Gibberellin Structure-Dependent Interaction between Gibberellins and Deoxygibberellin C in the Growth of Dwarf Maize Seedlings

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ABSTRACT
The effects of 3-deoxygibberellin C (DGC) on the growth-promoting actions of gibberellins A1, A2, A3, A4, A5, A7, A8, A9, A10, A13, A15, A20, and A32 (GA32) as well as 13-deoxygibberellin A3 (deoxy-GA3) were tested with seedlings of gibberellin-deficient dwarf mutants (d2 and d3) of maize (Zea mays L.). It was found that DGC promoted the actions of gibberellins having both C-1 double bond and C-3 axial hydroxyl group, and it inhibited the action of gibberellins having the saturated ring A and lacking the C-3 axial hydroxyl group, whereas it did not affect that of the ones having the hydroxyl group. The presence of C-2 double bond, as in GA20 and deoxy-GA20, diminished the inhibitory action of DGC. The DGC inhibition was alleviated by raising the doses of the relevant GAs, suggesting that it is a competitive inhibition. These results and the finding that the growth of normal maize and rice seedlings are inhibited by DGC indicate that GAs, GA3, GA3, GA3, or other gibberellins having ring A of the same structure are involved in the growth of these plants as active form(s) or as intermediate(s) leading to the active form(s).

Some compounds structurally related to plant hormones show an inhibitory activity against the relevant hormones, whereas these inhibitors per se show a weak or null hormone activity (1). As such compounds for gibberellins, epi-allogibberellic acid (14) and pseudogibberellic A1 (13) have been reported. The inhibitory activities of these gibberellin derivatives have been tested only with GA3,2 and their actions against other gibberellins are not known. In contrast, DGC, an GA20 isomer, has been shown to inhibit the growth of normal rice and maize seedlings and to promote that of the same plants induced by GA3 (8). The peculiarity of the DGC effect led us to test its interaction with various GAs, using GA-deficient dwarf mutants of maize to minimize the possible interference of endogenous GAs. The result of the tests was that DGC exerted inhibitory and promotive actions as well as null action depending on the structures of GAs combined with it, suggesting the usefulness of DGC as a selective inhibitor of the action of biosynthesis of GAs. The result of this study was presented at XI International Botanical Congress held at Seattle, Washington in 1969 (7), and its full description is made in the present paper.

MATERIALS AND METHODS

Chemicals. 3-Deoxygibberellin C (DGC) was chemically synthesized from GA3 (15) and was kindly supplied by Drs. K. Mori and A. Murofushi, Department of Agricultural Chemistry, University of Tokyo, Bunkyo-ku, Tokyo. This gibberellin derivative has C-D rings which were rearranged in a Wagner-Meerwein type, and the rest of its structure is the same as that of GAs and GA20 (Fig. 1). The sample of the derivative was rigorously purified so as to show no GA activity except the DGC zone on a thin-layer chromatogram. GAs, GA3, GA3, GA4, GA7, 13-deoxy-GA20, GA3, GA3, GA19, and GA20 were kindly supplied by Drs. N. Takahashi and A. Murofushi, Department of Agricultural Chemistry, University of Tokyo, Tokyo; GA3, GA3, GA3, and GA19, by Drs. J. MacMillan and B. E. Cross, then Akers Research Laboratories, Imperial Chemical Industries, Herts, England; and GA18 and GA23, by Dr. K. Koshimizu, Department of Agricultural Chemistry, Kyoto University, Kyoto. All these gibberellins were obtained from natural sources, except for 13-deoxy-GA3, which was synthesized by Dr. Murofushi.

Plants and Treatments. Seeds of normal maize (Zea mays L., cv Koshuromokoshi) were purchased from Yamato Seed Co., Tokyo, and those of dwarf mutants of maize, d2 and d3, were kindly supplied by Dr. B. O. Phinney, Department of Botany, University of California, Los Angeles. Seeds were germinated in vermiculite and grown for 8 or 9 d at 23°C under continuous lighting at a photon fluence rate of about 40 μmol/m² from white fluorescent lights in a growth chamber. When the first and second leaves appeared, 0.1 ml in total of test solutions were applied to the folded base of the second leaf. Plants were grown under the same conditions for further 7 or 8 d, and the lengths of the first and second leaf sheaths were determined according to Phinney and West (18), unless otherwise stated.

