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Contents and Recovery of Gibberellins in Monoecious and Gynoecious Cucumber Plants[1]

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Abstract. Diffusates from seedlings and root exudates from 6-week-old plants of a monoecious line of cucumber, *Cucumis sativus* L., contained considerably higher levels of gibberellin-(GA-)like substances than did those from plants of an isogenic gynoecious line. Most of the GA-like activity was found in a chromatogram region typical of GA_1 and GA_3; some activity, particularly in root exudates, appeared also at an R_F similar to that of GA_4 and GA_7.

When seedlings were treated with ^3H-labeled GA_1, more radioactivity was found in the diffusates from monoecious seedlings than from gynoecious ones. The same was true of biological activity in root diffusates from older plants which had been treated with gibberellin A_{4+7}.

In conjunction with evidence present in literature, these results support the idea that endogenous GAs play a part in the regulation of sex expression in cucumber, relatively high levels favoring the formation of staminate flowers.

Auxins have long been known to enhance the female sex "tendency" in cucumber, that is, to promote the formation of pistillate flowers (14). Gibberellins (GAs) have the opposite effect, favoring the formation of staminate over that of pistillate flowers (4) and moreover inhibiting the further development of the latter (Atsmon, unpublished data).

These findings were obtained with exogenous hormones. However, in the case of auxin, both, indirect studies concerning relations between growth rates and sex expression (2, 7) and direct analyses of the auxin content of plants with different sex expression (8), have provided evidence that this hormone participates in the endogenous regulation of sex expression in cucumber.

In the case of GAs, there is so far only indirect evidence for such an endogenous function. Part of it comes from studies on hypocotyl and internode length in plants of different sex types. These parameters can be used as indicators of the effective endogenous GA level in the plants, and the results show that this level increases with increasing male tendency (Atsmon, unpublished data). Another part of the evidence comes from work with the growth retardants AMO-1618 and CCC (Cycocel) which are known to inhibit GA biosynthesis in plants and which were found to reduce the male tendency in cucumber (Galun and Lang, unpublished data).

The experiments described below were designed to obtain more direct evidence for differences in GA content in cucumber plants of different sex types; and also to determine whether such plants differ with respect to recovery of applied GA, that is, presumably, in their metabolism of GAs.

Both types of experiments were done with 4-day-old seedlings and with 6-week-old plants. In the seedlings, we determined the quantities of GAs, either endogenous or applied, diffusing from the shoot into agar blocks. In the older plants, the GA content of root exudates was determined, this latter approach being based on the discovery that root exudates may contain significant levels of GA-like substances (16, 18) synthesized in the root (10, 11).

Materials and Methods

Plant Material. A homozygous gynoecious and a monoecious line of cucumber (*Cucumis sativus* L., cv. "Beit Alpha") were used throughout this study. The 2 lines are practically isogenic, differing only in the alleles of 1 of the 2 major genes (st^+/st and M/m; see 6) which determine sex expression in cucumber. The monoecious plants have the genic constitution st^+/st^+ M/M. They produce only staminate flowers on the first flower-bearing nodes of the stem; on the latter nodes, an increasing proportion of pistillate flowers are formed. The homozygous gynoecious plants, with the constitution st/st M/M, form exclusively pistillate flowers; formation of staminate flowers on the first flower-bearing nodes can be induced only by treating the plants with GA (15).

Collection of Diffusates and Exudates. The procedures for obtaining diffusates and exudates used

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in our work were essentially those described by Jones and Phillips (9), and Phillips and Jones (16), respectively.

Diffusates were obtained from seedlings which were grown from seeds soaked for 2 hours in water, then planted on moist facial tissue and kept, the first 48 hours, in the dark and at 32°, the following 48 hours in the greenhouse (natural light, extended to a total of 16 hours per day by light from Gro-lux fluorescent lamps). For each experiment, 50 seedlings of either sex type were selected for equal size, cut at the base, and placed with the cut ends into a layer of 1.5 % agar. After a diffusion period of 24 hours, the seedlings were discarded and the agar frozen and stored until further use.

