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Regulation of Growth in Rice Seedlings

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Abstract. Etiolated rice seedlings (*Oryza sativa* L.) exhibited marked morphological differences when grown in sealed containers or in containers through which air was passed continuously. Enhancement of coleoptile and mesocotyl growth and inhibition of leaf and root growth in the sealed containers ("enclosure syndrome") were accompanied by accumulation of CO_2 and C_2H_4 in and depletion of O_2 from the atmosphere. Ethylene (1 $\mu l\ l^{-1}$), high levels of CO_2, and reduced levels of O_2 contributed equally to the increase in coleoptile and mesocotyl growth. The effect of enclosure could be mimicked by passing a gas mixture of 3% O_2, 82% N_2, 15% CO_2 (all v/v), and 1 $\mu l\ l^{-1}$ C_2H_4 through the vials containing the etiolated seedlings. The effects of high CO_2 and low O_2 concentrations were not mediated through increased C_2H_4 production. The enclosure syndrome was also observed in rice seedlings grown under water either in darkness or in light. The length of the rice coleoptile was positively correlated with the depth of planting in water-saturated vermiculite. The length of coleoptiles of wheat, barley, and oats was not affected by the depth of planting. In rice, the length of coleoptile was determined by the levels of O_2, CO_2, and ethylene, rather than by light. This regulatory mechanism allows rice seedlings to grow out of shallow water in which the concentration of O_2 is limiting.

The effect of different gases on the growth of rice seedlings is not well defined, in spite of the large number of publications on this topic. It has long been known that rice seeds have the unique ability to germinate at very low levels of O_2 or even in the absence of it (Taylor 1942, Vlamis and Davis 1943). It has also been observed that coleoptile growth is stimulated and leaf and root growth inhibited in rice seedlings grown under water (Kordan 1977, Turner et al. 1981, Yamada 1954). These effects of submergence on the morphology of rice seedlings have been explained on the basis of reduced O_2 supply under water. Indeed, elongation of the rice coleoptile is enhanced at reduced O_2 tensions (Ohwaki 1967, Ranson and Parija 1955). Low concentrations of ethylene injected into sealed flasks containing rice seedlings also cause increased elongation of the rice mesocotyl and coleoptile (Imaseki et al. 1971, Ku et al. 1970, Miller and Miller 1974, Suge 1971). Indeed, Ku et al. (1970) have ascribed to ethylene the major role in the promotion of coleoptile growth. Recently, Atwell et al. (1982) have suggested that ethylene is mainly responsible for the stimulation of rice coleoptile elongation in stagnant water and that CO_2, an antagonist of ethylene, inhibits coleoptile growth. According to these same authors, elongation of the coleoptile is relatively insensitive to O_2 supply. With one exception (Ku et al. 1970, Table 2), all the above experiments on the effect of ethylene on growth of rice seedlings have been performed with plants in closed containers. Since enclosure leads to changes in the concentration of O_2 and CO_2 in the atmosphere, the true contribution of ethylene to the regulation of growth is difficult to ascertain from the literature.

In this paper, we evaluate the contributions of O_2, CO_2, C_2H_4, and submergence in water to the stimulation of growth of etiolated and light-grown rice seedlings. An attempt is also made to assign a physiological significance to the growth response induced by these gases in rice in comparison to other cereals. Finally, we have investigated whether the response of seedlings of different rice cultivars to altered gas atmospheres and to submergence is symptomatic for the later growth habit of these varieties. For example, deep-water rice plants that are at least 21 days old exhibit a dramatic growth response to ethylene (Métraux and Kende 1983). Do these same varieties at the seedling stage show a greater response to ethylene and altered CO_2 and O_2 atmospheres than do varieties not adapted to deep water?

