代謝阻害剤によるイネの葉身傾斜速度の調節

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Regulation of Lamina-inclination Rate by Selective Inhibitors in Excised Rice Leaves*

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Several reports have dealt with elongation of plant cells in relation to nucleic acid metabolism and energy supply[1,2,13,18,19]. The authors have previously shown that the lamina inclination of rice leaves is caused by cell elongation of adaxial side in the lamina joint[9,14]. In the previous experiments using the second leaves excised from etiolated rice seedlings, the IAA-induced increase in angle was inhibited by cytokinin and purine analogues[11]. Dinitrophenol, an uncoupler of oxidative phosphorylation, and antimycin A, also inhibited the lamina inclination[13].

In the present investigation the mechanism of lamina inclination were examined by determining the changes in inclination rate after the application of the metabolic inhibitors.

MATERIALS AND METHODS

Rice seeds (*Oryza sativa* L. var. Aichi-asahi and Kinmaze), submerged in water for 2 days at 30°C, were planted on 0.9% agar plate and cultivated in dark at 30°C, being illuminated occasionally with red light obtained from a 40 watt white fluorescent lamp covered with two layers of red cellophanes as described earlier[11,12]. The energy at the surface of trays containing the rice seedlings was about 5 μW/cm^2·10 nm at 635 nm. Seven days after the planting, explants, consisted of intact laminae, lamina joints and 2 cm of sheaths, were excised from the etiolated second leaves.

They were floated on distilled water for 24 hours, at the end of which they have curved about 40 degrees. The explants of this stage, which were used as experimental materials, are highly sensitive to auxin[14]. The curved explants were floated on auxin solutions containing various levels of the inhibitors for 48 hours at 30°C. The increased inclination caused by the treatments was measured with a protractor. The average increments in inclination of about 60 explants are indicated in the table and the figures.

The concentration of IAA (indole-3-acetic acid) at 5×10^{-5}M was used and 2.5×10^{-3}M of potassium maleate was added as a buffer solution in all the experiments (pH 5.6). As the selective inhibitors, dinitrophenol, antimycin A, chloramphenicol, 8-azaguanine, 2-thiouracil, 5-flu-

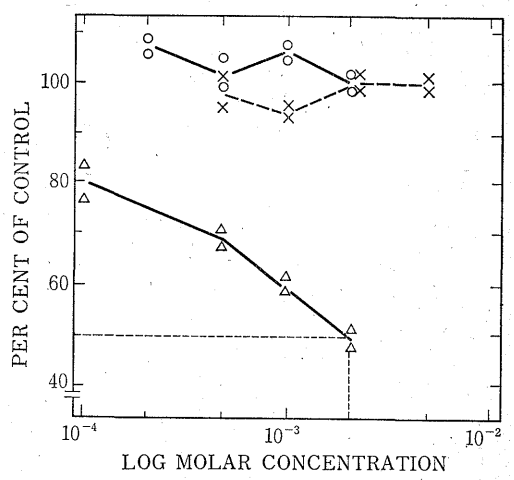

Fig. 1. Effects of 8-azaguanine, 2-thiouracil and 5-fluorouracil on the IAA-induced lamina inclination. Explants, pretreated for 24 hours, were floated for 48 hours on the solutions containing various concentration of inhibitors, 5×10^{-5} M of IAA and 2.5×10^{-3} M of potassium maleate.
—△— : 8-azaguanine, ···×··· : 2-thiouracil, —○— : 5-fluorouracil, dotted line (a): 50% inhibition.

* Received for publication on March 2, 1970.

Table 1. Reversal by guanosine of 8-azaguanine inhibiton of lamina inclination.