For exudate collections, plants were grown in the greenhouse, under the conditions described above, in plastic containers with vermiculite, and were watered with half-strength Hoagland nutrient solution. The culture period was 6 weeks. When ready for exudate collection, a plant was cut at the base of the main shoot, the stump provided with a tightly fitting rubber tube, and the exudate collected in glass containers over a period of 24 hours. After collection, the exudate was frozen and then lyophilized. Each determination is based on exudate collected from 15 plants and totalling *ca.* 900 ml.

GA Extraction from Diffusates and Exudates. The frozen agar from the diffusion treatments was allowed to thaw and was then extracted with methanol. The extract was filtered through a Buchner funnel, the methanol evaporated under vacuum, and the aqueous residue acidified to pH 2.7 and extracted 3 times with ethyl acetate. The ethyl acetate was dried with Na_2SO_4, reduced to dryness in a flash evaporator and taken up in a small volume of methanol for thin-layer chromatography.

The dried exudate was dissolved in phosphate buffer, pH 8.2, partitioned 3 times against ethyl acetate, then acidified and further treated the same way as the diffusate extract.

Chromatography and Bioassays. The final methanol solutions obtained from diffusates and exudates were streaked on Silica gel G plates and developed in chloroform:ethyl acetate:acetic acid (60:40:5), according to Sembdner *et al.* (17). The chromatogram fractions were eluted with water-saturated ethyl acetate, and the eluates reduced to dryness under vacuum and redissolved in 0.7 ml water. Subsequently, 0.2 ml of each fraction were tested by means of the barley half-seed α-amylase test (12) and 0.4 ml by the lettuce seedling test (5). The barley used was the cultivar "Himalaya", the lettuce, "Cabbage lettuce Arctic" (Carter's Tested Seeds, Ltd., London).

No attempt was made to identify the endogenous cucumber gibberellins chemically or by fluorescence.

Application of Exogenous GA. Short-term experiments were carried out with ^{3}H-labeled GA_1, prepared by Dr. H. Kende (13). The hormone was applied to the apices of 4-day-old, light-grown seedlings in 5-μl droplets, the concentration being 20 μM. The number of seedlings used, diffusion time, and further handling including chromatography were as described above for ordinary agar diffusion. The dried chromatogram fractions were redissolved in water and an aliquot was counted in a scintillation counter (Packard Tri-Carb Model 2211).

In long-term experiments, a mixture of GA_4 and GA_7 (approximately equal parts) at 10 μM in 0.05 % Tween was applied to plants grown in the greenhouse, as 10-μl droplets placed onto the youngest leaf, 3 times a week, starting with the first true leaf and continuing for a period of 6 weeks. Exudate was obtained and handled as described above and was tested for biological activity.

The aqueous phases remaining after the ethyl-acetate extractions were not tested for activity.

Results

Endogenous GAs in Monoecious and Gynoecious Cucumbers. Figure 1 shows the GA-like activity

