Comparative Effectiveness of Gibberellins A₁, A₂, A₃ and A₄, with Special Reference to That of A₄*

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The gibberellins, which are plant growth stimulators, were isolated as a crude crystalline mixture from culture filtrates of the pathological fungus *Gibberella fujikuroi* Wr. in 1935. Since that time four gibberellins, i.e., A₁, A₂, A₃ and A₄ (GA₁, GA₂, GA₃ and GA₄) were isolated.

Meanwhile it has been revealed that the gibberellins which were thus isolated as extensive growth stimulators of rice seedlings have many physiological effects such as stimulation of stem elongation and leaf expansion, growth promotion of dwarf mutants, removal of light inhibition in *Avena* first internodes, promotion of flowering, fruit setting, induction of dark germination of light sensitive seeds, etc. The historical background of discoveries and the physiological activities of the gibberellins have been reviewed by Stowe and Yamaki^{1,2)} and Brian³⁾.

The investigations of these physiological effects were performed by using GA₃ (gibberellic acid) or a mixture of GA₁ and GA₃. The next step would be to determine whether or not all of the four gibberellins have these physiological activities, and if active, how the comparative effectiveness is.

Bukovac and Wittwer⁴⁾ investigated the effects of GA₁, GA₂, GA₃, GA₄ and methylesters of GA₁ and GA₃ in inducing the vegetative elongation of epicotyls, flowering of facultative long-day annuals and fruit setting. According to their experiment, all of these gibberellins and the methylesters were effective in the investigated phenomena, and the order of activity was GA₃>GA₁≥GA₄>GA₂>methylester A₁≥methylester A₃. Sumiki⁵⁾ also reported that the effectiveness was in the order of GA₃, GA₁, GA₄ and GA₂ in promoting the elongation of rice seedlings. Phinney and Neely⁶⁾ obtained a similar order of effectiveness by the application of GA₁, GA₂ and GA₃ to the seedlings of dwarf mutant and normal maize.

We previously reported⁷⁾ in brief that all of the four gibberellins were effective in promoting leaf expansion and inducing the dark germination of light sensitive tobacco seeds, and that GA₄ was the most active in comparison with the other gibberellins. The present paper describes the detailed data about that experiment, and also gives the results obtained thereafter.

Materials and Methods

Comparative effectiveness of these gibberellins was studied with the elongation of intact rice seedlings, the expansion of radish leaf disks and etiolated bean leaf disks, and the induction of the germination of light sensitive tobacco seeds in the dark. The effects of GA₃ and of a mixture of GA₁ and GA₃ were already reported all these cases^{5,8,9)} except that of bean leaf disks.

The rice employed was *Oryza sativa* L. "Norin No. 25". The seeds were sown

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in shallow tap water in a large Petri dish and kept at 25–30° for three days until the coleoptiles appeared and became 2–3 mm. in length. The seedlings of uniform size were selected and lots of 12 seedlings each were transferred to small Petri dishes, 5.5 cm. in diameter, containing three layers of filter paper and 10 ml. of Boysen Jensen's culture solution with or without the addition of one of the gibberellins. Two dishes were prepared for each experimental condition. All these dishes were kept in an experimental room under diffuse light at 25–30°, being occasionally supplemented with distilled water. When the seedlings grew to reach the lids of the dishes, the lids were removed. Six days after the transference of the seedlings the length of the second leaf sheath was determined.

The radish used was *Raphanus sativus* L., "Riso-daikon". The experiment was made according to Kuraishi's method¹⁰).

In the case of bean leaf, the experimental method was almost similar to Miller's one¹¹); but 2 per cent sucrose was added to the incubation medium instead of glucose. Sucrose brings about better growth of bean leaf disks than glucose, and unlike glucose no lowering of pH of the medium during incubation was observed¹²).

Seeds of tobacco (*Nicotiana tabacum* L., "Bright Yellow") were obtained at Tateyama, Chiba Prefecture. These seeds ordinarily require exposure to light for germination. Seeds were sown on two layers of filter paper soaked with 0.02 M potassium nitrate solution containing various concentrations of gibberellins and were allowed to germinate for five days at 25° in the dark. Potassium nitrate intensifies the gibberellin-induced germination¹³).

