A Survey of Gibberellins in Shoots of Angiosperms by Rice Seedling Test

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Received June 19, 1970

Abstract

A survey of gibberellins in shoots (stem and leaves) from 86 species belonging to 82 families of monocotyledons and dicotyledons has been made. The extracts, fractionated by ethyl acetate, were purified by thin-layer chromatography and the eluates were applied to seedlings of the Tan-ginbozu and Waito-C dwarf varieties of rice by the microdrop method. With the Tan-ginbozu dwarf assay, zones having gibberellin activities were detected in the chromatograms of the extracts from 75 species. But Waito-C dwarf seedlings were almost insensitive to the same eluates except that from *Phytolacca americana*. From the specificity of the two dwarfs in response to gibberellins with different structures, it seems to be possible that gibberellins which have no β -hydroxyl group at the C-2 position in the A ring of gibbane might be widely distributed in shoots of monocotyledons and dicotyledons. *Aspidistra elatior* and *Aucuba japonica* had considerably higher concentrations of such gibberellins, and their contents were equivalent to 584 and 797 μg gibberellin A_3 per 100 g fresh weight of shoots, respectively.

In previous papers¹⁻³, I presented a new rice seedling test for gibberellins. In this method, gibberellin in a solution is applied to the coleoptiles of young seedlings in a microdrop. This bioassay has an advantage in detecting gibberellins from plant extracts, because rice plants are less susceptible to co-existing inhibitory substances in this method than other methods in which plant extracts are directly added to the rice growing medium. It also has an advantage that different dwarf varieties give marked differential responses to certain gibberellins as do dwarf mutants of maize⁴.

The present paper describes the results of a general survey of gibberellins in shoots of monocotyledons and dicotyledons by the application of this method.

Materials and Methods

Plant material

Plant materials were collected near Tokyo during the period from June to October in 1968 and 1969. They were 86 species each belonging to different genera covering 82 families as listed in Tables 1 and 2. After sampling, the shoots were weighed and either extracted immediately or stored at -15° until required for extraction. About 200 g fresh weight of shoots (stem and leaves) were used for gibberellin extraction. For comparison, moist mycelia (3 g) of *Fusarium moniliforme* (strain No. 4) were also tested. The fungus was grown for 10 days in 300 ml flasks

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each containing 100 ml of Czapek's solution at 30° without shaking. Sumiki and his co-workers⁵⁻⁶⁾ had isolated GA₁*, GA₂, GA₃, and GA₄ from cultures of the same strain.

Extraction and fractionation

Each material was ground in a blender with 5 times its fresh weight of 70% aqueous acetone, and this blended mixture was allowed to stand overnight at room temperature before filtration. After the acetone was evaporated, the resulting aqueous solution was adjusted to pH 2.5 with phosphoric acid and extracted three times with ethyl acetate. The combined ethyl acetate phase was extracted three times with 1 M potassium phosphate buffer of pH 7.0. The buffer phase was then adjusted to pH 2.5 with phosphoric acid and again extracted three times with ethyl acetate. The acidic ethyl acetate fraction was dried over anhydrous sodium sulfate and evaporated to dryness.

Each evaporated extract was taken up in a small volume of 70% acetone and applied as a band to the starting line of a 20×20 cm silica gel (G) thin-layer plate with 0.75 mm thickness. The plate was developed in isopropyl ether/acetic acid (95:5) by the ascending method. The plate was dried when the solvent front reached 10 cm from the starting line and was divided into 10 equal zones between the starting line and the solvent front (the first zone was further subdivided into two zones). Each zone was scraped off into a small beaker (10 ml) and eluted three times with 2 ml of 50% acetone. The eluates were taken up into a small glass bottle (1.8×3.5 cm), evaporated to dryness using a hair dryer, and dissolved again in 0.1 ml of 50% acetone for bioassay.

Reference chromatograms were sprayed with 5% sulfuric acid in ethanol and heated for 10 min at 120°. GA₁ and GA₃ were detected under a UV lamp as fluorescent spots at Rf 0.12.