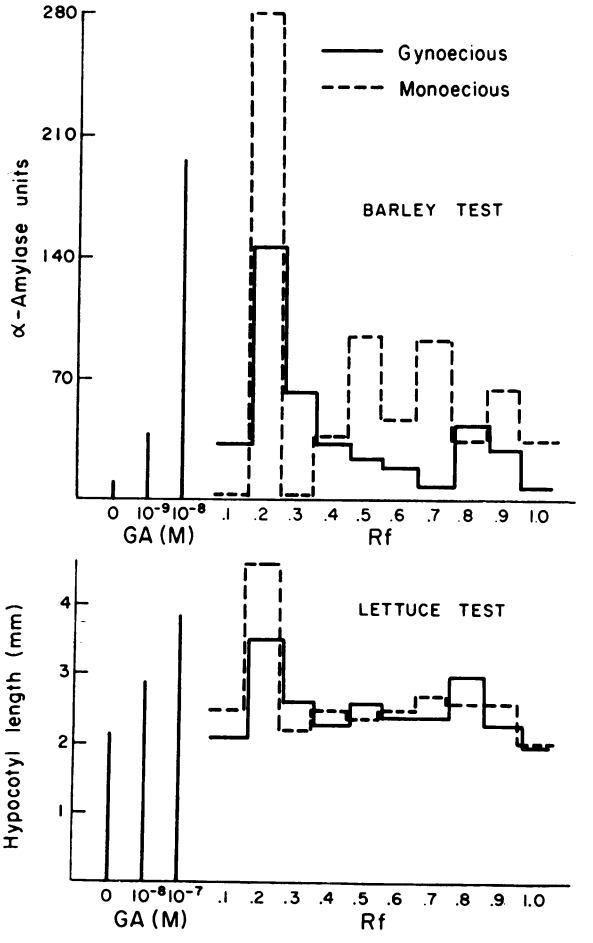

FIG. 1. Gibberellin-like substances in diffusates from seedlings of monoecious and gynoecious cucumber after separation by thin-layer chromatography, determined by the barley amylase and the lettuce seedling assay.

in diffusates from seedlings of the 2 sex types. Most of the activity appeared at R_F 0.2 which corresponds to the R_F value of GA_1 and GA_3 in the chloroform-ethyl acetate-acetic acid solvent used. Secondary peaks were found at R_F 0.5, 0.7 and 0.9, but only with diffusates from monoecious seedlings and with the barley-endosperm assay.

With either assay, the level of GA-like activity at R_F 0.2 obtained from monoecious seedlings was considerably higher than that obtained from the gynoecious ones. The difference was about one order of magnitude. This result was obtained consistently in 4 separate experiments.

The results with root exudates are summarized in figure 2. The chromatograms differ from those of the seedling diffusates by having a clear second peak of activity at R_F 0.6, a value similar to that of GA_4 and GA_7 in the solvent used (0.6–0.7). The slower peak appears to be broader than in the diffusate chromatograms but it is difficult to say whether this is because the active region in the exudate chromatograms happened to be shifted midway between the 2 R_F zones, or whether it has a genuine

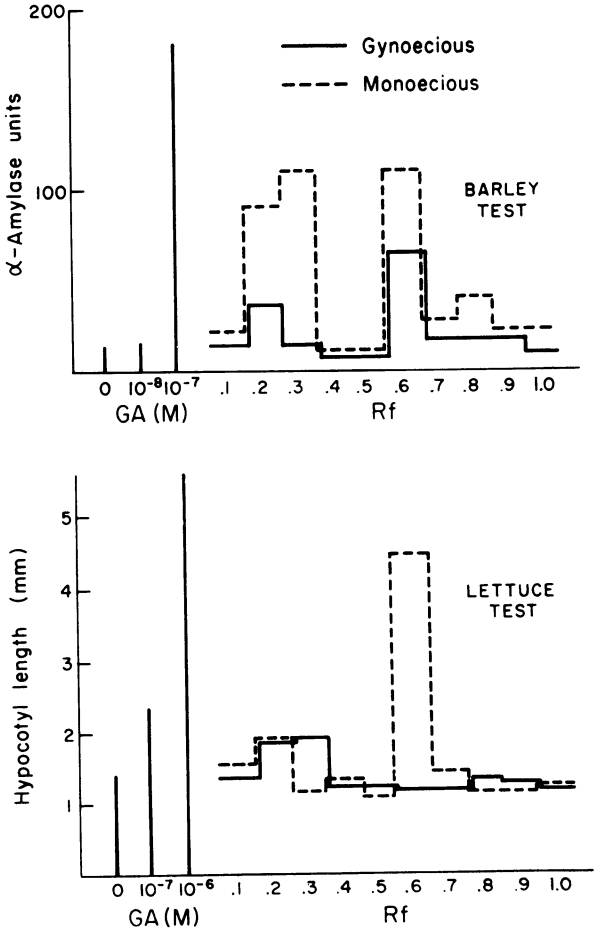

FIG. 2. Gibberellin-like activity in root exudates of the 2 sex types.

