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Changes in Responsiveness to Ethylene and Gibberellin during Corolla Expansion of *Ipomoea nil*

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Abstract: Corolla expansion in *Ipomoea nil* appears to be triggered by changes in gibberellin concentration and ethylene production during development. We investigated the role of responsiveness to GA and ethylene in corolla expansion. The effects of growth regulators applied in vitro were measured as a change in area of corolla segments from younger (15–17 mm) and older (18–20 mm) whole corollas. Applied gibberellic acid (GA_3) significantly ($p < 0.05$) promoted growth in the younger segments but was less effective in the older segments. Moreover, applications of the GA biosynthesis inhibitors, PP333 (paclobutrazol) AMO_{1618} (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride), chlorocholine chloride, and tetcyclasis had little effect on younger segments but inhibited growth of older segments. The older corollas have apparently synthesized and accumulated enough GA-like substances to become less responsive to additional applied GA_3. The amount of growth induced by applied or endogenous GA depended on the amount of ethylene simultaneously produced in the tissue. The younger corollas rapidly produced ethylene from endogenous 1-aminocyclopropane-1-carboxylic acid (ACC) and did not respond to applied ACC whereas the older corollas naturally produced much less ethylene and were significantly ($p < 0.05$) inhibited by applied ACC. When ethylene production was inhibited by applying aminoethoxyvinylglycine (AVG), growth was promoted in all segments. However, only the growth of the younger segments was further stimulated by simultaneously applied AVG and GA_3 over the GA_3 control. Thus the differential responses of segments from 15- to 20-mm long corollas to applied growth regulators reflect developmental changes in re-

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sponsiveness of the developing corolla. The change in responsiveness is attributed in part to the changes in production of endogenous growth regulators and to the effect of one endogenous plant growth regulator (PGR) on the responsiveness of the corolla to another PGR.

As the investigation of growth regulation in plants continues, alterations are being made on the original hormone concept (Went and Thimann 1937). Plant hormones have been renamed plant growth regulators (PGRs), because they often regulate growth in the same tissue in which they are synthesized. The origin of each PGR is not always limited to one tissue, making the regulation of its presence in responding tissues difficult to interpret. In many plant systems, PGRs applied in vitro have been effective over a 400-fold range in concentration, whereas the change in endogenous PGR concentration that elicited a response in vivo was only 10- to 100-fold. In fact, a PGR (at least in some cases) may be essential for growth, yet its concentration in a responding tissue may not change in a correlative manner during development. To explain this, Trewavas (1982a) proposed that plant tissues respond to PGRs according to the concentration of the PGR and the cell sensitivity to PGRs. Reanalyzing previous reports of plant responses to PGRs, Trewavas cited examples of what appear to be changes in tissue sensitivity (Barlow et al. 1957, Wright 1961, 1966, McGlasson et al. 1978, Trewavas 1982a).

Relatively few studies have thoroughly examined the role of PGRs in corolla growth (see Koning 1984). We have reported that the change in the balance of the PGRs (GA-like substances and ethylene) in developing corollas (16–18 mm in length) signaled the onset of expansion (Raab and Koning 1987). The present investigation examines whether the changing balance of the two PGRs is accompanied by a change in responsiveness to gibberellin (GA) and ethylene.

Materials and Methods

Plant Culture

Ipomoea nil (L.) Roth cv. Scarlett O'Hara (priority over the synonym, *Pharbitis nil*) seeds were a gift from W.A. Burpee Seed Co., Warminster, PA, USA. They were cultured as described previously (Raab and Koning 1987). The emerging seedlings were induced to flower under ambient, short-day conditions (less than 10 h). Thereafter flowering and vegetative growth were maintained under longer photoperiods (12 h). Unopened floral buds were picked and sorted according to corolla length. Plant growth regulators were applied to segments excised from whole corollas 15–20 mm in length. In this paper the stages of flower development are defined by the length of the corolla: "stage 15" refers to floral buds with 15-mm long corollas, etc.

Growth Substance Applications In Vitro

Within each bud stage, basal cylindrical corolla segments (having the same diameter) were excised and cut to equal lengths (Raab and Koning 1987).

