Difference in Vernalization Effect in Wheat under Various Temperatures*

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Though temperature is the most important factor that affects vernalization, relatively few studies have been established concerning the difference in vernalization effect of winter cereals due to variation of temperature of treatment. In winter rye, temperature ranging from 1°C to 7°C was the most effective for vernalization^{8,10)}. In winter wheat, temperature around 3°C was the most effective¹⁰⁾. In spring wheat, on the other hand, 10°C or a little higher was the most effective¹⁰⁾. In Japan, in the few studies reported concerning the difference in vernalization effects in wheat due to the variation of temperature of treatment, it was revealed that the degree of vernalization response in winter wheat treated at 19.3°C in a glasshouse was the same as in those treated at 10.5°C in the field⁹⁾, and in winter barley little difference was found in the similar effect, when the range of temperature of treatment was from 1°C to 10°C³⁾.

In this study, experiments were conducted to reveal the difference in vernalization effect due to the variation of temperature of treatment and due to the aging of plant, respectively, and also to make clear the most favourable temperature in vernalization process.

Experiment 1. Varietal difference in vernalization effect under various temperatures

MATERIALS AND METHODS

Sprouted seeds of wheat-varieties Akabōzu (winter habit: class III), Norin No. 4 (winter habit: class IV), Norin No. 27 (winter habit: class V), and Akakawaaka (winter habit: class VI)-were sown in clay pots. As an inductive treatment, temperature of 1°, 4°, 8°, 11°, 15°, and 18°C was respectively given to them for a different dura-

tion, immediately after sowing. During the treatment, growth of the plants was not halted. The plants were illuminated continuously by the artificial light of 1000 lx intensity at 1°C and of 2000 lx intensity at 4°C and at 8°C, respectively. The other inductive treatments were given in rooms covered with vinyl film, in which the plants were exposed to sunlight and illuminated by supplementary light of 200 lx intensity by night. Temperature was controlled with a margin of $\pm 1.5^\circ\text{C}$. The plants treated for different durations at various temperatures as described above were transferred into the glasshouse on March 15, 1964 and were kept under daylength of about 20 hours. Data on the days from the end of the treatment to final leaf emergence and on the final number of leaves on the main stem were recorded.

RESULTS

The number of main stem leaves of the variety Norin No. 27 at the end of the treatment is shown in table 1. The plants treated for 60 days at 1°C scarcely showed their growth, while those treated for 60 days at 18°C made a considerable growth. In the plants treated for the same duration, the number of main stem leaves increased consistently along with the increase of temperature

Table 1. Number of leaves in main stem of variety Norin No. 27 at the end of treatment in Experiment 1.

Duration of treatment (days)	Temperature of treatment (°C)					
	1	4	8	11	15	18
20	0.1	0.5	1.0	2.0	2.5	2.9
30	0.2	1.0	1.3	3.1	3.8	4.1
40	0.3	1.4	2.1	4.0	4.5	5.4
50	0.5	2.0	3.0	5.0	5.9	6.6
60	0.7	2.3	3.9	5.7	6.5	7.5

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form 1°C to 18°C. The rate of increase in the number of main stem leaves due to raising the temperature from 11°C to 18°C was slightly smaller than in the case when the temperature was increased from 1°C to 11°C.

The days required for final leaf emergence of the plants treated at various temperatures are shown in figs. 1 and 2. In the varieties Akabōzu, Norin No. 4, and Norin No. 27, temperature of 8°C or 11°C in almost all durations of treatment was the most favourable for final leaf emergence. Among the three varieties, which require different conditions of low temperature for heading, little difference was found in the trend of the days required for final leaf emergence. In the variety Norin No. 27, the plants treated for 20 days at 1°C or 4°C emerged their final leaves markedly late, and those treated at 18°C emerged earlier than them. Especially, for the final leaf emergence of the plants treated at 1°C, 114 days were required after the end of the treatment, while those treated at 15°C or 18°C required 80 or 97 days respectively. Consequently, no accelerating effect was found in the treatment for 20 days at 1°C. Even the plants treated for 30 days at 1°C showed remarkably late emergence of their final leaves. On the other hand, in the variety Akakawaaka, 4°C or 8°C was the most favourable and the plants treated at 11°C emerged their final leaves later than those treated at 4°C or 8°C, or could not have any emergence of final leaf. In the varieties Akabōzu, Norin No. 4, and Norin

No. 27, temperature of treatment at 8°C or 11°C was the most favourable, while the variety Akakawaaka showed different trend from the former.