Substances used*	Increment in angle	Per cent of control
IAA alone	82.4	100
IAA and 8-azaguanine	42.3	51.1
IAA, 8-azaguanine and sodium guanylate	46.6	56.6
IAA, 8-azaguanine and guanosine	67.9	78.5

* The concentrations of all the substances were 2.5×10^{-3} M except for IAA at 5×10^{-5} M.

orouracil, puromycin, chromomycin A_3 and actinomycin D were employed at various concentrations. The effect of kinetin was also investigated for comparison. Antimycin A and 5-fluorouracil were supplied from Kyowa Hakko Kogyo Co. and actinomycin D were supplied from Merck Sharp and Dohme Res. Lab.

To elucidate the kinetics of lamina inclination in the explants applied by inhibitors, the procedure described in the previous paper[14] was employed. The sheaths of 10 explants pretreated were adhered to a glass plate ($82\times15\times5$ mm) with α-cyanoacrylate (Alon alpha 202 produced by Toa Gosei Kagaku Co.) so that their laminae

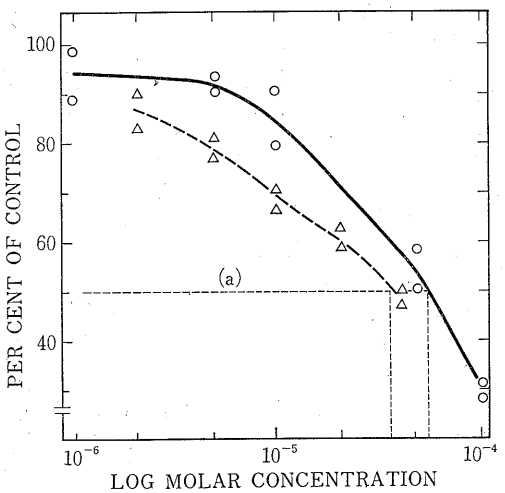

Fig. 2. Effects of dinitrophenol and antimycin A on the IAA-induced lamina inclination. Procedure and concentrations of the substances are the same as in fig. 1. —○— : dinitrophenol, ···△··· : antimycin A, dotted line (a) : 50% inhibition.

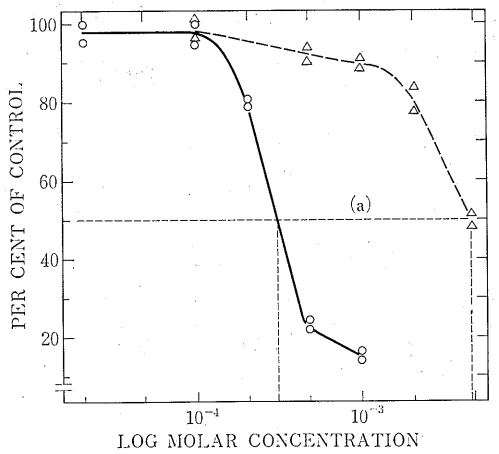

Fig. 3. Effects of puromycin and chloramphenicol on the IAA-induced lamina inclination. Procedure and concentrations of the substances are the sam as in fig. 1. —○— : puromycin, ···△··· : chloramphenicol, dotted line(a) : 50% inhibition.

inclinate at horizontal plane. The glass plate having the explants was placed in a Petri dish of 9 cm in diameter, and distilled water was poured into up to the level of explants. They

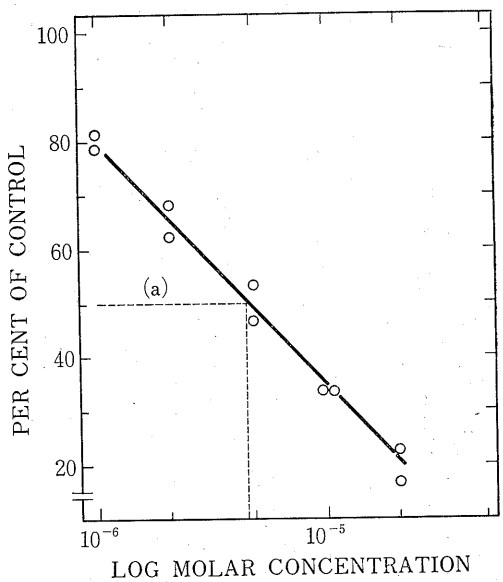

Fig. 4. Effects of actinomycin D on the IAA-induced lamina inclination. Procedure and concentrations of the substances are the same as in fig. 1, dotted line(a) : 50% inhibition.

