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GROWTH REGULATOR IN GERMINATING RICE SEED*

By

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Introduction

Several reports on the occurrence of some growth inhibitor not only in dormant seeds, buds and tubers, but also in the actively growing plant organs, such as expanding buds, leaves and young fruits have appeared in the literature (BENTLEY, 1958). Studying on germinating seeds, CARTWRIGHT et al. (1956) have extracted an acidic growth inhibitor from germinating peas, var. Alaska. It appeared at the earlier sampling times; its concentration decreased during the first 3 days of germination; none was detected in the 72- and 96-hour extracts. In the next few days of seedling growth, however, this inhibitor re-appeared. PFIRSCH (1956) extracted a neutral growth inhibitor which controls the development of the axillary bud, from cotyledons of Sinapis alba seedlings. POLJAKOFF-MAYBER et al. (1957) have also reported the appearance of an acidic growth inhibitor in germinating lettuce seeds at about 24-48 hours after incubation. MURAKAMI (1960) reported the occurrence of gibberellin in dry seeds and in growing shoots of rice plant. He (1961) also observed that gibberellin and acidic growth inhibitor are contained in the young seedlings of Pharbitis Nil germinated in the dark.

The present writers found an abundant accumulation of a neutral growth inhibitor in the endosperms of rice seeds during germination. As it was assumed that this inhibitor might be correlated with the sluggish growth at the early stage of rice germination, studies were made on the physiological role of this growth inhibitor obtained from germinating rice seeds and at the same time some observations were carried out to ascertain the effect of the gibberellin-like substance obtained from such rice seeds on the growth of wheat leaf sections.

* Dedicated to Prof. Hajime MATSUURA and Prof. Yukio YAMADA celebrating their Sexagenary birthdays.

Materials and methods

Oryza sativa var. 'Eiko' was used as experimental material. Unless otherwise stated hulled rice seeds were used in the present investigation for convenience of separation and extraction of the endosperms. All the processes of germination were carried out aseptically. The rice seeds were sterilized first with usplun solution, then rinsed thoroughly with sterile water and were sown in germinating dishes containing submerged sawdust which had been previously steam sterilized. The seeds were incubated either in a thermostat at 25°C in the dark, or in a green house under day light exposure. At various stages of germination samples were taken and separated into plant bodies and endosperms. Mainly the endosperms were used for the extraction of growth regulator, but sometimes young leaves were also used.

Samples were extracted with ethylalcohol-water (2:1) for about 24 hours at room temperature. Then the solution was filtered and the residue was washed thoroughly with alcohol-water and filtered again. The two filtrates were mixed together and alcohol was evaporated off at 40°C under reduced pressure. The aqueous residue was filtered and fractionated into neutral and acidic fractions with ether by the method described by Larsen (1955). After condensation of each fraction at 50°C, the samples were developed paper chromatographically with isopropanol-ammonia-water (10:1:1). The chromatograms were cut transversely into 10 equal sections, and each section was placed in a 6-cm. petri dish. As the control a section obtained from the plain chromatogram was used in the same way as stated above. One cc. of culture solution was added to each dish. The activity of this growth regulator was assayed by the wheat leaf section test developed by Radley (1958), with minor modification by the present writers. According to Radley, the growth of wheat leaf sections was stimulated by IAA only at higher concentration, while not stimulated by kinetin, but kinetin caused some inhibition at higher concentration. The present writers have reexamined these points and obtained similar results. To avoid any disturbance of the test due to IAA effect, IAA of 2 mg/cc was added to the culture solution. Summer wheat seeds (var. 'Norin' No. 75) sterilized previously with usplun solution were sown on 0.5 per cent agar medium and incubated in the dark at 25°C. They were harvested when 60 hours old. From each selected shoot one 5-mm. section was cut at 3 mm. above the base of the shoot. Primary leaves were removed from the coleoptile sections by gentle pressure with a fine glass rod, and ten sections were placed on each chromatogram strip in the test dish for incubation periods ranging from 18 to 20 hours in the dark at 25°C. After the desired incubation interval the elongations of the sections were measured and
results were expressed in terms of percentages compared with the control elongation. Taking into consideration its natural role in the plant in vivo, the concentration of the extract for bioassay was carefully adjusted not much to differ from that involved originally in the living tissues. Usually once to double concentration based on fresh weight (×1 to ×2) were used. For the extraction of endosperms 60 seed grains were used of which fresh weights were about 1.3 to 1.6 g.

