Rhythmic Variation in the Growth Rate of Embryo in *Triticum monococcum* L.

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The growth of embryo of *Triticum monococcum* L. was analyzed in detail. The caryopses were collected every two hours. At each time, the growth rate of embryo was calculated for the length and thickness, which showed an undulatory variation. Then the correlogram analysis was applied to the stationary growth rate. It also showed the apparent periodicity. In the next place, the correlation coefficient between the autocorrelogram and the cosine function was calculated as a periodogram. The periodogram showed two significant peaks in the thickness. Though only one peak was significant in the length, the second highest peak in the length existed at the same position as the significant one in the thickness. Furthermore, the other peaks near the significant one in the periodogram are considered to be of no biological meaning, resulted from the finiteness of the number of samples. Therefore, it is concluded that the growth rate of embryo both in the thickness and length are represented by the summation of two cosine functions with periods of 6.06 and 7.72 hr in thickness and 6.00 and 7.74 hr in length. But the variation of the growth rate at the very early stage did not agree well with the two rhythms. Embryo starts the dual rhythmic growth at the 100-cell stage. This suggests that at the 100-cell stage cells in embryo begin synchronous divisions at two regions which eventually develop into two apical meristems.

**Introduction**

Plant embryo differentiates two apical meristems during the development. One of the important problems in plant embryology is how these meristems are formed in early embryogenesis. In plants, morphological differentiation is advanced in company with the growth and cell divisions. This suggests that the quantitative analysis of growth is one of the effective means to answer how the morphogenesis occurs and to reveal how the morphological variation occurs.

In my previous paper (Nagato 1976), it is reported that rice embryo starts dual rhythmic growth at the very early stage (about 100-cell stage) and is suggested that this rhythmicity is brought about by the synchronous cell divisions at two regions in the embryo which develop into two apical meristems. There is no reason to consider that this rhythmicity is peculiar to rice embryo. It is rather reasonable to consider that the fundametal mechanism of meristem formation is common to many species and the rhythmic growth of embryo is also detected in other species than rice. Therefore, the growth of embryo in wheat was quantitatively analyzed in detail. So far, there has been no study which analyzed the growth of embryo in detail and made the relation of the growth and morphogenesis clear. Only morphological observation

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has been carried out on wheat embryo (ROTH 1957).

In the present experiment, *T. monococcum* was used as a material because the flowering of this species is restricted to a short period of a day. This enable us to obtain a large number of embryos starting their development simultaneously. The flowering time of other *Triticum* species vari widely in a day and hence they are not good for the time series analysis.

**Material and Methods**

*T. monococcum* var. *flavescens* KORN. was grown under usual conditions. Caryopses blossomed from 5:15 a.m. till 6:15 a.m. of the second of June, 1976 were marked and collected every two hours from 6:00 a.m. on the second day till 8:00 a.m. on the 12th day after flowering. At each sampling time, ten caryopses were randomly collected from more than five plants. They were fixed with ethanol-acetic acid mixture (3:1) for thirty minutes, then sectioned at 12~15 μm by the usual paraffin method and stained with Delafeld’s hematoxylin.

The length and thickness were measured on good samples. Measured sizes on seven to ten samples at each time were averaged to give the length or thickness at that time.

Detailed methods of correlogram analysis and periodogram analysis are the same as described in the previous paper (NAGATO 1976).

**Results**

Both embryo length and thickness showed sigmoidal growth curves which carried undulatory variations superimposed on general trends. Then, the growth rate *G*ₙ was calculated to make this undulation clear:

\[
Gₙ = \frac{\text{length (thickness) at time } i - \text{length (thickness) at time } i - 1}{\text{length (thickness) at time } i - 1}
\]

The time variation of *G*ₙ during development showed some kind of periodicity (Fig.1). Then, to clarify the periodical nature of the growth rate of embryo, correlogram analysis was applied. In order to apply the correlogram analysis, it is necessary to obtain a stationary time series. The variation of the growth rate is not stationary, because the embryo shows a sigmoidal growth curve and the growth rate converges to zero toward the maturing. Therefore, the growth rate was converted to the other time series *G ’ₙ* which is stationary on the mean value:

\[
G ′ₙ = Gₙ - G₀ₙ
\]

\[
G₀ₙ = (Gₙ₋₂ + 2Gₙ₋₁ + 3Gₙ + 2Gₙ₊₁ + Gₙ₊₂)/9
\]

That is, the stationary growth rate is given by the residue from the moving average. The autocorrelation function \( \gamma(T) \) was calculated for the stationary growth rate *G ’ₙ* (Fig. 2);

\[
\gamma(T) = \frac{\sum_{i=1}^{N} G ′ₙ · G ′ₙ₊T}{N(N−T)G ′²} \quad (N=121, \ T=0, 1, 2, \ldots \ldots N)
\]

\[
G ′² = \frac{\sum_{i=1}^{N} G ′ₙ²}{N}
\]

The correlogram showed a clear-cut periodicity. Then, to determine the exact period of the undulatory variation, the periodogram analysis was applied to the autocorrelogram.
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Fig. 1. Variation of the growth rate in thickness (a) and in length (b) of *T. monococcum* embryo.

