

Organs of Gibberellin Synthesis in Light-Grown Sunflower Plants

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Summary. The sites of gibberellin (GA) synthesis in light grown sunflower plants were studied. The results of organ excision and the exogenous application of indole acetic acid and gibberellic acid indicated that gibberellin synthesis occurred in the young leaves of the apical bud. This was substantiated using a combination of diffusion and extraction techniques. Diffusion of sunflower apical buds on agar for 20 hours revealed a level of gibberellin greater than that obtained by solvent extraction of a similar number of apices, indicating that synthesis of gibberellin was occurring in those apices during the diffusion period. The gibberellin level of apices extracted following a 20 hour diffusion period was the same as that obtained from buds extracted immediately following excision from the plant, again suggesting that apical buds are sites of gibberellin synthesis. A similar experiment was conducted with young internodal sections, the results indicating that they were not sites of gibberellin synthesis.

The ability of various parts of sunflower plants to produce gibberellin as measured by the agar diffusion technique was correlated to internode elongation, the region of maximum gibberellin production being associated with the region of maximum extension growth.

Root tips were also shown to be sites of gibberellin synthesis, this ability being confined to the apical 3 to 4 mm region of the root.

It was pointed out in an earlier paper (12) that the site or sites of gibberellin synthesis in higher plants have not been fully established. Translocation of applied gibberellin in plants occurs so freely (10, 17) that a comparison of extractable gibberellin levels in various parts of the plant is not necessarily meaningful. Thus it is possible that the presence of a high gibberellin level in an organ is a consequence not of in situ gibberellin synthesis, but rather of translocation into and accumulation within the organ of gibberellin produced elsewhere. There is however evidence showing that seeds are sites of active gibberellin synthesis (1, 16). Nevertheless, indirect evidence led Crane (5) to conclude that endogenous gibberellins important in peach fruit and pip growth originate in other parts of the plant.

Lockhart (9) on the basis of excision experiments with etiolated Alaska pea seedlings, considered the stem tip to be the organ of natural gibberellin production. He argued that if the response normally elicited by an organ could be reproduced by application of a pure hormone following excision of that organ, then the presumption is strong that the organ removed normally supplies to the plant a comparable substance. Similar methods to those

employed by Lockhart (9) were used in the present work in an attempt to determine the organ(s) of natural gibberellin production in light-grown sunflower.

The site of auxin synthesis in corn coleoptiles was demonstrated by van Overbeek (11) using a combination of extraction and diffusion techniques, showing that in such coleoptiles auxin was synthesized at the tip. An agar diffusion technique similar to that developed for auxin studies was used for the collection of gibberellins from sunflower apical buds (7), and experiments described in this paper provide further information as to the site(s) of native gibberellin synthesis in sunflower using methods similar to those described by van Overbeek (11).

Materials and Methods

Helianthus annuus (var. Tall Single) was used at various developmental stages. Nine week old plants were grown in 10 cm pots containing John Innes compost, while 10 to 16 day old seedlings were grown in the same mixture in wooden trays. Seedlings from which root tips were to be excised were grown from dehusked achenes which were allowed to germinate on moist tissue paper in covered plastic dishes. All plants except the seedlings from dehusked achenes were irrigated with a commercial

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nutrient solution. Nine to 10 week old plants were used when the fourth internode (numbered acropetally) was commencing elongation.

Both gibberellic acid (GA_3) and IAA were applied as suspensions in lanolin paste. These were prepared by dissolving the hormones in a minimal volume of methanol which was then beaten into lanolin to effect an even dispersion of the hormones throughout the paste. Analyses of variance were performed on growth measurements. Growth increments expressed as percentages were converted to angles, the analysis being performed on the transferred data. Treatment means were then compared by the Q test method described by Snedecor (14).

Agar diffusates were obtained from various organs using the technique described by Jones and Phillips (7). The preparation and subsequent extraction of the agar was accomplished in a manner similar to that described by the same workers.

Extracts of apical buds were obtained by homogenizing the tissue in a Waring blender, followed by extraction with 80% (v/v) methanol for 24 hours at 2°. The extract was filtered and reduced to dryness in vacuo at 35° on a rotary film evaporator prior to paper chromatography. Both agar block and tissue extracts were chromatographed on Whatman No. 3 paper, the chromatograms being developed in a descending manner in isopropanol:ammonia:water (10:1:1 V/V). Eluates were bioassayed with the dwarf pea epicotyl assay (12), and the lettuce hypocotyl assay (6).

