Stability of Embryo Growth in Rice

Yasuo NAGATO

Faculty of Agriculture, University of Tokyo, Tokyo 113

This study aims at investigating how temperature affects the growth of rice embryo developing under three temperature conditions (30~25°C, 25~20°C, 20°C). As expected, the increase in length and in cell number was delayed as the temperature fell. But the cell number at the start of exponential growth and that at the changing point of the rate of increase in cell number did not vary with the temperature. Also, the coefficient of allometry between the length and thickness changed at definite embryo lengths irrespective of the variation in temperature. The modification in embryo shape, estimated by the residual variance in the coefficient of allometry and by the coefficient of variation in the ratio of the thickness to length, was wide at the globular stage irrespective of the temperature conditions and increased as the temperature fell.

Treatment consisting of exposure to low temperature of 20°C was applied at various developmental stages when embryos were developing at 30~25°C. Embryo growth was delayed by about 12 hours immediately after such treatment. This delay was observed in every treatment until the embryo had almost reached maturity. Therefore, the pattern of embryo growth curve is considered to be stable. On the other hand, the shape of the embryo varied more widely than that of the control after exposure to a temperature of 20°C at the globular stage (especially in the late period). When such treatment was applied after the second leaf had differentiated, the variation in embryo shape was not wider than in the control. These results indicate that embryo shape is unstable at the globular stage and become stable after the embryo is differentiated morphologically.

Introduction

In the previous paper (NAGATO 1978), general characteristics of embryo growth were analyzed, revealing that embryo does not grow smoothly at the globular stage which may be divided into three periods. Moreover, at the globular stage, embryo shape varies more widely than at the stage following the "two point projection" (first morphological change from the globular embryo). These results suggest that the globular embryo is more unstable than the embryo after the "two point projection". Then, the effect of environmental conditions on the general characteristics of embryo growth must be investigated. YAMAKAWA (1962) studied the embryo growth when rice plants were cultivated in various seasons, but only growth retardation at low temperature was noted. In the present study, to clarify the response of embryo to the change of temperature and to evaluate the stability of embryo, embryo growth was monitored under various temperature conditions and the effect of exposure to low temperature for 24 hours on the embryo growth was recorded.

In biological phenomena, the term "stability" is not used strictly. Stability of a system is mathematically defined as follows: a system is stable, if a trajectory of the system does not change when the initial value of the system is perturbed. According to this definition, an embryo is stable if a trajectory of the development does not change when it is perturbed, for example, by exposure to low temperature. In the present study, the term "stability" is used in this sense.

Materials and Methods

Oryza sativa L. cultivar Nipponbare was used as material. Plants were cultivated under usual conditions. Two experiments were conducted using this material.

Experiment 1.

Caryopses which had blossomed from 9:15 a.m. till 11:15 a.m. on 6 September 1975 were sampled and developed under the following three temperature conditions: 30°C during daytime and 25°C during the night, 25°C during daytime and 20°C during the night and under a constant temperature of 20°C. Caryopses were collected every six hours from 0:00 a.m. on the first day till 6:00 p.m. on the eighth day, at 0:00 a.m. and at 12:00 a.m. on the ninth day, and at 12:00 a.m. each day from the tenth till the fifteenth day after flowering. Furthermore, caryopses developing at a constant temperature of 20°C were collected at 12:00 a.m. each day until the twentieth day after flowering.

Experiment 2.

Caryopses which had blossomed from 9:15 a.m. till 11:15 a.m. on 6 September 1975 were sampled and developed at 30°C during daytime and 25°C during the night. Though various combinations of temperature and duration of treatment might be considered, the combination of a temperature of 20°C to 24 hour treatment was selected in this experiment because of the duration of the middle and the late periods of development of the globular embryo (=about 24 hours respectively) and of the available experimental facilities. Exposure to low temperature (20°C) was as follows:

A (control): No exposure. That is, embryo remained at 30~25°C throughout the development.

