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Sexual differentiation in cucumber: Abscisic acid and gibberellic acid contents of various sex genotypes

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The AbA content of cucumber shoot tips was determined by GLC. Shoot tips of monoecious plants had a higher AbA content than those of gynoecious ones, and SD conditions caused higher AbA content than LD conditions. AbA content per unit dry weight of young floral buds was 5- to 9-fold higher than in older ones.

A GLC method was developed to allow parallel identification and quantitative determinations of GA_3 and AbA with the same sample. The shoot tip contents of both growth substances were determined at four developmental stages of the monoecious plant. Both significantly increased until the 12th day after planting then decreased; the GA_3 content decreased markedly.

A general hypothesis for the role of growth substances in regulating sex expression of cucumber is presented and discussed.

The flowering pattern in the main shoot of many cucumber cultivars consists of three flowering phases: male, mixed (male and female) and female (*9*, *14*). Monoecious cucumber cultivars usually show only the first and second phases, while gynoecious lines show only the third or second and third phases.

Endogenous growth substances have been tested in various cucumber sex types in order to elucidate their role in regulating sex expression. Sex genotypes homozygous for the allele *st* (acr^F) were found to contain more extractable auxin in the stem sections than parallel sections in genotypes being homozygous for the st^+ (acr^+) allele (*16*). Ethylene evolution showed the same trend as auxin content in both genotypes (*7*, *28*). Gibberellin content was higher in extracts, diffusates and exudates of monoecious cucumbers than in gynoecious ones (*5*, *18*). The highest gibberellin content was found in the apices at the time of differentiation of the first male bud (*19*). Thus, a high gibberellin content seems to be correlated with maleness in the cucumber plant. Leaves of monoecious plants were found to contain more extractable antagonists of auxin than leaves of gynoecious plants (*13*). The contents of gibberellin antagonists and AbA of gynoecious plants were higher than those of monoecious ones (*26*).

A number of studies showed that the balance of growth substances in the vicinity of the differentiating bud is closely correlated with sexual differentiation. Young leaves were found to have a high auxin content, and removal of young leaves from the

Abbreviations: AbA, ± abscisic acid; GA_3, gibberellic acid; GLC, gas-liquid chromatography; LD, long day; SD, short day; TLC, thin-layer chromatography.

stem tip of a monoecious cucumber increased the male tendency (*13*). Various studies showed that the exact location of the bud on the main stem of the monoecious cucumber influences its differentiation. The bud of a young plant develops in the axils of relatively mature leaves, while in the mature plant, a bud of the same ontogenetic stage develops in the axils of a younger leaf (i.e., nearer to the apex) (*2–4*).

We investigated in greater detail the interaction between sex expression and the endogenous contents of AbA and GA_3 of cucumber plants. We therefore measured their content in the shoot tips of various sex types, and at different sexual phases along the main stem. We also attempted to formulate a more general hypothesis for the role of growth substances in sex expression in cucumber and discuss its validity.

Materials and methods

Plant material

Stable and homogeneous sex types of cucumber (*Cucumis sativus* L.) were used throughout. The monoecious, gynoecious and heterozygous gynoecious types originated from the cv. 'Beit Alpha'; the andromonoecious and hermaphrodite types were derived from the cv. 'Richmond Green Apple'. Sex expression in cucumber is controlled by two alleles of two major genes (*14, 21, 22, 29*), thus the st^+ st^+ genotype is monoecious (i.e., st^+ st^+ *M M*) or andromonoecious (i.e., st^+ st^+ *m m*), and *st st* is gynoecious (i.e., *st st M M*) or hermaphrodite (i.e., *st st m m*).

Two plants were grown in each vermiculite-filled 20-cm pot in a greenhouse. Plants were irrigated alternately with a half-strength Hoagland solution and water. The average daily minimum and maximum temperatures were 15–30°C in the coolest month and 21–42°C in the hottest one. All experiments were performed under one of two light regimes: (1) short day–consisting of 8 hr light and 16 hr darkness; (2) long day–consisting of 16 hr light and 8 hr darkness. Natural light hours were supplemented with fluorescent illumination (Philips cool white, ca. 4500 lux at plant level).

AbA extractions and quantitative measurements

Extraction was carried out in dim light according to Milborrow's procedure (*23*) with some modifications (*10*).