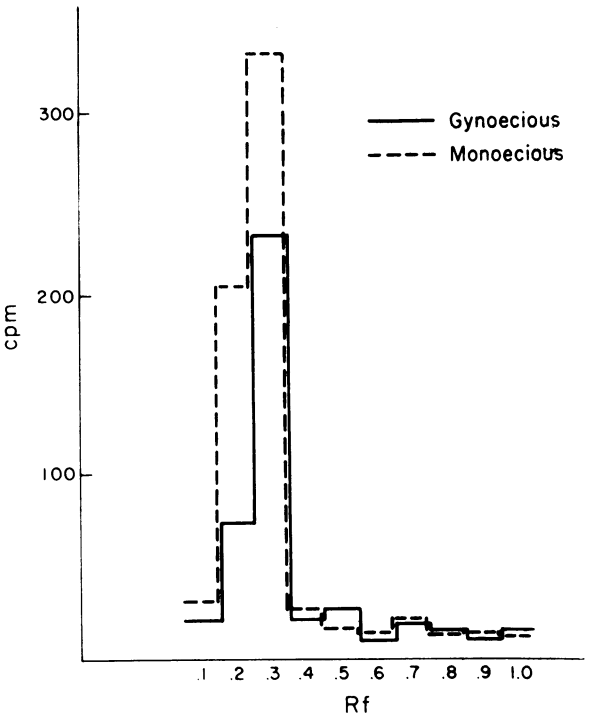

FIG. 3. Radioactivity in diffusates from seedlings of the 2 sex types, treated with ^3H-GA_1.

significance, such as different nature of the diffusate and the exudate factors, or presence in the exudates of an additional factor with a somewhat higher R_F value. Either assay demonstrated a higher level of GA-like activity for the monoecious plants, as compared to the gynoecious ones. This is in agreement with the results with diffusates from the seedlings of the 2 sex types.

GA-like Substances in Monoecious and Gynoecious Cucumber after Treatment with Exogenous GA. Figure 3 shows the amounts of radioactivity found in diffusates of seedlings treated with ^3H-GA_1. Those in diffusates from monoecious seedlings are significantly higher than in diffusates from gynoecious ones. In either case, practically all radioactivity found was concentrated in a single peak on the chromatograms, corresponding to the position of GA_1.

Figure 4 illustrates GA determinations in root exudates of 6-week-old plants which had been treated with GA_{4+7}. Two results can be seen: A) There is high activity in the exudate of the monoecious plants but no significant activity in that of the gynoecious ones. The trend is thus in quite the same direction as, although of even greater magnitude than, in the diffusates from the ^3H-GA_1-treated seedlings; B) the activity in the exudate of the monoecious plants is considerably higher (roughly, an order of magnitude) than in that from untreated plants of the same sex type (compare fig 4 and 2). This refers to all 3 peaks of activity that are evident