B: Exposure to 20°C lasted for 24 hours from 7:00 a.m. on the second day after flowering. Subsequently, embryo developed again at 30~25°C. Embryos were 45~50 μm long and were composed of 30~50 cells at 6:00 a.m. on the second day (= just before the onset of the treatment). They were 50~62.5 μm long with 80~160 cells at 6:00 a.m. on the third day (= just before the end of the treatment). Therefore, the exposure to 20°C may be regarded as having taken place in the middle period of the globular stage of the embryonic development.

C: Exposure to 20°C lasted for 24 hours from 7:00 a.m. on the third day after flowering. Subsequently, embryo developed again at 30~25°C. Embryos were 57.5~82.5 μm long and comprised 160~400 cells at 6:00 a.m. on the third day. They were 87.5~115.0 μm long with 400~900 cells at 6:00 a.m. on the fourth day. Therefore, this treatment may be regarded as having taken place in the late period of the globular stage of embryonic development.

D: Exposure to 20°C lasted for 24 hours from 7:00 a.m. on the sixth day. Subsequently, embryo developed again at 30~25°C. Embryos were 600~750 μm long at 6:00 a.m. on the sixth day and 780~980 μm long at 6:00 a.m. on the seventh day. Therefore, this treatment may be regarded as having taken place after the second leaf had differentiated.

In each treatment, ten caryopses were randomly collected as already mentioned in experiment 1. Caryopses collected were fixed with Farmer's fluid (100% ethyl alcohol

3: glacial acetic acid 1), sectioned after embedding in paraffin, and stained with Delafield's hematoxylin.

Average embryo length, thickness and cell number were measured on 7~10 samples each time. Cell number was estimated by counting the number of nuclei in the embryo.

Results

Experiment 1—Growth of embryo under three temperature conditions

1) Length increase

The increase of embryo length is shown in Fig.1. Embryo growth was delayed as the temperature fell. This figure also shows that under all conditions the embryo showed exponential growth only after completing a period of slow growth at the early stages. The regression line was calculated in the samples which appeared to have reached the stage of exponential growth to determine when the exponential growth started. Then the regression line was extrapolated and the point at which it intersected with the growth curve was estimated as the starting point of exponential growth. The cell number at that time was constant throughout the treatments (Table 1). Therefore, it is concluded that the embryo shows constant exponential growth at the 100~200 cell stage, though

Fig. 1. Increase of embryo length.

Table 1. Regression line and starting point of exponential growth in embryo length

Temperature	Regression line*	Starting point of exponential growth	
		Time after flowering	Cell number
30~25°C	$y=0.828x+1.584$	6:00 p.m. on the 2nd day ~6:00 a.m. on the 3rd day	100~180
25~20°C	$y=0.668x+1.475$	12:00 a.m. on the 3rd day ~0:00 a.m. on the 4th day	100~180
20°C	$y=0.413x+1.809$	6:00 a.m. on the 5th day ~6:00 p.m. on the 5th day	120~200

* $x=1$ at 0:00 a.m. on the 1st day, $x=2$ at 0:00 a.m. on the 2nd day after flowering and y =embryo length (μm) in logarithms.

Fig. 2. Regression lines of increase in cell number.

variance around the regression line was estimated as the changing point of the rate of increase in cell number. Table 2 shows the results of calculation. The cell number at the intersecting point was constant throughout the experiment (20~40 cells), showing values identical to those obtained in wheat and barley (NAGATO 1978). Therefore, it is concluded that the change in the rate of increase in cell number as well as the start of the exponential growth occur when the embryo comprises a definite number of cells common to many species.

Table 2. Regression line and the intersecting point of cell number increase

Temperature	Regression line*		Cell number at the intersecting point
	1st half	2nd half	
30~25°C	$y=2.936x-2.626$	$y=1.834x-0.349$	31
25~20°C	$y=2.186x-2.238$	$y=1.395x-0.185$	31
20°C	$y=1.309x-1.134$	$y=0.919x+0.174$	26

* $x=1$ at 0:00 a.m. on the 1st day, $x=2$ at 0:00 a.m. on the 2nd day after flowering and y =cell number in logarithms.