TLC plates (Kieselgel F-254) were developed in *n*-butanol, *n*-propanol, 0.880 M ammonium hydroxide, water (2 : 6 : 1 : 2, v/v). Fifteen plates were developed in one tank together with one standard plate to which a drop of authentic AbA was applied. The Rf of the standard AbA was located by quenching on a fluorescent background under UV irradiation. The Kieselgel from that specific Rf zone was scraped off the TLC plate and extracted three times with methanol. The methanol eluate was collected after sedimentation of the Kieselgel and evaporated to dryness. The 'dry residue' was used for qualitative identification and quantitative measurements.

A methylated AbA derivative was prepared according to Shlenk and Gellerman (*30*), using diazald (N-methyl-N-nitroso-*p*-toluene sulfonamide). The reagent was added to the dry residue and the excess was evaporated 24 hr later. The methylated AbA was dissolved in hexane and 1–4 μl was injected into a Model 7400

Packard Gas Chromatograph equipped with a 1.8 m × 3 mm glass column. Operation conditions were based partially on those of Mizrahi et al. (*24*). The column was filled with a stationary phase of QF-1,5–1 % on 60–80 mesh gas chrom Q. Dried nitrogen was used as the carrier gas at a velocity of 30 ml min^{-1}. The temperatures of the input, the column and the detector were 265°C, 200°C and 200°C, respectively, and the injections were isothermic. The electrometer worked at a sensitivity of 10^{-10} amp. An electron-capture detector with a tritium foil was used with a minimum sensitivity of 10 picograms AbA. Quantitative measurement was performed by calculating the area under the AbA peak. A standard calibration curve was prepared for the quantitative dertermination of each experiment.

Determination of GA_3

GA_3 was extracted from the plant material and separated on TLC as described above for AbA. The AbA moved to Rf 0.7–0.8 and the GA_3 to Rf 0.5–0.6. The Kieselgel from Rf 0.5–0.6 was scraped off the TLC and handled as described above for AbA. The GA_3 was measured quantitatively by the barley test system (*20*) and GLC. The barley test was calibrated with authentic GA_3. For the GLC measurement, the methyl ester of the GA: was prepared, then a silylic ether was derived from it. The silylating agent was BSA (bistrimethylsilyl acetamide), which also served as an injection solvent. The silylated plant extract was identified by comparing its retention time with that of silylated authentic GA_3. The temperatures of the input, the column and the detector were 265°, 240° and 200°C, respectively. Other conditions were similar to those used for AbA measurements. In a few cases, the Kieselgel of the TLC plates was scraped from the regions corresponding to both GA_3 (Rf 0.5–0.6) and AbA (Rf 0.7–0.8). The two fractions were combined and further extracted, dried, methylated and silylated together. Silylation of ABA did not interfere with its measurements.

Results

AbA content of cucumber plants

Cucumber shoot tips, which included the apex, floral buds and young folded leaves, were collected for hormone extraction. The results of two experiments presented in Table 1 show that shoot tips of monoecious plants had a higher AbA content than those of gynoecious ones under both LD and SD conditions, and that SD conditions caused higher AbA content than LD conditions. Although both

Table 1 *Comparison of AbA contents of monoecious and gynoecious cucumber shoot tips under short- and long-day conditions*

Exp. no.	Dry weight (mg)	Short day		Long day	
		Monoecious	Gynoecious	Monoecious	Gynoecious
1	200	45.0	39.5	31.2	22.9
2	75	28.6	16.7	19.0	11.3

Shoot tips were collected from plants having two expanded leaves. AbA was extracted and separated as detailed in **Materials and methods.** Results are expressed as ng AbA per g dry weight.

Table 2 *AbA content in floral buds of cucumber shoot tips*

Floral bud diameter	Monoecious	Gynoecious
Small buds (0.5–1.0 mm)	206	125
Large buds (1.0–2.0 mm)	23	23

Floral buds (20–70 mg) from plants kept on SD were extracted by a short procedure (the methanolic extract was directly developed on the TLC plates) and measured by GLC. Results are expressed as ng AbA per g dry weight.

experiments show these basic trends, they differ in absolute AbA content. The difference in AbA content in Experiment No. 1 may have been caused by a difference in the water deficit of the plants, which is known to influence AbA content (*32*), although sampling was always performed 1 hr after irrigation. Also, the floral buds within the shoot tips may have been of developmental stages between the two experiments. Since the tested tips contained buds of different stages, it seemed important to find out whether the AbA content differed in buds of different age. The results, presented in Table 2, showed that the AbA content per unit dry