Bioassay

The bioassay was carried out by the method of microdrop application¹⁾. Two dwarf varieties of rice, Tan-ginbozu and Wai-to-C, were used. Germinated seeds with 2 mm coleoptiles were planted in a group of five onto the surface of 0.9 per cent water-agar in a cylindrical bottle of 2.8 cm in diameter and 6 cm in depth. The bottles were placed in a tall Petri dish of about 18 cm in diameter and 12 cm in depth, and then they were incubated for 45 hours in a cabinet under continuous fluorescent light (about 5,000 lux) at 32°. When the second leaf emerged from the first leaf, eluates were applied in a single 1 μ l droplet onto the surface of each coleoptile with a micropipette. The treated seedlings were then grown for further 3 days under the same condition as before the treatment. The length of the second leaf sheath was then measured with a ruler. The results are shown in the form of histograms each indicating the average of 5 test plants. Assay responses were not regarded as positive unless they were greater than 10 per cent over the controls.

Results and Discussion

Response curves of two dwarf varieties for GA₃ are shown in Fig. 1. Both dwarf varieties responded almost equally to GA₃.

* Abbreviation: GA₁ stands for gibberellin A₁, for example.

Fig. 1. Responses of the second leaf sheath of rice seedlings to GA_3 .

●, Tan-ginbozu dwarf variety;
○, Waito-C dwarf variety.

The extract of *Fusarium moniliforme*, which has already been known to produce many kinds of gibberellin, gave almost the same pattern of histograms in the two dwarf varieties of rice (Fig. 2).

The histograms of gibberellin activities in the extracts from higher plants are summarized in Figs. 3 and 4. Fig. 3 is for monocotyledons and Fig. 4 for dicotyledons. The upper and lower histograms for each plant represent the results in Tan-ginbozu and Waito-C assays, respectively. Gibberellins were detected in 75 species belonging to 71 families among 86 plants when assayed by using Tan-ginbozu seedlings. But no clear gibberellin activity could be detected in the histograms when Waito-C seedlings were used except for *Phytolacca americana* (Fig. 4-I).

The growth-promoting zone which almost coincided with the position of an authentic sample of GA_3 was found in the upper histograms of many plant species examined. This activity was not thought to be due to GA_3 , since the lower histograms had no clear growth-promoting zone except that of *Phytolacca americana*. GA_{19} , isolated from young bamboo shoots by Murofushi *et al.*¹⁹, has the R_f value very similar to that of GA_3 in the solvent system used in the present survey and shows high activity on the Tan-ginbozu dwarf assay, but almost no activity on the Waito-C dwarf assay²⁰. The growth-promoting activity in this zone may probably be attributed to GA_{19} rather than GA_3 .

Fig. 2. Histograms showing gibberellin activities of the acidic ethyl acetate fraction from mycelia of *Fusarium moniliforme*.

The fraction was purified by ascending thin-layer chromatography in isopropyl ether/acetic acid (95:5). Gibberellin activities in the eluates from the chromatograms were determined by using two dwarf varieties of rice, Tan-ginbozu and Waito-C. These are mentioned as Tan-ginbozu assay and Waito-C assay, respectively. Dotted horizontal lines indicate the growth of control. The standard position of GA_3 on the chromatogram was determined by a blue fluorescent spot under ultraviolet light after spraying with sulfuric acid. This legend also holds for Figs. 3 and 4.

Fig. 3-I. Histograms showing gibberellin activities of the acidic ethyl acetate fraction from shoots of monocotyledons.
A, Tan-ginbozu assay; B, Waito-C assay.

Fig. 3-II. (Continued)

A zone with considerable growth-promoting activity was also observed near the solvent front in the histograms of several plant species, for example, *Aspidistra elatior*, *Rhodea japonica* (Fig. 3-I), and *Zephyranthes candida* (Fig. 3-II). This active zone may represent a non-polar gibberellin since its R_{GA_3} value of about 8 is higher than that of unhydroxylated GA₉ ($R_{GA_3}=6.8^{(8)}$).