3) Allometric growth between the length and thickness

The allometric growth between embryo length and thickness was analyzed in the same manner as in the previous paper (NAGATO 1978). Allometric growth applies not only to exponential growth but also to a logistic growth approximately. It shows the relative relationship between length and thickness growth rates and then the change in embryo shape. It is clear from Fig. 3 that under each condition the coefficient of allometry may be approximated by intersecting lines (= axes of the first principal components, not the

the growth rate decreases as the temperature falls.

2) Increase of cell number

Increase of cell number was examined after transformation into logarithms (Fig. 2). Fig. 2 shows that under each condition the cell number increased exponentially from the start, showing intersecting lines with one intersecting point, though the rate of increase in cell number declined as the temperature fell. The intersecting point was calculated by the following method. An intersecting point was moved successively and two regression lines each in the first and the second half were calculated. The intersecting point which minimized the residual

Fig. 3. Allometric growth between embryo length and thickness.

(a).....30~25°C (b).....25~20°C (c).....20°C

Table 3. Allometric growth between embryo length and thickness

Temperature	Coefficient of allometry			Changing point of coefficient of allometry (embryo length in logarithms)	
	1st section	2nd section	3rd section	1st	2nd
30~25°C	1.179	0.791	1.316	4.3~4.5 μm	6.6~6.8 μm
25~20°C	1.401	0.767	1.430	4.1~4.5 μm	6.9~7.1 μm
20°C	1.113	0.782	1.211	4.3~4.5 μm	6.7~6.9 μm

regression line) with two intersecting points. Two intersecting points were determined by the following method. Two intersecting points were moved by 0.1 or 0.2 μm of the length in logarithms and then the residual variance around the axes of the first principal components was calculated for each set of two intersecting points. The set of two intersecting points

which minimized the residual variance was defined as the changing points of the coefficient of allometry (Table 3). The positions of the intersecting points hardly varied with the change in temperature. The first intersecting point slightly preceded the "two point projection" and the second one followed the differentiation of the second leaf. That is, under each temperature condition, the embryo became slender after the "two point projection" and plumped towards maturity.

The residual variance of the coefficient of allometry around the axes of the first principal components was calculated for the globular stage and for the stage following the "two point projection" (Table 4). Under each condition, the residual variance was larger at the globular stage than at the stage following the "two point projection", and increased as the temperature fell. Therefore, it is considered that the globular embryo is sensitive to the change of the temperature.

4) Change in the ratio of the thickness to length

The ratio of the thickness to length changed according to the change in the coefficient

Table 4. Residual variance of coefficient of allometry around the axes of first principal component in the allometric growth between embryo length and thickness

Temperature	Globular stage	After "two point projection"
30~25°C	0.0017	0.0011
25~20°C	0.0017	0.0013
20°C	0.0030	0.0018

Fig. 4. Change in the ratio of embryo thickness to length.

of allometry, and this changing pattern did not vary with the temperature (Fig. 4). The variation in the ratio of the thickness to length was examined using a coefficient of variation. The coefficient of variation was calculated in dividing the embryo length so as to obtain 10~20 samples in each section. Embryo less than $110\mu\text{m}$ long was sectioned every $10\mu\text{m}$, embryos measuring from $110\mu\text{m}$ till $200\mu\text{m}$ in length were cut into two sections, $110\sim150\mu\text{m}$ and $150\sim200\mu\text{m}$ respectively. Embryos measuring from $200\mu\text{m}$ till $1500\mu\text{m}$ in length were sectioned every $100\mu\text{m}$. Then the coefficients of variation before and after the "two point projection" were averaged respectively and shown in Table 5. The coefficient of variation was larger at the globular stage than at the stage following the "two point projection". It was also larger at lower temperatures. At a constant temperature of 20°C , the coefficient of variation at the globular stage increased by 45% compared with that at $30\sim25^\circ\text{C}$. This indicates that lower temperatures increase the variation of embryo shape, especially at the globular stage.

