Rhythmic Variations of the Growth Rate in Rice Embryo Revealed by Time Series Analysis

Yasuo NAGATO

Faculty of Agriculture, University of Tokyo, Tokyo 113

To analyze the growth of embryo of *Oryza sativa* cultivar, "Nihonbare" in detail, caryopses were collected at every ninety minutes. At each time, (relative) growth rate was calculated for the length and the thickness of embryo, and the time series analysis was applied to the variations of the growth rate. At first, correlogram analysis was made on the stationary growth rate and the apparent periodicity was detected as a result of the calculation. In the next place, as a periodogram, the correlation coefficient between the autocorrelogram and the cosine function was employed. Periodogram shows two sharp peaks and reveals the existence of two rhythms in the variations of the growth rate. In other words, the variations of the growth rate are represented by the summation of two cosine functions. Periods of two cosine functions are 5.16 and 5.69 hrs for the growth rate in length and 5.13 and 5.67 hrs in thickness. The difference of periods between the growth rate in length and in thickness is not significant.

In the next place, to reveal the biological nature of two rhythms of the growth rate at the cellular level, the increasing rate in cell number on the central longitudinal section of embryo was calculated. On the careful comparison, it was confirmed that the two rhythms of growth rate agreed very well with the variations of the increasing rate in cell number except at the early stage of the development. At the early stage, both the growth rate and the increasing rate in cell number seem to show arrhythmicity.

Therefore, it is considered that the two rhythms of growth rate reflect the rhythmicity of cell divisions. However, the two rhythms do not exist at the beginning, and appear in the early period of the development. The existence of the two rhythms indicates that the cells divide synchronously in two regions. The author supposes that the two rhythms correspond to the synchronous cell divisions at the two growing points in plumule and radicle.

Introduction

In plant, morphogenesis is performed by the growing points which are formed in embryogenesis, and hence, it is considered that the variations in embryo bring about many morphological variations in mature plant. Therefore, the studies on embryogenesis is expected not only to reveal the fundamental mechanism of morphogenesis but also to make a contribution to our understandings how the variations arise.

In plant embryo, differentiation follows a certain growth such as a formation of a globular embryo, and the growth and differentiation are advanced in company with one another. Therefore, quantitative analysis on the growth of embryo seems to be one of the effective approaches to reveal the mechanism of embryogenesis. From this point of view, the author tried to analyze the growth of rice embryo in detail.

It is already known that a plant embryo grows following a sigmoid curve. But the

Received November 6, 1975

growth curve is not smooth everywhere and contains certain deviations from the sigmoid curve. Whether these deviations are produced due to the sampling errors or are intrinsic in the growth of embryo, is not clear. To answer this question, it is necessary to analyze the growth curve in detail. But so far the studies on the growth of embryo have been confined to plotting the sizes of embryo on a graph, measuring them every one or two days (SUETSUGU 1953, YAMAKAWA 1962). There has been no study in which the data on the growth of embryo are dealt as a time series and are made correspondence with a morphogenesis. The embryo sizes measured at the same interval form a time series. So, a time series analysis (correlogram analysis and spectral analysis) must be a very effective means to analyze the growth of embryo. As an example of the time series analysis on plant development, KUMAZAWA and KUMAZAWA (1971) applied this method successfully to analyze the phyllotaxis. In this study, a time series analysis is made on the variations of the growth rate in rice embryo.

Material and Measurement

Oryza sativa cultivar, "Nihonbare" was used in this experiment. Plants were grown in the glass house under usual cultural conditions. Caryopses blossomed from a.m.11:30 till 12:00 of 29th in August, 1974 were marked and collected at every ninety minutes from a.m.0:00 on the second day till a.m.10:30 on the eighth day after flowering. At each time, 10 caryopses were sampled randomly from more than five plants.

Collected caryopses were fixed with Farmer's fluid (100% ethyl alcohol 3:glacial acetic acid 1) for thirty minutes and then sectioned by the usual paraffin method. The thickness of the section was $10\mu\text{m}$ for the samples collected till the sixth day after flowering and $12\mu\text{m}$ for the samples collected after then. Sectioned samples were stained with Delafield's hematoxylin.