Tested concentrations of GA₁, GA₂ and GA₃ ranged from 10⁻¹² to 10⁻⁴ M, and those of GA₄ from 10⁻¹² to 3×10⁻⁶ M because of its insolubility in concentrations above 3×10⁻⁶ M.

Results

The elongation responses of the second leaf sheath of intact rice seedlings to GA₁, GA₂, GA₃ and GA₄ are indicated in Fig. 1. Growth promotion was obtained over the concentration ranges above 10⁻⁷ M for GA₁ and GA₃, above 6×10⁻⁷ M for GA₄, and above 10⁻⁶ M for GA₂. As seen in the figure, GA₁ and GA₃ were far more effective than GA₂ and GA₄; GA₄ has a little stronger effect than GA₂, and no significant difference in activity was noticed between GA₁ and GA₃. In this case the order of effectiveness was GA₁ = GA₃ ≫ GA₄ > GA₂.

In the case of application to leaf disks, on the other hand, the effectiveness order of the gibberellins was different from that for the leaf sheath elongation. Fig. 2 shows the results of the experiment about the effects of the

Fig. 1. Effects of GA₁, GA₂, GA₃ and GA₄ on the elongation of the second leaf sheath of rice seedling cultured for 6 days at 25–30°. Ordinate shows the length of the second leaf sheath as per cent of that of control (36.0 mm.).

gibberellins on the expansion of radish leaf disks in the light. Among the four gibberellins GA_4 produced the largest acceleration of leaf expansion and its activity was noticed even at as low a concentration as 10^{-11} M. GA_3 was more effective than GA_1 in high concentrations, but at lower concentrations the reverse was the case. GA_2 was the least effective among the four gibberellins.

Growth responses of etiolated bean leaf disks to the gibberellins were similar to those of radish leaf disks. The growth promotion of bean leaf disks was observed over the similar concentration ranges, and the order of effectiveness of the four gibberellins was identical.

Fig. 3 shows the germination rates of tobacco seeds induced by the four gibberellins in the dark. The germination was observed over the concentration ranges above 10^{-7} M, 3×10^{-6} M, and 3×10^{-5} M for GA_4 , GA_1 and GA_3 , and GA_2 , respectively. The concentrations required for 20 per cent germination were ca. 2×10^{-6} M, 9×10^{-5} M, 10^{-4} M and 3×10^{-4} M for GA_4 , GA_1 , GA_3 and GA_2 , respectively.

Fig. 3. Effects of GA_1 , GA_2 , GA_3 and GA_4 on the germination of tobacco seeds in the dark. Germination rates were determined 5 days after sowing. The medium contains 0.02 M potassium nitrate.

Fig. 2. Effects of GA_1 , GA_2 , GA_3 and GA_4 on the expansion of radish leaf disks. Ordinate shows the increase in diameter of treated disk as per cent of that of control disk (0.672 mm.) for 18 hours in the light. Initial average diameter of a disk was 4.33 mm.

It was convincingly demonstrated that GA_4 was the most active and GA_2 was the least active of the four in inducing the germination, too. The activities of GA_1 and GA_3 ranked between those of GA_4 and GA_2 . Though the difference in the activities of GA_1 and GA_3 was small, as seen in Fig. 3, GA_1 produced a little higher percentage of germination in all the repeated experiments. In the experiment in which Boysen Jensen's culture solution was used as the basal medium instead of 0.02 M potassium nitrate, corresponding results were obtained.

Discussion and Conclusion

As seen above, different concentration ranges of each gibberellin were required for the elongation of the second

leaf sheaths of rice seedlings, the expansion of leaf disks of radish and bean, and the germination of tobacco seeds, respectively. The leaf disks responded to lower concentrations of gibberellins, while the tobacco seeds required larger doses of gibberellins than the rice seedlings.

