Changes of the Daily Rhythm of Mitosis in Pinus nigra Arn. Caused by Gamma Rays")

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Introduction

On the occasion of our investigations into the chromosomes of the somatic cells of Picea abies KARST. (BEVIŁACZA and VAŠKOVIĆ, 1963) we noticed that it was not possible to find at any time of day a sufficient number of mitotic stages. Taking into consideration that in our further investigations into the mitoses and chromosomes of various species we had to obtain a larger number of cells in mitosis, we carried out investigations about the diurnal fluctuation of the mitotic frequence. The subject of these investigations was the Austrian Pine. We made the measurements of the frequency of mitosis in the root tips of the seed which were irradiated with gamma rays of 60Co. As controls nonirradiated material was used.

It should be emphasized that this work lays no claim to a quantitative study but only to a qualitative investigation for the purpose of guidance.

Material and Working Method

The seed we used for these investigations was supplied from Slovenia and collected in a natural stand [focality Divača-Pivko] in the autumn of 1961. The germinative capacity of the seed amounted to 90%: the irradiation of seeds with gamma rays was carried out in the spring of 1962 by means of a 60Co-source of 350 curies. The radiation doses were 100, 1000 and 5000 r respectively while the humidity of seeds during the irradiation was 8.41%.

The seed germinated at room temperature in Petri dishes. On achieving a 3–4 mm. length the roots were cut and fixed in acetic alcohol and stored into a refrigerator at 4°C. Immediately before the making of slides the roots were macerated in 1 N HCl at 60°C for 45–60 minutes. Then they were stained during 30 minutes in aceto-carmine on the slides. On covering the slides with the cover slip they were slightly pressed and weakly warmed over a gas burner. By covering the rims of the coverslips with molten paraffin we made semipermanent mounts.

Experimental Work

I. Programme of experiments

From each sample of the irradiated material and from the controls were taken every hour five roots. From each root we made one slide. The total number of preparations was 480. Each of these preparations was examined separately. The examination was carried out with an enlargement of 60×, and this always under the same conditions, i.e. in each preparation always the same area was examined (a total of 35 horizontal rows; read from the vernier scale for vertical shifting this interval was 0.5 mm.). Under the "prophase" we recorded only the advanced

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stages of this cell division phase, i.e. when the chromosomes were already formed and arranged, so that it was possible to enumerate and draw them.

Under the “metaphase” we understood all the stages from the moment when all the chromosomes began to group themselves into a spindle (prometaphase) up to the moment when all the chromosomes were correctly oriented within the spindle (proper metaphase).

Under the “anaphase” we recorded all the stages from the earliest anaphase when the chromatids began to separate at the centromeres up to the latest anaphase when the chromosomes reached the poles.

We included into the “telophase” all the stages from the moment the chromosomes began to loose their individuality at the poles of the newly formed cells up to the formation of the new cell plate and the transition of the nucleus from oval to round form.

By means of data for the particular hours (each time five slides taken together) graphs were made. We present the graphs with the absolute and relative values – the relative frequencies of the number of mitoses and of individual mitotic stages (the number of mitoses, and the number of individual mitotic stages divided by the total number of mitoses and the individual mitotic stages multiplied by 100). Because of lack of clearness in the presentation of these curves we have used the method of the sliding averages of relative frequencies (values for the individual points of the final curve were obtained, so that there were calculated the arithmetic means of the three successive values, e.g. for the 3rd hour was taken the sum of the values of the 2nd, 3rd and 4th hours and divided by 3).

Observed were also the particular hours, i.e. the relation of the individual stages of cell division in particular hours of day. The diagrammatic representation was given in the form of simple columns (histogram); the value of the individual column is 100%, and the individual mitotic stages are plotted into it with their corresponding values.

II. Mitotic frequency

Fig. 1 represents the curves of the absolute values of the total number of mitoses in the particular hours. Visible are three pronounced maxima in the control and in the 100r-dosis, a fairly marked morning maximum in the 1000r-dosis, and one pronounced maximum during midday hours in 5000r-dosis. In addition, in this graph are conspicuous also different levels of curves, the highest occurring in the control, and the lowest in the dosis of 5000r. If the total number of mitoses in the control is taken as 100%, then the number of mitoses in 100r-dosis is 50%, in 1000r is 30%, while in 5000r only 11%.

Fig. 2 represents the relative frequencies of the total number of mitoses expressed in percentages. These curves vary in clearness because they are interwoven.