Table 5. Coefficient of variation in the ratio of embryo thickness to length

Temperature	Globular stage	After "two point projection"
$30\sim25^\circ\text{C}$	0.0498	0.0390
$25\sim20^\circ\text{C}$	0.0530	0.0420
20°C	0.0723	0.0503

Fig. 5. Effect of exposure to 20°C for 24h on the increase of embryo length.

.....x..... treatment in the middle period of globular stage
 ---○--- treatment in the late period of globular stage
△..... treatment after the differentiation of the second leaf
 —●— control.

Experiment 2—Stability of embryo growth**1) Length increase**

In each treatment, embryo growth was delayed by about 12 hours immediately after exposure at 20°C (Fig. 5). For example, the time when embryo became about 270 μm long was 6:00 p.m. on the fourth day in the control and about 6:00 a.m. on the fifth day in treatments applied in the middle and late globular periods. Also, the embryo reached a length of approximately 1200 μm at about 0:00 a.m. on the eighth day in the control and at about 12:00 a.m. on the eighth day in all treatments. It is interesting to point out that this delay lasted till the maturity (about the twelfth day after flowering) whenever the embryo growth was perturbed. This indicates that the exposure at 20°C affects the embryo growth to delay immediately after the treatment but does not affect the pattern of the growth curve after the treatment. Therefore, the pattern of the growth curve appears to be stable.

The cell number at the start of the exponential growth when the treatment was applied in the middle period of the globular stage was 100~200, as in the control.

2) Allometric growth between the length and thickness

Allometric growth between the length and thickness was examined in embryos longer than 4.5 μm (logarithm) when the treatment was applied in the middle or late period of the globular stage. It was also investigated in embryos collected after exposure to

Table 6. Allometric growth between embryo length and thickness

Treatment	Coefficient of allometry		Changing point of coefficient of allometry (embryo length in logarithms)
	2nd section	3rd section	
Globular stage			
Middle period	0.765	1.275	6.7~6.9 μm
Late period	0.777	1.385	6.8~7.0 μm
After second leaf differentiation	0.769	1.312	6.7~6.9 μm
Control	0.791	1.316	6.6~6.8 μm

20°C following the differentiation of the second leaf. In each treatment, the allometric growth was approximated by intersecting lines with one intersecting point (Table 6). The coefficient of allometry and the position of intersecting point did not change when the treatment was applied. Table 7 shows the residual variance around the axes of the first principal components after the "two point projection". From the table, it is clear that when exposure at 20°C took place at the globular stage, the residual variance became larger than in the control (significantly larger in the late period treatment than in the control). On the other hand, the residual variance showed almost the same value as the control when the embryo was treated after

Table 7. Residual variance of coefficient of allometry around the axes of first principal component in the allometric growth between embryo length and thickness

Treatment	After "two point projection"
Globular stage	
Middle period	0.0013
Late period	0.0015
After second leaf differentiation	0.0009
Control	0.0011

the second leaf had differentiated. Therefore, it is considered that embryo shape is unstable at the globular stage and becomes stable after the morphological differentiation has occurred.

3) Change in the ratio of the thickness to length

Exposure at 20°C affected the change in the ratio of the thickness to length in the same way as the growth curve (Fig. 6). The change of the ratio was delayed by about 12 hours immediately after the treatment was applied as compared with the control, and the delay lasted until the twelfth day after flowering, when the embryo was nearly mature.

Thereafter, the variation of the ratio was examined using a coefficient of variation as in experiment 1. The results listed in Table 8 show that when the treatment was applied in the late period of the globular stage, the coefficient of variation increased by 60% at the globular stage and by 27% at the stage following the "two point projection" as compared with the control. On the other hand, when exposure at 20°C took place after the differentiation of the second leaf, the coefficient of variation after the treatment showed almost the same value as in the control. Therefore the embryo shape is unstable at the globular stage (especially in the late period) and becomes quite stable after the morpho-

Fig. 6. Effect of exposure to 20°C for 24h on the change in the ratio of embryo thickness to length.