The final number of main stem leaves of the variety Norin No. 27 treated for different durations is shown in fig. 3 (b). In this figure, the curve of the final number of main stem leaves showed different trend from that of the days required for final leaf emergence. The lower the temperature of the treatment, the fewer the final number of main stem leaves, except the variety Norin No. 27 treated for 20 or 30 days. The variety Norin No. 27 treated for 20 days at 1°C or 4°C had more leaves than those treated at the other temperature. This shows the same trend as in the days required for final leaf emergence described above.

The variety Norin No. 27 treated for 30 days at 4°C or 8°C markedly decreased their final number of main stem leaves, while those treated at 1°C gradually decreased the number of main stem leaves with the extension of duration of the treatment. The final number of main stem leaves showed minimum or nearly minimum in the plants treated for 30 days at 11°C or those treated for 50 days at 8°C, while in those treated for 60 days at 1°C or 4°C a trend of decreasing in the

Fig. 1. The effect of temperature on final leaf emergence in varieties Akabōzu (a) and Norin No. 4(b) treated for 10(○), 20(△), 30(×), 40(●), or 50(□) days.

Fig. 2. The effect of temperature on final leaf emergence in varieties Norin No. 27(a) and Akakawaaka(b) treated for 20(△), 30(×), 40(●), 50(□), or 60(·) days.

number of main stem leaves was found. These trends observed in the final number of main stem leaves were partially similar to those observed in the days required for final leaf emergence as shown in fig. 3 (a).

Experiment 2. Difference in vernalization effect due to the variation of age of plant

MATERIALS AND METHODS

The variety Norin No. 27 grown at 20°C under 20-hour daylength for different durations after

sowing were treated for 30 days at 0°, 5°, 10°, and 20°C. The number of leaves of main stem and the degree of differentiation of shoot apex are shown in table 2. The plants were illuminated continuously by the artificial light of 800 lx intensity at 0°C, 2000 lx intensity at 5°C, 6000 lx intensity at 10°C, and 7000 lx intensity at 20°C, respectively. The margins of temperature were within $\pm 1.5^\circ\text{C}$. After the treatment, the plants were grown at 20°C and were illuminated continuously by the artificial light of 7000 lx in-

tensity. Data on the days from the beginning of the treatment to final leaf emergence and on the final number of leaves of main stem were recorded.

RESULTS

The differences in the time of final leaf emergence and in the number of leaves of main stem due to the variation of age of plant are shown in figs. 4 and 5, respectively. The days required for final leaf emergence in the plants treated at each temperature decreased linearly in accordance with the increase of age of plant with a few exceptions. The 90-day-old plants treated at 0°C emerged their final leaves later than those treated at 20°C. The number of leaves of main stem expanded after the treatment showed nearly the same trend of decreasing as the days required for final leaf emergence. The plants

Fig. 3. Days required for final leaf emergence (a) and final number of leaves (b) in variety Norin No. 27 treated for various durations. Plants were treated at 1°C (○), 4°C (△), 8°C (×), 11°C (●), 15°C (□), or 18°C (●).

Table 2. Plant age of variety Norin No. 27 at the beginning of treatment in Experiment 2.

Days after sowing	0	5	10	15	20	30	60	90
Number of main stem leaves	0	1.0	1.9	3.0	4.1	5.5	8.9	12.6
Stage of spike differentiation*	I	I	I	I	I~II	II~III	IV	V

* Stage of spike differentiation

I: vegetative

II: initiation of spike primordia

III: initiation of bract primordia (early stage)

IV: initiation of bract primordia (middle stage)

V: initiation of bract primordia (late stage)

Fig. 4. The effect of age of plant on final leaf emergence in variety Norin No. 27 treated for 30 days at 0°C (○), 5°C (△), 10°C (×), or 20°C (●).