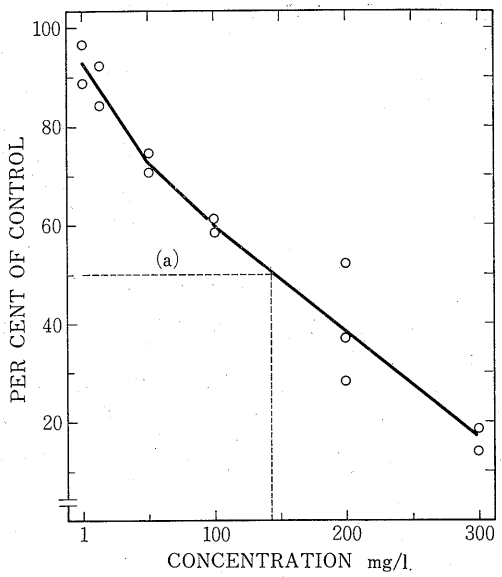

Fig. 5. Effects of chromomycin A_3 on the IAA-induced lamina inclination. Procedure and concentrations of the substances are the same as in fig. 1, dotted line (a) : 50% inhibition.

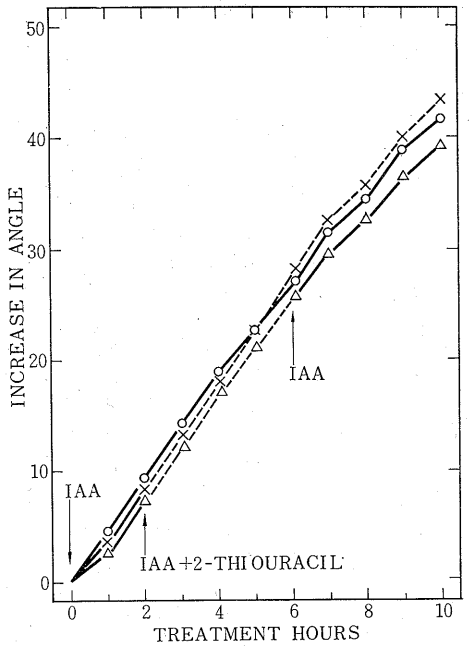

Fig. 7. Effect of 2-thiouracil on time course of lamina inclination. Procedures are the same as in fig. 6 except 5×10^{-3} M of 2-thiouracil was used.

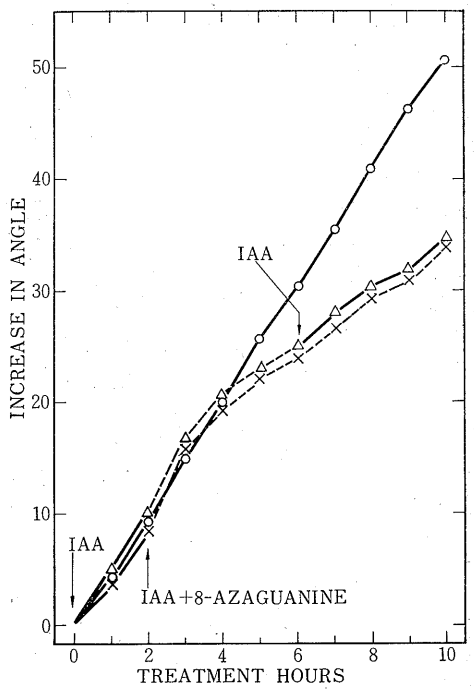

Fig. 6. Effect of 8-azaguanine on time course of lamina inclination. 8-Azaguanine at 2.5×10^{-3} M was added after 2 hours and removed after 6 hours of IAA treatment. — : IAA alone, - - - : IAA and 8-azaguanine. The arrows show time when IAA with or without 8-azaguanine were added.

were maintained for 4 hours at 30°C to nullify the inhibitory action of Alon alpha 202 on inclination rate.