Fig. 3 also represents the relative frequencies, but here the values for the individual points were obtained by means of the sliding averages. Thus the representation of these curves, is clearer and though their hourly maxima do not coincide completely with the values of the relative frequencies, they clearly exhibit the tendency of sliding of the individual maxima due to the gamma irradiation, i.e. the three marked maxima in the control, and the presence of the one most pronounced in the dosis of 5000r.

III. Frequency of the individual mitotic phases

1. Relative frequencies too are represented by means of the sliding averages, and they illustrate the diurnal course of the individual mitotic stages in irradiated material as well as in the nonirradiated one (controls). Which is represented in Fig. 4. Here we observe almost always three maxima, the first in the morning hours, the second in the midday hours, and the third in the evening, excepting in the prophase where two maxima exist. When applying a maximal dosis of radiation there occurred only one large maximum during the midday hours in all mitotic stages.

2. The relation of the mitotic stages in the individual hours is represented in Fig. 6 in the form of single columns whose total value amounts to 100%. The individual mitotic stages are plotted into the columns with their corresponding percentages. While in the controls one observes fluctuations of the frequency of the prophase and telophase, along with an equal number of metaphases and anaphases, after the radiation with different doses of gamma rays the situation changes. Thus already with a dosis of 100r one observes an increase of the number of anaphases, while at 1000r one seems an increased number both of anaphases and metaphases. In these cases the number of telophases is obviously reduced. In a dosis of 5000r one observes complete irregularity with a marked increase of telophases. Fig. 7
were made with the same data, only that here each mitotic stage was drawn separately, while the values for the individual hours were compared with the double value (2 x) and the single value (x) of the arithmetic mean for all 24 hours.
results agree completely with the data of Fadjejev (1962), Hollaender (1954), and Vidaković (1960).

On dividing the curve of the controls (total number of mitoses) into three parts, we obtain by summing up within the individual parts the results which show that the highest number of mitoses occur in the third part, i.e. from 17 to 24 hours. A gradual fusing of the three marked maxima occurring in the controls into one with the material irradiated with a dose of 5000 r confirms that the irradiation slows down the mitosis. This is corroborated also by the results of Amand (1856), Fadjejev (1962), Vaskjev (1962) and Hollaender (1954). The ultimate development of one pronounced maximum at 5000 r one could explain so that the cells in the mitosis of the first wave with regard to the controls divided with retardation and did not complete their division when the cells of the second wave already began to divide etc. Finally, it happened that at the highest dosage (5000 r) a very large number of cells were dividing simultaneously, and as a result there occurred the pronounced and wide maximum in the mentioned dosage. As regards the number of the maxima of mitoses the data in literature differ from one another. The most frequent results report the occurrence of one maximum which according to some authors appeared in the daytime and according to the others at night (Alov and Krasilnikova 1962, Dobrohotov, Bariejeva and Kuridzumova 1962, Mühlemann, Thomas, Marthaaler and Loutalot 1955, Zinecker-Brauer 1952, Harte and Zinecker-Brauer 1960). Alov and Krasilnikova, Dobrohotov, Bariejeva and Kuridzumova (1962), as well as Krasilnikova (1962), and Alov (1962) report the results about the appearance of the two maximum which occur usually within an interval of 12 hours, while a pronounced minimum lies between them. Kästner (1915 and 1918) succeeded to achieve besides a normal maximum also another one induced artificially through change of the time of illumination.

**Frequency of the individual mitotic stages**

Fig. 4 show that a maximum of mitoses usually occurs when the majority of mitotic stages show high frequencies. In the controls this occurs at 4 a.m., and 9 p.m. With the dose of 100 r the situation is very similar, only that the maxima are shifted, so that they occur at 7 a.m., 1 p.m., and 9 p.m. In the dose of 1000 r the first maximum sets in at 4 a.m., the second at 12 a.m., while the third stayed at 9 p.m. but was less pronounced. In dose of 5000 r the maximum occurs between 1 p.m. and 2 p.m., and at 9 p.m. In the latter two doses owing to large differences of their frequency maxima the morning maximum at 1000 r, and the midday maximum at 5000 r are more marked, while the others are hardly noticeable.

Observing each mitotic stage separately (Fig. 5) one can see the same tendency, i.e. that of the three primary maxima (only in the prophase are two maxima probably because the late prophase only was taken into consideration) by gradual increase of irradiation dose the number of maxima decreases. Here the dose of 1000 r is of interest, which looks like a transition from the lower doses to the higher ones. Here is pronounced one of the maxima but the two others can also be guessed, which cannot be said for the dose of 5000 r, where one sees always two maxima only one of which is actually clearly marked.

