Influence of Temperature upon The Fertilization of Wheat, Grown in Various Levels of Nitrogen*

Kiyochika HOSHIKAWA

(Faculty of Agriculture, the University of Tokyo)

Introduction

It has been well established that various environmental conditions, especially, the temperature influences on flowering^{1,2)}, pollen germination³⁾ and pollen tube elongation⁴⁾ and its rate⁵⁾. Despite its importance, our knowledge of the influences of various conditions on the process of fertilization or gametic fusion is highly meagre. In the cytological studies on the fertilization in wheat, the data related to the temperature are also lacking. SUETSUGU (1950)⁶⁾ observed that the early varieties of wheat required longer time to reach the first cell division of proembryo than in the late varieties, and he supposed that this difference is owing to temperature factor in their flowering periods. Generally, the flowering period of wheat is subjected to extreme climatic (temperature) change, and that the individual floret flowers at various times of the day, from dawn till midnight.

Besides, it is frequently stated that physiological functions of the reproductive organ of wheat are also influenced considerably by the nitrogenous fertilizers. Therefore, in agricultural standpoint, in order to examine the relation between fertilization and temperature, different levels of nitrogen are also considered indispensable as a connected factor.

In the previous report (HOSHIKAWA 1959)⁷⁾, normal process of double fertilization of common wheat at 20°C constant condition has been

mentioned cytologically. The present paper is in continuation to the previous one, and deals with the influence of various temperatures on the fusing phenomena of sex nuclei (the fertilization in narrow sense) in wheat grown at various levels of nitrogen.

Both aspects have been discussed.

Materials and Method

Six different varieties of common wheat, *Triticum aestivum* L. used as the materials in this experiment, are as follows:

Saitama-No. 27 (Nature is early, grain quality is mealy and tested in 1959), *Nōrin-No. 26* (early, mealy, 1956, 1957 and 1959), *Nōrin-No. 5J* (mid-season, mealy, 1958), *Nōrin-No. 67* (mid-season, glassy, 1958), *Nōrin-No. 42* (mid-season, glassy, 1959) and *Akasabi-shirazu* (late, mealy, 1958).

These wheats were cultivated at the experimental farm of the university of Tokyo, in three levels of nitrogenous fertilizer, shown in Table 1: Ammonium Sulphate was applied 131.4 kg per 10 ares as the excessive level, and 56.4 kg as medium level, and unmanured in low level plot.

Table 2 contains the observations of flowering at various temperatures in preliminary experiment made in 1957 (unpublished). The table shows that the maximum temperature at which flowering could take place was 32°C, minimum was 10°C and optimum was 18~20°C. Based upon these results, three separate rooms of 30°,

20°, and 10°C temperature were prepared, and every room was held constant conditions of light (inflorescence lamp), and humidity about 80 %.

Every ear was carried into these rooms a few hours before

Table. 1 Amount of Ammonium Sulphate(kg/10 ares) applied to different nitrogen levels.

Level of nitrogen	1958 5-Nov.	1959 9-Mar.	1959 28-Mar.	Total (Nitrogen)
Excessive	51.9	45.0	34.5	131.4 (26.3)
Medium	34.5	9.9	12.0	56.4 (11.3)
Low	0.0	0.0	0.0	0.0 (0.0)

Table. 2 Number of flowerings at various temperatures.
Variety: *Nōrin-No. 26*. (1957).

Temperature (°C)	8	10	12	14	16	18	20	22	24	26	28	30	32	34
Number of* flowering	0	3	3	16	—	43	41	35	41	29	30	24	9	0

* Sum of 8 ears; observation made from 10 a. m. to 3 p. m., 5 hours.

* Presented on the 125 th Meeting of the Crop Science Society of Japan. December 5, 1959.

Fig. 1 The illustration of the progressive processes of fertilization in common wheat, *Triticum aestivum* L. in 20°C constant condition.

m : male nuclei, p : protoplasm around polar nuclei.

flowering. Accordingly, the fertilization was examined at nine conditions (combinations of three temperatures with three nitrogenous levels).