After that IAA solution was added in the dishes instead of water. The treating solutions were exchanged quickly by pipettes and separating funnels. The induced angles were measured with vernier and rotary screen (×10) of Toshiba measuring projector MP Type 30B. In this method, changes in the inclination rate were recorded with the same explant at intervals of 1 hour during 10 hours of observation period. All the manipulation and recording were performed under red light at 30°C. Each experiment was repeated at least twice with three Petri dishes.

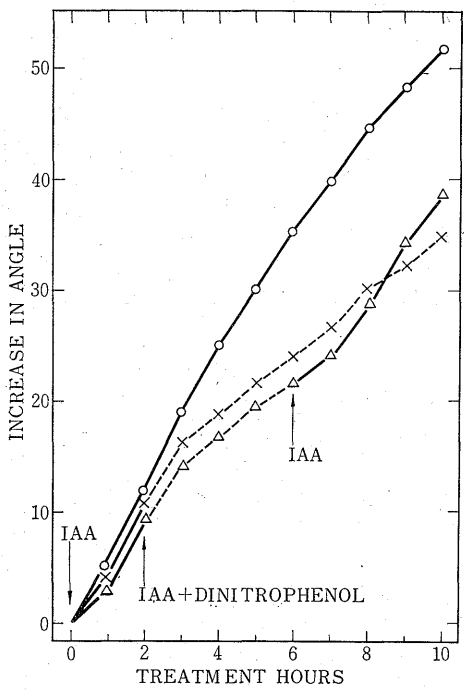

Fig. 8. Effect of dinitrophenol on time course of lamina inclination. Procedure are the same as in fig. 6 except 5×10^{-5} M of dinitrophenol was used.

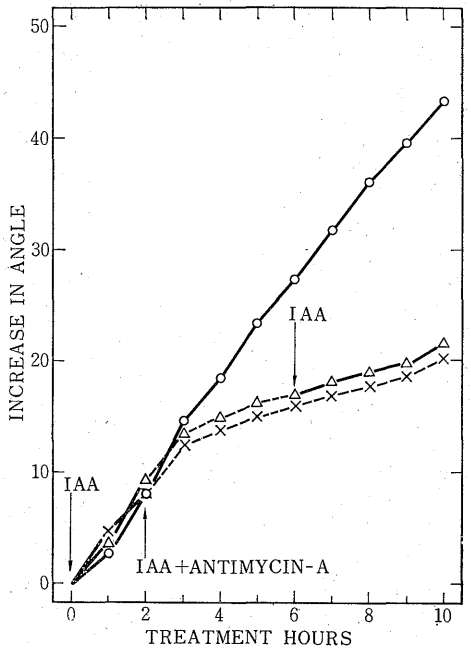

Fig. 9. Effect of antimycin A on time course of lamina inclination. Procedures are the same as in fig. 6 except 3.6×10^{-5} M of antimycin A was used.

Experimental Results

Fig. 1 shows the effect of various concentrations of purine and pyrimidine analogues on the IAA-induced lamina inclination in the excised rice leaves. The result reveals that 8-azaguanine is effective at high concentrations but 2-thiouracil and 5-fluorouracil is not, at any concentrations tested. The inhibition by 8-azaguanine was reversed by guanosine partially (table 1). This result is in agreement with that in artichoke tuber disk, in which 0.4 mM of guanosine prevents the inhibition by 0.4 mM of 8-azaguanine[18].

