Physiology of Flowering in Rice Plants

2. Inhibition of photoperiodic floral induction by antimetabolites of nucleic acid*

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The participation of nucleic acid metabolism in the flowering process was established by the experiments using antimetabolites in not only photoperiodic plants^{1-3,5,7,10}) but also in vernalizable plants^{6,11}) and even in an intermediate plant⁹) which induces flowering without any specific effect of photoperiod and temperatures but is rather easily influenced by nutritional conditions.

In this experiment, an attempt was made to reveal the role of nucleic acid metabolism in floral induction of rice plant as a physiological approach to the problem.

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MATERIAL AND METHODS

"Zuiho", a most photosensitive rice variety cultivated in Japan was used as material. Seedlings were prepared and successively transferred to solution culture equipments, adequate materials for photoperiodic induction so as to continuously supply.

A preliminary experiment has revealed that foliar application of 2-thiouracil, an antimetabolite of nucleic acid metabolism, does not affect flowering of rice plant. This is the reason why solution culture method was introduced in this experiment and test chemicals were applied through the root.

Chemicals were applied to the plants via the

root system certain days before or after the beginning of the short-day treatment. The root system was immersed in the test solution for 7 hrs (from 9 AM to 4 PM) and after the treatment, it was washed by water for several times, then the plants were returned to the nutrient solution. Short-day was consisted of 8 hrs of natural daylight and 16 hrs of darkness which was obtained covering the plant by dark box.

In some experiments, reversal of inhibition induced by 2-thiouracil or 5-bromouracil was tested by the application of corresponding normal metabolites.

Evaluation for the effect of chemicals on flowering was done by the number of days from the beginning of photoperiodic induction to heading in some cases, but detailed experiments were done by using floral stage system. In all experiments photoperiodic induction was started from 7-8 leaf stage.

RESULTS

As indicated in Fig. 1, 2-thiouracil in concentration of $1 \times 10^{-3}M$ and $5 \times 10^{-3}M$ have succeeded to inhibit flowering.

Results obtained in the attempt to reverse the inhibitory effect by certain metabolites are indicated in Fig. 1 and 2. Inhibition induced by 2-thiouracil was partially reversed by the application of uracil immediately after that of 2-thiouracil, but application 10 or more days after that of 2-thiouracil did not overcome the inhibition. On the other hand, inhibition induced by 8-azaguanine application was nearly completely reversed by guanine applied at any time during short-day treatment.

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Table 1. Inhibitory effect of 2-thiouracil on photoperiodic floral induction of a rice variety "Zuiho"

Period of treatment (Days before and after the beginning of inductive photoperiodic treatment)	Floral stage				
	Control	2-Thiouracil		2-Thiouracil $1 \times 10^{-3}M$ + Orotic acid $2.5 \times 10^{-3}M$	Orotic acid $2.5 \times 10^{-3}M$
		$2.5 \times 10^{-3}M$	$1 \times 10^{-3}M$		
-6		4.8	5.5	—	—
-4		3.8	4.5	—	—
-2		4.0	4.8	5.8	7.0
0		3.8	5.3	5.3	7.0
2		2.3	3.0	5.5	7.0
4		2.0	3.3	5.5	7.0
6		4.3	4.3	5.3	7.0
8		4.3	4.0	5.0	7.0
10		3.3	5.0	5.3	7.0
12		2.0	3.8	5.3	7.0
14		3.3	2.8	7.0	7.0
+2		3.5	3.0	—	—
	7.0

Fig. 1. Inhibitory effect of 2-thiouracil (TU) applied at 5 days after the beginning of photoperiodic induction on the flowering of rice plant "Zuiho" (upper), and reversion of the inhibition by uracil (U) (lower).
C: Control; Concentration of chemicals in the lower figure is $5 \times 10^{-3}M$.

Fig. 2. Effect upon flowering of an antimetabolite (5-bromouracil: BU) applied 5 days after the beginning of photoperiodic induction as compared to effect of the metabolite applied alone. Metabolites applied are uracil (U) (upper) and thymine (T) (lower).

Fig. 3. Effects on flowering of a representative inhibitor, 2-thiouracil applied at various times in relation to inductive photoperiodic treatment.

The translocation curve is obtained by defoliation experiment and the development curve is obtained by examining buds at various times after the beginning of induction. Except for the development curve, all points represent floral stage of plants examined 30 days after the beginning of induction. These three curves were cited from the first report of this investigation¹²⁾. Black bar indicates the period of inductive photoperiodic cycles.

Inhibitory effect of 5-bromouracil upon flowering, was reversed by uracil and thymine as indicated in Fig. 2.

Detailed experiments, using floral stage system, were carried out with 2-thiouracil as an inhibitor. Results obtained are shown in Table 1 and illustrated in Fig. 3. These experiments indicate that

2-thiouracil was effective in two phases of photoperiodic induction; period of floral stimulus synthesis and that of early stage of floral development.

DISCUSSION

If flowering is inhibited by the application of

certain antimetabolites which are known to inhibit specific metabolic step in other organisms, it may be considered that the suspected step is implicated in the flowering process. The participation of this suspected step can be further tested by attempts to reverse the effect of the antimetabolite by simultaneous application of a suspected corresponding metabolite.