In a previous paper²⁾ I reported that gibberellins such as GA₉, GA₁₉ and GA₂₀ which have no β -hydroxyl group at the C-2 position in the A ring of gibbane stimulate leaf-sheath elongation of Tan-ginbozu dwarf seedlings but they are slightly active on Waito-C dwarf seedlings. For example, GA₂₀ (a gibberellin which has been isolated from *Pharbitis* seeds by Murofushi *et al.*⁹⁾ and pea seeds by Komoda *et al.*¹⁰⁾) is 90 times more effective in promoting leaf-sheath elongation of Tan-ginbozu dwarf seedlings than Waito-C dwarf seedlings²⁾. However, both dwarf

Fig. 4-I. Histograms showing gibberellin activities of the acidic ethyl acetate fraction from shoots of dicotyledons.

A, Tan-ginbozu assay; B, Waito-C assay.

Fig. 4-II. (Continued)

Fig. 4-III. (Continued)

Fig. 4-IV. (Continued)

Family	Plant name	Date	Gibberellin : m μ g GA ₃ equiv. per 100 g fr. wt.
Typhaceae	<i>Typha latifolia</i> ガマ	Jul.	42
Alismataceae	<i>Alisma canaliculatum</i> ヘラオモダカ	Jul.	0
Gramineae	<i>Miscanthus sinensis</i> ススキ	Jul.	36
	<i>Phyllostachys bambusoides</i> マダケ	Jul.	25
Cyperaceae	<i>Cyperus microiria</i> カヤツリグサ	Jul.	103
Araceae	<i>Colocasia antiquorum</i> サトイモ	Jul.	4
Lemnaceae	<i>Spirodela polyrrhiza</i> ウキクサ	Jul.	5
Bromeliaceae	<i>Vriesia carinata</i> インコアナナス	Oct.	103
Commelinaceae	<i>Tradescantia fluminensis</i> シラフハカタツユクサ	Jul.	41
Pontederiaceae	<i>Eichhornia crassipes</i> ホテイアオイ	Aug.	43
Juncaceae	<i>Juncus effusus</i> イ	Jul.	40
Liliaceae	<i>Aspidistra elatior</i> ハラン	Jul.	584
	<i>Rhodea japonica</i> オモト	Jul.	160
	<i>Ophiopogon ohwii</i> ナガバジャノヒゲ	Jul.	13
	<i>Liriope graminifolia</i> フイリヤブラン	Jul.	61
Amaryllidaceae	<i>Zephyranthes candida</i> タマスダレ	Jul.	51
Dioscoreaceae	<i>Dioscorea batatas</i> ナガイモ	Jul.	5
Iridaceae	<i>Iris germanica</i> ドイツアヤメ	Jul.	5
Musaceae	<i>Musa basjoo</i> バシヨウ	Jul.	34
Zingiberaceae	<i>Zingiber mioga</i> ミヨウガ	Jun.	35
Cannaceae	<i>Canna generalis</i> ハナカンナ	Jul.	105
Orchidaceae	<i>Spiranthes sinensis</i> ネジバナ	Jul.	7

Table 2. The gibberellin content of shoots of dicotyledons.