The length of embryo and the thickness of embryo are defined by the maximal length of embryo from the base of suspensor and by the maximal thickness of embryo at the right angle to the length of embryo respectively. They were measured with an ocular micrometer on good sections. Using the same sections as used for measuring the embryo sizes, the cell number on the central longitudinal section of embryo was counted. Because counting the cell number was very laborious and the time series analysis was not applied, only the samples collected till a.m.3:00 on the fifth day after flowering were used. The measured sizes or cell number on 7-10 samples as each time were averaged to give the length or thickness of embryo and the cell number on the central longitudinal section of embryo at that time.

Application of Correlogram Analysis and Periodogram Analysis

The growth curve of the embryo shows the sigmoid type which carries a particular undulatory variation superimposed on a general trend. The smooth trend of the growth

Fig. 1. Variations of the growth rate in length (a) and in thickness (b) of rice embryo.

curve is out of interests, and attention is paid to the undulatory nature of the growth. This undulation is well manifested by the growth rate G_i , which is defined by

$$G_i = \frac{\text{length (thickness) at time } i - \text{length (thickness) at time } i-1}{\text{length (thickness) at time } i-1}$$

The time variation of the growth rate of the embryo in length and in thickness during the development is shown in Fig.1. It is noted that G_i apparently shows some kind of periodicity. Periodicity is studied by the correlogram analysis which is the common practice in the time series analysis. In order to apply the correlogram analysis, it is necessary to have the stationary time series. Because the variation of growth rate is not stationary (growth rate must converge to zero toward a maturing of embryo), the growth rate is converted into the other time series G_i' which is stationary on the mean value;

$$G_i' = G_i - G_{0i} \quad (1)$$

$$G_{0i} = (G_{i-2} + 2G_{i-1} + 3G_i + 2G_{i+1} + G_{i+2})/9$$

In other words, the stationary growth rate is given by the residue from the moving average. The series G_i' thus obtained maintains the fundamental characteristics on the periodicity of the original data, and hence they were used for the calculation of the correlogram. The autocorrelation function $r(T)$ of the growth rate G_i' defined by

$$r(T) = \frac{\sum_{i=1}^{N-T} G_i' \cdot G_{i+T}' / (N-T) \sigma_{G'}^2}{\sum_{i=1}^N G_i'^2 / N} \quad (N=103, T=0, 1, 2, \dots, <N) \quad (2)$$

$$\sigma_{G'}^2 = \frac{\sum_{i=1}^N G_i'^2}{N}$$

was calculated for G_i' and shown in Fig.2. The correlogram also shows a periodic nature. Since the autocorrelation function is an even function (the positive half ($T \geq 0$) alone is shown in Fig.2), it is represented by cosine functions only;

$$r(T) = \int_0^\infty f(p) \cdot \cos(2\pi T/p) d(1/p) \quad (3)$$

Fig. 2. Autocorrelogram of the growth rate in length (a) and in thickness (b).

where $f(p)$ is a spectrum amplitude at period p . Therefore, the ordinary Fourier cosine transformation of $r(T)$ represents the spectrum of $r(T)$ and thus that of G_i' . In the present case, however, we have two problems; (1) $r(T)$ is given at discrete values of T , and (2) $r(T)$ is not a series long enough to eliminate the truncation error in Fourier transformation. Further, the fine structure of the spectrum must be clarified.

Therefore, the author employed the correlation coefficient $f(p)$ between $r(T)$ and $\cos(2\pi T/p)$ as a periodogram;

$$f(p) = \frac{\sum_{T=1}^N [\bar{r}(T) - \overline{\bar{r}(T)}] \cdot [\cos(2\pi T/p) - \overline{\cos(2\pi T/p)}] / N}{\sqrt{\sum_{T=1}^N [\bar{r}(T) - \overline{\bar{r}(T)}]^2 / N} \cdot \sqrt{\sum_{T=1}^N [\cos(2\pi T/p) - \overline{\cos(2\pi T/p)}]^2 / N}} \quad (N=50) \quad (4)$$

where $\overline{\bar{r}(T)} = \sum_{T=1}^N \bar{r}(T) / N$, and $\overline{\cos(2\pi T/p)} = \sum_{T=1}^N \cos(2\pi T/p) / N$

The $f(p)$ in formula (4) is free from truncation error by the compensation introduced by