Results

Organ Excision. Lanolin preparations of both IAA and GA_3 were applied to intact and decapitated 9 week old sunflower plants. Decapitation describes excision of the apical bud and leaves at the node above internode 4 leaving this internode intact. Lanolin preparations were applied to the cut surface of the stem immediately following excision in decapitated plants and to the leaf pair above internode 4 in entire plants. The length of the fourth internode was measured every 2 days for 8 days and the growth increment expressed as a percentage of the original length (fig 1). Decapitation of mature sunflower plants resulted in a significant reduction of the length of this internode, in comparison with the intact control. This reduction in internode length was overcome by application of GA_3 at 5,000 and 1,000 $\mu\text{g/g}$ while application of IAA at the same concentrations had no stimulatory effect on the growth of the decapitated fourth internode. Similarly, application of GA_3 to intact plants stimulated elongation significantly as compared with intact control plants, but did not cause a significantly higher rate of growth than that seen in GA_3 treated decapitated plants. IAA did not stimulate internode extension in intact plants.

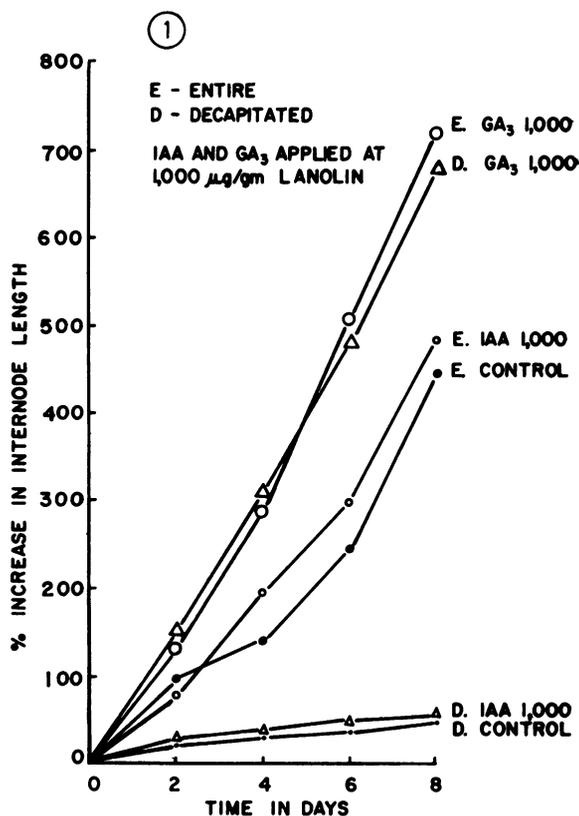


FIG. 1. Effect of decapitation and hormone application on the extension growth of internode 4 of mature sunflower plants.

From these results, it would appear that GA_3 was limiting internode extension to some degree in entire plants and that this was more evident in decapitated plants. It would also appear that auxin was not limiting extension growth in either intact or decapitated plants. The IAA was probably not supplied in supra-optimal amounts, for similar results were obtained with IAA concentrations as low as 10 $\mu\text{g/g}$ in lanolin. Adopting the argument put forward by Lockhart (9) it would seem reasonable to assume that as GA_3 completely replaces the need for the apical bud with respect to internode elongation, then GA_3 or a similar substance is produced in the apical bud and is necessary for the maintenance of internode elongation.

An attempt was then made to determine whether this substance was produced in the apical dome and leaf primordia, or in the young expanding petiole leaves of the apical bud and main stem. Excision of only the apical dome and leaf primordia from 9 week old sunflower plants did not result in a significant reduction in elongation of internode 4, whereas excision of the maturing and mature petiole leaves caused a significant reduction in internode elongation (fig 2). This result indicates that the petiole leaves of the apical bud, or the

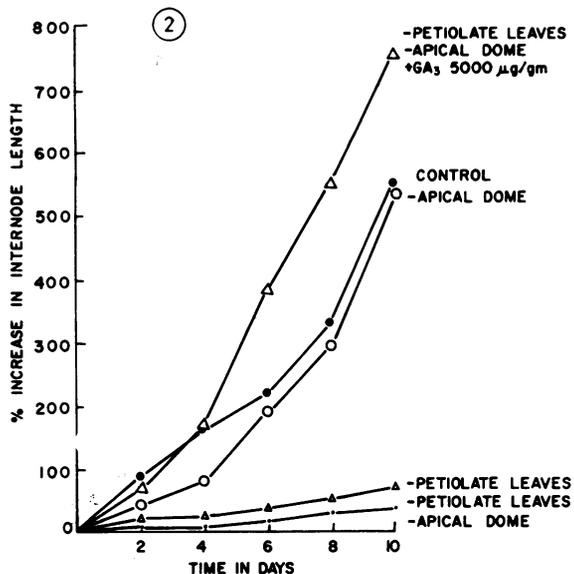


FIG. 2. Effect of excision of apical dome, petiolate leaves or both on the extension growth of internode 4 of mature sunflower plants.

stem leaves, or both, but not the stem apex proper are the sources of the factor necessary for internode elongation. It could be argued that excision of all the petiolate leaves resulted in a deprivation of carbohydrates and other nutrients necessary for the maintenance of extension growth. However, application of GA₃ to completely defoliated stems resulted in marked internode elongation (fig 2), indicating that in such internodes GA₃ was limiting extension growth, but that photosynthate was not.