Among stages, the number of epidermal cells along the length of each segment was kept constant by altering the segment length. Segments from corollas 15–17; 18–19; and 20-mm long were cut to 3, 3.5, and 4 mm, respectively. In this way, the same group of cells was consistently studied throughout bud development. Ten segments from buds of the same initial length were randomly distributed to each 10-cm Petri dish. Each dish contained a 9-cm filter paper disk and 6 ml of a 0.1 M sucrose solution (as a substrate for growth) containing a known concentration of the growth substance(s) to be tested. Sucrose, gibberellic acid (GA_3), chlorocholine chloride, and 1-aminocyclopropane-1-carboxylic acid (ACC) were obtained from Sigma (St. Louis, Mo, USA) and AMO_{1618} (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride) was acquired from Polysciences (Warrington, PA, USA). The following were gifts: aminoethoxyvinylglycine (AVG) (Maag Agrochemical Co., Vero Beach, FL, USA), paclobutrazol (PP333) (ICI Americas Inc., Goldsboro, NC, USA), and tetcyclasis (BASF, Parsippany, NJ, USA). The segments were incubated in the dark at 30°C for 96 h. The length and diameter of each segment were measured with a Finescale Comparator to within 0.1 mm (Finescale Tools, Orange, CA, USA). The average surface area of the abaxial epidermis was calculated for each treatment and compared to both the control treatments and the other corolla stages. Means were evaluated by one-way ANOVA and Duncan's New Multiple-Range Test.

Endogenous Growth Regulator Analyses

Ethylene production was measured by placing segments of a specific stage and treatment or freshly harvested whole corollas in a suitable septum vial, withdrawing 2 ml of atmosphere after a 15-min collection interval, and injecting it into a Varian 3700 gas chromatograph (Walnut Creek, CA, USA) fitted with a Porapak Q column and a flame ionization detector (Raab and Koning 1987). The amount of ethylene was calculated and expressed as $nl \cdot g\ FW^{-1} \cdot 15\ min^{-1}$.

Results

Corolla Segment Growth

Ipomoea nil corolla segments expand over a 2-day period in vivo and are fully opened by dawn (5–6 AM) of the third day (Fig. 1).

Response to Gibberellin

As we previously reported (Raab and Koning 1987), endogenous GA equivalents (ng/g FW) increased 100-fold (0.27 to 24.27 ng/g FW) in *Ipomoea nil* co-

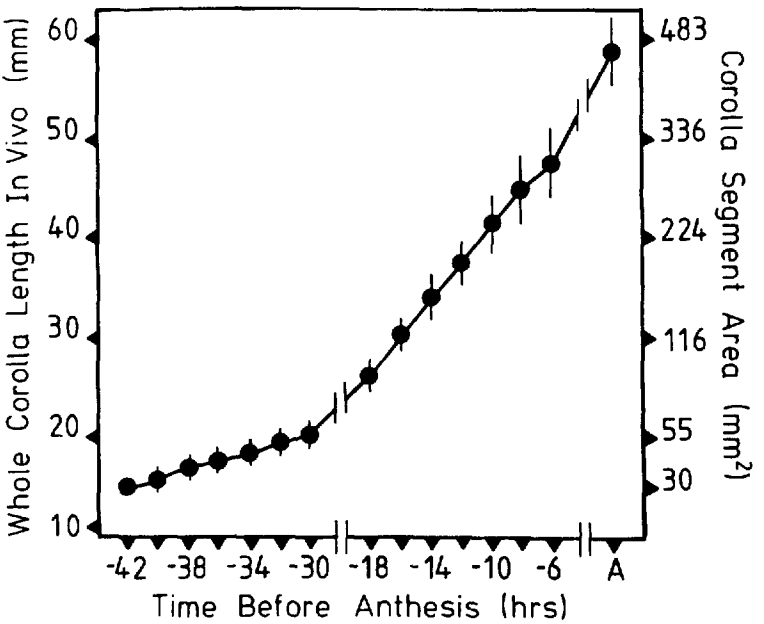

Fig. 1. The elongation of the whole corolla of *Ipomoea nil* and the expansion of the corresponding tubular corolla segment as the flower approaches anthesis (A) in vivo. The tubular segments were excised at the base of the corolla folds and just above the insertion point for the epipetalous stamens. The area of the abaxial epidermis was determined by multiplying the length by the circumference of the segment. Based on regression analysis, the growth rate (±95% confidence interval) of the corolla increased from 0.43 ± 0.04 mm/h in stages 15–20 (−48 to −30 h preanthesis) to 1.81 ± 0.16 mm/h in stages 26–48 (−18 to −6 h preanthesis).