.....×..... treatment in the middle period of globular stage
 —○— treatment in the late period of globular stage
△..... treatment after the differentiation of the second leaf
 —●— control.

Table 8. Coefficient of variation in the ratio of embryo thickness to length

Treatment	Treatment~"two point projection"	After "two point projection"
Globular stage		
Middle period	0.0565 (+24.0%)	0.0460 (+18.0%)
Late period	0.0677 (+59.3%)	0.0497 (+27.4%)
After second leaf differentiation	—————	0.0379* (-4.3%)
Control	—————	0.0390

* This value is for embryos longer than 700 μm .

Figures in parentheses indicate the comparison with control.

logical differentiation has taken place.

Discussion

In the present study, the interaction between embryo growth and temperature was investigated. Embryo growth was delayed as the temperature fell. The temperature conditions did not affect the cell number at the onset of the exponential growth, nor the cell number at the changing point of the rate of increase in cell number nor the length at the changing point of the coefficient of allometry between the length and thickness. Embryo form varied more widely as the temperature fell.

Exposure to a temperature of 20°C at various developmental stages enabled to demonstrate that the form of the growth curve and the changing pattern in the ratio of the thickness to length were stable while the embryo shape was unstable at the globular stage but became quite stable at the stage following the "two point projection".

The above results suggest that cells in the globular embryo (especially in the late period) do not interact strictly and that the globular embryo is not organized as an established system. Though the presence of an exponential growth and of two rhythms in the growth rate (NAGATO 1976) indicates that cells undergo active interaction at the 100~200 cell stage, intercellular interaction is not established at first and is easily affected by environmental perturbations. It is established only after the morphological differentiation has taken place.

Unstability of globular embryo can be found not only in rice embryo but also in wheat and barley embryos because as stated in the previous paper (NAGATO 1978), the above species show the same number of cells at the onset of the exponential growth and at the changing point of the rate of increase in cell number, suggesting that rice, wheat and barley share a common developmental mechanism. Moreover, unstability of globular embryo or stability of embryo following the "two point projection" should become an important clue to investigations pertaining to evolutionary process in plants.

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イネ胚生長の安定性について

長 戸 康 郎

(東京大学農学部)

イネ(品種日本晴)を用いて生育温度の変動に対する胚の生長反応を調べた。

変温: 30~25°C, 25~20°C 及び定温: 20°C の三つの温度条件下で生育させた場合, 胚の生長速度は高温になる程大きくなった。しかし, 指数生長開始時の細胞数及び細胞増加率の転換時の細胞数は温度条件にかかわらず一定である。従って, 上記のような生長様式の転換は生育温度と無関係に決定されていると考えられる。胚の形の変異を, 胚長と胚厚の相対生長係数及び胚厚/胚長値に基づいて調べたところ, 低温になるに従って胚の形の変異は大きくなった。特に球状胚段階での変異が大きくなり, この段階は生育温度の変動に対して不安定な時期であると考えられる。

次に, 30~25°C で生育させ, 球状胚中期・後期及び第2葉分化後にそれぞれ24時間の20°C温度パルスを与え, どの時期が不安定であるかを調べた。いずれの時期に温度パルスを与えても, 胚の生長はパルス後直ちに約12時間の遅延を示した。しかし, 生長の遅延からの回復はいずれの場合にも成熟後期の開花後12日目頃(胚長約1700 μ m)であって, 初期にパルスを与えても生長の遅延からの回復は早くならない。このことは, 低温パルスが生長の時間的遅れをもたらすだけであり, 生長曲線のパターン自体は変化せず安定なものであることを示している。また, 相対生長係数及び胚厚/胚長値に基づいて胚の形の変異を調べると, 球状胚中期及び後期での低温パルスによってパルス後の胚の形の変異は大きくなった。一方, 第2葉分化後に低温パルスを与えた場合には, 胚の形の変異は無処理区のものとほとんど変らなかった。従って, 球状胚段階, 特にその後期, は不安定な時期であるが, 形態的分化後の胚は安定なものとなる。