PHOTOPERIODIC STUDIES ON RICE

III. The Effect of Different Combinations of Light Periods and Dark Periods on the Floral Bud Initiation of a Short Day Rice Variety[1]

by

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Introduction

In the previous reports (Yü et al, 1962), it was pointed out that the turning point between short day and long day effects for the short day rice variety Wan-tze (蔓仔) was between the light period of 12 and 12.5 hours. That for the short day rice varieties Iwata-Asahi(磐田朝日), Shinriki (神力) and Shuang-chiang (霜降) was between the light period of 12 and 13 hours. Also they pointed out that the floral bud initiation of the variety Shuang-chiang was delayed when the dark period was interrupted by light. The nearer the turning point, the more marked was the effect of the light break.

In this paper, results will be presented on the differentiation of the growing points of the first tillers in a short-day rice variety as affected by various combinations of light periods and dark periods of different duration. Such a study is aimed at the further understanding of the turning point between short day and long day effects in short day varieties of rice.

Prior to this experiment, a preliminary experiment was conducted in 1954. The short day rice variety Shuang-chiang was grown continuously in a short day of 8 hours and the size and degree of differentiation of the growing points of three plants were determined every day. During the experimental period, the temperature was 29.23±0.59°C. The results thus obtained are shown in Table 1.

It was found from microscopic observation that the growing point of the variety Shuang-chiang had definitely arrived at an early stage of differentiation

(1) This is a report of a series of photoperiodic studies on rice done jointly with Prof C. J. Yü of Department of Botany, National Taiwan University, Taipei, Taiwan.

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of branch primordia of the first order or even had reached the middle stage on the 7th day of short day treatment. It was also found that the growing point had definitely started to differentiate effectively on the 4th day of the treatment.

Table 1. The size of the growing point of short day variety "Shuang-chiang" under the 8-hour daylength. (Average of three plants)

Days of treatment	Length (mm)		Width (mm)	
	Treated	Natural condition	Treated	Natural condition
1	0.097±0.024	0.100±0.019	0.067±0.016	0.065±0.014
2	0.101±0.020		0.072±0.015	
3	0.106±0.020		0.073±0.011	
4	0.121±0.020		0.082±0.009	
5	0.130±0.027		0.084±0.015	
6	0.152±0.069		0.093±0.031	
7	0.193±0.058		0.111±0.024	
8	0.229±0.107		0.156±0.083	
9	0.452±0.222		0.287±0.143	
10	0.789±0.472		0.503±0.246	
11	1.334±0.636		0.691±0.246	
12	1.493±0.444	0.127±0.023	0.759±0.012	0.086±0.012

Seeding	May 17
Transplantation	June 15
Beginning of treatment	June 15

With the preliminary knowledge so obtained, the author proceeded to the main experiment as will be described in this paper.

Material and Methods

Shuang-chiang, a short day variety of rice was used throughout this experiment.

Seeds were sown on July 26, 1955, and the seedlings were transplanted on August 15 at a rate of three per pot. Twenty-four hour illumination was applied to all the transplanted seedlings until the beginning of the experimental treatments.

The treatments consisted of all 36 possible combinations of light periods and dark periods of 4, 8, 12, 16, 20 and 24 hours duration respectively. There was one replication.

During the light period, day light was used in the day time and 200 Watts electric light bulbs were used during the night. The light intensities received by plants under artificial light were determined as follows:

	Average	Highest	Lowest
Light intensity at the top of the plant	12.57 f. c.	25 f. c.	—
Light intensity at the base of the plant	9.08 f. c.	—	4 f. c.

Pots requiring darkness were covered by a dark box made of asphalt paper and so sun light or electric light was entirely excluded.

The treatments were started at 12 noon on October 7 and continued until 10 a.m. October 21. The temperature during the period of treatment was 21.2±1.6°C. When the treatment was completed, the plants were dug up and the growing points of their first tillers were collected and preserved in 50% ethyl alcohol.

