

# Role of Gibberellin in the Growth Response of Submerged Deep Water Rice<sup>1</sup>

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## ABSTRACT

We have shown previously that ethylene, which accumulates in the air spaces of submerged stem sections of rice (*Oryza sativa* L. cv "Habiganj Aman II"), is involved in regulating the growth response caused by submergence. The role of gibberellins in the submergence response was studied using tetcyclacis (TCY), a new plant growth retardant, which inhibits gibberellin biosynthesis. Stem sections excised from plants that had been watered with a solution of 1 micromolar TCY for 7 to 10 days did not elongate when submerged in the same solution or when exposed to 1 microliter per liter ethylene in air. Gibberellic acid (GA<sub>3</sub>) at 0.3 micromolar overcame the effect of TCY and restored the rapid internodal elongation in submerged and ethylene-treated sections to the levels observed in control sections that had not been treated with TCY. The effect of 0.01 to 0.2 micromolar GA<sub>3</sub> on internodal elongation was enhanced two- to eight-fold when 1 microliter per liter ethylene was added to the air passing through the chamber in which the sections were incubated. GA<sub>3</sub> and ethylene caused a similar increase in cell division and cell elongation in rice internodes. Thus, ethylene may cause internodal elongation in rice by increasing the activity of endogenous GAs. In internodes from which the leaf sheath had been peeled off, growth in response to submergence, ethylene and GA<sub>3</sub> was severely inhibited by light.

When deep water rice plants become partially submerged, internodal elongation is greatly enhanced (1, 8). This submergence response is, at least in part, mediated by increased levels of C<sub>2</sub>H<sub>4</sub>, the synthesis of which is stimulated in submerged internodes (3). The increase in C<sub>2</sub>H<sub>4</sub> synthesis in submerged internodes is triggered by reduced concentrations of O<sub>2</sub> (7). This mechanism of growth regulation enables rice plants to adjust their height to the depth of the surrounding water.

This paper examines the involvement of GA<sub>3</sub> and light in the submergence response of rice using isolated stem sections. Tetcyclacis (TCY)<sup>3</sup>, a new growth retardant, which blocks GA biosynthesis at the oxidative reactions between ent-kaurene and ent-kaurenoic acid (6), has been used to lower the levels of endogenous GAs in the rice plant. Involvement of GAs has been suspected because they are known to promote elongation of *Avena* internodes (2). Gibberellins have also been shown to mediate the stimulatory effect of C<sub>2</sub>H<sub>4</sub> on stem elongation in the semi-aquatic plant *Callitriche platycarpa* (5).

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<sup>3</sup> Abbreviations: TCY, tetcyclacis; GA, gibberellin.

## MATERIALS AND METHODS

**Chemicals.** Gibberellic acid (GA<sub>3</sub>) was a gift from Merck and Co., Inc. (Rahway, NJ), TCY [5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,10<sup>2,6</sup>,0<sup>8,11</sup>-dodeca-3,9-diene] was a gift of BASF (Limburgerhof, FRG). All other chemicals were purchased from Sigma Chemical Co.

**Plant Material.** Seeds of deep water rice (*Oryza sativa* L. cv "Habiganj Aman II") were obtained from the Bangladesh Rice Research Institute (Dacca, Bangladesh). Rice was germinated