Family	Plant name	Date	Gibberellin : m μ g GA ₃ equiv. per 100 g fr. wt.
Saururaceae	<i>Houttuynia cordata</i> ドクダミ	Aug.	0
Salicaceae	<i>Salix babylonica</i> シダレヤナギ	Sep.	19
Juglandaceae	<i>Juglans sieboldiana</i> クルミ	Aug.	8
Fagaceae	<i>Castanea crenata</i> クリ	Sep.	0
Urticaceae	<i>Pilea hamaoi</i> ミズ	Sep.	6
Polygonaceae	<i>Polygonum thunbergii</i> ミゾソバ	Jul.	134
Chenopodiaceae	<i>Chenopodium ambrosioides</i> ケアリタソウ	Sep.	67
Amaranthaceae	<i>Amaranthus retroflexus</i> アオビユ	Aug.	61
Nyctaginaceae	<i>Mirabilis jalapa</i> オシロイバナ	Aug.	25
Phytolaccaceae	<i>Phytolacca americana</i> アメリカヤマゴボウ	Jul.	125
Molluginaceae	<i>Mollugo stricta</i> ザクロ	Aug.	5
Aizoaceae	<i>Aptenia cordifolia</i> ハナズルソウ	Aug.	39
Portulacaceae	<i>Portulaca oleracea</i> スペリヒユ	Aug.	30
Caryophyllaceae	<i>Stellaria aquatica</i> ウシハコベ	Sep.	60
Ranunculaceae	<i>Aconitum chinense</i> トリカブト	Sep.	0
Lardizabalaceae	<i>Stauntonia hexaphylla</i> ムベ	Sep.	17
Magnoliaceae	<i>Magnolia grndiflora</i> タイサンボク	Sep.	0
Papaveraceae	<i>Macleya cordata</i> タケニグサ	Sep.	0
Capparidaceae	<i>Cleome spinosa</i> セイヨウフウチヨウソウ	Jul.	50
Cruciferae	<i>Brassica pekinensis</i> ハクサイ	Sep.	58
Crassulaceae	<i>Graptopetalum paraguayensis</i> オボロツキ	Sep.	178
Saxifragaceae	<i>Hydrangea macrophylla</i> アジサイ	Sep.	57
Platanaceae	<i>Platanus orientalis</i> スズカケノキ	Sep.	0
Rosaceae	<i>Pyrus serotina</i> ナシ	Aug.	29
Leguminosae	<i>Arachis hypogaea</i> ナンキンマメ	Jul.	55
Geraniaceae	<i>Geranium nepalense</i> ゲンノシヨウコ	Sep.	0
Oxalidaceae	<i>Oxalis bowieana</i> ハナカタバミ	Oct.	10
Tropaeolaceae	<i>Tropaeolum majus</i> ノウゼンハレン	Oct.	8
Rutaceae	<i>Citrus unshiu</i> ウンシュウミカン	Aug.	30
Meliaceae	<i>Melia azedarach</i> センダン	Sep.	7
Euphorbiaceae	<i>Mallotus japonicus</i> アカメガシワ	Aug.	13
Celastraceae	<i>Euonymus japonica</i> マサキ	Sep.	44
Aceraceae	<i>Acer palmatum</i> タカオカエデ	Jun.	5
Balsaminaceae	<i>Impatiens balsamina</i> ホウセンカ	Aug.	6
Vitaceae	<i>Vitis vinifera</i> ブドウ	Jun.	6
Sterculiaceae	<i>Firmiana simplex</i> アオギリ	Sep.	20
Violaceae	<i>Viola tricolor</i> パンジー	Jul.	100
Theaceae	<i>Thea sinensis</i> チヤ	Sep.	0
Begoniaceae	<i>Begonia semperflorens</i> シキザキベゴニア	Oct.	37
Cactaceae	<i>Brasilopuntia brasiliensis</i> ハウチワサボテン	Sep.	24
Lythraceae	<i>Rotala indica</i> キカシグサ	Sep.	177
Punicaceae	<i>Punica granatum</i> ザクロ	Jul.	10

Table 2. (Continued)