Artificial pollination was done on the first florets of the sixth to tenth spikelets just before their lemmas rifted. Ovules were collected at several short intervals after pollination; every 15 minutes during first one hour, every 30 minutes during second to fifth hour and every one hour during fifth to twentyfourth hour after pollination.

Formalin acetic alcohol was used to fix and storage the sampling ovules. The dehydrated ovules were soaked in Crystal-violet-containing paraffin for about one week, and then stained ovules were cut into 10~14 μ thickness sections by microtome⁸⁾.

Through microscopic observations of about 1,000 embryo sacs, the progressive processes of fertilization have been classified into A, B, C, D, E, and F categories, illustrated in Figure 1. (cf HOSHIKAWA 1959)⁷⁾. By the application of the numerical values, such as A = 1, B = 2, C = 3, D = 4, E = 5, and F = 6, the calculation of the mean of every hour after pollination was done to express the progressive states. Fertilization curves thus obtained were utilized to determine the time necessary for the completion of fertilization.

Results and Discussion

Clear differences in the number of ears and spikelets, and the

first flowering day, appeared among the three nitrogen levels (Table 3).

Nine curves of progressive fertilization under various conditions are drawn in Figure 2, from which the time necessary for fertilization under nine conditions was obtained; the results are presented in Table 4. The six varieties used did not show any differences among each other, therefore, results of each variety are combined together.

1. The time necessary for fertilization.

Influence of temperature: It required 8.5 hours in 10°C, 5.0 hours in 20°C, and only 3.5 hours in 30°C to complete the fertilization with medium level of nitrogen (Figure 3). This tendency of shorter time required for fertilization associated with the higher temperature, is also observed similarly in both the excessive and low nitrogen levels.

Influence of nitrogen supply: It is clear from Figure 4, that the fertilization in both the excessive-N and low-N takes longer time than in medium-N, and this tendency is shown in

Table. 3 Some factors of growth at various nitrogen levels*.

Level of nitrogen	Number of ears per plant	Dry wt. of one ear at flowering time (gr)	Number of spikelets per ear	First ** flowering day(1959)
Excessive	11.0	0.404	21.5	28-April
Medium	8.7	0.394	20.5	27-April
Low	3.8	0.398	18.4	26-April

* Average value of three varieties, *Nōrin*-No. 26, 42, and *Saitama*-No. 27.

** Early varieties: *Nōrin*-No. 26 and *Saitama*-No. 27.

Fig. 2 Progressive processes of fertilization under various conditions.

M : Medium-N, E : Excessive-N, L : Low-N.

— : 30°C, ---- : 20°C, - · - · - : 10°C.

regardless of temperature. From the standpoint of agriculture, it is noticeable that, in 20°C, optimum temperature to flowering, the lag in time for fertilization in excessive and low-N is almost insignificant, that is to say, the delaying of fertilization caused by over or insufficient supply of nitrogenous fertilizer is come in evidence at somewhat abnormal temperature.

As stated above, the time necessary for fertilization gets shorter in inverse proportion to the temperature rises, within the range of 10°~30°C, and gets longer in both excessive or low-N than in medium-N.

2. The Progressive stage of fertilization.

In order to clarify the reason why these differences occur, the progressive fertilization curves in various conditions are compared from Figure 2. It is found that the fertilization makes rapid progress as temperature rises, regardless of the levels of nitrogen, and at any temperature the fertilization of medium-N progresses at more quicker pace than in any other nitrogen conditions. It is noticeable that the greater parts of the differences among the progressive paces of fertilization under various conditions, are made up during the early periods from A stage to C or D stage. All fertilization curves on and after D stage are rather parallel, hence there are little differences among their paces during the latter periods, namely, D, E, and F stages. The stages, when the embryo sac is in full activity, such as penetration of pollen tube into embryo sac, following its penetration into synergid, discharging of male nuclei and their penetration into

egg cell and polar nucleus, are strongly influenced by temperature and their own nitrogen levels. On the contrary, the later half processes such as the fusion of two sex nuclei and forma-

Table 4. Time necessary for fertilization under various conditions.

