Gonadotropin Promotion of Adventitious Root Production on Cuttings of Begonia semperflorens and Vitis vinifera[1]

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Abstract. **Adventitious rooting of *Begonia semperflorens* cv. Indian Maid and *Vitis vinifera* cv. Semillon stem cuttings was significantly promoted by human chorionic gonadotropin (HCG). Basal sections of HCG treated cuttings upon which promoted rooting took place had markedly less endogenous gibberellin (GA) activity than non-treated controls or apical sections of treated ones, while changes in auxin levels were not found. HCG also inhibited GA_3-induced reducing sugar release from embryoless barley endosperm halves. These findings are discussed in the light of a possible analogy to gonadotropin action in animal systems.**

Since in animal systems gonadotropic hormones of protein nature are known to induce steroid production, a hypothesis is presented that in plants certain morphogenetic processes, including rooting, may manifest a similar hormonal sequence. In a previous paper Leshem (14) has shown that certain steroids and the human chorionic gonadotropic hormone (HCG) have promotive effects on rooting and flowering of curd cuttings of broccoli-*Brassica oleracea* L. var. cymosa, and that the effects obtained were possibly via a steroid pathway. Since both steroids and gibberellins may be formed from a common precursor, mevalonic acid (2,7), their production may possibly be controlled by a similar triggering mechanism which could be a plant factor analogous to gonadotropin. The aim of the present study is to assess the effect of HCG on adventitious root development and its possible interaction, in the light of the above hypothesis, with endogenous levels of gibberellins and auxins which are known to be involved in rooting phenomena (1, 21, 23, 25, 27). Since *Brassica oleracea* cuttings have been shown to be atypical in response to the application of naphthalene acetic acid (9), the experimental plants selected for this study were *Begonia semperflorens* and *Vitis vinifera*.

Materials and Methods

In the first experiment, uniform ± 15 cm cuttings of *Begonia semperflorens* of the Indian Maid cultivar were taken and all flowers removed on 1.2.67. Each cutting was placed in a 50 ml Ehrlen Meyer flask containing Hoagland's nutrient medium aseptically prepared. Micro-organism development was checked by blackening the walls of the flask, frequent change and airing of solutions and addition of chloramphenicol to the solutions at 20 μg/ml, a concentration which has been shown to effectively inhibit bacterial growth without any known effect on plant metabolism (10, 28). The flasks were placed in a glass house bench and rooting temperatures were kept at a constant 18° (± 1.5°), by means of thermostatically controlled heating coils in the bench table. Temperature and photo-periodic conditions at the experimental location, Beit Dagan, Israel, have been described elsewhere (15). The HCG used was obtained from Ikapharm Company, Israel. No measurable pH change was experienced due to addition of HCG to nutrient solutions. Biological activity of the HCG was determined according to Loraine and Brown (16) and expressed as International Units (IU)/liter.

Each treatment was comprised of 10 cuttings. Experimental duration was 35 days, after which each plant was separately sheared of roots formed and dry weight root production of each determined. Preliminary trials indicated a significant correlation between root number and dry weight root production per cutting. Statistical analysis of rooting data was in this experiment conducted by the "Kruskall-Wallis one way analysis of variance" as described by Siegel (19).

A second rooting study on *Begonia* was commenced on 21.4.67., using the accepted horticultural practice for hormonal treatment for rooting whereby basal ends of the cuttings are immersed in test solutions for 24 hours. Controls were immersed in

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identical solutions without the hormone. After this initial absorption period, all cuttings were transferred to nutrient solutions in Ehrlen Meyer flasks and grown for 39 days under the same conditions as in experiment 1. At the end of the experimental period the number of roots per cutting was counted.

In a third experiment conducted on *Vitis vinifera* cv. Semillon cuttings the HCG application was also by means of an initial 24 hour immersion of cutting bases in test solutions. Cuttings were taken on 24.3.67. from the Mikveh Israel Agricultural High School vineyards according to the accepted practice for vegetative vine propagation, *i.e.* ± 25 cm long, somewhat lignified, including 3 nodes and, unlike *Begonia*, did not include stem tips. After this immersion period cuttings were vertically embedded in sterile river sand and kept at 18° ± 1½° for a period of 7 weeks, after which the number of roots per cutting was counted. Statistical analyses of root number data were according to Quenouille's test of monotonic association (18).