As shown in fig. 2, dinitrophenol and antimycin A also inhibited the IAA-induced inclination at the concentrations of 10^{-5}M or higher. Chloramphenicol, puromycin, actinomycin D and chromomycin A_3 certainly indicated inhibitory effect at levels higher than 10^{-3}M, 2×10^{-4}M, 10^{-6}M and 50 mg/*l* respectively (fig. 3, 4 and 5). It has been revealed that chromomycin A_3 is the inhibitor of DNA-dependent RNA polymerase reaction[5].

From these results and the preliminary test with kinetin, it was found that chloramphenicol, 8-azaguanine, puromycin, dinitrophenol, antimycin A, kinetin, actinomycin D and chromomycin A_3 could induce 50% inhibition of lamina inclination at 5×10^{-3}M, 2.5×10^{-3}M, 2.5×10^{-4}M, 5×10^{-5}M, 3.6×10^{-5}M, 2×10^{-5}M, 5×10^{-6}M and 150 mg/*l*, respectively. In some of these inhibitors, these concentrations were used in the following time-sequence experiment. 2-Thiouracil at 5×10^{-3}M was also employed for time-sequence experiment.

Effect of 8-azaguanine as a function of time is shown in fig. 6. Two hours after IAA application, some explants were applied by the IAA solution containing 8-azaguanine (△, ×) and others were remained in IAA solution till the end of experiment (○). The accelerated rate induced by IAA of lamina inclination preserved for 1 hour after the application of 8-aza-

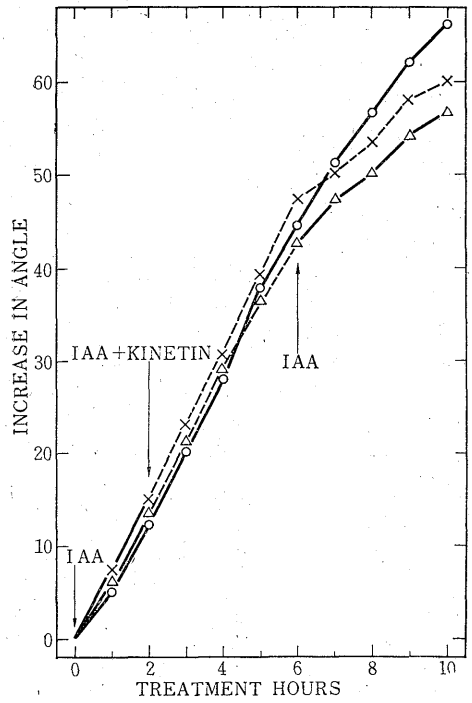

Fig. 10. Effect of kinetin on time course of lamina inclination. Procedures are the same as in fig. 6 except 2×10^{-5} M of kinetin was used.

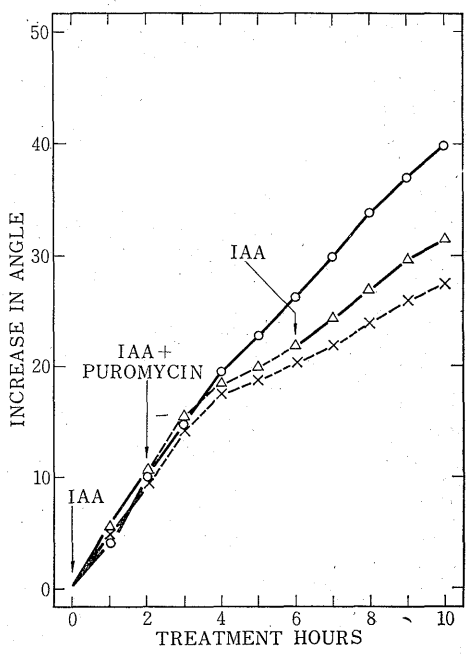

Fig. 11. Effect of puromycin on time course of lamina inclination. Procedures are the same as in fig. 6 except 2.5×10^{-4} M of puromycin was used.

guanine but afterwards began to decrease. The decreased rate continued over the observation period, even when the 8-azaguanine was excluded 4 hours after the application (△). 2-Thiouracil certainly failed to affect the inclination rate (fig. 7).