Materials and Methods

Plant Material. The following rice (*Oryza sativa* L.) cultivars were used in this study: M-9 (seeds provided by Dr. J. N. Rutger, University of California, Davis, California); Labelle (seeds provided by Dr. T. Johnston, University of Arkansas, Stuttgart, Arkansas); IR-8 (seeds provided by Dr. R. S. Bandurski, Michigan State University, East Lansing, Michigan); Habiganj Aman III and VII (seeds provided by Dr. S. M. H. Zaman, Bangladesh Rice Research Institute, Dacca, Bangladesh); Pin Gaew 56 and Thavalu (seeds provided by Dr. B. S. Vergara, International Rice Research Institute, Los Baños, Philippines). Most of the experiments were performed with the California semi-dwarf cultivar M-9. Seeds were sterilized in 2% (w/v) sodium hypochlorite solution, rinsed five times with sterile distilled water, and germinated in darkness at 30°C in sterile Petri dishes (9-cm diameter) containing 12 ml of sterile distilled water. After 2 days, seedlings with coleoptiles about 1 mm in length were selected for experimental treatments.

Growth in Closed and Flow-through Containers. Ten preselected 2-day-old seedlings were transferred under dim white light to one 40-ml shell vial containing 2 ml of distilled water, which formed a 5-mm-deep layer in the bottom of the vial. The shell vials were tightly stoppered with serum vial caps in order to study growth of seedlings in sealed containers. Alternatively, serum vial caps were fitted with a 3½-inch, 16-gauge hypodermic needle as an inlet and a 1½-inch, 16-gauge hypodermic needle as an outlet for experiments in which a continuous flow of air or a gas mixture was passed through the vial at 30 ml min^{-1}. Seedlings were handled under sterile conditions, all laboratory ware was sterilized, and the gases were passed through a Millipore filter (0.45 μm pore size, Millipore Corp., Bedford, Massachusetts). Unless mentioned otherwise, all experiments were carried out in complete darkness at 27°C.

The mixtures of N_2, O_2, and CO_2 were either purchased from Matheson Gas Products (Joliet, Illinois) in high-pressure gas cylinders or prepared with gas regulators and flow meters (Matheson Gas Products). Compressed laboratory air was used for all flow-through air treatments. The gas mixtures and air were humidified to 100% relative humidity by being bubbled through water and were dispersed to the vials containing the seedlings with a flowmeter board (Pratt et al. 1960).

C_2H_4 was added to the gas stream with a C_2H_4 diffusion apparatus (Saltveit 1978). To prepare C_2H_4-free gas mixtures or C_2H_4-free air, the gases were passed through a 25-cm-long column (7 cm I.D.) packed with Purafil (Purafil Inc., Atlanta, Georgia). For O_2 and CO_2 determinations, 2-ml gas samples were withdrawn with a gas-tight syringe and analyzed using a gas chromatograph equipped with a thermal conductivity detector (Model GC 8700, Carle Instruments Inc., Anaheim, California). C_2H_4 was determined by gas chromatography of 1-ml gas samples (Kende and Hanson 1976).

C_2H_4 Evolution in Different Gas Mixtures. Two-day-old etiolated rice seedlings were incubated in vials through which different gas mixtures were passed continuously for 6 days. At the end of the incubation period, the vials were tightly stoppered, and 1-ml gas samples were withdrawn through the serum vial caps with gas-tight syringes at hourly intervals for C_2H_4 determinations. A dim green flash light was turned on for less than 2 min during withdrawal of gas samples. Conditions of sterility were maintained as above.

C_2H_4 and Submergence Effects in Light. Two-day-old seedlings were individually sown in 20-ml plastic pots filled with fine Vermiculite and placed in an environmental growth chamber under the following conditions: day temperature 25°C, night temperature 22°C, relative humidity 60%, 16-h photoperiod with a light intensity of 350 μE m^{-2} s^{-1} at seedling level. Daily watering with ¼-strength Hoagland's solution kept the Vermiculite saturated with water. Six 8-day-old seedlings, 7 to 8 cm long, were selected and placed into two cylindrical glass containers (8 l volume) equipped with inlet and outlet tubing. Air with and without C_2H_4 was passed through the containers at a flow rate of 400

ml min^{-1}. C_2H_4 at a concentration of 5 μl l^{-1} was added to the air stream as described above. Each day, seedlings were taken out of the container for 5 min for length measurements.

