

1. BENNET, M. D., FINCH, R. A.: The mitotic cycle time of root meristem cells of Hordeum vulgare. - Caryologia 257 : 439 - 444, 1972.

2. FREIBERG, S. H., DAVIDSON, H.: Duration of S-phase and cell cycle in diploid and tetraploid cells of mixoploid meristems.- Exptl. Cell Res. 61: 216 - 218, 1970.

3. GICHNER, T., ONDŘEJ, M., LIM EN SE , VELEMÍNSKÝ, J.: The effect of methyl methansulphonate on mitosis and chromosomes of excised barley embryos. -Biol. plant. (Praha) 1976 (v tisku).

4. JENSEN, C. J.: Chromosome doubling techniques in haploids.In: KASHA, K. J. (Ed.): Haploids in Higher Plants. Advances and Potential. pp. 151 - 190. University of Guelph. Guelph 1974.

5. MIFLIN, B. J.: A technique for the sterile culture of germinating barley embryos.- J. Exptl. Bot. 20 : 805 - 809, 1969.

6. PRENSKI, W., SMITH, H. H.: The mechanism of 5-aminouracil induced synchrony in Vicia faba meristems.- Cell. Biol.24 : 409-414, 1965.

7. SAVIN, V. M., ABRAMOVA, V. V.: Sinchronizacija kletočnoj populjacii v apikalnoj meristěmě stěblja s pomoščju sovměstnovo dějstvija 5- aminouracila i poniženoj těmperatury. - Citologija i genetika 7(3) : 201-204, 1973.

8. SKULT, H.: Growth and cell population kinetics of tritiated thymidine labelled roots of diploid and autotetraploid barley. - Acta Acad. Aboensis Series B 29 : 1 - 15, 1970.

9. TARASENKO, N. D., MAJKĚVIČ, G. S., ABAŠEVA, L. M., ANTONNIK, A. A.: Synchronizacija populjacii kletok korněvoj i věrchněj meristěmy u rastěnij.- Citologija 14 (3):389-395, 1972.

10. VAN'T HOF, J.: Comarative cell population kinetics of tritiated thymidine labelled diploid and colchicine induced tetraploid cells in the same tissue of Pisum sativum. - Exptl. Cell Res. 41:274-288, 1966.

11. VAN'T HOF, J., WILSON, G., COULON, A.: Studies on the control of mitotic activity.- Chromosoma 11:313-321, 1960.

FIG. 1.

THE EFFECT OF 0.1 % AND 0.4 % COLCHICINE

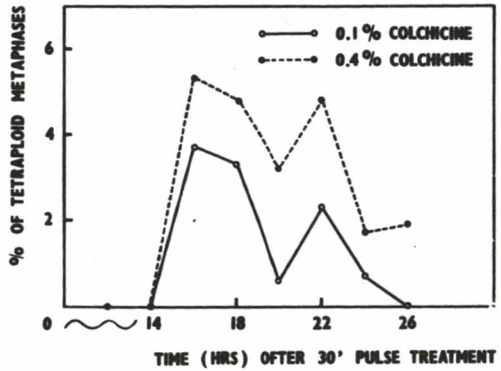


FIG. 2.

DIFFERENCES BETWEEN CENTRAL AND LATERAL ROOTS
– 0.1 % COLCHICINE

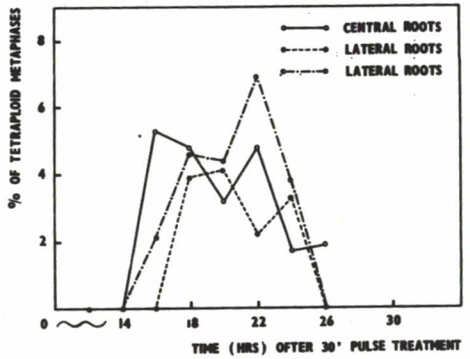


FIG. 3.

INFLUENCE ON 2 % DIMETHYLSULFOXIDE ON THE EFFECT
OF 0.1 % COLCHICINE

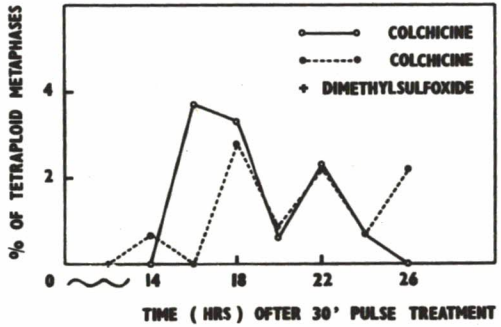


FIG. 4.

