*EVIDENCE FOR "GIBBERELLIN-LIKE" SUBSTANCES FROM FLOWERING PLANTS**

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Gibberellins are compounds that produce a wide variety of growth responses in flowering plants. Among these responses are stimulation of shoot growth for many plant species,[1] root elongation in maize,[2] induction of bolting and flowering in some biennial and long-day plants,[3–5] resumption of normal growth in certain genetically dwarfed genotypes of maize,[6] growth stimulation in dwarf strains of peas and beans,[7] and the overcoming of physiological dwarfing of apple seedlings.[8] Gibberellins also stimulate parthenocarpy in tomatoes,[9] promote the germination of light-requiring lettuce seed in the dark,[10] and reverse the light inhibition of pea stem growth.[11]

For the interpretation of these and other effects, it is important to know whether gibberellins or "gibberellin-like" substances occur naturally in flowering plants. The three known gibberellins, gibberellic acid (gibberellin A_3), $C_{19}H_{22}O_6$; gibberellin A (gibberellin A_1), $C_{19}H_{24}O_6$; and gibberellin A_2, $C_{19}H_{26}O_6$, are products of certain strains of the imperfect stage of the fungus *Gibberella fujikuroi* (Saw.) Wr.[12–16] No other organism has been found to contain these compounds.

The present paper will report evidence for the occurrence of "gibberellin-like" substances from species of several different families of flowering plants.[17] These substances produce the same growth response in maize dwarfs as do the known gibberellins. R_f values for these substances will also be reported and compared with those for the known gibberellins.

MATERIALS AND METHODS

Ten genetically different dwarf mutants of *Zea mays* L. were used in this study, Nine of them are simple recessives, while one is a simple dominant. Gibberellic acid, gibberellin A_1, and gibberellin A_2 produce normal growth for the five mutants *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, *dwarf-2*, and *dwarf-3*, the qualitative response to each of the three gibberellins being similar in the seedling stages. Four other mutants, *dwarf-(4963)*, *dwarf-(8043)*, *nana-1*, and *nana-2*, show only a very slight response in the seedling stages and little response or slight inhibition in later stages of growth. The other mutant, *dominant dwarf*, gives no response to any of the three gibberellins.

All ten mutants were used to compare the biological specificity of response of the known gibberellins with the "gibberellin-like" substances obtained from flowering plants (Angiosperms). The mutant *dwarf-1* was used for routine bioassays because of its specificity, sensitivity, and rapidity of response. This mutant does not respond in the bioassay to indole-3-acetic acid, indoleacetonitrile, indoleacetic acid ethyl ester, naphthaleneacetic acid, naphthoxyacetic acid, 2,4-dinitrophenoxyacetic acid, kinetin, leucoanthocyanins from *Aesculus* endosperm and *Arachis* seed, casein hydrolyzate, yeast extract, malt extract, or coconut milk. The mutant gives a positive response to 0.001 μg. of gibberellic acid per plant and a detectable response within 8 to 12 hours following application of 0.1 μg. of gibberellic acid per plant.

Material for bioassay was applied as a small drop to the first unfolding seedling leaf at the time of its emergence from the coleoptile. For semiquantitative work, the volume added for assay was arbitrarily standardized at a single application of 0.1 ml. per plant. For qualitative tests, larger volumes were often used, applications being repeated over a period of 24 hours. All assays were run in triplicate. Following treatment, the seedling was allowed to grow until the first leaf sheath had reached its final length, a period of from three to five days following application. Record of response was obtained by measuring to the nearest millimeter the length of this leaf sheath from the ligule to the coleoptilar node. Increases in length ranged from 0 to 400 per cent over that of the largest nontreated control plant (*dwarf-1*). Only those increases greater than 25 per cent were considered as evidence for activity in screening experiments. The sensitivity of the bioassay was increased by adding the detergent "Tween-20" (sorbitan polyoxyethylene monolaurate) at the level of two drops per 100 ml. of the aqueous solution to be tested. "Tween-20" gave no response by itself. Materials showing no activity were tested several times in aqueous solutions as well as in solutions of acetone-water (1:1) and ethyl alcohol–water (1:1).