**Relation of mitotic stages in the particular hours**

From Figs. 6—7 one can notice the changes due to gamma irradiation. While in the control only the stage of the late prophase and telophase changes, the number of metaphases and anaphases being equal, in the individual doses of radiation the whole situation changes, so that already in the dose of 100 r one notices an increase of the number of anaphases. In the 1000 r dosage besides the anaphase also the number of metaphases is increased, while the number of telophases is reduced. Which can be explained so that the irradiation weakens the functional activity of the spindle for with reason the chromosomes move more slowly and the mentioned stages last longer. In the dose of 500 r a completely irregular relationship is visible, which was always the case when examining this dosage, that is to say, a wholly different distribution of the cell-division maxima.

The results concerning the duration of the individual mitotic stages are in agreement with the generally known results from the literature. Sharp (1943), Küster (1954),
Fig. 7. — Relation of mitotic stages in the particular hours — each phase represented separately: (a) the control, (b) 100 r, (c) 1000 r, (d) 5000 r doses. 2X (——), X (— — —).
Dorbohotov, Barjjeva and Kurjumova (1962), Brachet and Minsky (1961) confirm that the prophase and telophase are longest, the metaphase and anaphase are shorter, and that only in some cases it was different. As to the effect of radiation on the individual mitotic stages it is stated that the radiation acts differently upon the individual stages (Amand, 1956), and that the relation among the individual stages within the various intervals after the radiation is different (Hollander, 1954), which could also be taken into consideration in these investigations.

Summary

Investigated was the diurnal rhythm of mitosis in the root tips which germinated from the irradiated and non-irradiated seed of Pinus nigra Arn. For each data of measurement were used five root tips 3-4 mm. long. The mitosis were watched after the fixing of specimens in acetic alcohol and staining with aceto-carmine. The performed investigations yielded the following results:

1. The total number of all mitotic stages examined during 24 hours decreased considerably in dependence of the amount of doses by which the seeds were irradiated. It was for the five root tips (with regard to the control taken as 100%): for 100 r 50%, for 1000 r 30%, and for 5000 r 11%.

2. The normal experimental material, i.e. the non-irradiated controls exhibited within the interval from 0 to 24 hours three maxima of the frequency of mitosis, which occurred at 4 a.m., 2 p.m., and 9 p.m.

3. In proportion to the intensity of the applied radiation dose there was disturbed the time sequence of maxima of mitosis frequency, and their number decreased so that in the material irradiated with a 5000 r dose became conspicuous a pronounced and wide maximum during mидday hours, and another one small in the evening.

4. The frequency maxima of the individual phases of mitosis (relative frequencies) confirm the existence of the three maxima in the control and a gradual transition (1000 r) towards a large very marked maximum (5000 r).

5. The particular hours (at which the individual phases occur) are different for various radiation doses, and, whereas in the control the prophase and telophase (the number of the metaphases and anaphases being equal) vary, in various doses this proportion changes, so that in 100 r dose also the anaphase varies, while in 1000 r this is valid for both, the metaphase and anaphase. In the dose of 5000 r an irregularity in the frequency of all cell-division stages is noticeable.

Résumé

Titre de l'article: Changement du rythme journalier de la mitose chez Pinus nigra Arn. causé par la radiation gamma.

On a fait des recherches sur le rythme journalier de la mitose dans les pointes des racines germissées des graines irradiées et non irradiées du Pin noir d'Autriche. Pour chaque donnée du mesurage on a utilisé cinq pointes de racines d'une longueur de 3 à 4 mm. Les mitoses ont été observées après la fixation avec l'acéol acétique et après la coloration avec l'acéto-carmine. Les recherches effectuées ont donné les résultats suivants:

1. Le nombre total de tous les stades mitogénétiques observés pendant 24 heures a subi une réduction considérable par rapport à la puissance du dosage des radiations gamma appliquée à des graines. Ce nombre est pour les cinq points de racines (le témoin pris comme 100%) de 50% à 100 r de 30% à 1000 r et de 11% à 5000 r.

2. Le matériel normal c'est-à-dire les témoins non irradiés ont montré à l'intervalle de 0 à 24 heures les trois maxima de la fréquence de mitose, savoir, à 4, à 14 et à 21 heures (Fig. 1).

3. En proportion de la puissance d'irradiation appliquée, la suite de temps des maxima de fréquence des mitoses fut dérégulée et leur nombre a diminué graduellement de manière qu'au matériel irradié s'est manifesté un maximum prononcé et élargié aux heures de midi ainsi qu'un autre maximum très réduit au soir (Fig. 4).