As the petiolate leaves of the apical bud and stem appear to be the major source of the gibberellin-like substance(s) necessary for internode

elongation, an examination was made of the dependence of internodes 3 and 4 on various leaf pairs (fig 3). Removal of leaf pair 2 had no significant effect on extension growth of internodes 3 or 4. Removal of leaf pair 3 caused a significant reduction in extension growth of internode 4, while removal of the apical bud caused a marked reduction in extension growth of both internodes. The petiolate leaves of the apical bud can therefore be considered to be the major sites of synthesis of the gibberellin-like substance(s) necessary for internode elongation, the leaf pair immediately below internode 4 contributing a significant but lesser amount.

Diffusible Gibberellin-like Substances from the Shoot. The data of Jones and Phillips (7) showed that gibberellin-like substances can be diffused in measurable amounts from apical buds of sunflower into agar gel. No evidence was presented to show whether the gibberellin-like substances obtained in this way represented what merely drained from the apical buds as was the case with auxin diffused from pea internode sections (13), or whether they were actually produced in the apical bud. As indicated earlier, van Overbeek (11) showed that auxin was produced in the apical region of corn coleoptiles. He argued that if the same amounts of auxin can be extracted from corn coleoptile tips immediately following excision and after a period of diffusion into agar blocks, then the amount of auxin in the agar blocks must represent the amount actively synthesized during the diffusion period. If the coleoptile tips were not synthesizing auxin, then the amount obtained by diffusion followed by extraction should approximate that obtained by extraction of apices following excision. This method of investigation was adopted to determine whether sunflower apical buds synthesized gibberellin-like substances. Figure 4 shows the bioassay results obtained following chromatography of both tissue and agar block extracts obtained from 2 comparable lots of 50 apical buds. One lot was extracted immediately after excision from the seedlings, the other was allowed to diffuse on agar blocks prior to extraction. The result suggests that the apical buds were sources of gibberellin which appears to have been unobtainable by extraction. It is possible that this gibberellin was present in the buds at the time of excision in an inactive or bound form. Notwithstanding this possibility, the results of this and several other similar unreported experiments indicate that the buds are sites of active gibberellin synthesis, rather than the activity being due to the draining of gibberellin present at the time of excision.

A similar experiment to that performed above was conducted to determine whether young internode sections are sites of gibberellin synthesis. Bioassay results following paper chromatography of both agar block and tissue extracts obtained from 2 similar lots of 200 internode sections are

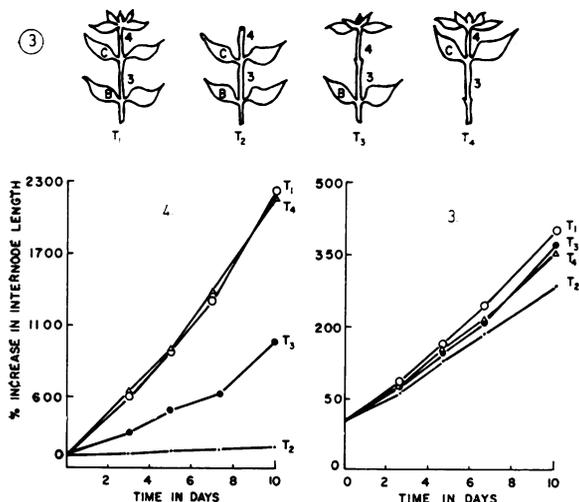


FIG. 3. Effect of excision of either apical bud, leaf pair C or leaf pair B on the extension growth of internodes 3 and 4 of mature sunflower plants.