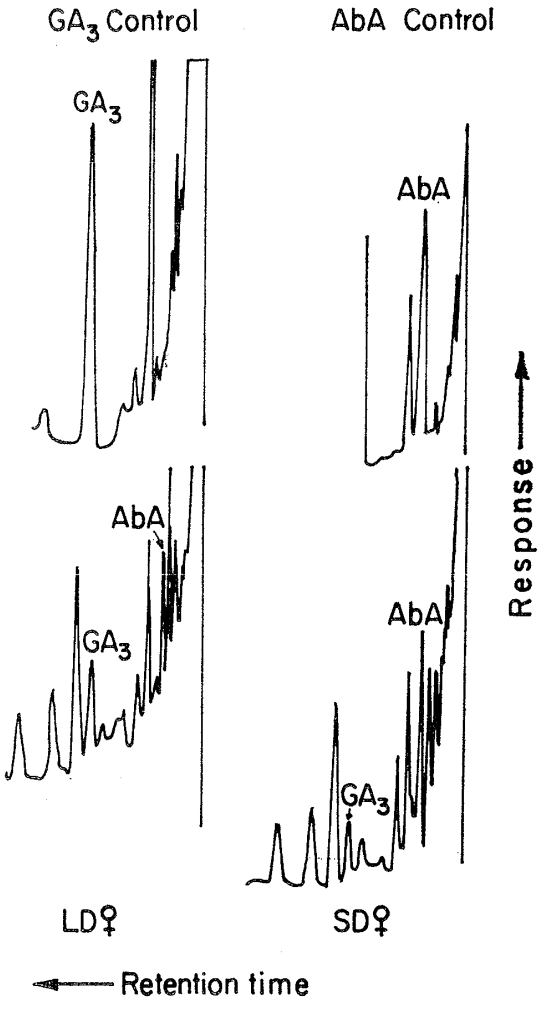

Fig. 1. *GLC measurement of AbA and GA_3 in extract of gynoecious cucumber plants.* The two upper graphs are GLC recordings of authentic AbA and GA_3 standards (5 ng and 50 ng, respectively); the two lower graphs are GLC records of plant extracts, which were prepared as described in Table 4. The temperatures of input, column, and detector were 265°, 240° and 200°C, respectively.

Table 3 *AbA content of various cucumber sex types*

Sex type	AbA
Monoecious	12.3
Heterozygous	10.3
Gynoecious	7.1

Exudates were collected, dried, extracted for AbA, and measured by GLC. Plants were grown under short-day conditions. Results are expressed as ng AbA per 100 ml exudate.

weight was 5- to 9-fold higher in young floral buds than in older ones, and higher in small floral buds of monoecious plants than in similar floral buds of gynoecious plants. These results indicate that small buds play a major role in the differences between AbA contents of tips, while the larger buds provide a uniform background which might blur such differences.

The AbA content of other sexual genotypes was also measured, either with shoot tip extracts or plant exudates. The exudate was collected from cut stems over 24 hr. The 'heterozygous gynoecious' sex type (st^+ *st M M*) is an F_1 product of a cross between the gynoecious and monoecious types. Table 3 shows that this sex type, which showed both monoecious and gynoecious elements in its flowering pattern, had an intermediate AbA content. The AbA contents of andromonoecious (st^+ st^+ *m m*) and hermaphrodite (*st st m m*) plants were respectively 3.4 and 13.5 ng per 1 g dry weight of shoot tips. This is the reverse of the relativd AbA content of the respective sex types which carry *M M* rather than *m m* genes.

Combined measurements of AbA and GA_3

Available information on the role of endogenous gibberellins in sexual regulation of the cucumber plant has been obtained mainly by bioassays. In the present study, GA_3 was measured quantitatively by GLC, and a method was developed to allow parallel determinations of GA_3 and AbA in the same sample.

Fig. 1 shows the GLC recordings of extracted plant material with authentic AbA and GA_3 standards. Table 4 shows the calculated values for the same plant material. These results indicate that the AbA content of leaves of gynoecious SD-treated plants was higher than that of the same genotype under LD. Fig. 1 also indicates that the GA_3 content is higher in leaves of LD-treated plants than in those

Table 4 *AbA and GA_3 contents of young fully extended leaves of gynoecious cucumbers*

Light regime	AbA	GA_3
Short day	160	600
Long day	55	1100

Leaves were collected from plants having four unfolded leaves. One gram of dried material was extracted, and developed with the first TLC mixture. Next the dried material was methylated and silylated before GLC measurement then identified according to the retention time of authentic standards. Results are expressed as ng AbA or GA_3 per 1 g dry material.