N-level	Excessive	Medium	Low
Temperature	hrs.	hrs.	hrs.
30°C	4.8	3.5	4.7
20°C	5.5	5.0	5.4
10°C	10.4	8.5	10.2

Fig. 3 Time necessary for fertilization at various temperatures.

Fig. 4 Time necessary for fertilization at various levels of nitrogen.

tion of the proembryo cell and the primary endosperm nucleus, seem to be unaffected by conditions any more. It is concluded that the fertilization of wheat is influenced by the tested conditions mainly during its first half processes rather than in its later half processes.

By many workers, it is mentioned that the growth of the pollen tube is affected to an appreciable degree by the temperature conditions, consequently, the interval between pollination and completion of fertilization may also be affected by temperature. As mentioned earlier, within the range of flowering temperature, the fertilization is carried out rapidly as temperature rises. This present result is an exhibition on the problem, and supports the SUETSUGU's speculation, reviewed in introduction of this paper.

However, it can not be said that an optimum temperature is the one at which the fertilization is carried out most rapidly. In a different experiment, the author examined the influences of temperature upon the pollen germination and growth of pollen tube, and drew influences that the pollen tube may arrive at embryo sac at 30°C earlier than at 20°C, and may be delayed considerably at 10°C⁹⁾. Such deduction is proved by the results of this experiment. The author rather thinks that temperature 20°C is the optimum for flowering, and that the variance of time necessary for fertilization caused by nitrogen levels is the least of all, therefore, we must give our attentions to the temperature 20°C. IKEDA et al (1959)¹⁰⁾ fertilized wheat and barley experimentally at various temperatures and observed the increasing pace of the ovule weight and the sterility percents, and have estimated that the optimum temperature for fertilization lies in the range of 15°C~20°C. TOGARI et al (1958)¹¹⁾ reported that the occurrence of sterility of rice plant increased with an increase in the level of nitrogen. No observation on the occurrence of sterility were made in this experiment. The author, however, succeeded to throw light on the behaviour of sex nuclei at various levels of nitrogen.

In the genetical and crop-science studies on wheat, it is required frequently to know, how far the fertilization has progressed in pollinated ovary at a given time and under certain circumstances. But there were very little experimental data existing on this problem up to date. Figure 2 graphed in this paper, may satisfy the afore-mentioned requirements, and this technical contribution is also one of the purposes of this paper.

Summary

The influences of various temperatures (10°C, 20°C, and 30°C) and nitrogen levels (excessive-N, medium-N, and low-N) upon the double fertilization of common wheat, *Triticum aestivum* L. were studied cytologically.

Through microscopic observations of about 1,000 embryo sacs, collected at several short intervals after pollination, the progressive processes of fertilization under various conditions were brought to light. According to these fertilization curves shown in Figure 2, the time necessary for fertilization under nine conditions was determined respectively.

Within the range of flowering temperature (10~30°C), the time necessary from pollination to the completion of double fertilization got shorter in inverse proportion to the temperature rises regardless of nitrogen levels. The fertilization in both the excessive-N and low-N took longer time than in medium-N, however, in 20°C this difference was almost insignificant.

The results obtained by comparative consideration of the progressive pace of fertilization curves under various conditions, illustrated the cause of the variation of time necessary for fertilization among nine conditions. Moreover, it was shown that the fertilization of wheat is influenced by the tested conditions mainly during its first half processes (A, B, and C stages in Figure 1) than in its later processes (D, E, and F stages).

Acknowledgement

The author is indebted to Dr. Y. TOGARI, Professor of Agriculture, and Assistant professor, Dr. T. TAKEDA, the University of Tokyo, for valuable advice and encouragement.