Fig. 1. Histograms showing activities of the neutral growth inhibitor and gibberellin-like substance, which were obtained paper chromatographically with ammoniacal isopropanol from endosperms of dry rice seeds and from those germinated in the dark. Broken lines indicate the growth of controls.

A, B, C and D, neutral fractions; E, F, G and H, acidic fractions. A and E dry seeds; and the others, germinated seeds: B and F, for 2 days; C and G, for 4 days; D and H, for 6 days after incubation.
Experimental results

(A) Growth regulator in the endosperm of germinating rice seed.

The germination of rice seeds had started in the majority of cases within 24 hours after incubation. But the early development of the sprout was slow; after 2 days the coleoptile was only 1-2 mm. in length and the fresh weight of the sprout was about 10 per cent of that of the endosperm. However, after the emergence of the 2nd leaf, the rate of growth increased considerably. Samples of seeds were taken at the 2nd, 4th and 6th days after incubation and the contents of the growth regulator in the endosperms were determined by the method stated above.

The results are given in figure 1. In the neutral fraction the growth inhibitor was found between Rf values 0.8 and 1.0. The activity of the growth inhibitor was not so significant before germination, but it increased rapidly soon after the germination had advanced, and thereafter it increased steadily with the progress of the sprout development. In the acidic fractions extracted from the dry seeds, the growth inhibitor was found to be present, but it decreased and then disappeared entirely after the onset of germination. Acidic fractions obtained from the seeds on the 4th and 6th days after incubation showed a growth stimulation in the wheat leaf section test. In the present bioassay no growth stimulation due to IAA or kinetin could be detected, therefore, such growth stimulation might be attributed to some certain gibberellin-like substance contained in this acidic fraction.

(B) Effect of light on the formation of growth inhibitor.

When rice seeds germinated in the dark, coleoptiles and leaves became much elongated compared with those germinated in the light. In order to examine the effect of light on the formation of inhibitor in the endosperms of germinating seeds, the following experiments were made. The rice seeds were divided into two groups; the one group was raised in the green house under natural light exposure and the other was raised under the same conditions, but covered with black cloth.

The results are shown in figure 2. The activity of the neutral growth inhibitor in the endosperms cultured under the light condition was higher than that in the dark. Its activity was still significant even at the stage when the development of the third leaf and the liquefaction of starch in the endosperm of the light germinated seed were recognized (see fig. 3). With the advance of the growth stages, the gibberellin-like substance was found also to appear in the acidic fraction obtained from the light-germinated seed, as well as in that obtained from the dark-germinated seed. The activity of the gibberellin-like
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Fig. 2. Histograms showing activities of the neutral growth inhibitor which was obtained from endosperms of rice seeds germinated in the dark and in the light.
A, B and C: dark germinated seeds;
D, E and F: light germinated seeds.

Fig. 3. Top. Histograms showing activities of the neutral growth inhibitor and gibberellin-like substance, obtained from endosperms of rice seeds germinated in the light at the stage of 3rd leaf development.
A: neutral fraction; B: acidic fraction.

Fig. 4. Bottom. Histograms showing activities of the neutral growth inhibitor and gibberellin-like substance, obtained from the basal 5 mm. of the 2nd leaves of light germinated rice seeds showing active growth.
C: neutral fraction; D: acidic fraction.
substance became more intense, when the liquefaction of the endosperm was recognized.

(C) **Growth regulator in the actively growing region of the leaf.**

As stated above, activity of the neutral growth inhibitor obtained from the endosperms was ascertained to be still significant even at the advanced stages of germination. The point to be considered next is the physiological role of this growth regulator in growth. The grand period of growth of the 2nd leaf was found to be at the time when the upper part of the 2nd leaves emerged about 5 to 6 mm. out of the first leaf sheath. So the basal 5 mm. of the 2nd leaf at this actively growing stage was taken as materials; the growth regulator was extracted from these samples of 1 g. in fresh weight, and bioassayed for its activity.