The following procedures were typical for the preparation of extracts and diffusates from higher plants for bioassay. Shelled market peas, fresh weight 250 gm., were shaken for 24 hours in 300 ml. of acetone-water (1:1). The solvent was filtered and an aliquot of the filtrate evaporated to dryness on a steam cone. The oily residue was resuspended in a small volume of water containing "Tween-20" and the suspension assayed for activity. Extracts were obtained from maize by grinding kernels in the milk stage in a Waring Blendor with acetone-water (1:1). The resulting slurry was shaken for 48 hours and the extract processed as described above. In certain cases the endosperm dissected from young seed was applied directly to the test plants.

For paper-partition chromatography, the active extracts or diffusates were applied as a narrow band through a 10-cm. width of 14 × 43-cm. Whatman No. 1 filter paper. A spot of gibberellic acid (5 μg.) was applied as a reference compound in the remaining 4-cm. zone. The chromatogram was then developed by descending solvent flow in a closed tank at room temperature. After the solvent had migrated to a point 30 cm. from the origin, the chromatogram was dried, and the 4-cm. zone containing the gibberellic acid was cut from the chromatogram. The 10-cm. strip was then cut into 2-cm. sections at right angles to the direction of the solvent flow, and the 15 sections thus obtained were extracted separately with a total of 5 ml. of acetone-water (1:1). The eluants were evaporated to dryness and the residues dissolved or suspended in a minimum amount of water containing "Tween-20." The position of the active zone for the plant extract was determined by bioassay, that of the gibberellic acid control by a fluorescence test (see below). R_f values were calculated from these data.

The fluorescence test described by Cross[13] was adapted for the detection of gibberellic acid on the chromatograms. The chromatograms to be tested were soaked with concentrated sulfuric acid and exposed to ultraviolet light. Under these conditions, as little as 0.1 μg. of gibberellic acid in a spot 1 cm. in diameter gave a characteristic blue fluorescence. Gibberellin A_1 and gibberellin A_2 did not give a detectable response to this test. Ehrlich's aldehyde reagent[18] was used as a spray

test for the detection of indole derivatives on the chromatograms. Vanillin reagent[19] was used as a spray test for the detection of leucoanthocyanins and similar compounds.

PRESENTATION OF DATA

Screening of Plant Materials for Activity in the Bioassay.—Methanol extracts from maize kernels in the milk stage were active in the bioassay, as were extracts and diffusates from the seed or fruit of a number of species of flowering plants. The endosperm from *Echinocystis*, *Aesculus*, and *Persea* gave evidence of activity when tested directly. The activities of the various plant sources are given in Table 1. The response of *dwarf-1* seedlings to *Echinocystis* endosperm and to diffusates of *Phaseolus* are shown in Figures 1 and 2.

TABLE 1

"GIBBERELLIN-LIKE" ACTIVITY FROM EXTRACTS OR DIFFUSATES OF FLOWERING PLANTS

(Activity Is Evidenced by a Growth Response in *dwarf-1* Seedlings)