rollas from stage 15 to stage 18–20. These stages are separated by less than 10 h during normal development in vivo (Fig. 1). Applied GA_3 decreased ethylene production of stage 16–20 corolla segments (Table 1). Applied GA_3 promoted corolla segment growth as compared with the water and sucrose controls, particularly in stage 15–18 segments (Raab and Koning 1987). Applied GA_3 was most effective on stage 16–17 segments, stimulating growth fivefold. Statistical analysis indicated that by stage 20, GA_3 did not significantly ($p > 0.1$) promote growth (Raab and Koning 1987) (Fig. 2). Applied PP333, a GA biosynthesis inhibitor, inhibited the stage 18–20 segments as compared to sucrose controls; simultaneously applied GA_3 negated its effect (Figs. 2, 3, 4). Younger (stage 15–17) corolla segments were not affected by application of PP333 (Fig. 2). AMO_{1618}, chlorocholine chloride, and tetcyclasis had similar effects (data not shown).

Response to Ethylene

Ethylene production (nl/g FW) was relatively high in stage 15 whole corollas but declined more than twofold as the corollas developed to stage 20 (Raab and Koning 1987).

Table 1. Ethylene production (nl · g FW^{-1} · 15 min^{-1}) from stage 15–20 segments treated for 96 h with sucrose (Suc) alone and in the presence of GA_3, AVG, or GA_3 + AVG[a]

Treatment	Corolla length (mm)					
	15	16	17	18	19	20
0.1 M Suc	34.9	34.7	13.4	12.4	20.6	16.9
10^{-4} GA_3	46.4	25.4	11.3	8.4	2.5	0.5
10^{-5} M AVG	6.3	2.8	1.3	0.9	0.6	ND
10^{-4} M GA_3 + 10^{-5} M AVG	10.8	1.3	1.1	0.4	0.8	1.1

[a] Ten segments were pooled for each stage and analyzed as a group. Ethylene production was measured after a 15-min collection interval using a gas chromatograph fitted with a flame ionization detector. Each experiment was repeated twice, and the results shown here represent the mean production rates.

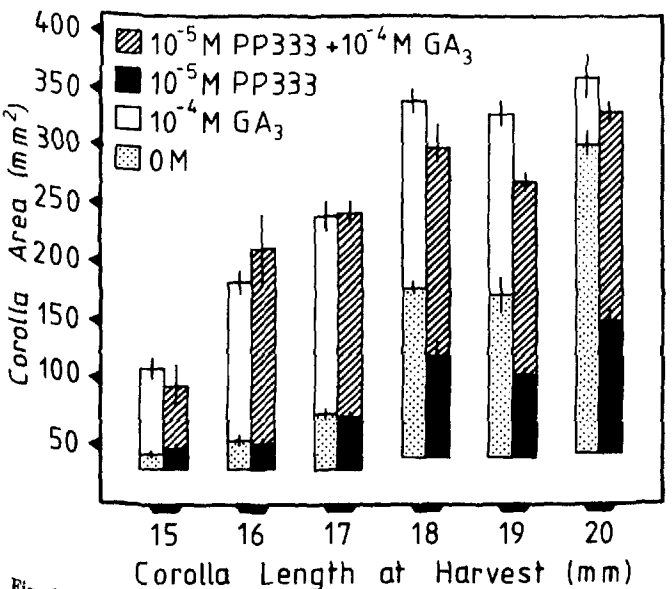

Fig. 2. Responsiveness of stage 15–20 corolla segments to PP333, a GA biosynthesis inhibitor. The surface area of the corolla segments was measured before and after treatment with GA_3, PP333, or PP333 + GA_3 in vitro. All treatments contained 0.1 M sucrose. The lower and upper ends of the bar for each treatment represent the initial (before treatment) and final (after treatment) segment areas, respectively. Vertical lines represent ± SE.