THE TREATMENT OF 20 HRS AND 60 HRS CULTIVATED EMBRYOS
– 0.1 % COLCHICINE

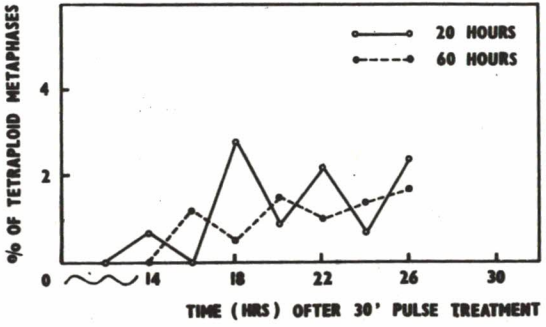


FIG. 5.

THE FIRST PEAKS OF TETRAPLOID CEELS IN DIFFERENT EXPERIMENTS

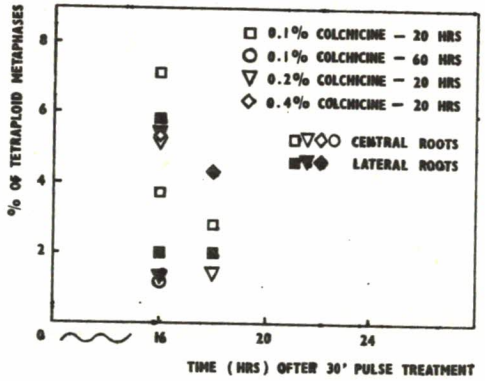


FIG. 6.

VARIABILITY OF THE MITOTIC INDEX WITH DURATION OF CULTIVATION

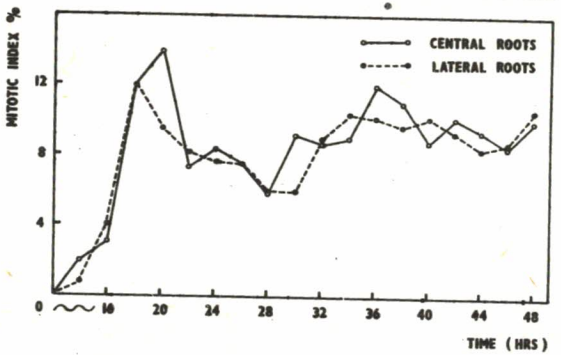


FIG. 7.

THE FRACTIONATION OF MITOTIC INDEX INTO INDIVIDUAL MITOTIC STAGES (CENTRAL ROOTS)

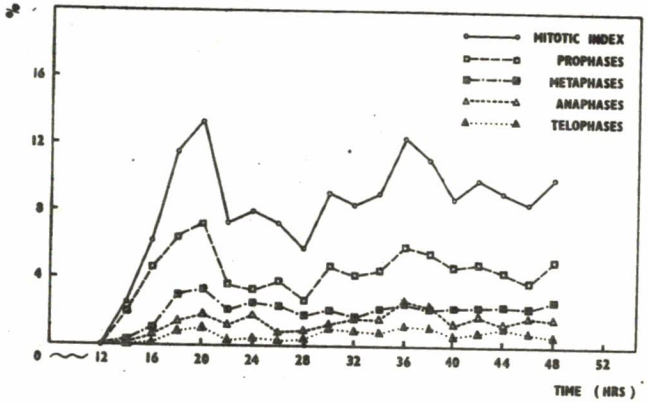
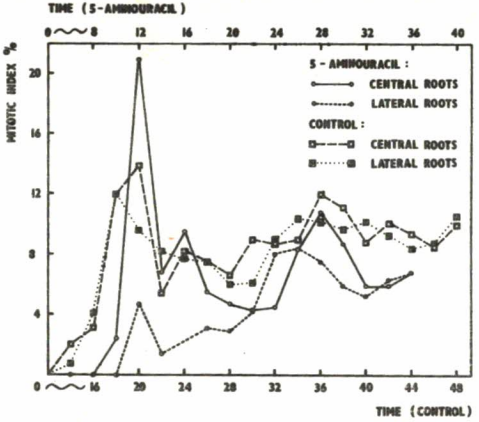


FIG. 8.

SYNCHRONISATION OF MITOSIS BY 5 - AMINOURACIL



TAB . I.

INDUCTION OF ANAPHASE ABERRATIONS BY 5 - AMINOURACIL

	NORMAL ANAPHASES	ABERRANT ANAPHASES*	% OF ABERRANT ANAPHASES	χ^2
CONTROL	508	3	0.6	13.9
5 - AMINOURACIL	513	22	4.1	

* WITH FRAGMENTS, BRIDGES AND / OR LAGGARDS

TAB . II.

THE DURATION OF INDIVIDUAL STAGES OF MITOSIS

	MINUTES
PROPHASE	44
METAPHASE	22
ANAPHASE	15
TELOPHASE	7
MITOSIS	88