Table 5 *AbA and GA_3 in exudates of cucumber plants*

Sex type	AbA	GA_3
Monoecious	24.8	1300
Gynoecious	18.4	42

Exudates were collected from plants having four unfolded leaves which were grown under long-day conditions. The volume of the exudates was measured then they were dried. AbA and GA_3 were extracted together without TLC development. AbA was measured by GLC and GA_3 by the barley test. Results are expressed as ng AbA or GA_3 equivalents per 100 ml exudates.

from SD-treated ones. In another experiment, both growth substances were extracted and purified together from exudates of monoecious and gynoecious cucumbers. In this experiment, AbA was measured by GLC and GA_3 was estimated by the barley test (Table 5). These results agree with our above-mentioned data which show differences in AbA content between the two sex genotypes.

AbA and GA_3 extraction from shoot tips at different developmental stages

Younger floral buds contained more AbA content (Table 2); therefore, the higher AbA content of shoot tips of monoecious vs. gynoecious plants (Table 1) could reflect the relatively more advanced stage of floral buds in the latter (*15*). Thus,

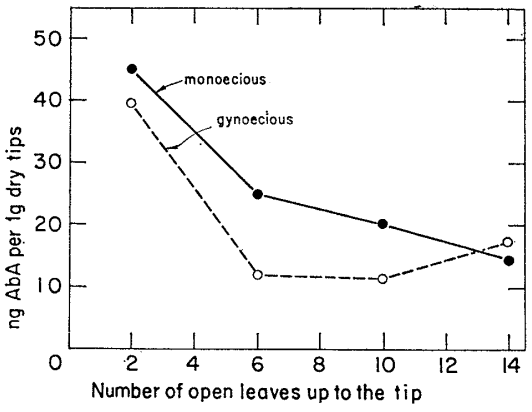

Fig. 2.

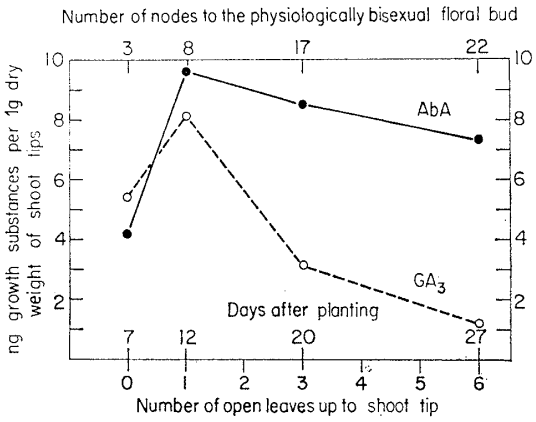

Fig. 3.

Fig. 2. *Changes of AbA content of shoot tips of monoecious and gynoecious plants during maturation.* Plants were sown under short-day conditions. The plants of each sex type were divided into four groups. The shoot tips of each group (120 mg dry weight) were collected at a specific plant age. The age was determined by the number of unfolded leaves. AbA was extracted as usual and measured by GLC.
Fig. 3. *Changes with age in AbA and GA_3 contents of shoot tips of monoecious plants.* Plants were sown under short-day conditions. Plants for each treatment were divided into four groups. The shoot tips of each group (650 mg dry weight) were collected at different ages, starting from the cotyledon stage. The age was determined by the number of unfolded leaves (stage zero designates spread cotyledons) and by the number of days after sowing. AbA and GA_3 were extracted from the shoot tips as usual and measured by GLC. The position of the physiologically bisexual stage of the floral buds was determined in control plants, which were sown seven days earlier and used as references; plants were dissected in order to determine the position of the floral bud which was at the morphologically bisexual stage.

Table 6 *AbA content of cucumber exudate from plants of different ages*

Sex type	Plant age	
	10 days	15 days
Monoecious	67.5	6.0
Gynoecious	20.2	4.0

Plants were grown under short-day conditions. One ml of exudate, collected with a pipette above the highest unfolded leaf, was frozen and dried immediately. The dry material was methylated and measured by GLC. Results are expressed as ng AbA per 100 ml exudate.

a gradual change in the AbA content during ontogenetic development is expected. To test this possibility, shoot tips were collected periodically from monoecious and gynoecious plants. Plant age was defined by the number of unfolded leaves. Fig. 2 shows that within a certain developmental range of 2–4 or 2–6 unfolded leaves in monoecious or gynoecious plants, respectively, there is a gradual reduction in the AbA content of the shoot tips. These results were supported by the determination of the AbA content of exudates at two different developmental stages (Table 6).