In order to gain further insight on the line of events leading to the HCG effect, a series of bio-assays was conducted, whereby HCG at different concentrations was added to barley endosperm halves before incubation with 5 ppm GA_3. Results obtained from this experiment could possibly indicate how HCG affects the GA activated endosperm hydrolysis. Parallel controls with the same HCG concentrations without GA were also run in order to determine any hydrolytic activity of the HCG itself. In all experimental work on barley endosperm the HCG used was in purified form prepared by the Tel-Hashomer Institute of Endocrinology, and contained no additives.

In the experiment dealing with determination of endogenous levels of growth substances, *Begonia* plants were grown, as in experiment 1, for a period of 23 days, when root primordia were macroscopically discernible but elongation had not yet occurred, *i.e.* a period of 12 days elapsed between macroscopic appearance of primordia and their subsequent elongation. In this experiment only control and HCG treatments were included. Upon termination, cuttings were immediately plunged into liquid air, divided into basal and apical halves, ground into a fine powder with mortar and pestle and lyophilized. Division into basal and apical halves was considered necessary since Vardjan and Nitsch (26) have shown that apical and basal sections of *Cichorium* cuttings may vary greatly in auxin and kinin content during the process of regeneration. Subsequent determinations of growth substances were individually made upon 250 mg samples of lyophilized material.

Possible sources of error could be reducing substances originally present in the extract, or the gibberellinic-like activity of ethyl acetate residues (4). Thus chromatographic sections of plant extracts were checked for reducing substances in the former case by leaving barley halves out, and in the latter by chromatographing ethyl acetate without plant extracts and determining activity by the usual procedure, including endosperm. All data obtained were accordingly corrected.

Auxin activity was determined by ether extraction as described by Larsen (13) and chromatography and bio-assay by the Wheat Straight Growth Test with the modifications of Sirois (20). Gibberellin (GA) activity was determined by the Barley Endosperm Test according to the procedure of Coombe *et al.* (5, 6). Statistical analyses of chromatography and bio-assay data were according to the "Range of Sample Test" of Kurtz *et al.* as described by Snedecor (22).

Results

A) *Rooting Studies.* Table I presents rooting responses expressed as mg dry weight per plant of the first experiment in which treated plants grew in nutrient solutions containing various concentrations of HCG, while control plants grew in nutrient solution alone.

Table I. *Dry Weight Root Production of* Begonia semperflorens *Growing in Nutrient Solutions With and Without HCG*

mg/Cutting – mean of 10 plants.

Treatment	Root production	Statistical significance at $p<0.05$[1]
	mg/dry wt	
Control	9.1	a
HCG 500 I.U./l	54.4	b
HCG 750 I.U./l	56.0	b
HCG 1000 I.U./l	49.4	b

[1] Calculated by "Kruskall-Wallis one way analysis of variance" (22). Different letters in column indicate statistically significant differences. Identical letters indicate no statistically significant differences.

From this table it can be seen that HCG has a clear promotory effect—dry weight root production was about 6 times more than control. In this

Table II. *Number of Roots Produced on Cuttings of* Begonia semperflorens *Immersed for 24 Hours in Solutions With and Without HCG and Subsequently Grown on Nutrient Solutions From 11.4 to 19.5.67*

The data are the means of 10 plants.

Treatment	No. of roots/cutting
Control	9.0
HCG 750 I.U./l	14.0
HCG 1500 I.U./l	14.5
HCG 3000 I.U./l	18.1
Level of significance (p)[1]	< 0.05

[1] According to an adapted "test of monotonic association" for one tailed alternatives (18).

Table III. *Number of Roots Produced in Cuttings of* Vitis vinifera *Immersed for 24 Hours in Solutions With and Without HCG and Subsequently Rooted in Sterile River Sand from 24.3 to 5.5.67*

The data are the means of 10 plants.

Treatment	No. of roots/cutting
Control	28.9
HCG 500 I.U./l	32.5
HCG 1500 I.U./l	42.6
HCG 3000 I.U./l	44.6
Level of significance	< 0.05

[1] According to an adapted "test of monotonic association" for one tailed alternatives (18).

experiment no significant differences were found between the various HCG concentrations.

The results of the second experiment with *Begonias* in which HCG was applied by a 24 hour immersion and subsequent growth in nutrient solutions not containing hormone are shown in table II. From this table it can be seen that the number of roots produced by HCG treated cuttings was significantly higher than control and that the root number increases with concentration of HCG.