The results are shown in figure 4. The concentration of the neutral growth inhibitor for the assay was $\times 1$. The neutral fraction obtained from this sample showed a remarkable growth inhibition, while in the acidic fraction high activity of gibberellin-like substance was recognized. That the high activity of the neutral growth inhibitor was ascertained in the tissue which shows rapid growth is at any rate highly suggestive that this substance acts not simply as a growth inhibitor only.

(D) **Influences of some growth conditions on the formation of the growth regulator.**

1) Germination at low temperature: Seeds were allowed to germinate in the dark in the laboratory. The room temperature was 12–18°C; the growth was very retarded and the coleoptile was only 6–9 mm. in length even after 15 days. Samples were harvested on the 7th, 11th and 15th days after incubation and the growth regulator in the endosperms was extracted for assay.

The activity of the neutral growth inhibitor was found to increase steadily with the development of the seedlings, namely, the growth of the test plant was depressed to about 30 per cent of the control by the application of the extract obtained 15 days after incubation.

2) Germination under water: When a rice seed was germinated under water, the elongation of the coleoptile was much accelerated, while the root growth was suppressed. However, after the upper part of the coleoptile grew above the surface of water, root emergence was recognized soon (YAMADA, 1954). As such a change in growth behavior is assumed to be due to the change of the activity of the growth regulator, the following experiments were carried out. Sterilized rice seeds were germinated under sterile tapwater of about 7 cm. depth in the dark at 25°C. To avoid the influence of alcohol fermentation
during germination, the medium was renewed twice a day. Practically roots and leaves did not develop on the submerged seedlings. Samples were harvested on the 3rd, 5th and 7th days after incubations and assay was made of the activity of the neutral growth inhibitor. In the 3 day old plant the activity was considerably higher than that in the dry seed. This indicated that the formation of neutral growth inhibitor has happened in the early stage of growth in the case of submerged germination. In the subsequent 5th and 7th day samples the activities had wholly disappeared. In the next experiment the rice seeds were germinated for 4 days under water, then the part of water was discarded, and the seedlings were exposed partially to air for 2 days. By such treatment the roots and leaves were caused to develop soon; the neutral growth inhibitor was found to be formed notably in the endosperms. In the control seedlings, continuing submerged germination, the development of the root and leaf and formation of growth inhibitor were all negative as ever.

3) Growth regulator in germinating rough seed: All the results noted above were obtained with hulled rice seeds. It appeared desirable to ascertain whether the formation of neutral growth inhibitor in endosperms was special to the hulled rice seeds. Accordingly the following experiments were carried out with rough seeds. Such seeds were germinated in the light and the neutral growth inhibitor in the endosperms was measured. The root growth of the seedlings from rough seeds was somewhat better than that from hulled seeds. The formation of neutral growth inhibitor in the endosperms was clearly indicated in this case too, however, its activity was lower than that of the hulled seeds.

ELLIOTT and LEOPOLD (1953) and KÖVES (1957) reported that Avena seed hulls contained ether and water soluble growth inhibitor for seed germination and amylase action. The present writers have also proved (unpublish) that the alcohol extract of the rice seed hulls contained both neutral and acidic growth inhibitors.

(E) Properties of neutral growth inhibitor.

The neutral growth inhibitor was extracted from rice seeds germinated in the light and its properties were assayed. Rf values of the neutral growth inhibitor on paper chromatography developed with several different solvents were as follows; isopropanol-ammonia-water (10: 1: 1), Rf=0.8—1.0; ethanol (80 per cent), Rf=0.8—0.9; ethanol-3N.ammonia (8: 2), Rf=0.7—0.9; n.butanol-acetic acid-water (95: 5: 30), Rf=0.9—1.0; water, Rf=0.5—0.6.