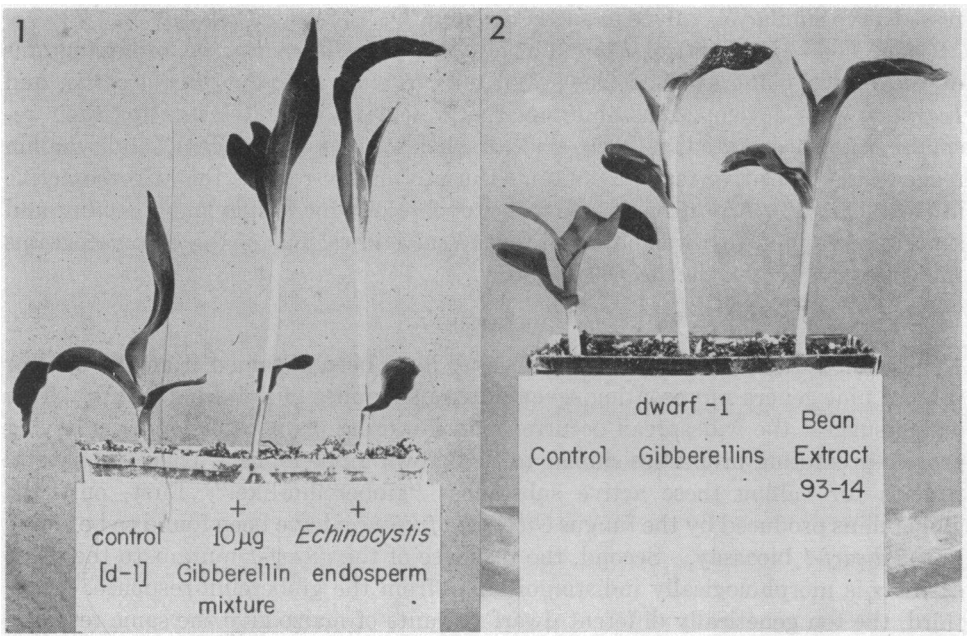
Plant Source	Part	Extraction Solvent	Activity
Phaseolus vulgaris L. (bean)	Green seed, from small to full size	Ethyl ether	+
		Acetone-water	+
		Acetone	+
Pisum sativum L. (pea)	Green seed, from small to full size	Acetone-water	+
		Ethyl ether	+
Aesculus californica (Spach.) Nutt. (California buckeye)	Endosperm	*	+
Echinocystis macrocarpa Greene (wild cucumber)	Endosperm	*	+
Lupinus spp. (lupine)	Green seed, full size	Ethyl ether	+
Zea mays L. (Maize)	Fruit, milk stage	Ethyl ether	+
		Methanol	+
		Acetone	±
Persea americana Mill. (avocado)	Endosperm	*	+
Prunus domestica L. (plum)	Endosperm, young seed	Ethyl ether	+
Prunus armeniaca L. (apricot)	Endosperm, young seed	Ethyl ether	+
Prunus amygdalus Batsch. (almond)	Endosperm, young seed	Ethyl ether	+
Datura stramonium L. (Jimson weed)	Young fruit	Ethyl ether	−
Juglans regia L. (English walnut)	Endosperm, young seed	Ethyl ether	−
Juglans californica Wats. (California walnut)	Endosperm, young seed	Ethyl ether	−
Nicotiana glauca Graham (tree tobacco)	Young fruit	Ethyl ether	+

* Liquid endosperm tested directly.

Specificity of Response of the Ten Dwarf Mutants to the Plant Extracts.—Extracts or diffusates from *Echinocystis*, *Aesculus*, *Persea*, *Lupinus*, *Phaseolus*, and *Pisum* were tested with each of the ten dwarf mutants to see whether the specificity of response followed that of the gibberellin response. It was found that these extracts were active for the five mutants that responded to the gibberellins, slightly active for the four mutants that showed a slight response to the gibberellins, and inactive for the mutant, *dominant dwarf*, that showed no response to the gibberellins.

Chromatographic Comparison of Active Plant Extracts with the Gibberellins.—Extracts or diffusates prepared from *Phaseolus*, *Pisum*, *Aesculus*, *Echinocystis*, and *Lupinus*, and were compared with each other and with the known gibberellins by

means of paper-partition chromatography. The results from four different solvent systems are shown in Table 2. The three known gibberellins could not be distinguished from each other on the basis of R_f values in any of the solvent systems used.



FIGS. 1 AND 2.—Response of *dwarf-1* seedlings to gibberellins and to the "gibberellin-like" substances from *Echinocystis* (wild cucumber) and from *Phaseolus* (bean). *From left to right: dwarf-1* nontreated control, *dwarf-1* treated with a mixture of gibberellic acid and gibberellin A_1, and *dwarf-1* treated with plant extract. Plant extract in Fig. 1 is the endosperm of one half-developed *Echinocystis* seed. Response is confined to the second leaf sheath, because of the age of the assay plants. Plant extract in Fig. 2 is a diffusate from young bean seed.