Fig. 2. Autocorrelogram of the growth rate in embryo thickness (a) and in length (b).
Fig. 3. Periodogram analysis of the autocorrelogram based on the correlation coefficient between the autocorrelogram and the cosine function.
(a): in thickness (b): in length

In the present case, the author employed the correlation function between the autocorrelation function and cosine function \( \cos(2 \pi T/P) \) as a periodogram where \( P \) was the period of the cosine function. From the periodogram (Fig. 3), it is obvious that in the thickness two peaks significant at 5% level exist at \( P = 3.03 \) and 3.86. On the other hand, there exists only one significant peak \( (P = 3.00) \) in the length. But the second highest peak in the length is located at \( P = 3.87 \) which is the same as the position of the second significant peak in the thickness. Furthermore, in periodogram, several side peaks appear inevitably on both sides of a real peak, when the time series is finite. And the side peaks are gradually damped as the distances from the real peak increase. This indicates that the several peaks near \( P = 3.00 \) of the length in Fig. 3 are of no biological meaning, resulted from the finiteness of the time series. But the peak at \( P = 3.87 \) will not be incidental as it is relatively high and does not show any damping nature. This indicates that the periodogram of the length also has two real peaks at \( P = 3.00 \) and 3.87. Therefore, it is reasonably concluded that the variation of the growth rate is expressed by the summation of two cosine functions with periods of 6.06 hr \( (= 3.03 \times 2 \text{ hr}) \) and 7.72 hr \( (= 3.86 \times 2 \text{ hr}) \) for the thickness and 6.00 hr \( (= 3.00 \times 2 \text{ hr}) \) and 7.74 hr \( (= 3.87 \times 2 \text{ hr}) \) for the length;

growth rate of embryo in thickness
\[ = A \cos(2 \pi t/6.06) + B \cos(2 \pi t/7.72) + \epsilon \]
growth rate of embryo in length
\[ = A \cos(2 \pi t/6.00) + B \cos(2 \pi t/7.74) + \epsilon \]
where \( t \) is time in hours, \( A \) and \( B \) are the amplitude of the cosine functions and \( \epsilon \) denotes a random fluctuation.

Thus far, correlogram analysis and periodogram analysis revealed that the embryo of *T. monococcum* showed dual rhythmic growth. Next, to fit these rhythms to the original data, the phases of the two cosine functions at 6:00 a.m. on the second day after flowering were calculated by the following method; the correlation coefficient between the stationary growth rate and \( \cos(2 \pi (t - \tau)/P) \) were calculated for \( 0 \leq \tau < P \),
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Fig. 4. Fitting of the estimated rhythm to the stationary growth rate of embryo. The amplitude of the cosine function was determined by a visual fitting.

- : stationary growth rate in thickness
- - - : 0.08 \{0.5 \cos \left[2 \pi (t-2.92)/3.03\right] + 0.3 \cos \left[2 \pi (t-1.82)/3.86\right]\}

where $t=1, 2, \ldots, 121$ and $P=3.03, 3.00, 3.86$ or 3.87. The results of numerical calculation are summarized in the next equation;

growth rate of embryo in thickness

\[= A \cos \left(2 \pi \left(t - 5.84\right)/6.06\right) + B \cos \left(2 \pi \left(t - 3.64\right)/7.72\right) + e\]

growth rate of embryo in length

\[= A \cos \left(2 \pi \left(t - 1.72\right)/6.00\right) + B \cos \left(2 \pi \left(t - 2.96\right)/7.74\right) + e\]

where $t$ is converted into hours and $t=0$ at 6:00 a.m. on the second day after flowering.

In Fig. 4, the estimated rhythm of the growth rate in thickness is fitted to the stationary growth rate. It is confirmed from Fig. 4 that the estimated rhythm agrees well with the original growth rate from 6:00 a.m. on the fourth day after flowering. But in the earlier stage of the development, the growth rate shows no agreement with the estimated rhythm and seems to vary randomly. Embryo at 6:00 a.m. on the fourth day after flowering comprises 80~110 cells. Therefore, wheat embryo is considered to start the dual rhythmic growth at the 80~110-cell stage of the globular embryo.