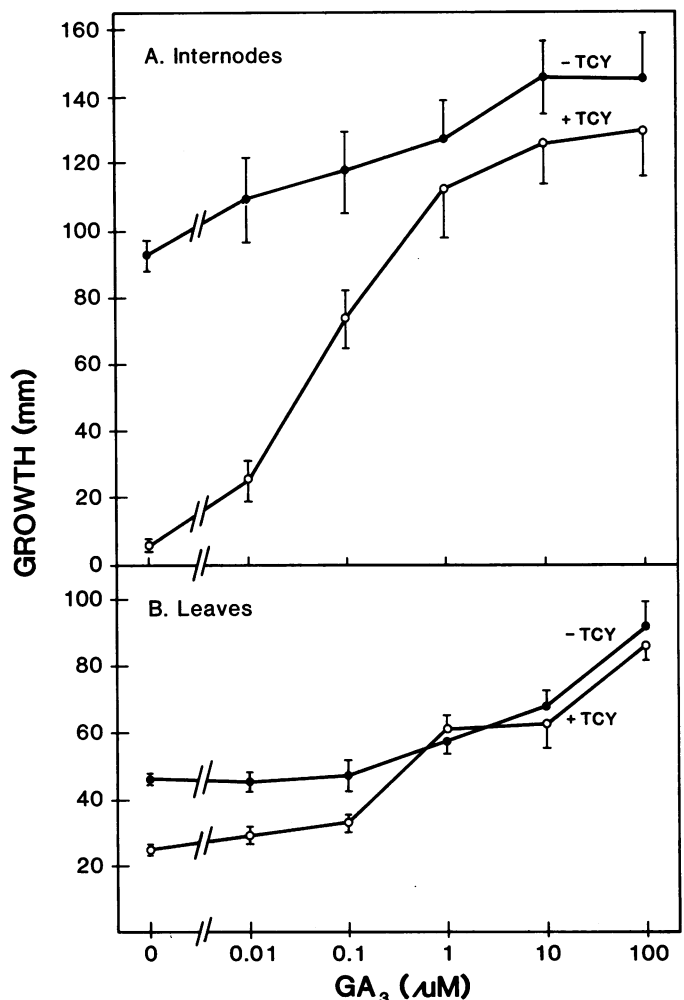


FIG. 1. Effect of GA<sub>3</sub> on the growth of internodes (A) and leaves (B) of rice stem sections submerged in 1 μM TCY solution (O) or distilled water (●) for 3 d in continuous light. Each point is the average of 14 sections. Vertical bars denote ±SE. The lower or upper part of the bar is omitted when it would interfere with another SE bar.