Fig. 5. The effect of age of plant on number of leaves expanded after the beginning of treatment in variety Norin No. 27 treated for 30 days at 0°C (○), 5°C (△), or 10°C (×). Solid circle indicates final number of main stem leaves of the plants treated at 20°C.

treated at 20°C were raised at 20°C during their entire period of growth. The days to their final leaf emergence decreased linearly with the increase of age of plant, and the final number of

Fig. 6. Hastening of final leaf emergence by vernalizing treatment for 30 days at 0°C (○), 5°C (△), or 10°C (×) in variety Norin No. 27 of different ages.

their leaves of main stem were nearly the same in spite of the difference of their age. The days from sowing to final leaf emergence in the plants treated at 20°C were within the range from 143 to 154 days showing a little difference. Fig. 6 shows the vernalization effect of the variety Norin No. 27 treated at 0°C, 5°C, and 10°C which imply the difference between the days required for final leaf emergence when the plants treated at 20°C are compared with those treated at various other temperatures on the assumption that 150 days are needed from sowing to final leaf emergence when the plants are grown at 20°C.

From fig. 6, it is evident that the plants which were treated at 0°C at the age of 0, 5, 10, and 15 days and those which were treated at 5°C at the age of 0, 5, and 10 days had nearly the same vernalization effect, and also that the vernalization effect decreased with the increase of age of plant except for those described above. When treated at 10°C, the vernalization effect in the 0-day-old plants was smaller than that of 5-day-old one and in the case of those over 5-day-old the effect decreased with the increase of age of plant. No effect was revealed in the plants aged 90 days, even when treated at any temperature. In each

age of plant, the plants treated at 0°C showed less vernalization effect than those treated at 5°C or 10°C. This fact confirms the result obtained in Experiment 1.

Experiment 3. Favourable temperature of treatment during vernalization process

MATERIALS AND METHODS

Plants of variety Norin No. 27 were grown for 36 days immediately after sowing under the temperature of 0°, 4°, 8°, and 12°C respectively. On the other hand, treatment at 0°, 4°, 8°, or 12°C for 12 days was respectively intercalated at the time of 0, 12, 24, or 36 days after sowing. So the total duration of the treatment was 48 days. The plants were illuminated continuously by the artificial light of 1000 lx intensity at 0°C and at 4°C, 2000 lx intensity at 8°C, and 4000 lx intensity at 12°C, respectively. The temperature allowance in each case was within $\pm 1.5^\circ\text{C}$. After these treatments, the plants were grown under the condition illuminated continuously by the artificial light of 6000 lx intensity at 18°C. Data on the days from sowing to final leaf emergence and on the final number of leaves of main stem were recorded.

RESULTS

Fig. 7 shows the days required for final leaf emergence of the plants grown at each temperature. The plants grown at 12°C for 48 days showed the earliest emergence of final leaf, and those grown at 12°C for 0, 24, and 36 days before the treatment at 0°C, 4°C, and 8°C for 12 days emerged their final leaves later than those grown at 12°C for 12 days before the same treatment, as shown in fig. 7 (d). When the plants were treated at various temperatures for 12 days immediately after sowing or after 36

days of growth, the higher the temperature was, the earlier the plants emerged their final leaves. There was, however, little difference in the days required for final leaf emergence due to the variation of the temperature during the treatment for 12 days intercalated at the time of 12 days after sowing with an exception of the one treated at 0°C.

The plants grown at 4°C or 8°C showed little difference in the days required for final leaf emergence in spite of the variation in the intercalating treatment, with the exception of those grown at 4°C or 8°C for 36 days and then treated at 12°C. Final leaf emergence of the latter was earlier than the former. In the plants grown at 0°C, those treated at 8°C for 12 days immediately after sowing emerged their final leaves earlier than those grown at 0°C for 48 days. As the

Fig. 7. The effect of intercalated temperature on final leaf emergence in variety Norin No. 27. The plants were grown at 0°C(a), 4°C(b), 8°C(c), or 12°C(d) and were exposed to various temperatures for 12 days as an intercalating treatment at the time of 0(○), 12(△), 24(×), or 36(●) days after sowing.

temperature of treatment given after 36 days of growth at 0°C, temperatures of 4°, 8°, and 12°C were more favourable than 0°C.

In the plants grown at 0°C, those treated at 12°C at each stage had more final number of main stem leaves than others. In the plants grown at 12°C, those treated at various temperatures after 12 days of growth had less final number of leaves of main stem than those treated after 0, 24, and 36 days of growth, and this trend of decreasing resembled to that in the days required for final leaf emergence. The plants treated at higher temperature grew more rapidly and had more final number of leaves of main stem. Then the curve of the final number of leaves of main stem showed somewhat different trend from that of the days required for final leaf emergence.