In another experiment, AbA and GA_3 contents of shoot tips were determined at four stages of plant maturation (Fig. 3), and correlated with the developmental stage of buds within the tip. The nodal site of the bisexual floral bud was recorded by dissecting monoecious plants at the following developmental stages: expanded cotyledons, one unfolded leaf, three unfolded leaves, and six unfolded leaves (i.e., 7, 12, 20 and 27 days after planting, respectively). Fig. 3 clearly shows that during plant ontogeny, the bisexual floral bud moved acropetally much 'faster' than the unfolding of the leaves.

The variation of growth substance content with age can be summarized thus: the content of both growth substances significantly increases until the 12th day then decreases from that stage on. This decrease is more pronounced for GA_3 than for AbA.

Discussion

The new findings of this study are based on the assumption that the relative concentrations of growth substances relevant to the differentiating process are those located in the vicinity of the differentiating buds. We found (Table 1) that high and low AbA levels in the shoot tips were correlated with male and female flower differentiation, respectively. The data fit the effect of applied AbA: increase of the 'node number' in the monoecious type (*11*). The shoot tips which were used contained buds and folded leaves of various sizes. The AbA content of small buds was more than five times that of large buds (Table 2). According to Atsmon and Galun (*3*), this means that monoecious shoot tips which contained the relatively small male buds had more AbA than gynoecious shoot tips which contained the relatively large female buds. The shoot tips which contain the differentiating floral buds seem to be effectively in contact with the stream of growth substances which, when tested as exudate, also showed a higher AbA level in the monoecious type than in the gynoecious one (Tables 5 and 6). On the other hand, the AbA

content of mature cucumber leaves, which was higher in gynoecious than in monoecious plants (*26*), does not seem to be related to sex determination of the differentiating buds. The AbA content of the 'bisexual' types (andromonoecious and hermaphrodite, i.e., having the *m m* genotype) showed the reverse relationship, when compared to the 'Beit Alpha' types (*M M* genotype). A different balance of growth substances in these types is probably the reason for their reversed AbA content. The change in AbA content as a result of light regime seems irrelevant to sexuality, and may be result from overall differences in the balance of growth substances of these plants (Table 1). High AbA contents under short-day conditions have also been reported elsewhere with no connection to sex expression (*12*, *25*).

The different endogenous AbA contents (Tables 1–3, 5, 6) and ethylene evolution (*27*) in the above-mentioned sex types, and the opposite responses of these types to treatments with AbA and ethephon (*11*) may be explained by an optimum-curve hypothesis. This hypothesis is based on a scheme which was first presented by Thimann (*31*) to explain the role of growth substances in plant organ growth. According to this scheme, sex expression ('node number') may be considered a

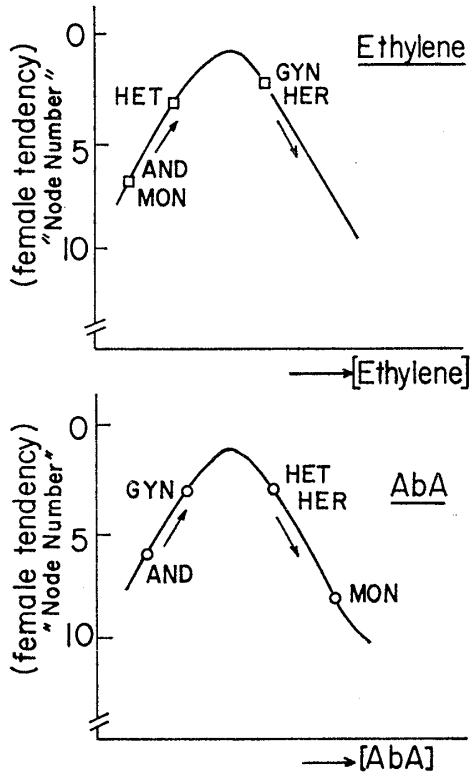

Fig. 4.

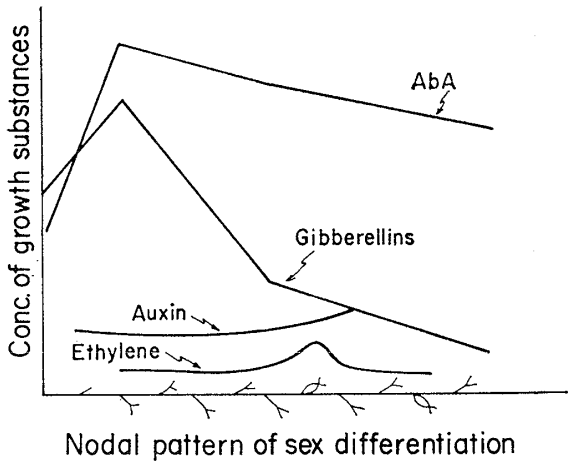

Fig. 5.