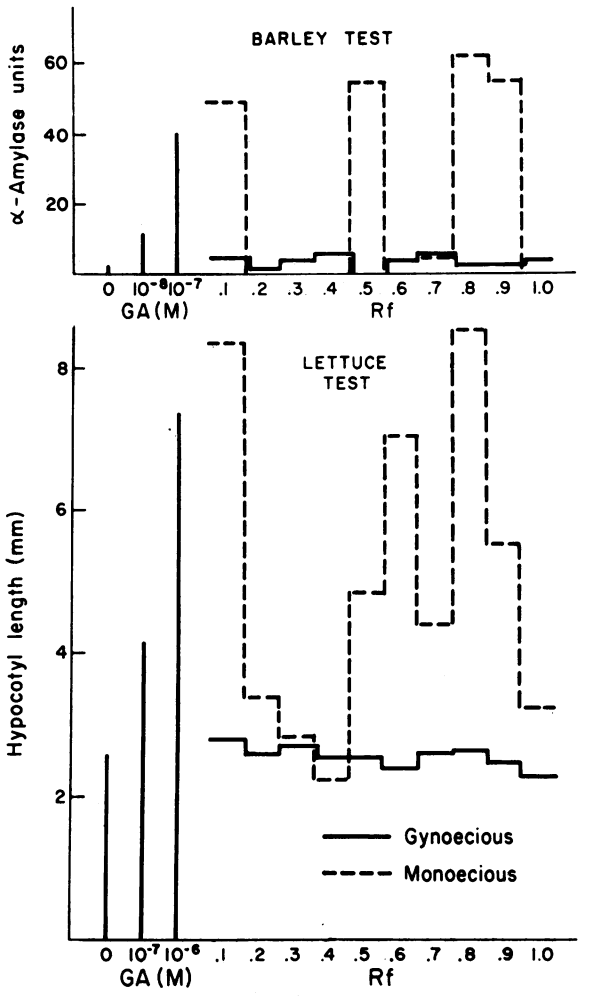

FIG. 4. Gibberellin-like substances in root exudates of monoecious and gynoecious cucumber plants treated with a mixture of GA_4 and GA_7.

(R_F 0.1–0.3, 0.5–0.6, 0.8–0.9) and is particularly striking in the barley endosperm assay. The increase at R_F 0.5 to 0.6 is most probably due to unchanged GA_4 and GA_7 but the increases in the 2 other peaks show either that a substantial part of the applied GA_4 and/or GA_7 was converted to other GA-like substances, or else that the treatment with GA_{4+7} modified the production of endogenous GAs. The R_F value of 0.8 to 0.9 is, with the solvent used, that of GA_9.

Discussion

Our experiments demonstrate the existence of higher levels of GA-like substances in diffusates from seedlings and exudates from the root system of older plants in monoecious cucumbers than from gynoecious plants, and also a considerably greater recovery of applied GA from monoecious plants, as compared to gynoecious ones. Taken in conjunction with the well-known effect of applied GA on sex expression in cucumber, and with the indirect lines of evidence which were summarized in the Introduction, these results lend additional, strong support to the concept that endogenous GAs are an integral part of the regulatory system for sex expression in this plant. Similar evidence is available for auxins which affect sex expression in cucumber in the opposite way than do the gibberellins, that is, they enhance the female tendency. It thus appears probable that the endogenous regulation of sex expression in cucumber involves either type of hormone, and that it may be the balance between the two, probably in the immediate vicinity of the differentiating flower bud, which determines whether the latter becomes a staminate or a pistillate flower. (It may be useful to recall that the flowers of cucumber are initiated as bisexual organs, with both stamen and pistil primordia. It is only during further development that the one kind of sex organs continue to develop while the others do not; see 1).

Different levels of endogenous GAs, when determined by means of bioassay, can be explained by differences in synthesis, or in destruction or inactivation (*e.g.*, small changes in the structure of the molecule or combination with another substance), or in levels of antagonistic substances which are extracted together with the hormone and reduce its effect in the assay. Differences in the recovery of an applied substance may be caused by several factors, namely, differences in uptake into the tissue, in transport, in binding within the tissue, and again in destruction or inactivation and in the level of inhibitors. Among these various possibilities, inhibitors as critical factors are unlikely since inhibitors would not affect determinations of radioactive GA as carried out in the recovery experiments with seedlings. In addition, preliminary experiments in which extracts of diffusates from monoecious and gynoecious seedlings were combined did not provide proof for the presence of substances inhibiting the bioassays. Other preliminary experiments on transport of ^3H-GA_1 through hypocotyl sections indicate no great differences of transport rates and of binding in the tissue between monoecious and gynoecious seedlings. Lesser uptake by gynoecious plants than by the monoecious ones is also improbable since applied GA causes relatively more growth in the former than in the latter, reducing the differences in height characteristic of the 2 sex types (Atsmon, unpublished data). Thus, differences in synthesis and differences in destruction or inactivation of GAs remain as the 2 most likely reasons to explain the differences in GA levels and GA recovery observed between monoecious and gynoecious cucumbers. The results on recovery of applied GA are a strong indication that differences in destruction or inactivation are actually present. Such differences would also suffice to explain the differences found in the levels of endogenous GAs, both in seedling diffusates and in root exudates. However,

differences in synthesis can by no means be excluded, and further studies on this question are needed.