TABLE 2

CHROMATOGRAPHIC COMPARISON OF ACTIVE PLANT EXTRACTS AND THE GIBBERELLINS*

MATERIAL TESTED	SOLVENT SYSTEM A	B	C	D
Gibberellic acid	0.25	0.58	0.85	0.74
Gibberellin A_1	0.24	0.55	0.89	0.72
Gibberellin A_2	0.29	0.61	0.87	0.73
Phaseolus (bean)	0.27	0.65	0.85	0.76
Pisum (pea)	0.43	0.65	0.91	0.73
Aessclus (buckeye)	0.43	0.66	0.86	0.74
Echinocystis (wild cucumber)	0.51	0.77	0.87	...
Lupinus (lupine)	0.11	0.48	0.67	...

* R_f values are the average of two or more determinations which agreed within ±5 per cent. Solvent systems consisted of the upper phase of an immiscible system prepared by mixing 3 volumes of *n*-butanol and 1 volume of 1.5 *N* ammonium hydroxide for solvent A; 35 volumes of *n*-amyl alcohol, 35 volumes of pyridine, and 30 volumes of water for solvent B; and 95 volumes of *n*-butanol, 5 volumes of glacial acetic acid, and 30 volumes of water for solvent C. Solvent D consisted of 8 volumes of ethanol and 2 volumes of 3 *N* ammonium hydroxide.

Bean extracts gave R_f values similar to the gibberellins in three of the solvent systems but gave a consistently higher R_f value in solvent system B. When bean extract and gibberellic acid were co-chromatographed in this latter system, an active zone was found in front of the gibberellic acid zone. The active materials from

Pisum and *Aesculus* were similar to each other in migratory properties but were different from those of *Phaseolus* and the gibberellins. The active components of *Echinocystis* and *Lupinus* gave migratory properties differing from each other and from the other active components tested, including the gibberellins. All materials tested gave similar R_f values in solvent system D.

Color Tests of the Active Plant Extracts and the Gibberellins.—Chromatograms of the active plant extracts from *Phaseolus, Pisum, Aesculus, Echinocystis,* and *Lupinus,* were developed in *n*-butanol–acetic acid–water and tested for their response to concentrated sulfuric acid, Ehrlich's aldehyde reagent, and vanillin reagent. No positive test was obtained in any of the regions found to be active in the bioassay. A positive indole test was obtained for *Pisum* and *Aesculus,* and a positive vanillin test was obtained for *Aesculus* in regions of the chromatograms that were inactive in the *dwarf-1* bioassay.

DISCUSSION

Substances active in the *dwarf-1* bioassay have been obtained from the seed or fruit of nine genera representing seven different families of flowering plants. This would suggest the widespread occurrence in flowering plants of substances having growth-promoting properties similar to the known gibberellins. There are several reasons for calling these active substances "gibberellin-like." First, only the gibberellins produced by the fungus *Gibberella fujikuroi* have been found to be active in the *dwarf-1* bioassay. Second, the response of the *dwarf-1* mutant to the plant extracts is morphologically indistinguishable from the gibberellin response. And, third, the ten genetically different dwarf mutants of maize give the same response to the plant extracts as to the gibberellins. That is, the plant extracts are active for the five mutants that respond to the gibberellins, and inactive or only slightly active for the five mutants that do not respond or respond only slightly to the gibberellins.

The negative test from extracts from four other species of flowering plants could be due to the conditions of extraction and testing or could mean that such substances may be absent from the seed or fruit of these species or present only in minute amounts.

The evidence for the chemical relationship of the "gibberellin-like" substances to the known gibberellins is indirect. The regions of the chromatograms active in the bioassay give no color tests for indole compounds and for leucoanthocyanins, nor do they fluoresce in sulfuric acid as does gibberellic acid. Indole compounds, leucoanthocyanins, kinetin, and sources known to be rich in growth factors are not active in the bioassay. Finally, R_f values of the plant extracts are similar in some but not all of the solvent systems tested. In fact, the R_f values from the plant extracts vary depending on the source, suggesting that there may be a number of "gibberellin-like" substances in flowering plants.