Fig. 4. *The optimum curve hypothesis*: *'node number' as a function of growth substance concentration.* MON—monoecious, GYN—gynoecious, HET—'heterozygous' (F_1: gynoecious × monoecious), AND—andromonoecious, HER—hermaphrodite.

Fig. 5. *Hypothetical scheme of the changes with age in the content of plant growth substances in shoot tips of monoecious cucumber plants.* The horizontal axis expresses a stem which first has (I) a phase of sterile nodes, then (II) a phase of male nodes, and finally (III) a mixed phase with both male and female nodes.

function of AbA and ethylene concentration and described by an optimum curve (Fig. 4). The location of each sex type on the curve is determined according to the growth substance content of its shoot tips and its response to exogenous treatments. Support for such a regulation hypothesis appears in other developmental systems: promotion and inhibition by AbA of lettuce root development, cucumber hypocotyl and root growth, and destruction of spinach chlorophyll in detached leaves (*1*).

A new quantitative procedure for simultaneously measuring AbA and GA_3 was developed and used to confirm previous results (Tables 4 and 5). This system has not yet been developed for gibberellins other than GA_3, which may, of course, take part in regulating sex expression. Previous studies by others include combined GLC measurements of growth substances, such as IAA, AbA and gibberellins (*8*), AbA and phaseic acid (*17*); but we are not aware of any report on combined measurement of GA_3 and AbA in the small quantities (20–40 nanograms) measured by us. Whether or not the GA_3 GLC peak contains other substances is still an open question. Low gibberellin content in the gynoecious type under short-day conditions (Tables 4 and 5) seems to be correlated with a marked response to external addition of gibberellin: a significant change in the 'node number' has been recorded (*11*, Galun and Lang, unpublished). On the other hand, in plants having a high gibberellin content, addition of this growth substance caused only a small change in sex expression. These data suggest that the 'node number' as a function of endogenous gibberellin concentrations may be expressed as a saturation curve. Other support for this hypothesis stems from the observation that AbA inhibited the gibberellin effect on 'node number' in the gynoecious but not the monoecious type, presumably because the latter type is saturated with gibberellins (*11*).

The findings concerning the differences in AbA and GA_3 contents in the shoot tips of gynoecious and monoecious cucumber plants suggested that the contents of the growth substances may change during development. Fig. 2 and 3 and Table 6 show that the decrease in the AbA and GA_3 contents with age in the monoecious type is correlated with the increase of the female tendency. The first female flower, which appeared approximately in the 15th node, differentiated when the plant had 7–9 unfolded leaves. On the other hand, the gradual change in AbA content in the gynoecious type may be correlated with flower development (Fig. 2 and Table 6). In this type, the first female flower buds appeared in the 2nd or 3rd node, but the first flower which reached anthesis did not develop before the 9th node, because preceding buds degenerated. One may assume, therefore, that the optimal level of AbA for female differentiation in 'node numbers' 2–3 was suboptimal for their development into mature flowers. This level might of course change in each developmental stage. The development from the cotyledonary phase to the first unfolded leaf, which was accompanied by differentiation of the first male buds in 'node numbers' 3–5, was correlated (Fig. 3) with a sharp increase in the GA_3 and AbA contents. This increase is apparently one of several factors which induce reproduction.

The above-mentioned data may contribute to understanding the general balance of growth substances in the monoecious plant. Rudich et al. (*27, 28*) showed an increase in ethylene evolution with age in monoecious cucumber tips. The auxin content of the plant, which is generally correlated with that of ethylene (*6*), showed a qualitatively similar increase in monoecious cucumbers (*16*). Measurements of

gibberellin content showed an increase in GA_3 content until about 10 days after germination and then stabilization at a lower level (*18*, *19*). Although these data were not based on tests of plant tips, they are similar to the GA_3 content found in the present study (Table 3). A scheme of the changes in the balance of growth substances with age is shown in Fig. 5. The combination of these and other growth substances which presumably affect bud differentiation in every node should be tested by simultaneous determinations with the shoot tips of the relevant sex types.

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