The ethylene precursor, ACC, applied alone had no significant effect ($p > 0.1$) on younger segments but strongly inhibited the effects of simultaneously applied GA_3 (Fig. 3). Older corolla segments, however, were greatly inhibited by applied ACC alone (Fig. 3). Simultaneously applied GA_3 partially relieved this inhibition in older corollas.

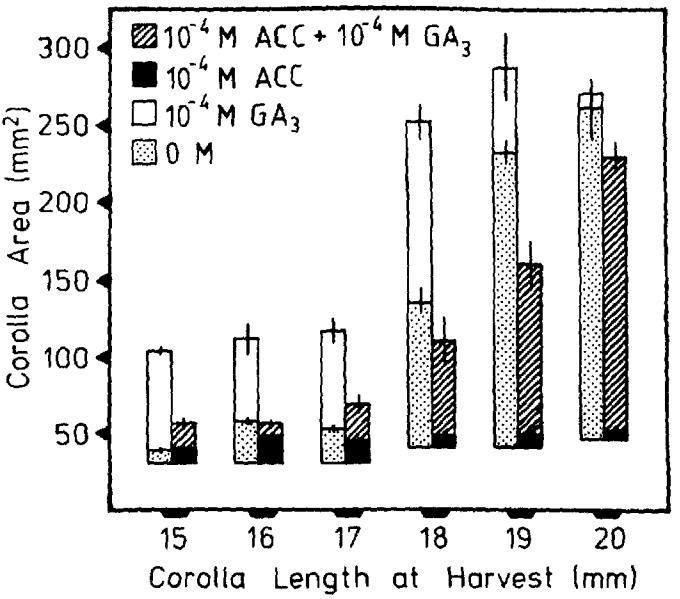

Fig. 3. Responsiveness of stage 15–20 corolla segments to ACC, the precursor to ethylene. The surface area of the corolla segments was measured before and after treatment with GA_3, ACC, or ACC + GA_3 in vitro. All treatments contained 0.1 M sucrose as substrate. The lower and upper ends of the bar for each treatment represent the initial (before treatment) and final (after treatment) segment areas, respectively. Vertical lines represent ± SE.

The ethylene biosynthesis inhibitor, AVG, stimulated growth not only in the younger segments but also in the older corolla stages (Fig. 4). Ethylene production from stage 15–20 corolla segments treated in vitro with 0.1 M sucrose (Table 1) was much greater than that from whole corollas in vivo (Raab and Koning 1987). However, ethylene production from both segments in vitro (Table 1) and whole corollas in vivo declined during growth (Raab and Koning 1987). Applied AVG reduced ethylene production of segments in all stages and thus promoted some growth even in stage 19–20 segments (Fig. 4). In the presence of simultaneously applied GA_3, AVG still reduced ethylene production in all stages (Table 1), but growth was promoted only in the younger stage 15–17 segments (Fig. 4). Similar results were observed with cobalt chloride (10^{-6} to 10^{-3} M) treatment (data not shown).

Discussion

The role of growth regulators in plant development is a controversial subject. Theories for the role of PGRs include the traditional direct growth regulator (Went and Thimann 1937), a nonspecific Ca^{+}/K^{+} trigger (Trewavas 1982a), a nonlimiting but essential factor (thus a nonregulating factor) (Trewavas 1982a), or a nonessential factor (Reeve and Crozier 1980). If PGRs are indeed involved with growth in vivo, three mechanisms for growth regulation could be (1)