The neutral growth inhibitor was separated paper chromatographically with ammoniacal isopropanol, then eluted with distilled water. The properties of this
partially purified aqueous solution of the neutral growth inhibitor were tested. This aqueous solution could be stored in a refrigerator for one month without showing any change of activity. It was diffusible through a cellophane membrane and could be recovered completely from its diffusate. Active material was adsorbed on blood charcoal and then eluted out readily with alcohol. Its activity was largely destroyed by treatment with dilute hydrogen peroxide solution; the initial inhibiting activity of 85 per cent of the control decreased to 59 per cent after such treatment.

Aqueous, 0.1 N.HCl and 0.1 N.NaOH solutions of neutral growth inhibitor were prepared and heated at 100°C for one hour, then neutral fraction was recovered by refractionation, and their activities were reexamined. The inhibiting activities of aqueous and HCl solutions showed little change, but NaOH solution lost almost all its activity. In the latter case gibberellin-like substance was found to be formed in the refractionated acidic fraction. However, as will be shown below, it seems highly probable that this substance might be derived

![Graph](image)

**Fig. 5.** Effect of neutral growth inhibitor of various concentrations on the elongation of wheat leaf sections in the culture solution containing IAA (2 mg/l) with or without gibberellin.

- with gibberellin (2 mg/l), \(\times\)
- without gibberellin, \(\circ\)

Concentration of the neutral growth inhibitor was expressed on a fresh weight basis of endosperm used for the extraction.
from some substance contained in the neutral growth inhibitor solution, and
supposed to have no relation with the latter. As both neutral growth inhibitor
and gibberellin-like substance are contained in the same organs, such as endo­
sperms or leaves, it is assumed that these two may function antagonistically as
the growth regulator. To ascertain this point neutral growth inhibitor in
various concentrations was added to the culture solution containing IAA (2 mg/l)
and gibberellin (2 mg/l), and preparates were bioassayed. Culture solutions
containing the same series of inhibitor concentrations without gibberellin were
used as control.

The results are shown in figure 5. With increase in the concentration of
the inhibitor, the rate of growth inhibition increased in both series of exper­
iments, but the rate of the inhibition was higher in the series with gibberellin
than in that without gibberellin; in the concentration where about 50 per cent
inhibition of the growth resulted, the accelerating effect of gibberellin was com­
pletely antagonized with inhibitor. The neutral growth inhibitor seems to have
no effect on the germination of rice seeds themselves even in concentration where
about 50 per cent growth inhibition in the wheat leaf section test was caused.

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**Fig. 6.** Effect of the treatment of partially purified solution of the neutral
growth inhibitor with dilute NaOH solution, on the activity of the
neutral growth inhibitor and on the production of gibberellin-like
substance from some impurity contained in the original neutral
growth inhibitor solution.

Upper: treated at 100°C for one hour.
Lower: treated at 45°C for 30 min.
A and C are refractionated neutral fractions after the treatment.
B and D are refractionated acidic fractions after the treatment.
(F) Formation of gibberellin-like substance from neutral fraction.

When the neutral growth inhibitor solution was heated at 100°C in alkalin solution as mentioned above, the inhibiting activity was lost, but gibberellin-like substance made its appearance in the refractionated acidic fraction of such treated solution. If this original solution was treated, however, at 45°C for 30 min. in the same NaOH solution, the activity of the neutral growth inhibitor was retained without showing any changes, while a gibberellin-like substance was found to be formed in the refractionated acidic fraction (Fig. 6). Similar result was obtained in the case when the same NaOH solution of the neutral growth inhibitor was allowed to stand at room temperature of 15–20°C for 2 days. It seems reasonable to assume that the gibberellin-like substance may be derived from some neutral substance other than the neutral growth inhibitor, and that this gibberellin-like substance may be formed in the germinating rice seeds. However the precursor and the mechanism for the formation of gibberellin-like substance in vivo remain to be investigated.

(G) Growth regulator in germinating wheat seed.