Thus the treatment of antimetabolites revealed the participation of nucleic acid metabolism in the flowering process in *Xanthium*^{1,2,9)}, *Pharbitis*^{7,13)}, *Lolium*⁸⁾, *Streptocarpus*⁵⁾, *Lycopersicum*⁸⁾, *Cannabis*⁴⁾ and *Triticum*¹⁰⁾.

The present experiment has clearly shown that the nucleic acid metabolism plays an important role in photoperiodic floral induction also in a photosensitive rice variety. Recently INOUE⁴⁾ has reported that the inhibitory effect of 2-thiouracil on the flowering of photoinensitive rice variety.

Though problems are still remained on what part of flowering process including hormone synthesis, translocation, differentiation or development, is most strongly inhibited by antimetabolites of nucleic acid, the present experiment has suggested that nucleic acid metabolism plays an important role in hormone synthesis and floral development in apical meristem. We can not draw final conclusion from only these results but participation of nucleic acid metabolism in these two phases of photoperiodic induction was revealed in this experiment.

LITERATURE CITED

1. BONNER, J., and J. A. D. ZEEVAART 1961. Ribonucleic acid metabolisms in the bud, an essential component of floral induction in *Xanthium*. *Plant Physiol.* **37**: 43—49.
2. COLLINS, W. T., F. B. SALISBURY and C. W. ROSS 1963. Growth regulators and flowering. III. Antimetabolites. *Planta* **60**: 131—144.
3. EVANS, L. T. 1964. Inflorescence initiation in *Lolium temulentum* L. VI. Effect of some inhibitors of nucleic acid, protein and steroid biosynthesis. *Aust. Jour. Biol. Sci.* **17**: 24—35.
4. INOUE, J. 1965. Effects of chemicals on flower bud initiation in cereals. I. Effect of 2-thiouracil on rice plants grown under aspeptic conditions. *Plant and Cell Physiol.* **6**: 131—134.
5. HESLOP-HARRISON, J. 1960. Suppressive effect of 2-thiouracil on differentiation and flowering in *Cannabis sativa*. *Sci.* **132**: 1943—1944.
6. HESS, D. 1959. Die selektive Blockierung eines ander Blühinduktion beteiligten Ribose-nucleinsäure-Eiweiss-Systems durch 2-Thiouracil (Untersuchungen an *Streptocarpus wendlandii*). *Planta* **54**: 74—94.
7. KATAYAMA, T. C. 1963. A survey of botanical studies on the genus *Oryza* especially of photoperiodic studies. *Seiken Zihō* **15**: 98—109.
8. MARUSHIGE, K. and Y. MARUSHIGE 1962. Effect of 8-azaguanine, ethionine on floral initiation and vegetative development in seedlings of *Pharbitis nil* Chis. *Bot. Mag. (Tokyo)* **75**: 270—272.
9. PHATAK, S. C. and S. H. WITTEW 1964. Origin and nature of the flowering stimulus in tomato. *Plant Physiol.* **39** suppl. XXXVII.
10. SALISBURY, F. B. and J. BONNER 1960. Inhibition of photoperiodic induction by 5-fluorouracil. *Plant Physiol.* **35**: 173—177.
11. SUGE, H. and N. YAMADA 1965. Effect of nucleic acid and its antimetabolite on induction of flowering in winter cereals. *Proc. Crop. Sci. Soc. Japan* **33**: 324—329.
12. SUGE, H. and A. OSADA 1966. Physiology of flowering in rice plants. 1. Synthesis and translocation of floral stimulus. *Proc. Crop. Sci. Soc. Japan* **36**: 32—36.
13. ZEEVAART, J. A. D. 1962. DNA multiplication as a requirement for expression of floral stimulus in *Pharbitis nil*. *Plant Physiol.* **37**: 296—304.

〔和 文 摘 要〕

水稻の開花生理に関する研究

2. 花成誘導におよぼす核酸代謝阻害物質の影響

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感光性品種瑞豊を用いて、花成におよぼす核酸代謝阻害物質の影響をしらべた。供試した2-チオウラシル、8-アザグアニン、5-ブロモウラシルなどのうちでは2-チオウラシルおよび5-ブロモウラシルが強い阻害作用を示し、他の物質でも処理時期、濃度によつて多少の花成阻害がみられた。2-チオウラシルの花成阻害は処理後直ちに対応する正常な代謝物であるウラシルを与えるとある程度回復し、また8-アザグアニンによる阻害は同様にグアニンによつて回復する。また5-ブロモウラシルによる阻害はウラシルおよびチミン両者により完全に回復した。このようにウラシルおよびチミンの両者で回復するため5-ブロモウラシルの阻害がDNA代謝を阻害するのか、RNA代謝に関連するのかは明らかでない。

なお、2-チオウラシルによる阻害は、誘導処理開始後2～4日目と、12日目以後に強く現われる。この時期は、第一報の結果から推定すると前者の時期は花成刺激生成期にあたり、後者は花芽発育初期にあたる。この2-チオウラシルによる阻害は、2-チオウラシル処理と同時にオロチン酸を与えるとかなりの回復を示した。

これらの結果から水稻では花成刺激生成と花芽発育に核酸代謝が関与しているものと推定される。