Dinitrophenol, uncoupler of oxidative phosphorylation, also inhibited the inclination rate after 1 hour of its application. The decreased rate recovered to the initial rate 1 hour after the exchange with auxin solution lacking dinitrophenol (fig. 8). Antimycin A, respiratory inhibitor, decreased the inclination rate after 1 hour of the application. The decreased rate was always preserved regardless of the removal of the antimycin A (fig. 9).

In fig. 10, the effect of kinetin is shown. The inclination rate was decreased after 4 hours of the application. This result is the same as in the previous paper in which 2, 4-D (2, 4-dichlorophenoxyacetic acid) was employed instead of IAA[11]. The decreased rate did not altered even when kinetin was excluded from the medium.

Puromycin indicated the inhibitory action 1 hour after the application and after the removal the inclination rate was slightly recovered (fig. 11). The rate was decreased 2 hours after the application of actinomycin D, but the decreased rate continued even after its removal (fig. 12). This observation is in agreement with those of Penny and Galston[19], in whose work green pea stem segments were employed. And this is not essentially different from those of Nooden[18] who made use of corn coleoptile sections.

DISCUSSION

The agronomical merits of lamina inclination in the cultivation of rice plants have been discussed previously[10,15]. In this work, there are the effects of metabolic inhibitors on the inclination.

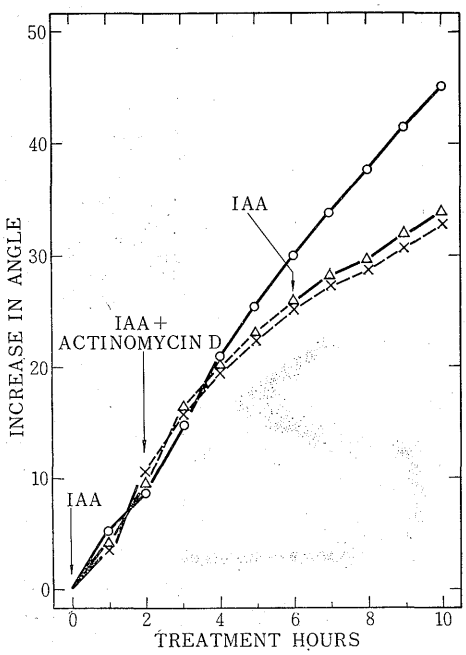

Fig. 12. Effect of actinomycin D on time course of lamina inclinntion. Procedures are the same as in fig. 6 except 5×10^{-6} M of actinomycin D was used.

8-Azaguanine inhibits the growth and increases incorporation of ^{32}P into RNA, in the artichoke tuber disks but not in corn coleoptile[18]. Although the activity of 8-azaguanine varies with the different tissues[6,16], it is assumed from our results regarding this substance that the IAA-induced inclination of laminae may be related to RNA synthesis in the cells of lamina joints.

5-Fluorouracil suppressed RNA synthesis in soybean hypocotyl section[6,7], oat coleoptile[17], corn mesocotyl[7], corn coleoptile and artichoke tuber disk[18]. The growth of these tissues and also of lamina joint of rice leaves was not inhibited by 5-fluorouracil. From these facts it may be postulated that a little amount of specific RNA, which is not affected by this substance, participates in the lamina inclination.

Although 2-thiouracil is not effective upon lamina inclination, the findings that 2-thiouracil stimulates both growth and oxygen uptake in pea root segments[3,4] but decreases the growth of lettuce seedlings[8] and artichoke tuber disk[16] have been reported.

The lamina joint test, which is usually used to study the effect of various substances on the inclination angle, is not suitable for the experiment to assertain the primary site of auxin action on the inclination, because of the necessity of long term. However, the new method revised for the short term experiment seems to be appropriate for the experiment to research the kinetics of auxin action on lamina inclination.