In the present investigations wheat leaf sections were used for bioassay of the growth regulator, therefore, by the interpretation of the results of the bioassay, it is clearly important to gain information on the growth regulator in test plant used. Some difference in growth regulator content between germinating wheat seeds and germinating rice seeds being anticipated, as the former seedlings develop more rapidly than the latter, some observations were made on the growth regulator in germinating wheat seeds.

The results are graphed in figure 7. Both neutral growth inhibitor and gibberellin-like substance are contained in larger amounts in wheat section used

![Histograms showing activities of the neutral growth inhibitor and gibberellin-like substance, which were obtained from the wheat leaf sections used for bioassay of the active substances. A: neutral fraction; B: acidic fraction.](image-url)
for bioassay, than in rice leaf sections. In a separate experiment carried out using ether extract of wheat leaf sections, it was indicated that IAA was also contained in high level in the wheat leaf sections. In the endosperms of dry wheat seeds (30 seed grains were used for each extraction), inhibitor was found to be contained both in neutral and acidic fraction, but the content was very small in the germinated seed. Gibberellin-like substance was shown clearly to be present in the acidic fractions of the endosperms of germinated seeds. That the wheat leaf sections which showed active growth and were used for bioassay, contained all: IAA, gibberellin-like substance and neutral growth inhibitor, in pretty high concentrations, suggested that any of them may have some relation to the active elongation of the sections. The remarkable difference between the seedlings of rice and wheat is that the neutral growth inhibitor was never detected in the endosperms of wheat, while it is contained in rather high level in wheat leaves which elongate more rapidly than those of rice.

Discussion

The neutral growth inhibitor was found to be contained in significant amount in the endosperms of germinating rice seeds, however, it was detected also notably in the actively growing zone of rice leaves. The coleoptile of rice seedling germinated under water, developed better than that germinated in air, but the development of roots and leaves was suppressed. At the early stage of rice seed germination, on the other hand, the growth inhibitor was found to be contained in the endosperm, but with the advance of the germination stage it disappeared entirely. When the leaves and roots have commenced to develop as a result of exposing such submerged seedlings to air, the neutral growth inhibitor appeared again in high level in the endosperms. The present writers have found in other experiments the accumulation of neutral growth inhibitor in large amounts in the apices of etiolated young stems of potato or pea plant, but in the subsequent lower regions of the same stems, where the grand growth period was observed, a little or no accumulation was recognized. Libbert (1955) has reported two kinds of growth inhibitors, correlative inhibitor and inhibitor precursor, to be present in the pea seedling. Judging from their properties, it seems highly probable that the rice inhibitor might be similar to the inhibitor precursor. Varga and Köves (1959) have measured the changes of activities of β-inhibitor contained in different organs of bean plants throughout their ontogeny, and concluded that they are contained in the highest level in the most functional organs of middle age. According to Van Steveninck (1959), acidic growth inhibitor is contained in very high activity in the young growing pods of Lupinus plant. From the facts stated above, it is very
reasonable to assume that the neutral growth inhibitor is contained generally in young tissues where the cell division and cell expansion are going on actively. Bentley (1958) suggested also that the inhibitor would be the regulator for the normal growth of plants. Bonde (1953) extracted an active neutral inhibitor from leaves of cocklebur and assayed by Avena coleoptile curvature test; he found that this inhibitor suppressed the plant growth when applied in high concentration, while it accelerated clearly at low concentration; these facts were found to be true even when IAA was added to the test medium in various concentrations. Varga and Kőves (1959) examined the function of inhibitor obtained from bean plants by means of coleoptile section test and proved similar facts.

The present writers could not confirm the stimulating action of the rice neutral growth inhibitor on the growth of wheat leaf section when applied in low concentration, however, this might be interpreted by supposing that this inhibitor is contained already in so high concentration in the test sections, that the exogenous application of the inhibitor caused no growth acceleration.

Bonde (1953) reported that leaves of cocklebur contained neutral growth inhibitor in heavy doses, and van Guttenberg and Zetsche (1956) have found that neutral inhibitor is also contained in large amounts in the leaves of Helianthus annus and Solanum Lycopersicum. According to Sunderland (1960) the cell division and cell expansion of the leaves of Lupinus and Helianthus ran parallel until the leaves attained to 1/2 to 2/3 of their full mature size. This seems to offer the most reasonable explanation for the long continuation of cell division under the influence of growth inhibitor in high concentration in the leaves.