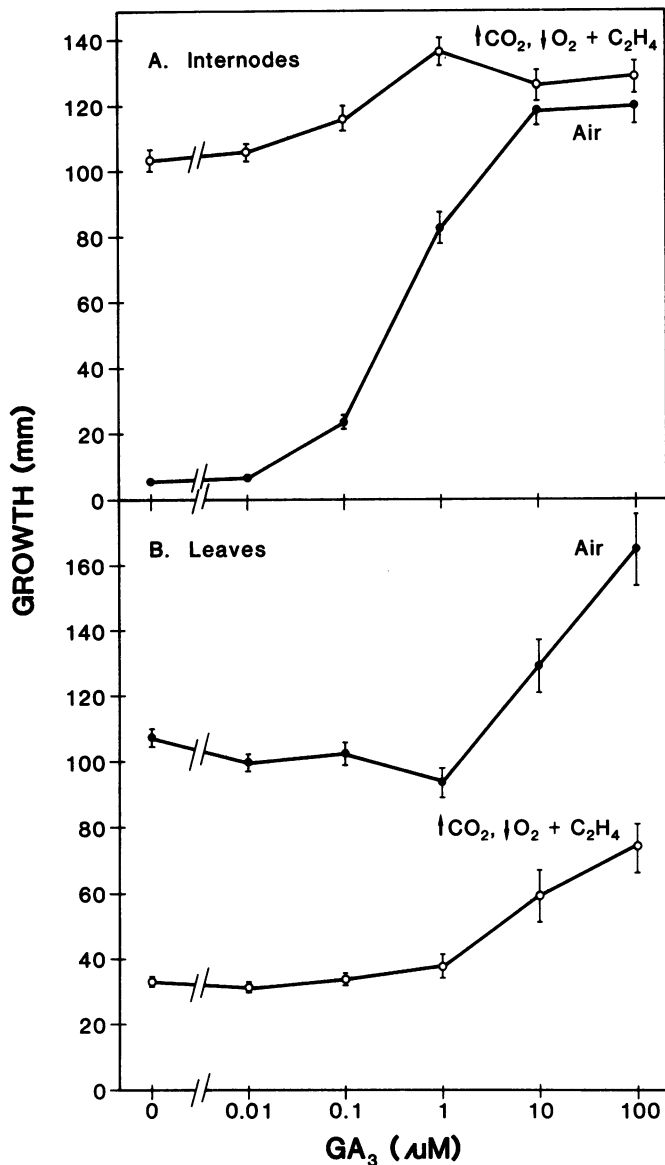


FIG. 2. Effect of  $GA_3$  on the growth of internodes (A) and leaves (B) of rice stem sections incubated in a stream of air (●) or 3%  $O_2$ , 6%  $CO_2$ , 91%  $N_2$  (by volume), and  $1 \mu\text{l l}^{-1}$   $C_2H_4$  (○) under continuous light for 3 d. Sections standing upright in 100-ml glass beakers containing 40 ml of different  $GA_3$  concentrations in distilled water were placed in 2.5-L plastic cylinders through which air or the above gas mixture was passed at  $80 \text{ ml min}^{-1}$ . Each point is the average of 25 sections  $\pm$ SE (see legend Fig. 1). When no bars are given, the SE is smaller than the symbol used.

and grown as described by Métraux and Kende (3). Twenty-cm-long stem sections containing the top-most internode were excised, subjected to submergence or gas treatments, and measured as described by Raskin and Kende (7). All experiments were performed at  $27^\circ\text{C}$  either in continuous light (cool-white fluorescent tubes;  $70 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) or in darkness. Stem sections used for TCY treatments were excised from rice plants which had been watered daily for 7 to 10 d with  $1 \mu\text{M}$  TCY in half-strength Hoagland solution. The term 'leaf' as we use it, includes the leaf sheath and the bases of the leaf blades that grow out of the original 20-cm-long stem section during the course of the experiment.

**Microscopy.** For the determination of cell numbers in the elongation zones of rice internodes, stem sections were incubated