DGC and GAs were individually dissolved in 25% (v/v) aqueous acetone containing Tween 20 (polyoxyethylene sorbitan monolaurate) at 0.25 mg/ml, and 0.05 ml each of DGC and GA solutions were consecutively applied. When a single compound was applied, an equal amount of the plain acetone-Tween solution was supplemented. For control of no treatment, 0.1 ml of the plain acetone-Tween solution was applied.

RESULTS AND DISCUSSION

Effective Dosage Level and Time Course of the Action of DGC. DGC inhibited the growth of normal maize seedlings (Fig. 2).
2. When applied in combination with GA3, however, it promoted the GA3-induced growth of dwarf maize seedlings (Fig. 3), confirming our previous results obtained with normal maize and rice seedlings (8). The effective dosage of DGC ranged from 10 to 100 µg/plant for both actions, and the optimum dose for promotion of the GA3-induced growth was 30 µg/plant. Since, although the optimum dose may not be identical for the interaction with other GAs, application of DGC at 10 to 30 µg/plant gave a sufficient inhibitory effect to the growth of normal maize seedlings considered to be due to endogenous GA(s), this dosage level was used in subsequent experiments on DGC-GA interaction.

Time-course studies showed that the appearance of the DGC action accompanied that of the GA action. This was the case with the interactions with GA3 and GA7 (promotion) as well as with GA8 and GA10 (inhibition, see below). The case of GA3 is shown in Figure 4.

![Figure 2](image2.png)

**Fig. 2.** Effect of DGC doses on growth of normal maize seedlings (cv Koshutomorokoshi). Leaf sheath lengths were determined 8 d after treatment.

![Figure 3](image3.png)

**Fig. 3.** Effect of DGC doses on GA3-induced growth of dwarf maize (d2) seedlings. GA3, 0.3 µg/plant. Leaf sheath lengths were determined 7 d after treatment.

![Figure 4](image4.png)

**Fig. 4.** Time-course of the promotive effect of DGC on GA3-induced growth of maize (d2) seedlings. At d 0, 0.3 µg GA3 alone (—) or in combination with 30 µg DGC (— — — —) was applied to each seedling and the resulting growths of the first and the second leaf sheaths were followed. Eight seedlings for each treatment; small bars indicate twice SE.

![Figure 5](image5.png)

**Fig. 5.** Interactions between DGC and various C19 GAs. Shaded and nonshaded bars represent the sums of first and second leaf sheath lengths treated with or without 30 µg DGC/plant, and a horizontal broken line in each bar, the value of control which was not given GA. Vertical small lines at the top and on the horizontal broken line of each bar represent twice a SE, respectively. Dwarf maize d2 was used. The lengths of leaf sheaths were measured 7 d after treatment.
Differential Effects of DGC on the Actions of Various GAs.

The effects of DGC on the growth-promoting actions of GAs in dwarf maize seedlings varied with the GAs combined (Figs. 5 and 6). The actions of GA\textsubscript{2}, GA\textsubscript{3}, and GA\textsubscript{4} were promoted by DGC, whereas those of GA\textsubscript{5}, GA\textsubscript{19}, and GA\textsubscript{20} were inhibited. The inhibition for GA\textsubscript{19} was particularly striking and almost complete. The actions of GA\textsubscript{1}, GA\textsubscript{2}, GA\textsubscript{4}, GA\textsubscript{6}, GA\textsubscript{13}, GA\textsubscript{18} and GA\textsubscript{23} were not affected by DGC. Although Figures 5 and 6 show the results obtained with d\textsubscript{5} except for GA\textsubscript{23}, tests with d\textsubscript{5} also gave the same results (cf. Fig. 7).