4. Les maxima de fréquences des phases mitogénétiques particulières (les fréquences relatives) confirment la présence des trois maxima chez les témoins ainsi qu'une transition graduelle (1000 r) vers un maximum très prononcé (5000 r) (Figs. 4-5).

5. Les heures particulières (où se produisent les stades individuels) varient selon les différentes doses d'irradiation et pendant que la prophase et la télaphase (le nombre de métaphases et d'anaphases étant égal) varient, cette proportion change aux différentes doses de manière qu'à 100 r l'anaphase varie aussi, alors qu'à 1000 r change tant la métaphase que l'anaphase. Enfin à l'application de la dose de 5000 r on constate une irrégularité de la fréquence de toutes les stades mitogénétiques (Figs. 6-7).

Zusammenfassung

Titel der Arbeit: Änderungen im Tagesrhythmus der Mitosen bei Pinus nigra Arn., die durch Gamma-Strahlung verursacht wurden.

Die Tagesrhythmik der mitotischen Teilungen wurde bei Wurzelspitzen untersucht, die aus bestrahlten und unbestrahlten Samen der Schwarzkiefer erhalten worden waren. Für die Angabe aller Meßdaten wurden 5 Wurzelspitzen mit einer Länge von 3-4 mm benutzt. Die Wurzeln wurden in Essig-Alkohol und mit Essig-Karmin gefärbt. Die dann durchgeführten Untersuchungen ergaben folgende Befunde:

1. Die Gesamtzahl aller mitotischen Stadien, die innerhalb von 24 Stunden beobachtet werden konnten, sank beträchtlich in Abhängigkeit von der Bestrahlungsstärke und betrug für die 5 Wurzelspitzen (die Kontrolle als 100% angenommen) bei 100 r = 50%, bei 1000 r = 30% und bei 5000 r = 11%.

2. Das normale unbestrahlte Kontrollmaterial wies in der Zeit von 0 bis 24 Uhr 3 Maxima in der Mitosenfrequenz (um 4, 14 und 21 Uhr) auf (Fig. 1).

3. Im Verhältnis zur Stärke der angewandten Bestrahlungsdozen wurden auch die Zeitfolgen der Maxima der Mitosenfrequenzen gestört, so daß sich beim Versuchsmaterial der Dosis 5000 r ein ausgeprägtes und breites Maximum während der Mittagsstunden und ein weiteres unbedeutendes am Abend zeigten (Fig. 3).

4. Die Frequenzmaxima einzelner Mitosestadien (relative Frequenzen) bestätigen das Vorhandensein von 3 Maxima bei den Kontrollen, einen allmählichen Übergang bei 1000 r zu einem ausgeprägten Maximum bei 5000 r (Fig. 4-5).

5. Die einzelnen Uhrzeiten, bei denen die individuellen Stadien erreicht werden, liegen bei verschiedenen Bestrahlungsdozen verschieden. Während bei den Kontrollen die Prophasen und Telophasen (bei gleicher Anzahl Meta-phasen und Anaphasen) variieren, ändern sich diese Verhältnisse bei verschiedenen Dosen so, daß bei 100 r auch die Anaphasen fluktuieren und bei 1000 r zusätzlich noch die
Vollständige Varianzen und Kovarianzen in Pflanzenbeständen

I. Ein Modell für Konkurrenz zwischen Genotypen

VON K. STERN

(K eingegangen am 15. 1. 1965)


1. Genetische Varianz ohne Konkurrenz

Nehmen wir an, die in allen Pflanzenbeständen auftretenden Direktstandesfekte könnten irgendwo ausgeschaltet und die genotypischen Werte der drei möglichen Genotypen unserer Population direkt bestimmt werden; genetische Konkurrenzfekte können ebenfalls nicht vor. Dann könnte man die genetische Varianz der Population in der üblichen Weise erhalten:

\[
\text{Var} = \sum_{i=1}^{3} n_i (x_i - \bar{x})^2
\]

Hierin stelle \( a \) und + \( a \) die Abweichungen der Merkmalwerte der beiden Homozygoten von ihrem gemeinsamen Mittelwert dar und \( d \) den Abstand des Heterozygoten von diesem Mittelwert. Bezüglich weiterer Erklärungen wird auf die Literatur verwiesen, etwa auf KEMPTHORNE (1957) oder FALCONER (1960). p und q bezeichnen die Häufigkeiten der Allele A bzw. a. Es ist \( p + q = 1 \).

Das Mittel einer solchen Population wird erhalten zu

\[
M = a(p^2 - q^2) + 2pq
\]

Für die Varianz zu

\[
\text{Var} = 2pq (a + d) (p - q)^2 + 2pq^2
\]