Seedlings used in submergence experiments were grown in the same environmental chamber under the same conditions. Five 9-day-old seedlings were completely submerged in a 40-l glass tank filled to the top with distilled H_2O. The tanks were kept in the same environmental chamber in which the seedlings had been grown. Seedlings remained submerged during growth measurements.

Growth of Seedlings at Different Water Depths. Ten 2-day-old seedlings were incubated in 40-ml shell vials filled with distilled H_2O to the depth of 0.5, 2, 4, 6, and 8 cm. Vials were incubated at 27°C in darkness or in continuous light (intensity 70 μE m^{-2} s^{-1}). Air was passed at a flow rate of 30 ml min^{-1} through the head space above the water of all vials except for those that were used to examine the effect of enclosure.

Growth of Seedlings at Different Depths of Vermiculite. Sixteen 2-day-old seedlings of rice, cv. M-9, of wheat, cv. Ionia, of barley, cv. Lakeland, and of oats) cv. Korwood, were planted at depths of 1, 2, 4, 6, and 8 cm in fine Vermiculite in 800-ml, square plastic pots with holes in the bottom. The pots were kept in darkness at 27°C in plastic trays filled with water to keep the Vermiculite saturated with water for the duration of the experiment.

Results

Etiolated rice seedlings (cv. M-9) grown in aerated or sealed containers exhibited marked differences in morphology (Fig. 1A–C). The altered composition of gases in the atmosphere caused by enclosure of the seedlings stimulated growth of the mesocotyl and the coleoptile and inhibited growth of the leaves. Enclosure also led to inhibition of root development (data not shown), which was in agreement with earlier observations (e.g. Kordan 1976a). The levels of O_2, CO_2, and C_2H_4 inside the sealed vials containing the seedlings were measured daily (Fig. 1D). After 8 days of incubation, the sealed vials contained 3% O_2, 21% CO_2, and 0.9 μl l^{-1} C_2H_4 (all v/v).

The growth of etiolated rice seedlings of different varietal groups incubated for 7 days in sealed and aerated containers was compared to that of M-9 (Fig. 2). Enclosure caused a similar increase in coleoptile and mesocotyl growth and inhibition of leaf growth in all varieties tested. The rice cultivars used in this experiment included a Texas lowland variety Labelle, the semi-dwarf cultivar IR-8 from the Philippines, the Sri Lanka flood-tolerant variety Thavalu, the Bangladesh deep-water rice variety Habiganj Aman VII, and the Thai deep-water rice variety Pin Gaew 56. Results similar to those shown in Fig. 2 were also observed with the deep-water cultivars Habiganj Aman III, Leb Mue Nahng, and Kalar Harsall (data not shown).