Experimental results

The length and width of the growing points preserved in 50% ethyl alcohol were measured and the results are shown in Table 2. The mean sizes are expressed graphically in Figure 1.

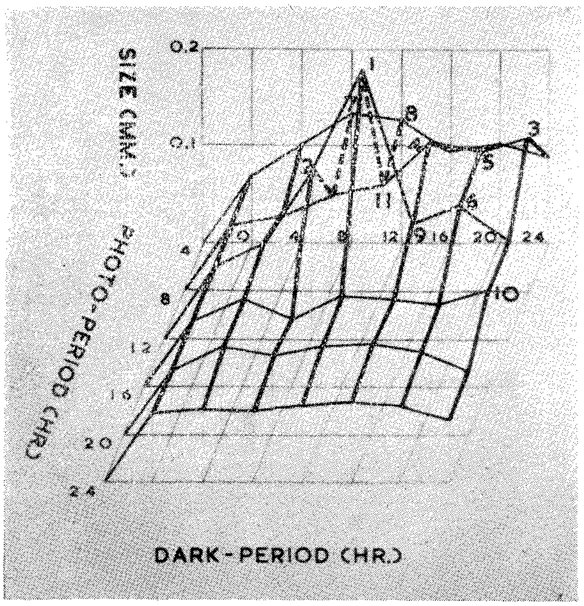

Fig. 1. The mean size of the growing point of short day variety "Shuang-chiang" in relation to various combinations of light-periods and dark-periods.

At the start of the treatments, the length or width of the growing points of the first tillers was invariably below 0.069 mm. The growing points of plants which had been grown in continuous light for 14 days increased very little in size. They were on the average 0.074 mm long and 0.065 mm wide. They were still vegetative.

From Table 2, it can be seen that the duration of light period had marked effects on the floral bud initiation of the short day variety of rice. The growing points were largest under the 12-hour light period with an average of their mean length and width at 0.153 mm. The next largest were those under the 8-hour light period with an average of their mean length and width at 0.124 mm, followed by those under the 4-hour light period with an average of their mean length and width at 0.109 mm. The smallest were those under the 16-, 20- or 24-hour light period with an average of their mean length and width at 0.092 mm,

Table 2. The size of the growing point (mm) of short day rice variety "Shuang-chiang" under varied combinations of light-periods and dark-periods.

Dark-period (hr.) / Size (mm.) / Light-period (hr.)	0		4		8		12		16		20		24		Average
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	
4			.111±.013	.099±.014	.144±.016	.126±.018	.136±.036	.119±.045	.101±.015	.090±.013	.108±.013	.098±.011	.093±.015	.083±.017	
			0.105		**0.135**		**0.128**		**0.096**		**0.103**		**0.088**		**0.109**
8			.082±.014	.073±.015	.104±.015	.090±.014	.113±.022	103±.021	.172±.042	.143±.043	.154±.042	.134±.038	.161±.077	.156±.073	
			0.078		**0.097**		**0.108**		**0.158**		**0.144**		**0.159**		**0.124**
12			.104±.035	.095±.037	.190±.089	.162±.051	300±.101	258±.081	.125±.025	.115±.031	.149±.044	.129±.040	.108±.029	.094±.019	
			0.100		**0.176**		**0.279**		**0.120**		**0.139**		**0.101**		**0.153**
16			.097±.013	.086±.013	.076±.014	.067±.013	.106±.017	.087±.018	.099±.019	.090±.016	.093±.012	.082±.022	113±.017	.104±.015	
			0.092		**0.072**		**0.097**		**0.095**		**0.088**		**0.109**		**0.092**
20			.096±.013	.088±.010	.088±.023	.079±.018	.100±.012	.091±.011	.104±.024	.088±.018	.090±.027	.083±.025	.075±.011	.063±.012	
			0.092		**0.084**		**0.096**		**0.096**		**0.087**		**0.069**		**0.087**
24	.074±.014	.065±.013	.084±.014	.070±.012	.081±.013	.070±.012	.087±.013	.077±.016	.094±.014	.077±.015	.085±.018	.075±.024	.068±.014	.064±.018	
	0.070		**0.077**		**0.076**		**0.082**		**0.086**		**0.080**		**0.066**		**0.078**
Average	**0.070**		**0.091**		**0.106**		**0.132**		**0.109**		**0.107**		**0.099**		

0.087 mm or 0.078 mm respectively.