Table III represents results of the experiment with *Vitis vinifera*. Here again HCG treatment causes a statistically significant increase in root number above controls. Root number per cutting increases with HCG concentration.

B) *Endogenous Growth Substance Determinations.* i) *Auxins.* Figure 1 shows results of auxinic activity of the various R_F sections of the chromatograms run with extracts of basal and apical halves of cuttings of *Begonia semperflorens* grown in nutrient solutions with and without HCG at a concentration of 750 I.U./l. It can be seen that, in general, auxin activity did not differ significantly in the various cutting sections and treatments and that the minor differences obtained were well below the $p<0.05$ difference level.

ii) *Gibberellins. Effect of HCG on GA_3-induced Barley Endosperm Hydrolysis.* Table IV shows a marked and significant HCG inhibition of GA_3-induced reducing sugar production in embryoless barley endosperm halves, the inhibition increasing progressively with increase of HCG concentration. (HCG controls were essentially similar to those obtained from incubation of endosperm halves with sterilized distilled water.)

Table IV. *HCG Inhibition of GA_3 Induced Reducing Sugar Production in Embryoless Barley Endosperm Halves*

Treatment	mg Reducing sugar/vial
GA_3 5 mg/l	4.20
HCG 500 I.U./l + GA_3 5 mg/l	3.62
HCG 750 I.U./l + GA_3 5 mg/l	3.15
HCG 1000 I.U./l + GA_3 5 mg/l	2.87
Level of significance (p)[1]	<0.05

[1] According to an adapted "test of monotonic association" for one tailed alternatives (18).

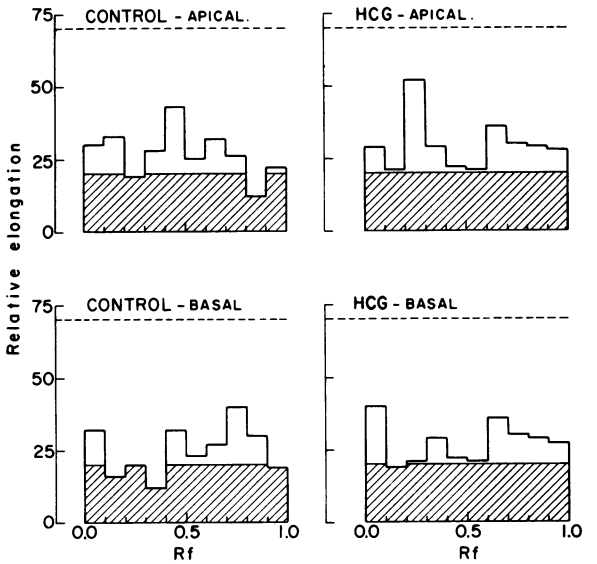

FIG. 1. Auxin-like activity of apical and basal halves of extracts of *Begonia semperflorens* cuttings growing in nutrient solutions, as shown by the Wheat Straight Growth Test. Left-control in nutrient solution. Right-nutrient solution also containing human chorionic gonadotropin (HCG) at a concentration of 750 I.U./l. Relative elongation of wheat coleoptiles incubated on sections of paper chromatograms. Shaded area indicates relative elongation under identical conditions without plant extracts. Broken line indicates level of significance at $p<0.05$.

Endogenous Levels of GA Activity in Various Sections of HCG Treated and Non-treated Begonia *Cuttings.* Figure 2 shows gibberellin-like activity of the various R_F sections of the chromatograms run with extracts of basal and apical halves of cuttings of *Begonia semperflorens* grown in nutrient solutions with and without HCG at a concentration of 750 I.U./l.

From this figure it can be seen that in control cuttings growing in nutrient solution alone the R_F zone 0.6 to 0.8 has marked gibberellin activity and, while occurring both in apical and basal cutting halves, in the latter it is much greater. The active substances in this zone may be related to gibberellins A4 and A7, which under identical chromatography conditions have similar R_F's (17). In HCG treated cuttings gibberellinic activity disappears from the basal halves of the cuttings, while it is considerably increased in the apical halves in the identical R_F zone—0.6 to 0.8—as the cuttings grown in nutrient solution alone. Some activity was also obtained in the R_F 0.0 to 0.1 zone.