Two additional points may be made. A) While the chemical nature of the endogenous GAs of cucumber plants remains unknown, it can be stated that the majority of the GA activity, at least in the seedlings, is not due to the gibberellins A_4, A_7, and/or A_9 which, when applied exogenously, are the gibberellins known to be the most active ones in cucumber (3).

B) Sex expression in monoecious cucumbers can be modified by various environmental conditions. Long days, high light intensities and high temperatures generally favor the male tendency, the opposite conditions favor the female one. Applied GAs cause a shift to the male side, inhibitors of gibberellin biosynthesis, a shift to the female one. It therefore appears likely that those environmental factors are acting by way of modifying the endogenous GA level. However, it remains unknown how this is accomplished, whether by increased synthesis, reduced inactivation, or otherwise. Our present results, obtained with lines in which the differences in sex expression are genetically controlled, should not be extrapolated, with no additional tests, to situations where the control of sex expression is purely physiological.

Literature Cited

1. ATSMON, D. AND E. GALUN. 1960. A morphogenic study of staminate, pistillate and hermaphrodite flowers in *Cucumis sativus* L. Phytomorphology 10: 110–15.
2. ATSMON, D. AND E. GALUN. 1962. Physiology of sex in *Cucumis sativus* L. Leaf age patterns and sexual differentiation of floral buds. Ann. Botany London 26: 137–46.
3. BRIAN, P. W. AND H. G. HEMMING. 1961. Promotion of cucumber hypocotyl growth by two new gibberellins. Nature 189: 74.
4. BUKOVAC, M. J. AND S. H. WITTWER. 1961. Gibberellin modification of flower sex expression in *Cucumis sativus* L. In: Gibberellins. Advan. Chem. Ser. 28: p 80–88.
5. FRANKLAND, B. AND P. F. WAREING. 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature 185: 255–56.
6. GALUN, E. 1961. Study of the inheritance of sex expression in the cucumber. The inheritance of major genes with modifying genetic and non-genetic factors. Genetica 32: 134–63.
7. GALUN, E. AND D. ATSMON. 1962. The leaf-floral bud relationship of genetic sexual types in the cucumber plant. Bull. Res. Council Israel 9D: 43–50.
8. GALUN, E., S. ITZHAR, AND D. ATSMON. 1965. Determination of relative auxin content in hermaphrodite and andromonoecious *Cucumis sativus* L. Plant Physiol. 40: 321–26.
9. JONES, R. L. AND I. D. J. PHILLIPS. 1964. Agar-diffusion techniques for estimating gibberellin production by plant organs. Nature 204: 497–99.
10. JONES, R. L. AND I. D. J. PHILLIPS. 1966. Organs of gibberellin synthesis in light-grown sunflower plants. Plant Physiol. 41: 1381–86.
11. JONES, R. L. AND I. D. J. PHILLIPS. 1966. Effect of CCC on the gibberellin content of excised sunflower organs. Planta 72: 53–59.
12. JONES, R. L. AND J. E. VARNER. 1967. The bioassay of gibberellins. Planta 72: 155–61.
13. KENDE, H. 1967. Preparation of radioactive gibberellin A_1 and its metabolism in dwarf peas. Plant Physiol. 42: 1612–18.
14. LAIBACH, F. AND F. J. KRIBBEN. 1950. Der Einfluss von Wuchsstoff auf die Bildung männlicher und weiblicher Blüten bei einer monözischen Pflanze. Ber. Deut. Botan. Ges. 62: 53–55.
15. PETERSON, C. E. AND L. D. ANHDER. 1960. Induction of staminate flowers in gynoecious cucumbers with GA_3. Science 131: 1673–74.
16. PHILLIPS, I. D. J. AND R. L. JONES. 1964. Gibberellin-like activity in bleeding sap of root systems of *Helianthus annuus* detected by a new dwarf pea epicotyl assay and other methods. Planta 63: 269–78.
17. SEMBDNER, G., R. GROSS, AND K. SCHREIBER. 1962. Die Dünnschichtchromatographie von Gibberellinen. Experientia 18: 585–86.
18. SKENE, K. G. M. 1967. Gibberellin-like substances in root exudate of *Vitis vinifera*. Planta 74: 250–62.