In the present investigations wheat leaf sections obtained from the growing zone where the cell division is proceeding actively, were used for bioassay of the inhibitor. However, it is also conceivable that the wheat coleoptile section test exclusively based on the cell expansion, may show some appreciable difference in sensitivity to the growth inhibitor. An attempt was made to get some light on this problem; it was confirmed that the wheat coleoptile is twice as sensitive to the neutral growth inhibitor as the leaf.

That the neutral growth inhibitor is contained abundantly in the plant tissues where cell division occurs actively, as indicated above, makes it seem very likely that this inhibitor may play some special role in the cell division. In view of these considerations, this growth inhibitor should be referred to as a growth regulator. It remains to be confirmed in future, by finding some proper method of bioassay for this substance, whether it may be really responsible for the cell division itself.
By assuming that the neutral growth inhibitor was produced from some reserve material in the endosperm of rice seeds and was transported to the zone of growth, the accumulation of this inhibitor in the endosperm may be the resultant of a balance between the production and consumption of this inhibitor in tissue.

Summary

1. A remarkable accumulation of neutral growth inhibitor in the endosperm during the germination of hulled rice seeds was ascertained. This inhibitor was extracted from the endosperms with ethyl alcohol, and then fractionated into neutral and acidic fractions with ether. Each of them was developed paper chromatographically with ammoniacal isopropanol mixture, and their activities were bioassayed by wheat leaf section test.

2. The neutral growth inhibitor is contained also in the dry rice seeds; its content increased during the germination. The accumulation of this inhibitor was enhanced by light exposure. Gibberellin-like substance was found to be contained in the acidic fraction of the extract obtained from the endosperms of germinating rice seeds.

3. The neutral growth inhibitor was also contained significantly in the basal parts of leaves where active growth was going on. Gibberellin-like substance was also found to be contained in the acidic fraction obtained from these leaves.

4. The content of the neutral growth inhibitor in rice seeds germinated at low temperature was high in the endosperms. When the rice seed was germinated under water, the development of coleoptiles was good, while that of roots and leaves was much suppressed. In this case the neutral growth inhibitor was never detected in the endosperms. If the submerged seedlings were exposed to air, leaves and roots commenced to develop soon concomitant with the prompt appearance of the neutral growth inhibitor there.

5. The production of the neutral growth inhibitor was also recognized in the endosperms of germinated rough rice seeds, but its activity was lower than that of the hulled rice seeds.

6. Rf values of the neutral growth inhibitor on the paper chromatography developed with several solvents were as follows: isopropanol-ammonia-water (10:1:1), Rf=0.8-1.0; ethanol (80 per cent), Rf=0.8-0.9; ethanol-3N.ammonia (8:2), Rf=0.7-0.9; n.butanol-acetic acid-water (95:5:30), Rf=0.9-1.0; water, Rf=0.5-0.6.

7. Some properties of partially purified aqueous solution of the neutral growth inhibitor were examined. This inhibitor could be stored for about one
month in a refrigerator without showing any change in activity. It can be dialyzed and is adsorbed easily with blood charcoal. It was heat resistant and stable even with boiling in hot dilute HCl solution, while inactivated by heating in dilute NaOH solution. By treatment with dilute hydrogen peroxide solution, the greater part of its activity was caused to be lost. The inhibiting action of this inhibitor on the leaf growth is increased with increase in its concentration; the rate of inhibition was higher in presence of gibberellin. The accelerating effect of gibberellin on the growth was quite antagonized with the inhibitor. The inhibitor showed no effect on the germination of rice seeds themselves.

8. In the neutral fraction obtained from the endosperms of germinating rice seeds, there was found to be contained a certain substance which produced gibberellin-like substance by treatment with dilute NaOH solution.

9. The possible role of the neutral growth inhibitor in the natural development of plants was considered.

Literature