DISCUSSION

When wheat plants are treated at different temperatures, the difference in growth occurs during the treatment due to the variation of temperature. The differentiation of shoot apices continues during the treatment when the plants are treated at comparatively high temperature. It may be, therefore, unsuitable to estimate their vernalization effect by the days required for heading, when the plants are treated at comparatively high temperature.

Purvis (1948) reported that the same vernalization effect was observed in Petkus winter rye treated at temperature ranging from 1°C to 7°C for 2 to 6 weeks either by the limited moisture method or by the unlimited moisture method. If, then, all the plants treated at different temperatures can be placed on the same starting line by limiting their growth during the treatment, the vernalization effect could be compared strictly with each other. It is difficult, however, to control the growth completely for a long time under such comparatively high temperature ranging from 10°C to 20°C. Vernalization response, moreover, may be affected by such a growth limiting method¹³⁾. As discussed later, the most sensitive vernalization response may be obtainable when

wheat plants are treated under conditions which allow their growth. The plants grown in field or a glasshouse continue their growth and the differentiation of their shoot apices during their vernalizing period. It is impossible to discuss the vernalization response of wheat plants without considering their growth and differentiation during the treatment. The same may be said about the final number of main stem leaves. For example, plants treated at 0°C have less final number of main stem leaves than those treated at high temperature. This may not be due to the effectiveness of the vernalizing treatment but due to the extremely small amount of growth of the plants during the treatment. In this paper, therefore, comparing the earliness of final leaf emergence is emphasized.

In Experiment 1, the varieties of Akabōzu, Norin No. 4, and Norin No. 27 treated at 8°C or 11°C emerged their final leaves early, while those treated at 1°C emerged late. Especially in Norin No. 27 treated for 20 or 30 days at 1°C or 4°C, not only final leaf emergence was later, but also final number of leaves was larger than when treated at 8°C or 11°C for the same duration. Moreover, when the plants treated at 1°C for 20 days were compared with those treated at 15°C or 18°C for the same duration, in the former the vernalization effect was not observed at all. These facts suggest that the vernalization treatment at 1°C or 4°C, especially at 1°C, is less effective than that at 8°C or 11°C. In this case, the retardation of final leaf emergence at low temperature may rather result from devernization effect due to exposure to the high temperature in a glasshouse after planting than from less effectiveness of the treatment.

According to the unpublished data of the author, vernalization effect at 1°C for 30 or 40 days was neutralized completely by the heating treatment which followed, while the effect at 4°C, 8°, or 11°C for the same duration scarcely indicated any retardation. Table 3, on the other hand, presents the days required for final leaf emergence and the final number of main stem leaves:

Table 3. Days required for final leaf emergence and final number of leaves in main stem of wheat varieties grown under constant temperature during their entire period of growth. Final number of leaves in main stem are shown in bracket.

Temperature during growth	Variety			
	Akabōzu	Norin No. 4	Norin No. 27	Akakawaaka
15°C	65(9)	69(9)	98(13)	146(17)
20°C	67(12)	93(15)	151(18)	196(24)

of the plants grown at 15°C or 20°C under long day condition. In experiment 2, the variety Norin No. 27 grown at 20°C emerged their final leaves in about 150 days after sowing under long day condition without any low temperature treatment. When these data were compared with the days required for final leaf emergence and the final numbers of main stem leaves of the plants treated at various temperatures obtained in Experiment 1, devernization due to the exposure as indicated above after planting would be small, if any. From this fact, it appears that the retardation of final leaf emergence of the plants treated at 1°C or 4°C did not result from the devernization but mainly from the less effectiveness of the treatment.

The variety Akakawaaka treated at 11°C or above did not emerge its final leaf showing the same trend as in winter rye^{6,14}. The low-temperature requirement for heading in varieties is ranked as follows: Akabōzu < Norin No. 4 < Norin No. 27 > Akakawaaka. In the former three varieties, the upper limit of vernalizable temperature was 11°C or 12°C or more above, while in the other, around 8°C. It may be deduced from the above mentioned that modification of the upper limit of vernalizable temperature in accordance with their low temperature requirements for heading seems necessary.

As shown in fig. 3, the final leaf emergence of the plants treated for 30 days at 4°, 8°, or 11°C were markedly hastened, while the final leaf emergence of those treated at 1°C is gradually accelerated with the extension of the duration of treatment. The final number of main stem leaves

showed the same trend as the days required for final leaf emergence. These facts suggest that vernalization response progresses more rapidly at 4°, 8°, or 11°C than at 1°C.