When attention is paid solely to the order of effectiveness of these four gibberellins, we find out that the order differs with the phenomena investigated here. $GA_1 \doteq GA_3 \gg GA_4 > GA_2$ for the growth promotion of the second leaf sheaths of rice seedlings, $GA_4 \gg GA_3 > GA_1 > GA_2$ for the acceleration of the leaf expansion of radish and bean, and $GA_4 \gg GA_1 > GA_3 > GA_2$ for the induction of the dark germination of tobacco seeds were observed. The order of the effectiveness in the rice seedlings generally corresponded to the results obtained by Bukovac and Wittwer⁴⁾, Phinney and Neely⁶⁾, and Sumiki⁵⁾, except that no difference in activity between GA_1 and GA_3 was found. On the other hand, in the case of the leaf expansion and of the dark germination of tobacco seeds, the order of the effectiveness was quite different, especially in the fact that GA_4 was the most active. This is to be noted, since GA_4 had relatively little activity in promoting the elongation of rice seedlings⁵⁾, the epicotyl elongation of bean, the stem elongation and the flowering of lettuce and dill⁴⁾.

Though what causes such different effectiveness of GA_4 can not be mentioned from the present data, the particular characters of GA_4 for permeability and affinity to the probably existing active site in the cell and the special action mechanism for different physiological phenomena may be conceivable as the cause.

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Summary

Comparative effectiveness of gibberellins A_1 , A_2 , A_3 and A_4 (GA_1 , GA_2 , GA_3 and GA_4) in promoting the elongation of rice seedlings, in accelerating the expansion of green radish leaf disks and of etiolated bean leaf disks, and in inducing the dark germination of tobacco seeds, was investigated. These four gibberellins were all active in every investigated phenomenon, but different in comparative activity. In the leaf expansion and the tobacco seed germination, unlike in the elongation of rice seedlings, GA_4 was especially active and new orders of effectiveness such as $GA_4 \gg GA_3 > GA_1 > GA_2$ or $GA_4 \gg GA_1 > GA_3 > GA_2$ were observed. It appears that gibberellins show different effectiveness with different physiological phenomena.

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摘 要

ジベレリン A_1 , A_2 , A_3 , A_4 の生理作用の比較, とくに A_4 の作用について

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ジベレリン $A_1 \cdot A_2 \cdot A_3 \cdot A_4$ の生理作用の強さをつぎの 3 種類の生理現象について比較した.

1. 明所で育てたイネ幼植物の第二葉の葉鞘の伸長生長.
2. 明所で育てたダイコンの第一葉の切片および暗所で育てたウズラ豆第一葉の切片の生長.
3. 発芽に光を必要とするタバコ種子の暗黒における発芽.

イネ葉鞘の伸長促進においては, GA_1 と GA_3 の強さがほとんど等しく, $GA_2 \cdot GA_4$ よりはるかにつよい. GA_4 は GA_2 よりやや強い. すなわち, $GA_1 \approx GA_3 \gg GA_4 > GA_2$ で表わされるような強さの順序を示した. しかし, ダイコンやウズラ豆の葉切片の生長を促進する作用は, GA_4 がもつとも強く, そのつぎに GA_3 , つぎに GA_1 が続き (比較的低濃度では GA_3 より GA_1 の方がわずかに強い作用を示す), GA_2 は最も弱い. すなわち $GA_4 \gg GA_3 > GA_1 > GA_2$. タバコ種子の発芽でも GA_4 がもつとも強い作用を示し, そのつぎに GA_1 , GA_3 が続き GA_2 はもつとも弱い. すなわちその効果の順序は $GA_4 \gg GA_1 > GA_3 \gg GA_2$ で表わされる.

イネの葉鞘の伸長促進における 4 種のジベレリンの強さの順序は, これまで報告されたチジャやヒメウイキョウの茎の伸長や花芽形成におよぼす促進作用の強さの順序と大体一致するが, 葉の生長やタバコ種子の発芽では全く異なっている. 最も注目すべき差異は, イネ葉鞘の生長の場合には, 非常に弱い促進作用しか示さなかつた GA_4 が, 葉の生長やタバコ種子の発芽においては, 他の 3 種のジベレリンにくらべてはるかに強い作用を示すことである.

このように 4 種のジベレリンの生理作用の強さの順位, 特に GA_4 の順位は, ジベレリンによつて影響される生理現象の相違によつて異なることが示された. (東京大学教養学部生物学教室)