Fig. 3. Periodogram analysis of the autocorrelogram based on the correlation coefficient between the autocorrelogram and the cosine function with period p .
(a) : in length (b) : in thickness

$\overline{r(T)}$ and $\overline{\cos(2\pi T/p)}$. Further, formula (4) gives $f(p)$ at any value of p . The result of the numerical calculation of formula (4) is shown in Fig.3. From the periodogram (Fig.3), it is obvious that the two sharp peaks with almost the same height exist at $p=3.44$ and 3.79 for the length of embryo and $p=3.42$ and 3.78 for the thickness of embryo. The presence of two peaks stated in the above is certain with a risk less than 1%. The error involved in the numerical values of these periods is less than 1.5%, and hence, the difference of the periods between the length and the thickness is not significant. Consequently, the undulatory variations of the growth rate of embryo in the length and the thickness can be expressed by the summation of two cosine functions with periods of 5.16 hrs($=3.44 \times 1.5$ hrs) and 5.69 hrs($=3.79 \times 1.5$ hrs) for the length and 5.13 hrs($=3.42 \times 1.5$ hrs) and 5.67 hrs ($=3.78 \times 1.5$ hrs) for the thickness;

growth rate of embryo in length

$$= A[\cos(2\pi t/5.16) + \cos(2\pi t/5.69)] + e$$

growth rate of embryo in thickness

$$= A[\cos(2\pi t/5.13) + \cos(2\pi t/5.67)] + e$$

where t is time in hours, A is the amplitude of the cosine function and e denotes a random fluctuation.

Thus far, the correlogram analysis and the periodogram analysis were applied successfully and it was revealed that the variations of the growth rate showed two rhythms. Now, to fit these estimated rhythms to the original data, the phases of the two cosine functions at a.m. 0:00 on the second day after flowering were calculated for both the length and the thickness of embryo by the following method; the correlation coefficient between the stationary growth rate and $\cos[2\pi(t-\tau)/p]$ were calculated for $0 \leq \tau \leq p$, where $t=1, 2, 3, \dots, 103$ and $p=3.44, 3.42, 3.78$ or 3.79 . The phases of each cosine function at a.m. 0:00 on the second day after flowering are summarized in the next equations;

growth rate of embryo in length

$$= A\{\cos[2\pi(t-3.36)/3.44] + \cos[2\pi(t-2.90)/3.79]\} + e$$

growth rate of embryo in thickness

$$= A\{\cos[2\pi(t-0.14)/3.42] + \cos[2\pi(t-3.16)/3.78]\} + e$$

or, if converted into hours,

growth rate of embryo in length

$$= A\{\cos[2\pi(t-5.04)/5.16] + \cos[2\pi(t-4.35)/5.69]\} + e$$

growth rate of embryo in thickness

$$= A\{\cos[2\pi(t-0.21)/5.13] + \cos[2\pi(t-4.74)/5.67]\} + e$$

where $t=0$ at a.m. 0:00 on the second day after flowering.

The phase differences between the rhythms in length and in thickness will not be significant and both the growth rates in length and in thickness show the same rhythmicities. In Fig.4, the estimated rhythm of growth rate in length is fitted to the stationary growth rate in length. From Fig.4, it is confirmed that the estimated rhythm agrees well with the original growth rate. But at the very early stage of the development, the variations of the growth rate seem to show arrhythmicity.

Fig. 4. Fitting of the estimated rhythm to the stationary growth rate of embryo. Amplitude of the cosine function was determined by a visual fitting.

— : stationary growth rate in length

----- : $0.03\{\cos[2\pi(t-3.36)/3.44] + \cos[2\pi(t-2.90)/3.79]\}$

Foot Note

In 1973, similar experiment was done. The samplings were done at every three hours in this case. As a result of the time series analysis, periods of 5.67 hrs and 6.36 hrs were obtained. These were different from the periods obtained in 1974. It is not clear whether these differences were resulted from the differences of the environmental conditions or the difference of the sampling interval. In the first place, the time series analysis was made on the data collected at every three hours from the data in 1974 and the periods of 5.67 hrs and 6.36 hrs were calculated. Therefore, it is concluded that the sampling interval affects the calculated period. The next question is which period is real. To solve this question, the growth of an embryo was assumed to have a growth rate of $\{\cos(2\pi t/5.1 \text{ (hrs)}) + \cos(2\pi t/5.7 \text{ (hrs)})\}$. Then, the time series analysis was made on the data sampled at every three hours (i.e., samplings were 1.7 and 1.9 times in each period), and periods of 5.7 hrs and 6.3 hrs were obtained. On the other hand, by the time series analysis based on the data sampled at every 1.5 hrs (i.e. samplings were 3.4 and 3.8 times in each period), periods of 5.1 hrs and 5.7 hrs were obtained.