In *Hyoscyamus niger*, the most favourable temperature to obtain the earliest flowering became lower in accordance with the increase of days of treatment¹². In winter wheat, on the other hand, 3°C was the most favourable temperature for heading of the plants when treated for 14 days, while temperature ranging from 5°C to 25°C was favourable for heading of the plants treated for 7 days¹⁰. In this paper, however, the most favourable temperature to obtain the earliest heading was 8°C and 11°C in the plants treated for different durations.

In the above-mentioned, unpublished data of the author, the vernalization effect at comparatively high temperature was not neutralized by heating. In Experiment 1, the vernalization response proceeded rapidly at such a temperature. These facts suggest that the vernalization effect is large and the vernalization response is rapid, when the growth of plants is possible during the treatment.

In Experiment 2, the variety Norin No. 27 grown at 20°C for 90 days had 12.6 leaves, but no vernalization effect was found. When the plants were divided into two groups, and one is grown under the temperature of 15°C, while the other under 20°C, some difference was found between these groups in the days required for final leaf emergence and in the final number of main stem leaves, as shown in table 3. These differences were also found in accordance with the varietal difference of the plants. Accordingly it appears that the age of plant, at which the plants become to show no vernalization effect, varies due to their requirement for low temperature and also to the temperature of the period before vernalization treatment.

When an Australian winter wheat was grown at 12°C or above and then was treated at 3°C for 48 days, its vernalization effect became smaller with the increase of age of plant, and the plants

7 weeks old or more had no vernalization effect⁵⁾. Though the requirement of low temperature for heading in the Australian winter wheat is not yet obvious, the temperature condition before the vernalizing treatment seemed to be too low as compared with the result given in this paper. Many reports by other authors concerning vernalization effect and that modified by the age of plant were insufficient in the determination of growth temperature before the vernalization treatment. But most of them agree with the result that vernalization effect became smaller with the increase of age of plant.

As described above, wheat plants grown at 20°C showed smaller vernalization effect with the increase of age of plant and finally headed without any low temperature treatment. This fact may be attributed either to gradual progress of vernalization induction even under high temperature⁵⁾ or to aging effect apart from vernalization process.

In Experiment 3, some plants were treated for 12 days at different temperatures preceding the growth at 12°C for 36 days. Under such condition, the lower the temperature of treatment, the later the time of final leaf emergence. This acceleration of final leaf emergence by comparatively high temperature at the first stage of vernalization may be attributed to either the small response of vernalization in extremely young plants or more sensitive response of vernalization at comparatively high temperature, or both of them. In another experiment carried out by the author, in which the plants were grown for 5 days at 20°C and then grown at 0°C, final leaf emergence was accelerated by the treatment at 5°C or 10°C before placing at 0°C. It may be, therefore, more possible that vernalization response proceeds more rapidly at comparatively high temperature at its early stage.

When the treatment at temperature ranging from 0°C to 12°C was given at the growth period 12 days after sowing, there was little difference in the days required for final leaf emergence. At this stage, the vernalization response may proceed under low temperature at the same rate as

under comparatively high temperature. When the treatment at various temperatures was given 24 days after sowing or was given following 36 days of growth after sowing, the plants emerged their final leaves more early at higher temperature during the treatment. The vernalization response in the late stage may proceed more rapidly at higher temperature. There was, however, no difference in their final number of main stem leaves in spite of their difference in the days required for final leaf emergence when the treatment was given following 36 days of growth after sowing. It appears that their final number of main stem leaves has already been decided at this stage. The dissection of the plants of 36 or 48 days of age revealed their shoot apices to be vegetative and could not find any formation of the double ridge.

The fact described above was true only when the plants were grown at 12°C. There was a different trend when they were grown at 0°, 4°, and 8°C. When the plants were grown at 4°C or 8°C, they emerged their final leaves early at higher temperature at the late stage of vernalization, but this trend could not be observed when they were grown at 0°C. If the plants were grown at 0°C for more extended duration than used in this investigation, they might also emerged their final leaves earlier at higher temperature given at the later stage. As discussed already, the vernalization response proceeded slowly at 0°C, and as the result the plants may have been unable to be vernalized at the late stage of growth when the effect of high temperature brings about the hastening of heading.