**Discussion**

Rhythmicity of embryo growth has been reported only in rice (*Nagato* 1976). Rice embryo also shows dual rhythmicity with periods of 5.16 and 5.69 hr in length and 5.13 and 5.67 hr in thickness. Further, rice embryo at the very early stage of the development grows arhythmically and starts the rhythmic growth at the 90~110-cell stage. Accordingly wheat and rice embryos start the dual rhythmic growth at the same stage (about 100-cell stage). This suggests that the rhythmicity of embryo growth is universal one at least in Gramineae, though the period differs with species and/or environmental conditions.

As discussed in the previous paper (*Nagato* 1976), the appearance of dual rhythmic
growth in the early globular embryo gives suggestions on the mechanism of the formation of two apical meristems. The rhythmicity of growth rate reflects that of cell divisions (NAGATO 1976). That is, synchronous cell divisions at two regions in embryo bring about two rhythms of the growth rate. Therefore, the formation of apical meristems will be explained as follows. Zygote divides repeatedly to make an early globular embryo in which cells does not show any synchronous division except the proembryonic stage and probably there exists a gradient of the frequency of cell division in the embryo. When the development is advanced to the 100-cell stage, two pacemaker cells which entrain neighboring cells appear in two regions, as a result of intercellular interaction. This results in the synchronous cell divisions at two regions which eventually develop into two apical meristems. This mechanism is suggested by the phase-shift model of GOODWIN and COHEN (1969).

At about 100-cell stage, some drastic changes other than the start of dual rhythmic growth also occur. Exponential growth starts at the 100~200-cell stage (NAGATO 1978) and the active incorporation of $^3$H-uridine also starts at the same stage in rice and barley (NAGATO 1979). At the earlier stage, embryo grows very slowly and shows almost no uridine incorporation. These facts suggest that the 100-cell stage is a critical point in the embryonic development.

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**Literature Cited**


**コムギ（Triticum monococcum）における胚生長率の周期的変化**

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イネの胚生長率に見られた 2 つのリズムがコムギにも存在するか否かを確かめるために、*T. monococcum* を用いて胚生長率の時系列解析を行った。

1976 年 6 月 2 日午前 5 時 15 分から 6 時 15 分までの 1 時間以内に開花した穂花を、開花後 2 日目午前 6 時から 12 日目午前 8 時まで、2 時間ごとに 10 個ずつ採取し、胚長および胚厚を測定した。各時刻 7～10 個の
胚の平均値をもってその時刻の胚長・胚厚とした。
胚長および胚厚の生長率の変化に周期性が見られたので、生長率の平均値に関して定常化を行った後、生長率のコロログラム解析を行った。次に正確な周期を求めるために、コロログラムとcos関数との相関係数によるベリオドグラム解析を行った。その結果、胚厚においては6.06時間と7.72時間に有意なピーグが見られた。
従って胚厚の生長率には2つのリズムが存在する。ところが胚長については有意なピークが6.00時間のみであった。しかし、胚長での2番目に高いピークの位置は7.74時間であり、これは胚厚において2番目に有意なピーグの位置とほぼ一致している。また6.00時間付近に数個のピークが見られたが、これは時系列が有限であるために本来のピークのまわりに必然的に出現する意味のないピークであると考察される。それらの意味のないピークは本来のピークから離れに従い減衰するのであるが、7.74時間のピークは減衰性を示さない。以上のことから、胚長での7.74時間に見られるピークは無意味なものではなく、生長率が本来持っているものであると考えられる。従って、胚の生長率には2つのリズムが存在すると言える。

次に、この2つのリズムを定常化された生長率にあてはめたところ、開花後4日目午前8時頃（約100細胞期）までは生長率の変化に周期性は認められず、ランダムに変化しているようであった。従って、生長率の2つのリズムは球状胚の約100細胞期になって出現すると考えられる。この生長率の2つのリズムは、胚の2つの領域での同調した細胞分裂を反映したものであろう。そしてその2つの領域が将来2つの頂端分裂組織に分化すると考えられる。
この100細胞期は生長率のリズムが始まるだけでなく、指数生長が始まる時期でもある。またイネやオオムギではこの時期にuridineの活発な取り込みが始まる。従って、球状胚の約100細胞期は胚の発育にとって極めて重要な時期であると考えられる。