The duration of the dark period also markedly affected floral bud differentiation. After the experimental period of 14 days, the size of the growing points, based on the average of their mean length and width, was 0.070 mm under the 24-hour light period and varied between 0.091 to 0.132 mm, depending on the length of the period. The differences between continuous light and any of the treatments with a dark period were significant at 1% level. Among the dark periods, the 12-hour dark period demonstrated the largest difference from the 24-hour light period, followed by 16, 20, 8, 24 and 4 hours in that order.

Table 3. The size of the growing point of rice under fifteen combinations of light and dark periods which stimulate floral differentiation of the growing point.

Order	Light and dark period (hr)	The size of growing point (mm)	Differentiation Stage†
1	12-12	0.279**	A middle stage of the differentiation of branch primordia of the first order
2	12- 8	0.176**	An early stage of the differentiation of branch primordia of the first order.
3	8-24	0.158**	
4	8-16	0.157**	
5	8-20	0.144**	
6	12-20	0.139**	
7	4- 8	0.135**	
8	4-12	0.128**	
9	12-16	0.120**	
10	16-24	0.108**	A stage of bract primordia increase.
11	8-12	0.108**	
12	4- 4	0.105*	
13	4-20	0.103*	
14	12-24	0.101*	
15	12- 4	0.100*	

** and * Showing significant difference as compared with the plant under the 24-hour light period at 1% and 5% level respectively.

† According to Akimoto and Togari (1939)

Among the different combinations of light periods and dark periods, the combination of a 12-hour light-period with a 12-hour dark-period caused the growing points to grow largest. The average size was 0.279 mm. This treatment also gave the largest degree of floral bud differentiation. The growing points had reached the so-called middle stage of the differentiation of branch primordia of the first order (Akimoto and Togari, 1939). The differences in average size of the growing points between this combination and all others were significant at 1% level. Fourteen other combinations could also induce differentiation of the growing points, causing them to form the primodia of the floral buds. The average size of the growing points was between 0.100 and 0.179 mm under those treatments. The duration of light periods and dark periods in the 15 effective combinations and the size of the growing points and their degree of differentiation under those combinations are shown in Table 3.

The 15 effective combinations can be arranged according to the durations of light periods and dark periods as shown in Table 4.

Table 4. The distribution of 15 effective combinations in the various combinations of light periods and dark periods. (The number indicates the order of effectiveness.)

Light-period (hr.) / Dark-period (hr.)	4	8	12	16	20	24
4	12	—	15	—	—	—
8	7	—	2	—	—	—
12	8	11	1	—	—	—
16	—	4	9	—	—	—
20	13	5	6	—	—	—
24	—	3	14	10	—	—

The following results may be noticed from Table 4.

a) As far as the duration of light period is concerned:

i) When the light period was 16 hours long, floral bud initiation could be induced to some extent in a 24-hour dark period in the experimental period of 14 days, but could not be induced at all when the dark period was shorter than 24 hours.

ii) When the light period was 20 hours or longer, floral bud initiation could not be induced within the experimental period under any of the dark periods used in this experiment, i. e., up to 24 hours in length.

iii) The optimal photoperiod was 12 hours. When the photoperiod was 12 hours, floral bud initiation could be induced under any of the dark periods tested in the experiment. The degree of differentiation varied with the length of the dark period. As mentioned before, floral bud initiation was most strikingly promoted by the combination of a 12-hour light-period plus a 12-hour dark-period.