The plants, which were grown for 36 days at 8°C and then treated for 12 days at 12°C, emerged their final leaves earlier than those which were grown for 48 days at 12°C or 8°C. This fact suggest that vernalization response may proceed more rapidly at 8°C than at 12°C.

The result in Experiment 3 suggests that the most favourable temperature for vernalization process, especially at its early stage, varies with the temperature under which the plants are

grown. In wheat plants, low temperature at the early stage, medium temperature at the middle stage, and high temperature at the late stage were favourable for the progress of their vernalization⁹⁾. This trend in the effect of temperature is partially different from that given in this paper. However, as the temperature conditions of this experiment were different from that in this paper, it is impossible to compare the latter with the former.

SUMMARY

Wheat seeds or wheat plants were kept under constant conditions of various temperatures permitting their growth in order to study the difference in vernalization effect resulting from the variation of temperature of treatment.

(1) Sprouted seeds of wheat varieties such as Akabōzu, Norin No. 4, Norin No. 27, and Akakawaaka, each of which requires different intensity of low temperature for heading, were treated by various temperatures ranging from 1°C to 18°C for different durations. In varieties Akabōzu, Norin No. 4, and Norin No. 27, the plants treated at 8°C or 11°C emerged their final leaves early and those treated at 1°C emerged late. In variety Akakawaaka, 4°C and 8°C were the most favourable temperature. It is suggested from this experiment that the vernalization response proceeds more slowly at 1°C than at 4°, 8°, or 11°C.

(2) Variety Norin No. 27 of different age of plant was treated at 0°, 5°, and 10°C for 30 days. The vernalization effect decreased with the increase of age of plant. The plants aged 90 days had no vernalization effect. In each plant age, the plants treated at 0°C showed less vernalization effect than those treated at 5°C or 10°C.

(3) Variety Norin No. 27 was grown under the temperature of 0°, 4°, 8°, or 12°C respectively for 36 days after sowing. On the other hand, treatment at 0°, 4°, 8°, or 12°C for 12 days was respectively intercalated at the time of 0, 12, 24, or 36 days after sowing. When the plants were grown under 0°C, there was a little difference in the time of final leaf emergence in

spite of the variation in temperature of intercalating treatment. When the plants were grown under 4°C or 8°C, the final leaf emergence were markedly hastened by the treatment at 12°C following the growth period of 36 days. When the plants were grown under 12°C, the plants, in which the treatment of higher temperature was intercalated at early or late stage of growth period, emerged their final leaves earlier, but when the treatment was intercalated at middle stage of growth period, there was little difference in the time of final leaf emergence in spite of the variation in temperature of treatment.

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[和 文 摘 要]

種々の温度での小麦のバーナリゼーション効果

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小麦を種々の温度の下で処理し、バーナリゼーション効果の差異について研究した。

1) 秋播性程度の異なる小麦品種赤坊主、農林4号、農林27号、赤皮赤の催芽種子を $1^{\circ}\sim 18^{\circ}\text{C}$ の温度で種々の期間処理した。赤坊主、農林4号、農林27号では 8°C または 11°C で処理された時にバーナリゼーション効果が最も大きく、赤皮赤では 4°C または 8°C で処理された時に効果が大きであった。農林27号の 4°C 、 8°C 、または 11°C で処理されたものでは30日間の処理により止葉抽出が著しく促進され、主稈総葉数も著しく小となったが、 1°C で処理されたものの止葉抽出は処理日数の増加とともに徐々に早くなり、主稈総葉数も徐々に少なくなつた。これらの事実からバーナリゼーション反応は 4°C 、 8°C 、 11°C では 1°C に比べて進み方が速いものと考えられる。

2) 農林27号の種々のageのものを 0°C 、 5°C 、 10°C で30日間処理した。バーナリゼーション効果はageが大となるに従つて減少し、播種後90日のものでは効果が認められなかつた。いずれのageのものでも 0°C での処理効果は 5°C 、 10°C での効果よりも小であつた。

3) 農林27号を播種直後より36日間 0°C 、 4°C 、 8°C 、 12°C で生育せしめ、播種後0、12、24、36日目より上記の温度での処理を12日間挿入した。 0°C で生育した場合には挿入処理による止葉抽出期の差が少なく、 4°C または 8°C で生育した場合には後期に 12°C で処理した時止葉抽出が著しく促進された。 12°C で生育した場合には生育期間の初期または後期は挿入処理温度が高い方が止葉抽出が早く、中期は挿入処理温度による差が小であつた。