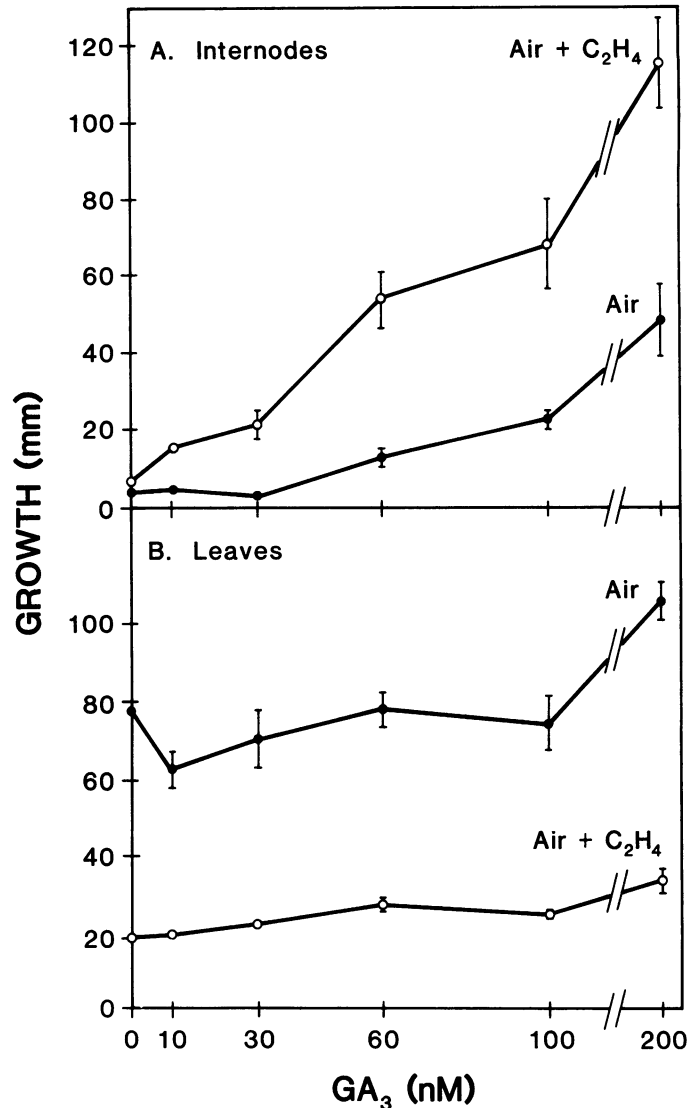


FIG. 3. Effect of low  $GA_3$  concentrations on the growth of internodes (A) and leaves (B) of rice stem sections treated with  $1 \mu\text{M}$  TCY and incubated in a stream of air (●) or air containing  $1 \mu\text{l l}^{-1}$   $C_2H_4$  (○). The sections, standing upright in 100-ml glass beakers containing 40 ml of  $1 \mu\text{M}$  TCY solution with different  $GA_3$  concentrations, were placed in 2.5-L plastic cylinders through which air or air with  $C_2H_4$  was passed at  $80 \text{ ml min}^{-1}$  and were incubated in continuous light for 3 d. Each point is the average of 14 sections  $\pm$ SE (see legend Fig. 2).

for 2 d in air or in a gas mixture consisting of 3%  $O_2$ , 6%  $CO_2$ , 91%  $N_2$  (by volume), and  $1 \mu\text{l l}^{-1}$   $C_2H_4$ . Stem sections kept in this gas mixture were standing in 40 ml of distilled  $H_2O$  while the stem sections incubated in air were either standing in 40 ml of distilled  $H_2O$  or  $5 \mu\text{M}$   $GA_3$  solution. The regions of the internodes which grew during the last 20 h of treatment were excised with a razor blade. Thin, freehand longitudinal sections were cut from the surface of each internode to cover the whole length of the elongated region. The sections were stained with methylene violet, and the number of subepidermal cells in files of 1.1 mm length was counted in six different regions distributed evenly along the newly elongated region of each internode. The total number of cells was calculated from the average number of cells in each region examined.

## RESULTS

Stem sections excised from TCY-treated and control plants were submerged in  $1 \mu\text{M}$  TCY solution or distilled  $H_2O$ , respec-

Table I. *Number of Subepidermal Cells in the Growing Regions of Rice Internodes*

Rice stem sections were incubated for 2 d in flow-through chambers (2.5 L) through which air or a mixture of 3% O<sub>2</sub>, 6% CO<sub>2</sub>, 91% N<sub>2</sub> (by vol) and 1 μl l<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> were passed at 80 ml min<sup>-1</sup>. The sections kept in air or the above gas mixture had their cut ends 2 cm deep in 40 ml of water while sections treated with GA were standing in 40 ml of a 5 μM GA<sub>3</sub> solution. Cells were counted in the regions that had elongated during the last 20 h of incubation.

Treatment	Increase in Internodal Length	Total No. of Cells in one File of the Newly Elongated Region	Average Cell Length
	mm		μm
Air + H <sub>2</sub> O <sup>a</sup>	1.1 ± 0.4	18.7 ± 5.7	57.3 ± 2.6
Air + 5 μM GA <sub>3</sub> <sup>b</sup>	41.3 ± 2.9	308.4 ± 25.1	135.0 ± 3.8
3% O <sub>2</sub> , 6% CO <sub>2</sub> , 91% N <sub>2</sub> + 1 μl l <sup>-1</sup> C <sub>2</sub> H <sub>4</sub> <sup>b</sup>	39.0 ± 1.8	316.5 ± 11.4	123.3 ± 3.7

<sup>a</sup> n = 3 ± SE.  
<sup>b</sup> n = 6 ± SE.

Table II. *Effect of Leaf Sheath Removal on Internodal Elongation in Rice Stem Sections*

The air and gas mixture (all by vol) were passed through the 2.5-L incubation cylinders at 80 ml min<sup>-1</sup>.