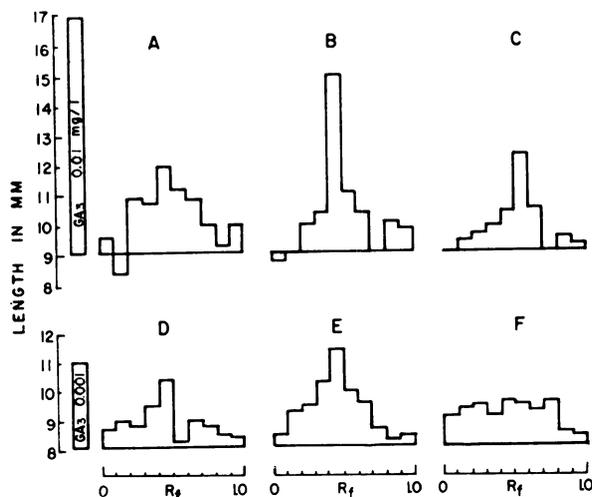


FIG. 4. Concurrent diffusion and extraction of sunflower seedling root tips and sub-apical root sections. A) Extract from 200 root tips, B) Diffusate from 200 root tips, C) Extract from 200 root tips following a diffusion period, D) Extract from 200 sub-apical root sections, E) Diffusate from 200 sections following diffusion. Values ± 0.8 mm of controls are significant at the 1% level of risk.

shown in figure 4. Although these results do not allow conclusions to be drawn as to whether internodes synthesize gibberellins, the level of gibberellin activity obtained in the agar blocks (fig 4E) indicates that internodes contain considerably lower levels of gibberellin than apical buds (fig 4B).

By the use of excision experiments the presence of young leaves, particularly those of the apical buds, was found necessary for the maintenance of internode extension in *H. annuus*. The function of these leaves would appear to be that of synthesizing gibberellin-like substances. The following experiment was therefore conducted to evaluate the relative amounts of diffusible gibberellin-like (GA-like) substance which could be obtained from apical buds, leaves and internodes, and the extension growth of comparable internodes.

Sixty mature sunflower plants were selected. Ten of these were used for measurement of internode elongation rates; the remaining 50 were separated into apical buds, leaves, and internodes as indicated in figure 5. Measurements of the lengths of internodes 1 to 5 were made 24 hours prior to the diffusion period and final measurements taken 24 hours following termination of this (the diffusion) period. Diffusates were obtained from the various organs as previously described, although certain modifications were made to the basic technique by altering block size and by securing the agar to the internodes by means of a fine glass rod passing through the center of the block into the pith, thus drawing both surfaces into close contact. There was a positive correlation between the rate

of extension growth in individual internodes and the quantities of diffusible GA-like substances obtained from them (fig 5). Similarly the amounts of these substances obtained from leaves and internodes decreased with age. The quantity of gibberellin obtained from leaves was always found to exceed that obtained from the internodes immediately below the point of insertion of the leaves; in fact, only internodes 5 and 4 yielded substantial amounts.

Diffusible Gibberellin-like Substances from Root Tips. Although experiments reported here indicate that leaves donate physiologically significant levels of GA-like substances to the internodes, they cannot be considered to be the only sites of native gibberellin production. It has been previously reported that the root system in several plant species seems to supply GA-like substances to the shoot system (3, 12). The presence of gibberellins in root exudate or bleeding sap does not permit the conclusion that roots are sites of gibberellin production, although the finding that excised tomato roots synthesize gibberellin-like substances following 4 years in isolated culture (2) indicates that roots have the ability to synthesize these substances. An examination was therefore made, using the agar diffusion technique, of the ability of certain parts of sunflower seedling roots to synthesize gibberellin-like substances.

The method used for diffusion of sections of sunflower roots is similar to that used for shoot apices. Dehusked sunflower achenes were sown on moist tissue paper in covered plastic dishes. Two

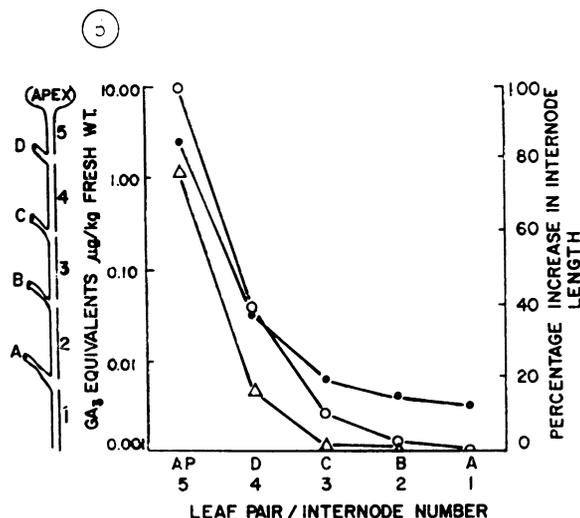


FIG. 5. Relationship between the amounts of diffusible GA-like substances obtained from leaf pairs A to D, apical buds and internodes 1 to 4 of mature sunflower plants and the growth rates of comparable internodes. \circ — \circ diffusible GA-like material from apical buds and leaf pairs D to A. \triangle — \triangle GA-like material diffused from internodes 5 to 1. \bullet — \bullet Percentage increase in internode length.