Using this method, we found the followings : (I) A constant rate in the lamina inclination is obtained over a period of 10 hours in the presence of IAA at 5×10^{-5} M. (II) The rate is about 5 degrees per hour. In this inclination rate, the adaxial cells of lamina joint elongate about 4 μ per hour. (III) By removal of IAA from the medium, the rate is reduced by 50%, and again it is recovered to 5 degrees per hour by the reapplication of IAA[14]. However, in the adult rice plants cultivated in the field, the laminae inclinate so very slowly that the rate of about 4 degrees per day at maximum value is occurred[15].

The length of lag period before the appearance of inhibition by the inhibitors was various in the experiments using the revised method. This period was 1 hour at the case of 8-azaguanine, dinitrophenol, antimycin A and puromycin. It was 2 hours at actinomycin D and 4 hours at kinetin. From these results we could propose one possibility that both DNA-type RNA synthesis and the following protein synthesis participate in the lamina inclination induced by auxin.

And also, the simplest hypothesis consistent with these facts is that the inclination is regulated by the substances decreased by actinomycin D for 2 hours after its application. An evaluation of this hypothesis cannot be made until the difference in penetration rate into the joint cells of the inhibitors used is revealed.

It has been suggested that the 2 hour-lag period before the inhibition by actinomycin D

is necessary for the substances to be used up, which interact with auxin in cell elongation[19]. That IAA exerts an effect on DNA-dependent RNA synthesis has been reported in *Avena* coleoptile section[2]. Furthermore, it should be noted that auxin acts by induction of mRNA, allowing cell expansion[18]. An evidence that the growth inhibition by kinetin in excised soybean hypocotyl correlated with the inhibition of ribosomal (but not DNA-type) RNA synthesis has been reported[20].

It is known that the growth of rice coleoptiles depends on the energy released by the respiratory oxidation[21]. This may be true also of the growth of lamina joints in excised rice leaves. From the effect of dinitrophenol, it can be said that the inclination rate of laminae seems to associate with the energy supply into the adaxial cells in lamina joints. Particularly, it is very interesting that the inhibition by dinitrophenol is completely recovered when this substance was removed and the laminae of the explants inclinate the same as those kept in IAA.

SUMMARY

Selective inhibitors were applied to excised rice leaves in the presence of IAA, and the effects on lamina inclination were noted as a function of time.

Dinitrophenol, antimycin A, 8-azaguanine, puromycin, chloramphenicol and actinomycin D inhibited IAA-induced inclination of laminae. But 2-thiouracil and 5-fluorouracil did not affect the inclination at any concentrations tested.

The decrease in the inclination rate was actually caused by dinitrophenol, antimycin A, 8-azaguanine and puromycin after 1 hour, by actinomycin D after 2 hours, and by kinetin after 4 hours of inhibitor application. The decreased rate was not recovered by the removal of the inhibitors except dinitrophenol.

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〔和 文 摘 要〕

代謝阻害剤によるイネの葉身傾斜速度の調節

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（名古屋大学農学部 生化学制御研究施設）

イネの切断第2葉片に IAA とともに，各種の代謝阻害剤を処理し，葉身傾斜速度の経時的変化をしらべた．葉身傾斜に対し，dinitrophenol, antimycin A, 8-azaguanine, puromycin, chloramphenicol および actinomycin D は，阻害作用を示したが，2-thiouracil と 5-fluorouracil では阻害作用が見られなかった．葉身傾斜速度の減少は，dinitrophenol, antimycin A, 8-azaguanine, puromycin で処理1時間後，actinomycin D で処理2時間後，kinetin で処理4時間後にあらわれた．阻害剤を除去したのちでも，dinitrophenol 以外の場合には，傾斜速度がもとの速度にもどることがなかった．以上の結果にもとづき，イネの葉身傾斜機構について若干の考察を加えた．