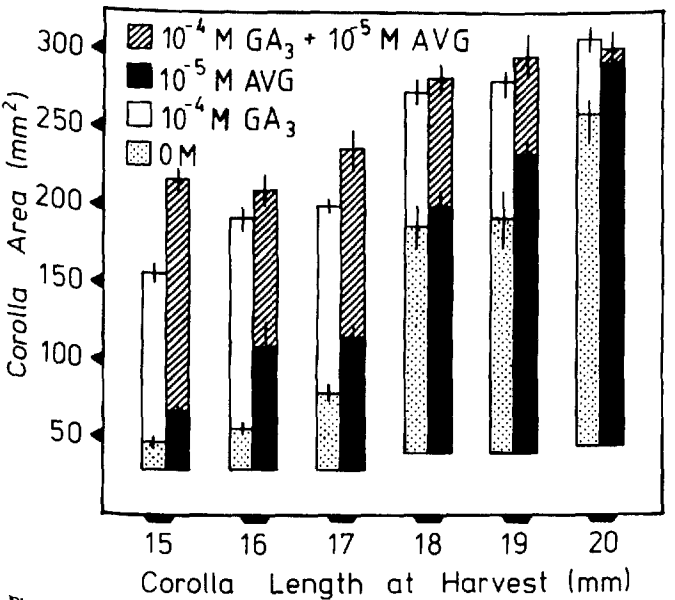

Fig. 4. Responsiveness of stage 15–20 segments to AVG, the ethylene biosynthesis inhibitor. The surface area of the corolla segments was measured before and after treatment with GA_3, AVG, or AVG + GA_3 in vitro. All treatments contained 0.1 M sucrose as substrate. The lower and upper ends of the bar for each treatment represent the initial (before treatment) and final (after treatment) segment areas, respectively. Vertical lines represent ±SE.

change in the concentration of the PGR delivered to responding cells, (2) change in the sensitivity of responding cells by changes in the cell receptor sites, and (3) change in the cells' ability to respond to the PGR-receptor complex:

1. The concentration of PGRs has been measured in responding tissues for many years; most investigations were unable to correlate changes in PGR concentrations with parallel or inverse variation in growth (Trewavas 1982a).

2. Trewavas (1982a) proposed that the response of cells to PGRs critically depends on the number of cell receptors for the PGRs, which he called sensitivity. Firn (1986) has added that changes in sensitivity could also be regulated by changes in receptor affinity for PGRs. In attempting to clarify the language of growth substance mechanisms, he rejected the term "sensitivity." Our ideas of appropriate terminology have been developing simultaneously (Raab and Koning 1985, 1987) and in our opinion "sensitivity" should be synonymous with Firn's term "receptivity" and should be limited to discussions involving enzyme-type kinetic analyses (Salisbury et al. 1986, and personal communication) of receptors (V_{max} changes = changes in number of receptor sites; K_m changes = changes in receptor affinity). To date, very few studies have addressed the question of changes in the number of PGR binding sites (Ray 1977, Ray and Dohrmann 1977, Walton and Ray 1981, Vesper 1986) but instead have measured changes in the final response of plant tissues (Wright 1961, 1966, Barlow et al. 1957, McGlasson et al. 1978, Trewavas 1982a, among others).

3. In this paper we emphasize that the final plant response may depend not

only on PGR concentrations and adequate sensitivity but also on the ability of the cells to respond to the PGR-receptor complex. The latter may also limit growth and may change in a developmentally significant manner. These changes in response capacity to one PGR may be influenced by responses to other PGRs. The changing ability of a cell to respond may depend on transcriptional, translational, or posttranslational changes including those involved in the production of enzymes and structural proteins important for cell wall growth. In this paper we use the term "responsiveness" to describe developmental changes in the response of tissues to PGRs. This integrates the components of sensitivity (receptivity) and ability to respond (response capacity), but cannot distinguish between them.

Response to Gibberellin

We have proposed that corolla growth is regulated by a balance of a growth promoter, GA, and a growth inhibitor, ethylene (Raab and Koning 1987). Increase in the concentration of GA-like substances and a concomitant decrease in ethylene production comprise a signal (Raab and Koning 1987) for the corolla to fully expand (Fig. 1). The increases in GAs were found almost entirely in fractions corresponding to retention times for nonhydroxylated, relatively nonpolar GAs (Raab and Koning 1987). We are currently collaborating with R. Pharis to rigorously identify the specific GAs present in stage 18–20 corollas. The increase in GA-like substances may be involved in the decline of ethylene production (Table 1). GA treatments in vitro reduced ethylene production as compared to sucrose-treated control segments (stages 16–20). This has also been observed in *Ipomoea nil* filaments (Raab and Koning 1987). Further investigations must determine the apparent mechanism of the interaction between GA and ethylene biosynthesis.