iv) When the light period was 8 hours long, floral bud initiation could only be induced within the experimental period under a dark period of 12 hours or more. But a dark period of 16 hours or longer was more effective than 12 hours.

v) When the light period was 4 hours long, floral bud initiation could not be induced within the experimental period by a dark period of 16 or 24 hours. 8-and 12-hour dark periods were most effective.

b) As far as the duration of dark period is concerned:

Floral bud initiation could be induced in the experimental period under dark periods of all the different durations as specified in the present experiment as long as they were combined with light periods of appropriate durations. But combinations with the 4-hour light period were the least effective in photoperiodic induction.

Discussion

Hamner (1940) subjected soybeans (*Glycine max*) to a 16-hour dark period combined with various photoperiods. He found that 7 cycles induced the formation of floral buds when the light period was shorter than 18 hours. No floral buds were formed when the light period was longer than 20 hours. Also, when the length of the light period was 16 hours, the formation of floral buds was not induced by 7 cycles when the dark period was 10 hours or shorter. Floral buds were formed when the dark period was 10½ hours or longer.

Thus Hamner suggested that in the soybean, a substance A was formed in the light and a substance B in the dark. A substance C would be formed when A and B in a certain ratio due to a suitable combination of light period and dark period. This substance C would induce the formation of floral buds.

In examining Hamner's experimental results it is clear that floral bud formation occurred in soybeans when the dark period was longer than the light period or when they were about equally long. When the dark period was shorter than the light period there was a minimal length of the dark period which still caused floral induction. If the dark period was shorter than the minimal one, no floral bud formation would take place. (For example, when the light period was 16 hours long, no floral bud would be formed when the dark period was shorter than 10½ hours).

In short, in Hamner's experiments, the dark period was fixed at 16 hours and the duration of the light period was varied, or alternately, the light period was fixed at 16 hours long and the length of the dark period was varied. His experimental results indicated that the light period was as important as the dark period for the formation of substances which induced floral bud initiation.

According to the results of the present experiment with rice no floral bud initiation could be induced during the experimental period when the 20- or 24-hour light period was combined with any one of the dark period of various durations. While the combination of 12-hour light period plus 12-hour dark period was the most effective one in photoperiodic induction, the combination of 24-hour light period plus 24-hour dark period which has the same ratio of light period to dark period could not induce floral bud initiation. Therefore, Hamner's hypothesis that substance A and B are formed in the light period and dark period respectively, and that they combine to form a substance C which induces floral bud initiation depending on the proper ratio does not seem to apply to the short day variety of rice.

From the foregoing experimental results two possible interpretations concerning the photoperiodic induction of the floral buds of rice may be suggested: First, the substance which induces floral bud initiation is formed in the dark period. If the dark period is too short, the substance will not be formed in adequate amounts. If the light period is too long, the substance formed in the dark period may be destroyed. Second, a precursor of the substance which induces floral bud initiation is formed in the light period and its transformation takes place in the dark period. If the dark period is too short or the light period is too long, the time for transformation may become insufficient.

To sum up, disregarding both assumptions, when the dark period is too short or the light period is too long, the substance which induces floral bud initiation will not be formed or will be formed very slowly and will not accumulate to the extent necessary for the induction, and therefore floral bud initiation fails to take place or it takes place but slowly.