Literature cited

- 1) LIGHTY, C.E., et al, Pistillody in wheat flowers. Jour. Hered. 15 : 1924. (cited from KIHARA, H., Studies on Wheat. 1954)
- 2) MINAMI, T., et al, Observations on the flowering of cereal crops. Tokubetsu-Hōkoku, Farm att. Hokkaidō Imp. Univ. 4 : 1933. (in Japanese).
- 3) TABATA, K., et al, Proc. Crop Sci. Soc. Japan 1 : 1929. (in Japanese)
- 4) TERAOKA, H., et al, Physiological studies of the rice plant with special reference to the failure caused by the occurrence of unseasonal low temperature. Proc. Crop Sci. Soc. Japan 12 : 1940. (in Japanese).
- 5) POPE, M.N., The temperature factor in fertilization and growth of barley. Jour.

- Agr. Res. 66 : 1943.
- 6) SUETSUGU, I., Developmental morphology of the embryo in wheat varieties. Jour. Nat. Agric. Expt. St. (Tokyo). 4 : 1950. (in Japanese).
- 7) HOSHIKAWA, K., Cytological studies of double fertilization in wheat (*Triticum aestivum* L.). Proc. Crop Sci. Soc. Japan. 28 : 1959.
- 8) HOSHIKAWA, K., A simplified method for staining preparation of plant tissue. Agr. and Hort. 34 : 1959. (in Japanese).
- 9) HOSHIKAWA, K., Studies on the pollen germination and pollen tube growth in relation to the fertilization in wheat. Proc. Crop Sci. Soc. Japan 28. (in Print. 1960).
- 10) IKEDA, T., et al, Studies on the ripe-hastening of wheat and barley in the warmer regions in Japan. VII. Jour. Agric. Res. Tōkai-kinki Reg. Japan. 10 : 1959. (in Japanese).
- 11) TOGARI, Y., et al, Studies on the sterility in rice plant by superabundant nitrogen supply and insufficient light intensity. Proc. Crop Sci. Soc. Japan. 27 : 1958. (in Japanese).

〔和 文 摘 要〕

小麦の受精に及ぼす温度ならびに窒素施用量の影響

星 川 清 親

(東京大学農学部作物学研究室)

本実験は、小麦農林 26, 42, 50, 67 号、埼玉 27 号および赤錆不知の各品種を硫酸施用量の異なる 3 区の圃場 (N 過剰区 [反当 N 7 貫], 標準区 [反当 N 3 貫] および不足区 [無施用]) に栽培し、各開花期に 30°, 20°, および 10°C の温度下において人工的に受粉せしめて得た総数約 1000 の試料子房について、各区の受精過程の様相を細胞学的に鏡検し、小麦の受精過程に及ぼす温度ならびに窒素レベルの影響を明らかにする事を目的とした。

本実験の結果は、第 2 図にまとめられ、これにもとづいて受精所要時間及び受精進捗状況を比較検討した。なお一般に供試した小麦の生育した N レベルと、受粉・受精時の温度さえわかっているならば、受粉してから一定時間後の子房内での受精進捗状況を、第 2 図を利用することによつて察知し得る便宜が得られた。

受精所要時間 (受粉から雌雄両性核の融合完了まで) は窒素施用量の如何にかかわらず、開花温度域 (10°~30°C) 内においては高温ほど短く 10°C では 30°C の場合の 2 倍以上を要した。また何れの温度条件下でも標準 N 施用区は最も受精所要時間が短かく、それより N レベルが高すぎても又低すぎても受精に長時間を要した。

各処理区の受精の進捗状況を比較検討してみるに、いずれの N レベルでも高温ほど進捗が著しく、低温ほど遅く、而して其の進捗は受粉から精核の卵細胞質内侵入完了までの諸過程の期間に主として差異を生じるのであつて、それ以後の雌雄性核の融合の完了および胚乳原核形成に至る過程では、温度による進捗の影響が比較的少ないことが明らかになつた。同様に何れの温度条件下でも常に標準 N 区のものものの受精の進捗が、N 過剰、N 不足両区に比して早く、且つ其の差が受精前半諸過程の進捗状況の差異に起因していることが判つた。これらの事から小麦の受精においては温度ならびに窒素レベル等の影響が受精の前半の諸過程に特に強く影響するものであることが明らかになつた。

なお供試全品種間には差異が認められなかつたから、結果は総合して検討した。