Family	Plant name	Date	Gibberellin : m μ g GA ₃ equiv. per 100 g fr. wt.
Oenotheraceae	<i>Oenothera lamarckiana</i> オオマツヨイグサ	Jul.	4
Araliaceae	<i>Tetrapanax papyriferum</i> カミヤツデ	Sep.	29
Umbelliferae	<i>Daucus carota</i> ニンジン	Sep.	330
Cornaceae	<i>Aucuba japonica</i> アオキ	Sep.	797
Ericaceae	<i>Rhododendron indicum</i> サツキツツジ	Sep.	285
Primulaceae	<i>Primula obconica</i> トキワザクラ	Oct.	3
Oleaceae	<i>Ligustrum japonicum</i> ネズミモチ	Aug.	0
Gentianaceae	<i>Gentiana makinoi</i> オヤマリンドウ	Sep.	3
Apocynaceae	<i>Nerium indicum</i> キョウチクトウ	Aug.	8
Convolvulaceae	<i>Ipomoea batatas</i> サツマイモ	Aug.	295
Polemoniaceae	<i>Phlox paniculata</i> クサキョウチクトウ	Jul.	13
Verbenaceae	<i>Clerodendron trichotomum</i> クサギ	Aug.	20
Labiatae	<i>Isodon japonicus</i> ヒキオコシ	Jun.	5
Solanaceae	<i>Lycium chinense</i> クコ	Aug.	3
Boraginaceae	<i>Symphytum officinale</i> ヒレハリソウ	Sep.	3
Bignoniaceae	<i>Paulownia tomentosa</i> キリ	Jul.	28
Gesneriaceae	<i>Sinningia speciosa</i> オオイワギリソウ	Sep.	60
Rubiaceae	<i>Rubia cordifolia</i> アカネ	Jun.	17
Dipsacaceae	<i>Scabiosa japonica</i> マツムシソウ	Aug.	20
Caprifoliaceae	<i>Viburnum awabuki</i> サンゴジュ	Aug.	0
Cucurbitaceae	<i>Luffa cylindrica</i> ヘチマ	Aug.	65
Compositae	<i>Helianthus tuberosus</i> キクイモ	Jul.	39

varieties respond equally well to GA₃ as shown in Fig. 1. These facts and present results suggest that most of the gibberellins found in the chromatograms of the extracts from shoots do not possess the C-2 β -hydroxyl group. In other words gibberellins such as GA₁, GA₃ and GA₄, which are main gibberellins in *Fusarium moniliforme*, may not be predominant in the extracts from shoots.

There are several reports¹¹⁻¹⁶⁾ which attempted to demonstrate the presence of GA₁ and/or GA₃ in shoots by thin-layer chromatographic comparison with the authentic samples of the gibberellins. The isolation of GA₁ from water sprouts of citrus trees was reported by Kawarada and Sumiki¹⁷⁾. In the present survey Waito-C dwarf seedlings did not respond to the extract of citrus shoots while Tan-ginbozu dwarf seedlings responded well (Fig. 4-II). This means that GA₁ was absent or present only in a trace amount in citrus shoots. The discrepancy will be the problem of future research.

The approximate concentrations of gibberellins present in various plants were expressed as GA₃ equivalents per 100 g fresh weight of tissue (Tables 1 and 2). The amount was calculated by comparing the responses of Tan-ginbozu dwarf seedlings to the eluates (Figs. 3 and 4) with those to standard solutions of GA₃ (Fig. 1). In considering the estimates it should be noted that the activity of other gibberellins in the bioassay differs from that of GA₃ as already mentioned. The shoots of *Aspidistra elatior* and *Aucuba japonica* contained considerably higher concentrations of gibber-

ellins as compared with other plants and gave values equivalent to 600–800 μg GA_3 per 100 g fresh weight. Even in these plants the gibberellin content was less than 1/1,000 of that of the mycelium of *Fusarium moniliforme*. No activity could be found on the chromatograms of the extracts from 11 plant species. This does not necessarily imply the absence of gibberellin in the shoots of these plants. The presence of a much higher concentration of growth inhibitors in the extract cannot be excluded. In such a case more thorough purification of large amounts of plant extracts is necessary.

Acknowledgement

This work was supported in part by a Research Grant from the Ministry of Education of Japan (6011).

I thank Mr. K. Ikuma for his excellent technical assistance.

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