Lang and Melchers (1943) observed that floral initiation could be induced by a supply of carbon compounds when the long day plant *Hyoscyamus* was grown under short day conditions. Tashima and Imamura (1953), and Tashima (1953) were able to induce floral bud initiation by the same means in the short day plant *Pharbitis nil* and in the long day plant *Raphanus sativus* grown under continuous dark conditions. The light period had no effect when carbon dioxide was removed according to Parker and Borthwick (1941), and Harder, Bode and Witsch (1944). All these observations seem to suggest that the light period is essential for the formation of the precursor (a carbon compound) of the floral bud formation substance. In other words, of the two interpretations concerning photoperiodic induction as mentioned above, the second one is the more plausible. The combination of a 12-hour light period with a 12-hour dark period was the most effective in promoting the formation of the precursor of the floral bud formation substance and the transformation of the former into the latter in the short day variety of rice. It also seems evident from the experimental results that the best combination of light period and dark period is the actual 12 hours of light period plus 12 hours dark period and that this combination cannot be replaced by other combinations with the same ratio of light and darkness.

Furthermore, according to the results obtained previously the short day varieties of rice can still head, although very late, under 24-hour continuous illumination (Yü and Yao, 1962 and unpublished data). Therefore, the author believe that the most appropriate dark period as needed by the short day varieties of rice essentially speeds up the transformation of the precursor into floral bud formation substance to the maximal extent, but that such a transformation is not necessarily impossible in the light period.

By comparing the results of the present experiment with those of the preliminary one (see Table 1), it was found that the floral bud initiation was generally slower in the present experiment than in the preliminary one. As far as the degree of floral bud initiation is concerned, the growing points with an average size of 0.279 mm on the 14th day under the most effective treatment of 12-hour light plus 12-hour darkness in the present experiment are comparable to those between the 8th day with an average size of 0.193 mm and the 9 th day with an average size of of 0.369 mm in the preliminary experiment. The growing points with an average size of 0.158 mm on the 14th day under the treatment of 16-hour dark period plus 8-hour light period in the present experiment are about comparable to those between the 6th day with an average size of 0.152 mm and the 7th day with an average size of 0.192 mm in the preliminary experiment. The author believes that this difference in the rate of floral bud initiation is due to the temperature factor. The preliminary experiment was conducted from May to July when the temperature was high (29.23± 0.54°C) while the present experiment was conducted in October when a lower temperature prevailed (21.20±1.60°C). It has been recognized by a number of workers that temperature will influence the effect of short day treatment or more specifically, low temperature will cause a delay in the effect of short day treatment. This was first reported by Hamner and Bonner (1938). In this connection, rice is certainly no exception as demonstrated by the experimental results presented in this paper.

Summary

1. By subjecting the Formosan short day variety Shuang-chiang to all possible combinations (36) of light periods and dark periods of 4, 8, 12, 16, 20 and 24 hours duration respectively, it was found that the most effective combination for inducing floral bud initiation in this variety was a 12-hour dark period plus a 12-hour light period. This indicates that the combination of a light period and a dark period which is similar to that of the turning point, is more effective than any of the other combinations of light and dark periods.

2. On the basis of these experimental results, Hamner's experiments and deductions were discussed. Doubts were cast on the validity of Hamner's

hypothesis for rice. Because of insufficient experimental results, other hypotheses related to the substance inducing the formation of floral buds were not discussed.

3. By comparing the results of the present experiment with those of preliminary ones, it was found that low temperature would suppress the effect of short day teratment in rice.

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水稻光週性的研究

III. 各種不同光期與暗期之組合對於短日性水稻品種花芽分化的影響

姚潤德

作者分光期暗期各爲4, 8, 12, 16, 20, 24小時等六組，以之互相配合，獲36個組合，以水稻品種霜降，分別置此36組合下，並以1區置24小時照明下，以爲對照。其目的是要觀察水稻主桿及第一次分蘗之生長點在各種不同組合下的分化狀態。每區重複一次，對照區重複二次。

實驗結果，知有促進花芽分化效果者，共有15組合，而最有效者爲"12小時光亮加12小時黑暗"的一組。即最接近於長短日效果轉捩點之組合，在促進花芽分化上，最爲有效。

作者據此水稻方面之實驗結果，對於 Hamner 之開花學說，略作批評，認爲其學說至少對於水稻並不適用，作者又根據二次實驗結果，推測低溫對於短日效果有抑制作用。