The action of GA\textsubscript{2} at relatively low doses was suppressed slightly in every experiment with d\textsubscript{5}, but not in d\textsubscript{2} (Fig. 5, Table I, compare 90% of treatment 4 with 113% and 114% of treatments 8 and 10 in d\textsubscript{2} and with 128% of treatment 5 in d\textsubscript{5}). The action of 13-deoxy-GA\textsubscript{3} was not affected by DGC in either d\textsubscript{2} or d\textsubscript{5}.

It is readily seen that the promotive action of DGC is associated with the C-1 double bond and the C-3 axial OH group of GAs, and that the inhibitory action of DGC requires the absence of the C-3 axial OH group in the saturated ring A of GAs. If one admits the following speculation, the requirement of ring A being saturated for the DGC inhibition to occur would have to be deleted. The speculation is: GA\textsubscript{3} and deoxy-GA\textsubscript{3} may be rapidly hydrated in tissues, respectively, to GA\textsubscript{4} and GA\textsubscript{5}. The conversion of GA\textsubscript{1}, in d\textsubscript{2}, however, may be slow. Thus, GA\textsubscript{1} in d\textsubscript{5} and deoxy-GA\textsubscript{3} in d\textsubscript{2}, as well as in d\textsubscript{2} may escape possible DGC inhibition.

Mode of Interaction of DGC and GAs. The inhibitory effects of DGC on the actions of GA\textsubscript{5}, GA\textsubscript{19}, and GA\textsubscript{20} were alleviated by raising the dose of relevant GAs (Fig. 7, b–d). The data were based on nonlinear growths of maize leaf sheaths, and Line-weaver-Burk plots were not useful. But the results in this figure suggest that DGC might interact competitively with these GAs at the action site(s). When applied alone, DGC slightly promoted the growth of d\textsubscript{3} as well as d\textsubscript{2} (Figs. 5–7 and Table I). This effect also corresponds to one of the properties of true anti-gibberellins (1). DGC did not cause either toxicity or abnormality in treated seedlings.

On the other hand, DGC may block the possible conversion of GA\textsubscript{5}, GA\textsubscript{19}, and GA\textsubscript{20} to more active or truly functional forms of GAs as kaurene analogs and a GA analog inhibit the GA biosynthesis (2, 6). In pea GA\textsubscript{5} (19, 22), GA\textsubscript{9}-open-lactone and GA\textsubscript{19} (12) have been shown to be converted to GA\textsubscript{20}, and GA\textsubscript{19}, in turn, to GA\textsubscript{1} (10, 11, 21) or much less active GA\textsubscript{20} (4, 5, 12, 19, 22, 25) and then to an inactive GA\textsubscript{29} catabolite (23). In maize also, a conversion of GA\textsubscript{20} to GA\textsubscript{19} has been shown (24). Thus, this action mechanism of DGC may be possible.

The relative effectiveness of the promotive effect of DGC on GA\textsubscript{2} and GA\textsubscript{3} decreased as the dose of the GAs increased (data not shown). But this result may give no clue, since it is natural that no further increase of growth could be expected if the GA response capacity of plants be saturated by these GAs. Conceivable mechanisms of the promotive action of DGC are (a) to promote the conversion of these GAs to more active forms, if any; (b) to inhibit the destruction of these GAs, and (c) to increase the fitness of GA receptor proteins to these GAs, e.g. by allosteric effect. GA\textsubscript{2} and GA\textsubscript{3} are among the most active GAs in most bioassay systems including the dwarf maize seedling system (3); therefore, the possibility (a) seems unlikely.

As such GA synergists, isourea and triazinone derivatives have
been reported (16, 17). But their action mechanism is not known, nor seems to be the same as that of DGC, since the action of the N-compounds is observable only in rice, but not in maize plant (16), and they have no GA-specificity (20) as observed with DGC.

DGC inhibited the growth of normal maize and rice seedlings (8) (Fig. 1). This finding suggests that GAs such as GA_3, GA_19 and GA_20 are involved as either active forms or biosynthetic intermediates leading to an active form(s) in the growth of these normal plants. It is noteworthy that the major GAs in a normal maize plant were identified to be GA_17, GA_19, GA_20, GA_44, and GA_53 (9), all of which had the structures to be inhibited by DGC according to the rule stated above.