Based on these findings, stage 15–20 corolla segments were examined for changes in response to GA and ethylene. Corolla segments from all stages of bud development grew minimally in water (Raab and Koning 1987). Sucrose (0.1 M) stimulated growth in vitro primarily in older segments and applied GA_3 stimulated growth primarily in younger segments (Raab and Koning 1987). Endogenous levels of GA-like substances in the corollas were significantly increased by stages 18–20 (Raab and Koning 1987). The older segments were less responsive to applied GA_3, indicating saturation with endogenous GA-like substances (Raab and Koning 1987). Further supporting this hypothesis, GA biosynthesis inhibitors had no effect on younger segments but reduced growth twofold in older (stage 18–20) segments (Fig. 2). Moreover, the presence of these inhibitors restored responsiveness to applied GA_3 in stage 20 segments, which had been greatly reduced by endogenous GAs in the absence of the inhibitors. These data suggest that GA biosynthesis begins before the corolla reaches stage 19–20 but then saturates the response mechanism of the corolla to additional GAs (Raab and Koning 1987).

Response to Ethylene

Even though the younger segments responded to applied GA_3 in vitro, they did not achieve the same size as the older segments in vitro (Raab and Koning 1987). The younger segments were not as responsive to applied GA_3 as the older segments were to sucrose alone (which may be a function of endogenous GA). We propose that this can be partially explained by the inhibitory effects of rapid ethylene release from younger segments (Raab and Koning 1987). By the time the corolla developed to stage 18–20, the level of ethylene production dropped to significantly ($p < 0.05$) lower levels than at stage 15. Since applied ACC elicited a response only in older segments (Fig. 3), the endogeneous level of ethylene production in younger segments is apparently sufficient to eliminate the response to whatever endogenous GAs are present. Applied ACC, known to stimulate ethylene production in *Ipomoea nil* corollas (Raab and Koning 1987), inhibited both the effects of endogeneous GA (when present in stage 18–20 segments) and applied GA_3 (in both younger and older segments) (Fig. 3). The natural reduction in ethylene biosynthesis correlated with the corolla becoming more responsive to applied GA (Raab and Koning 1987). As stated above, the response to applied GA_3 in older segments was inversely related to the level of endogenous GAs already accumulated.

AVG effectively reduced the ethylene production rates of segments (Table 1) below the production rates of whole corollas (Raab and Koning 1987). Control segments in vitro produced much more ethylene (Table 1) than whole corollas in vivo (Raab and Koning 1987). These data indicate that wound ethylene is induced by the in vitro conditions of the 96-h experiments. Applied AVG stimulated growth in segments at all of the stages examined (Fig. 4). Even in the presence of simultaneously applied GA_3, AVG further reduced ethylene production in all but stage 20 segments (Table 1) but stimulated growth ($p < 0.05$) (Fig. 4) only in younger segments, which usually produce ethylene rapidly (Raab and Koning 1987).

Even though growth of the young segments was optimally induced by applying both a growth promoter (GA_3) and a synthesis inhibitor (AVG) of the growth inhibitor (ethylene), the segments still did not achieve the total size of sucrose-treated older segments even after 96 h in vitro (Fig. 4). In *Ipomoea nil*, the responsiveness to PGRs probably includes more than just interactions between the changing levels of the growth promoter and inhibitor. Perhaps "sensitivity," the affinity (Firn 1986) or the number of cell receptors (Trewavas 1982a), for one or both of these PGRs must change in the young stages before the corolla can maximally expand. Alternatively, differences in the ability of younger and older segments to expand may be caused by different spectra of nutrients supplied by the plant prior to excision, differential wounding during excision, and differences in the proportion of the segment immersed in the incubation medium. Yet the difference in size and the time between stage 15 and stage 20 segments are small (Fig. 1).

Corolla expansion in *Ipomoea nil* thus seems to be regulated by many interrelated factors, but the effect of changing levels of one PGR on the responsiveness of the tissue to another PGR plays a major role in regulation of growth.

These factors, as a whole, apparently serve to coordinate the growth of the flower parts in a very specific temporal sequence.

In summary, this paper supports a "combinational" theory of plant growth regulation involving changes in PGR concentrations, the interaction between the responses to PGRs, and changes in the responsiveness to these PGRs.

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