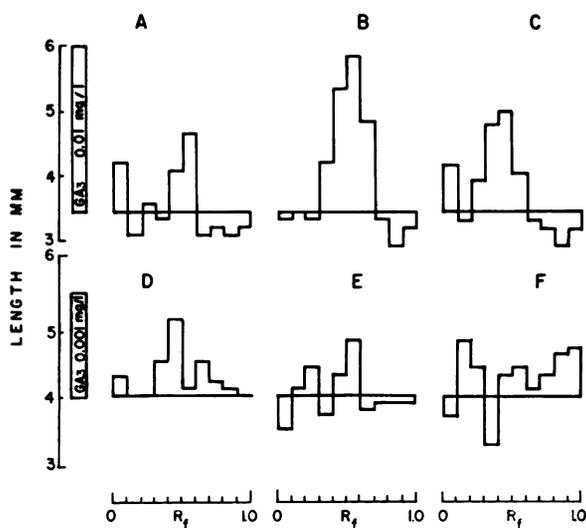


FIG. 6. Concurrent diffusion and extraction of sunflower seedling apical buds and internode sections. Both extracts and diffusates bioassayed with dwarf pea epicotyls. A) Extract from 50 sunflower seedling apical buds, B) Diffusates from 50 sunflower seedling apical buds, C) Extract from 50 sunflower apical buds following diffusion, D) Extract from 200 seedling first internode sections, E) Diffusate from 200 internode sections, F) Extract from 200 internode sections following diffusion. Values ± 2.3 mm of controls are significant at the 1% level of risk.

days after sowing, 3 to 4 mm long portions were excised from the apical part of such roots and allowed to diffuse on agar blocks, 4 mm in diameter. Although several experiments indicated that such root tips contained gibberellin-like substances which could be collected in agar blocks, the results did not indicate whether the tips actively synthesized these substances. Experiments similar to those performed with shoot apical buds were repeated using root tip sections (fig 6). The results indicate that these organs are in fact sites of synthesis of GA-like substances. A similar experiment was performed using root segments obtained from a zone 4 to 8 mm behind the root tip, this region having ceased elongation and bearing root hairs. The results of this experiment (fig 6D, 6E, 6F) differ markedly from those with the apical portion of the root (fig 6A, 6B, 6C), and, adopting the argument of van Cverbeek (11), this region of the root cannot be considered to be a site of gibberellin synthesis.

Discussion

The results presented here lend support to the conclusion reached by Lockhart (9) that the apical bud is an important source of gibberellin-like substances which are involved in the control of stem extension growth. The experiments reveal further that it is the young leaves of the apical bud, rather

than the apical meristem, which serve as sites of gibberellin synthesis. There also seems little doubt that older but not fully grown leaves supply gibberellins to the stem, although in relatively smaller quantities.

The results of experiments using the agar diffusion technique confirm the observations made above that synthesis of a gibberellin-like substance necessary for the maintenance of internode elongation occurs principally in the young leaves of the apical region. The gibberellin recovered from agar blocks following a 20 hour diffusion period is present as a result of active synthesis by the diffusing organ. Although the technique is not performed under aseptic conditions, the possibility that the gibberellin present in the agar arises from microbial contaminants has been excluded. The apparent ineffectiveness of young internodal tissue as sites of gibberellin synthesis, supports the observation that internodes are dependent on an external supply of gibberellin necessary for elongation. It could be argued that the apparent lack of gibberellin-like substances in diffusates from older leaves (fig 5) and internode sections (fig 4) is due not to a decrease in gibberellin levels but to an increase in the amount of natural gibberellin inhibitors which would mask the gibberellin-induced response during bioassay. Diffusates have been assayed with barley half seeds, an assay method proven to be specific to some natural GA-inhibitors (4), but no inhibitory substances were detected.

There seems little doubt that sunflower roots are also sites of gibberellin synthesis, and that this ability is localized in the apical portion of the root. Kinins have also been shown to be produced in sunflower roots (8) and as is the case for gibberellin, the locus of kinin production is confined to the apical portion of the root (15). It cannot be concluded, however that all the GA-like substance(s) present in root exudate (3, 12) is produced in the root tip. Nevertheless, the roots can now be described as organs of gibberellin synthesis, although the physiological significance of the GA thus produced can be determined only by further experimentation.

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