In conclusion, DGC inhibits the growth-promoting action of giberellins such as GA_3, GA_19, and GA_20, the ring A of which is saturated and lacks a C-3 OH group. The mechanism of the inhibition is likely to be a competitive one at the action site of these GAs, or at the sites of their conversion to active form(s). DGC promotes the action of GAs, such as GA_3 and GA_19, which have a C-1 double bond and a C-3 axial OH group. The mechanism of the promotion is open to question. The GA structure-dependent actions of DGC may serve as a tool to discriminate the types of GAs involved as active forms or as biosynthetic intermediates leading to active forms of GAs.

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**LITERATURE CITED**


18. Phinney BO, CA West 1967 Metabolism of tritiated giberellin

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**Table 1. Effects of DGC on the Actions of GAs and Deoxy-GAs in Dwarf Maize d_2 and d_3 Seedlings**

Values are means of second leaf sheath lengths and SE (n). Percentages are relative to the respective controls without DGC.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>d_2</th>
<th>d_3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 670426</td>
<td>No. 670511</td>
<td>No. 670621</td>
</tr>
<tr>
<td>No. 70426</td>
<td></td>
<td>mm</td>
</tr>
<tr>
<td>1. None</td>
<td>31.7 ± 1.7</td>
<td>100</td>
</tr>
<tr>
<td>2. 30 µg DGC</td>
<td>38.4 ± 1.6</td>
<td>121</td>
</tr>
<tr>
<td>3. 0.16 µg GA_3</td>
<td>69.1 ± 4.5</td>
<td>100</td>
</tr>
<tr>
<td>4. 0.16 µg GA_3 + 30 µg DGC</td>
<td>62.6 ± 3.4</td>
<td>90</td>
</tr>
<tr>
<td>5. 1.6 µg GA_3</td>
<td>79.4 ± 3.5</td>
<td>100</td>
</tr>
<tr>
<td>6. 1.6 µg GA_3 + 30 µg DGC</td>
<td>77.7 ± 4.5</td>
<td>97</td>
</tr>
<tr>
<td>7. 0.16 µg deoxy-GA_3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. 0.16 µg deoxy-GA_3 + 25 µg DGC</td>
<td>68.1 ± 2.6</td>
<td>113</td>
</tr>
<tr>
<td>9. 1.6 µg deoxy-GA_3</td>
<td>68.6 ± 4.7</td>
<td>100</td>
</tr>
<tr>
<td>10. 1.6 µg deoxy-GA_3 + 25 µg DGC</td>
<td>78.8 ± 2.4</td>
<td>114</td>
</tr>
</tbody>
</table>
20. SATOH Y, H YAMANE, N TAKAHASHI, M OGAWA 1981 Synergistic effect of
isourea and triazinone derivatives on activity of various gibberellins. Plant
Cell Physiol 22: 1603–1606
21. SPONSEL VM 1986 Gibberellins in dark- and red-light-grown shoots of dwarf
and tall cultivars of Pisum sativum: the quantification, metabolism and
biological activity of gibberellins in Progress No. 9 and Alaska. Planta 168:
119–129
22. SPONSEL VM (née FRYDMAN), J MACMILLAN 1977 Further studies on the
metabolism of gibberellins (GAs) A_9, A_20 and A_29 in immature seeds of
 gibberellin-catabolite in maturing seeds of Pisum sativum cv. Progress No.
9, Planta 150: 46–52
24. SPRAY C, BO PHINNEY, P GASKIN, SJ GILMOUR, J MACMILLAN 1984 Internode
length in Zea mays L. The dwarf-1 mutation controls the 3β-hydroxylation
of gibberellin A_20 to gibberellin A_1. Planta 160: 464–468
25. YOKOTA T, N MUROFUSHI, N TAKAHASHI, M KATSUMI 1971 Biological activi-
ties of gibberellins and their glucosides in Pharbitis nil. Phytochemistry 10,
2943–2949