Recently, Radley[20] has reported evidence for substances like gibberellic acid obtained from purified extracts of young pea shoots. On paper chromatograms, using chloroform: ethanol: water: formic acid (20:4:2:1), an active zone was found having the same R_f value as gibberellic acid. Radley[20] has suggested that the phytohormone reported by Mitchell *et al.*[21] in 1951 was probably a gibberellin. Mitchell had found that ether diffusates from young bean seed would give an 879 per cent in-

crease in length for the first internode, with additional growth stimulation of the internodes above this region. Indole-3-acetic acid also gave a 310 per cent increase with this test, but the IAA response was confined to the first internode of the bean seedlings. The unknown phytohormone was best obtained from very young seed. Activity disappeared by the time the seed were relatively mature. Our *dwarf-1* bioassay shows the presence of a "gibberellin-like" substance in bean seed from shortly after pollination to *green* seed that have reached full size. Mature green seed of other species were also found to be active.

Our data demonstrate the widespread occurrence in flowering plants of substances having properties similar to those of the gibberellins. These "gibberellin-like" substances must be isolated and purified before the precise chemical relation to the known gibberellins can be established.

SUMMARY

1. Extracts which give a "gibberellin-like" response in the *dwarf-1* bioassay have been obtained from nine genera representing seven different families of flowering plants.

2. Five genetically different dwarf mutants of maize respond both to the gibberellins produced by the fungus *Gibberella fujikuroi* and to the "gibberellin-like" substances obtained from the seed or fruit of flowering plants. Four other dwarf mutants respond only slightly in the seedling stage to the gibberellins and to the "gibberellin like" substances. Another mutant, *dominant-dwarf*, gives no growth response to the gibberellins or to the "gibberellin-like" substances.

3. The morphological response of the mutants to the "gibberellin-like" substances is indistinguishable from the response to the known gibberellins.

4. A comparison of R_f values for the "gibberellin-like" substances from six plant species and for the known gibberellins suggests that (*a*) these "gibberellin-like" substances are not *chemically identical* with the known gibberellins and (*b*) there may be a number of "gibberellin-like" substances present in flowering plants.

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*THE EFFECT OF HETEROZYGOSITY FOR NEW MUTATIONS ON VIABILITY IN DROSOPHILA: A PRELIMINARY REPORT**

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Geneticists are nearly unanimous in their belief that the vast majority of mutations are deleterious. This belief rests primarily upon the effect of mutations in the homozygous condition. The standard with which these homozygous individuals are compared is generally provided by individuals heterozygous for the mutations under investigation; the relatively excellent viability of these latter individuals is ascribed to the dominance of normal, "wild-type" alleles.

There exists in the literature a sizable record of well-documented instances of heterozygotes being superior in viability or fitness to both homozygotes; many of these are the cases of balanced polymorphism observed in natural populations. Some observations suggest that heterosis can be produced in individual instances by mutations with a marked deleterious effect when homozygous as well as an average deleterious effect in the heterozygous condition. Thus, Wallace and King,[1, 2] Cordeiro,[3, 4] and Dobzhansky *et al.*[5] have studied the relative viabilities of individuals homozygous and heterozygous for chromosomes, including lethal chromosomes, from experimental and natural populations and have found ample evidence for the occurrence of heterotic chromosomal combinations. Stern *et al.*,[6] in reporting on the average decrease in viability of *Drosophila* females heterozygous for sex-linked lethals, call attention to two lethals among the seventy-seven tested which appear heterotic; an analysis of these data by a technique reported by Wallace and Madden[7] suggests that the proportion of heterotic lethals among those tested may even exceed 20 per cent. Morton, Crow, and Muller[8] refer to another study of the viability of individuals heterozygous for "recessive" lethals. They point out that the variance between viabilities of individuals heterozygous for different lethals in this study is not significantly greater than that between replicate tests of the same lethal; the observed variances (F-ratio of 1.76) differ by an amount, however, which indicates that these data, also, are not inconsistent with the occurrence of a sizable proportion of heterotic lethals.