Treatment	Leaf Sheath	Condi- tions	Increase in Internodal Length
			mm
Submerged	+	Light	95.4 ± 5.8
	-	Light	2.9 ± 0.5
Submerged, wrapped <sup>a</sup>	+	Light	95.1 ± 6.3
	-	Light	48.3 ± 4.2
10 μM GA <sub>3</sub> , air	+	Darkness	81.5 ± 9.5
	-	Darkness	86.6 ± 9.4
	+	Light	121.8 ± 6.5
	-	Light	17.5 ± 5.0
3% O <sub>2</sub> + 6% CO <sub>2</sub> + 91% N <sub>2</sub> + 1 μl l <sup>-1</sup> C <sub>2</sub> H <sub>4</sub>	+	Darkness	66.6 ± 5.7
	-	Darkness	46.0 ± 5.9
	+	Light	99.8 ± 9.1
	-	Light	12.0 ± 2.1

<sup>a</sup> The lower 10 cm of the stem sections were wrapped in a 10 × 4 cm piece of aluminum foil.

tively, in the light for 3 d. Submergence stimulated internodal elongation in control sections but not in TCY-treated sections (Fig. 1A). Gibberellic acid added to the solution in which the sections were submerged promoted internodal growth, especially in TCY-treated sections. At about 0.3 μM, GA<sub>3</sub> restored the submergence response of TCY-treated internodes to the level of control (-TCY) internodes. Saturating concentrations of 100 μM GA<sub>3</sub> increased internodal growth of submerged control sections by 54% while the internodal growth of the sections submerged in TCY was promoted 22-fold at the same concentration of GA<sub>3</sub>. In contrast to internodes, rice leaves were less inhibited in growth by TCY (Fig. 1B). Addition of GA<sub>3</sub> increased leaf length in control and TCY-treated sections to a similar extent.

We showed earlier that the submergence response could be mimicked by exposing nonsubmerged sections to a gas mixture which was similar to the gaseous atmosphere in the internodal lacunae of submerged sections, namely 3% O<sub>2</sub>, 6% CO<sub>2</sub>, 91% N<sub>2</sub> (all by volume) and 1 μl l<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> (7). Internodes of stem sections incubated in this gas mixture for 3 d responded similarly to GA<sub>3</sub>

as did submerged sections (Fig. 2A), while internodes of stem sections kept in air showed a much greater response. Internodal elongation of sections incubated in 3% O<sub>2</sub>, 6% CO<sub>2</sub>, 91% N<sub>2</sub>, and 1 μl l<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> and treated with 100 μM GA<sub>3</sub> was promoted by 23% while the internodal growth of sections incubated in air was enhanced 18-fold by the same concentration of GA<sub>3</sub>. Leaf growth was 2- to 3-fold inhibited by 3% O<sub>2</sub>, 6% CO<sub>2</sub>, 91% N<sub>2</sub>, and 1 μl l<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> when compared to leaf growth in air (Fig. 2B). The amount of GA<sub>3</sub>-induced leaf growth was similar in both treatments. Ethylene did not enhance internodal elongation in sections standing in 40 ml of 1 μM TCY solution for 3 d (Fig. 3A). When low concentrations of GA<sub>3</sub> were added to the TCY solution, the increase in internodal elongation was 2.4 to 8 times larger with ethylene in the atmosphere than without. Again, C<sub>2</sub>H<sub>4</sub> inhibited leaf growth at all GA<sub>3</sub> concentrations, and only a small enhancement of leaf growth by GA<sub>3</sub> was observed in both treatments (Fig. 3B).

Cell division in the internodes of stem sections that had been submerged or incubated in 3% O<sub>2</sub>, 6% CO<sub>2</sub>, 91% N<sub>2</sub>, and 1 μl l<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> for 3 d was greatly enhanced (4). We found that both 5 μM GA<sub>3</sub> and a gas mixture of 3% O<sub>2</sub>, 6% CO<sub>2</sub>, 91% N<sub>2</sub>, and 1 μl l<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> increased the number of cells in the growing region of rice internodes 17-fold (Table I). Both GA<sub>3</sub> and C<sub>2</sub>H<sub>4</sub> promoted cell elongation 2-fold over that in control internodes. Also, comparable amounts of [<sup>3</sup>H]thymidine were incorporated into DNA of the newly elongated region of internodes in sections treated with 5 μM GA<sub>3</sub> in air or with the above gas mixture (data not shown).