The above calculation shows that (1) there is almost no difference between the real period and the calculated period, if samplings are done more than 3.4 times in each period, but (2) the real period is distorted by the calculation, if samplings are done only 1.7 times in each period. Therefore, it is concluded that (1) periods in 1973, though distorted by the calculation, will be the same as the periods in 1974, and (2) periods calculated in 1974 do not differ from the real periods.

Rhythmicity at the cellular level

The above results were obtained only through the extraction of the statistical properties of the data. But the biological nature of the two rhythms is not obvious. It is necessary to reveal what correspond at the cellular level to the two rhythms of growth rate.

The growth of embryo is attained by cell divisions and the cell enlargement. But it is difficult to suppose that the cell enlargement shows its intrinsic rhythmicity, because there

Fig. 5. Time variation of the increasing rate in cell number. Cell number was counted on the central longitudinal section of embryo.

Fig. 6. Comparison of the rhythm of embryo growth in length with the stationary increasing rate in cell number.

— : increasing rate in cell number

----- : $0.03\{\cos[2\pi(t-3.36)/3.44] + \cos[2\pi(t-2.90)/3.79]\}$

is an upper limit in the cell size and the cells in embryo are small. Accordingly, it is conjectured that the two rhythms of growth rate will be resulted from the rhythmicity of cell divisions. To confirm this conjection, the rhythmicity of cell divisions was examined by counting the cell number on the central longitudinal section of embryo. The increasing rate in cell number was calculated and shown in Fig.5. In Fig.6, the rhythms of growth rate in length is fitted to the stationary increasing rate in cell number. It is obvious that the close agreement between the rhythm of growth rate and the variations of the increasing rate in cell number is obtained. Therefore, though the time series analysis is not made on the variations of the increasing rate in cell number, the rhythms of growth rate must reflect the rhythmicity of cell divisions. From Figs.5 and 6, it is also apparent that at the early stage of the development (probably before 18:00 on the second day after flowering), the variations of the increasing rate in cell number do not agree well with the rhythms of growth rate and even show arrhythmicity.

Therefore, it is considered that the two rhythms of growth rate are resulted from the rhythmicity of the increasing rate in cell number. In addition, the two rhythms do not exist from the starting point (=zygote), but appear at the early stage of the development.

Discussion

The rhythmicity of the growth of embryo has never been reported. But the rhythmic phenomena are very common in plants and animals. It is not surprising that the rhythmicity also exist in th growth of embryo. In this study, the two rhythms were detected

in the growth of embryo and were revealed to reflect the rhythmicity of cell divisions. The existence of the two rhythmicity in cell divisions indicates that the cells in embryo divide synchronously in two regions. This will be possible if cells in embryo interact with one another, though the synchronous cell division in plant embryo has not been reported. As to this problem, GOODWIN and COHEN (1969) indicated one of possibilities of the synchronization of cells and the partitions of cells into several regions by "phase-shift model". According to their model, cells are partitioned into regions and the cell with the highest frequency, called "pacemaker cell", entrains other cells in each region, if the following conditions are satisfied; (1) each cell has an autonomous "s-event", (2) cells interact with one another, i.e., each cell receives signals on "s-event" from the other cells, (3) a gradient of the frequency of "s-event" exist between cells, (4) a refractory period exists in which a cell cannot receive signals on "s-event" from the other cells. If "s-event" is replaced by cell division, this model will be applicable to the present case. Based on this model, the formation of two rhythms may be interpreted as follows; at the early stage of the development, a gradient of periods of cell divisions exists on a globular embryo, but with the increase of cell number, cells cannot interact with all other cells and the interactions are confined to the neighbouring cells and embryo is partitioned into two or more regions. After then, cells begin to divide synchronously in each region.