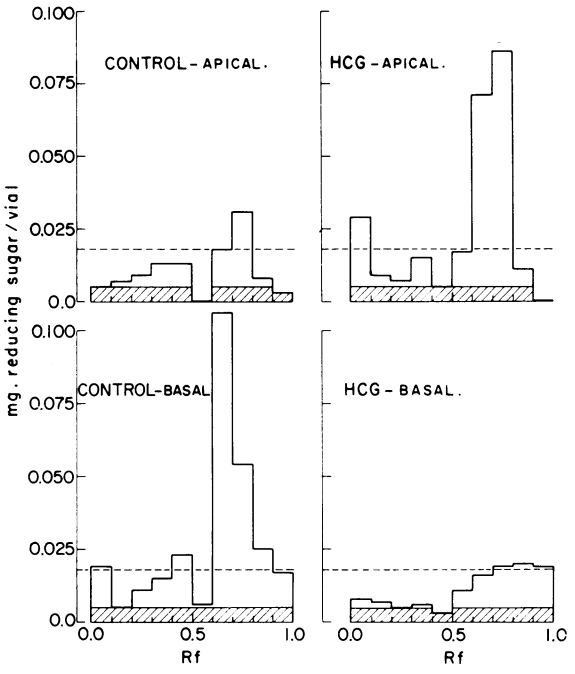

FIG. 2. Gibberellin-like activity of apical and basal halves of extracts of *Begonia semperflorens* growing in nutrient solutions, as shown by the Barley Endosperm Test. Left-control, in nutrient solution. Right-nutrient solution also containing human chorionic gonadotropin (HCG) at a concentration of 750 I.U./l. Mg reducing sugar per vial produced by barley endosperm incubated on sections of paper chromatograms. Shaded area indicates reducing sugar produced under identical conditions without plant extracts. Broken line indicates level of significance at $p<0.01$.

Discussion

From results in tables I, II, and III it can be seen that the gonadotropic hormone HCG has a marked and statistically significant promotory effect on adventitious root production of cuttings of both *Begonia* and *Vitis*. In this respect the response elicited by HCG was similar to that previously reported by Leshem (14) for *Brassica oleracea* L. var. cymosa. The promotory effect could be direct, the HCG acting as a plant hormone and performing the function of an endogenous hormone within plant tissues or, alternatively, via regulation of equilibrium between known phyto-hormones active in rooting, *e.g.* the auxin-kinetin equilibrium reported by Skoog and Miller (21), and this in turn possibly regulated by GA effects on auxin levels (11, 12).

The effect of HCG on endogenous levels of auxin and gibberellin activity is represented in figures 1 and 2 and table IV respectively. It can be seen that, while no changes in auxin levels were detected (fig 1), a marked change in gibberellin-like activity occurred (fig 2 and table IV). HCG application causes a significant decrease in the endogenous level of gibberellin in basal sections of cuttings and an increase in apical halves, *i.e.* an apparent depletion or transfer from the basal half upon which enhanced rooting occurred, and an accumulation in the apical zone. Furthermore, the results presented in table IV may corroborate the GA depletion or inactivation noted in the HCG treated cuttings.

This table shows that GA_3-induced hydrolysis of barley endosperm is checked with an increase of HCG concentration. These findings may indicate that gonadotropin promotion may be primarily by means of lowering endogenous gibberellin levels or by inhibiting biological activity of GA already present.

While promoting effects of GA on main root development have been reported (23, 25), it has been found by Brian, Hemming, and Lowe (3), Fernqvist (8) and Stuart and Cathey (24) that this hormone considerably depresses lateral root initiation. The results here obtained indicate that adventitious rooting in the *Begonia* resembles the latter response. Bastin (1), in rooting studies of balsam, *Impatiens balsamina*, has proposed that the mechanism of GA effect does not involve inhibitors of IAA-oxidase but the synthesis of auxin. The effect of GA depletion or inactivation on auxin-like activity was not found here, but it is possible that changes, not detected by procedures employed, of auxin precursors such as tryptophan or anthranilonitrile (29) occurred. These changes could have altered the auxin/kinetin equilibrium (21) and mediate the enhanced rooting obtained 12 days later (table I), during which time precursors could have formed auxin, and discernible root primordia produce roots.

The results of this study seem to indicate that endogenous GA production does not follow the hormonal pathway known in animal systems, whereby gonadotropin triggers increased steroid hormone production. Since in plant tissues both steroids and GA are present and both are formed via different metabolic pathways from a common precursor, mevalonic acid (2, 7), it is possible that gonadotropin in plant, as in animal systems, caused increased utilization of this precursor in the direction of steroids, and this at the expense of lowered mevalonic acid conversion to diterpenes of which GA is a derivative (7).

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