The internode within the rice stem sections used in our experiments is covered by a single leaf sheath which originates at the lower of the two nodes of the section. When these leaf sheaths were removed and the exposed internodes illuminated, growth of the internodes in response to submergence, 10 μM GA<sub>3</sub> and 3% O<sub>2</sub>, 6% CO<sub>2</sub>, 91% N<sub>2</sub>, and 1 μl l<sup>-1</sup> C<sub>2</sub>H<sub>4</sub> was severely inhibited (Table II). In darkness, the inhibition of growth of exposed internodes was much less pronounced or, in the case of GA<sub>3</sub>-treated internodes, no inhibition was evident at all. Wrapping the lower 10 cm of submerged stem sections with aluminum foil to darken the exposed internodes counteracted, in part, the inhibitory effect of light (Table II).

## DISCUSSION

Figures 1 to 3 indicate that C<sub>2</sub>H<sub>4</sub> is likely to cause internodal elongation in rice by increasing the activity of endogenous GAs. Ethylene may either increase the sensitivity of internodal tissue to endogenous GAs or increase the concentration of physiologi-

cally active GAs in the rice internode. This hypothesis is supported by the following results: (a) stem sections excised from plants that had been watered with  $1 \mu\text{M}$  TCY did not elongate when submerged in the same solution or when exposed to  $1 \mu\text{l l}^{-1}$   $\text{C}_2\text{H}_4$  in air (Figs. 1, 3). Addition of  $\text{GA}_3$  at concentrations above  $0.1 \mu\text{M}$  restored rapid internodal growth in submerged and  $\text{C}_2\text{H}_4$ -treated internodes. (b) Low  $\text{GA}_3$  concentrations ( $0.01$ – $0.2 \mu\text{M}$ ) were much more effective in promoting internodal elongation when  $1 \mu\text{l l}^{-1}$   $\text{C}_2\text{H}_4$  was added to the air (Fig. 3). (c) Saturating concentrations of  $\text{GA}_3$  ( $10$ – $100 \mu\text{M}$ ) enhanced internodal elongation in the sections incubated in air to the levels observed in the gas mixture containing 3%  $\text{O}_2$ , 6%  $\text{CO}_2$ , 91%  $\text{N}_2$ , and  $1 \mu\text{l l}^{-1}$   $\text{C}_2\text{H}_4$  (Fig. 2). (d)  $\text{GA}_3$  stimulated cell divisions in the intercalary meristem of rice internodes to the same extent as did the gas mixture of 3%  $\text{O}_2$ , 6%  $\text{CO}_2$ , 91%  $\text{N}_2$ , and  $1 \mu\text{l l}^{-1}$   $\text{C}_2\text{H}_4$  (Table I).

Growth of internodes from which the leaf sheath had been peeled off was severely inhibited by light (Table II). Since  $\text{GA}$ - and  $\text{C}_2\text{H}_4$ -induced growth was greater in light than in darkness with the leaf sheath present and since growth in wrapped sections was greater with the sheath than without, the leaf sheath might affect growth also by means other than only protecting the internode from light. It may, *e.g.*, supply assimilates to the growing internode.

The following chain of events appears to take place following submersion of rice stem sections. The level of  $\text{O}_2$  in the tissue is greatly reduced as a result of submergence, and lowered  $\text{O}_2$  concentrations stimulate ethylene synthesis (7). Ethylene accu-

mulates in the submerged internode because its diffusion in water is 10,000 times slower than in air. Ethylene promotes rapid internode elongation by enhancing the activity of endogenous GAs. In this respect, the situation in rice may be similar to that in *Callitriche platycarpa* where promotion of stem elongation by ethylene is also dependent on the presence of GAs (5).

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