In other words, using the terminology of catastrophe theory (THOM 1972), a gradient of periods of cell divisions exists in the beginning (early globular embryo) and no attractor exists, but with the advance of the development, two attractors appear and the embryo is partitioned into two regions, each of which is controlled by one attractor. The author supposes that the two attractors correspond to the two growing points in the plumule (shoot) and the radicle (root), and the two rhythms of growth rate correspond to the synchronous cell divisions in the plumule and in the radicle. It is naturally supposed that the two rhythms of the growth of embryo exist because of the existence of the two growing points, and that the stability of the shoot and the root formation is provided by the rigid interactions of cells in these regions which bring about the synchronous cell divisions. Of course, whether the two rhythms of the growth of embryo indeed correspond to the synchronous cell divisions in the plumule and the radicle or not must be solved in the future study.

Acknowledgement

The author wishes to express his thanks to Prof. T. NAKAJIMA and Dr. K. YAMAMOTO for their helpful discussions.

Literature Cited

- GOODWIN, B. C. and M. H. COHEN 1969. A phase-shift model for the spatial and temporal organization of developing systems. *J. Theoret. Biol.* **25** : 49~107.
- KUMAZAWA, M. and M. KUMAZAWA 1971. Periodic variations of the divergence angle, internode length, and leaf shape, revealed by correlogram analysis. *Phytomorphology* **21** : 376~389.

- SUETSUGU, I. 1953. Developmental morphology of the embryo in rice variety. Bull. Nat. Inst. Agr. Sci., Ser. D. No. 6 : 23~52.
- THOM, R. 1972. Stabilité structurelle et morphogénèse. Benjamin, New York
- YAMAKAWA, Y. 1962. Studies on the ecological variations of the growth of rice plant caused by the shifting of cultivation season in warm region in Japan. Agr. Bull. Saga Univ. 14 : 23~159.

イネ胚生長の時系列解析

——生長率の周期的変化——

長 戸 康 郎

(東京大学農学部)

植物においては、形態形成の基本的な型が胚発生に集約されているので、植物体にみられる形態的変異の多くは、胚発生における変異に基づくと考えられる。したがって胚発生についての詳細な研究によって、形態形成の基本的な機構が明らかにされるだけでなく、さまざまな変異の成因についても多くの基本的な知見が得られるものと考えられる。このような考えのもとで、イネの胚発生に関する研究を進めるため、水稻品種日本晴を供試して、胚の生長についての詳細な解析を行った。

1974年8月29日午前11時30分から12時までに開花した穎花を、開花後2日目の午前零時から8日目の午前10時30分まで1時間半ごとに5個体以上の植物体からランダムに10個ずつ採取した。その後パラフィン切片を作製し、胚長、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。

まず各時刻における胚長・胚厚の(相対)生長率を求めたところ、周期的な変化が観察された。そこで、時系列解析を行なうために、移動平均からの残差を求めることによって、生長率の平均値に関する定常化を行なった。この定常化された生長率を用いてコレログラム解析を行なった。コレログラムもある種の周期性を示したので、その周期を求めるためにペリオドグラム解析を行なった。ここでは、コレログラムとCOS関数との相関係数によるペリオドグラム解析を行なった。その結果、胚長・胚厚ともに、約5.15時間と約5.68時間に著しい有意なピークが見られた。従って、胚長・胚厚の生長率は、ともに二つのCOS関数の和として表わすことができる。また胚長と胚厚のCOS関数の位相のずれもわずかであった。従って、胚の生長率には二つのリズムが存在する。

次に、胚生長のリズムを細胞レベルでの変化と対応づけるために、開花後5日目の午前3時までの材料を用いて、胚の中央部縦断面の細胞増加率を求めた。細胞増加率の変化は、生長率の変化とほぼ一致しており、生長率のリズムは、細胞増加率のリズム、即ち、細胞分裂のリズムの反映であると考えられる。

しかし、生長率及び細胞増加率は、胚発生のごく初期(開花後2日目の午後6時ぐらいまで)では周期性を示さず、ランダムに変化している。従って、生長率の二つのリズムは、最初から存在しているのではなく、胚発生の途中で形成されるものと考えられる。

また、その二つのリズムは、胚に存在する二つの生長点——幼芽及び幼根の生長点——における細胞分裂のリズムであろうと推察される。