Six different mixtures of N_2, O_2, CO_2, and C_2H_4, in comparison to air, were

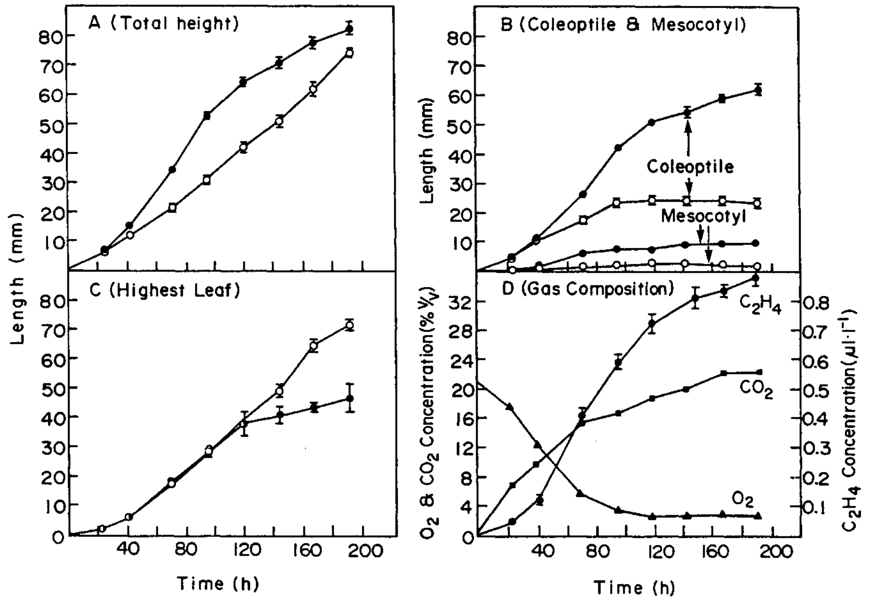
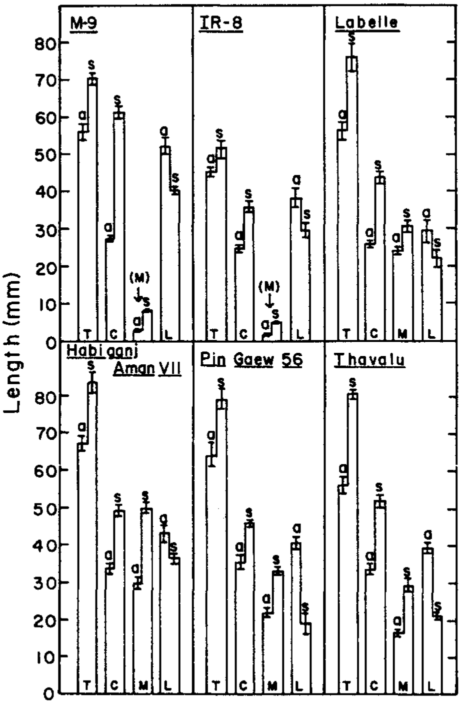


Fig. 1. A–C: The time course of growth of etiolated rice seedlings (cv. M-9) in sealed (●) and aerated (○) containers. D: The time course of changes in O_2, CO_2, and C_2H_4 concentrations inside the containers. Twenty-four 40-ml shell vials, each containing 10 2-day-old seedlings, were sealed, while 24 other vials were continuously flushed with air at a flow rate of 30 ml min^{-1}. Every 24 h, seedlings from three randomly chosen sealed and aerated vials were measured with a ruler and discarded. Gas samples for CO_2, O_2, and C_2H_4 determinations were withdrawn from the sealed vials with gas-tight syringes before the vials were opened. A–C—each point is the average value for 30 plants. D—each point is the average value for three vials. Vertical bars denote S.E. When no error bar is given, the S.E. is smaller than the symbol used.

used to evaluate the contribution of high concentrations of CO_2 and C_2H_4 and low concentrations of O_2 on the growth of etiolated rice seedlings. In the artificial gas mixtures, the concentrations of O_2, CO_2, and ethylene were adjusted singly or in combination to the concentrations found in sealed vials containing seedlings after several days of incubation, namely 3% O_2, 15% CO_2, and 1 µl l^{-1} ethylene (all v/v). The total length of the seedlings and the length of their coleoptiles, mesocotyls, and leaves were measured after 7 days of incubation (Table 1). The results demonstrated that enclosure increased coleoptile length by 160%, mesocotyl length by 200%, and inhibited leaf growth by 44%, compared to seedlings incubated in containers that were continuously flushed with air. High CO_2 (15%), low O_2 (3%), and C_2H_4 (1 µl l^{-1}) applied individually in the gas stream were responsible for about one-third of the total stimulation of coleoptile growth that was observed in the sealed containers. Combined treatment with any two of these gases elicited about two-thirds of the response caused by enclosure. The combination of C_2H_4, high CO_2, and low O_2 closely mimicked the effect of enclosure on the growth of etiolated rice seedlings (Table 1). Ethylene had some stimulatory effect on leaf growth in air and high CO_2 but inhibited leaf growth in low O_2. While the relative length of



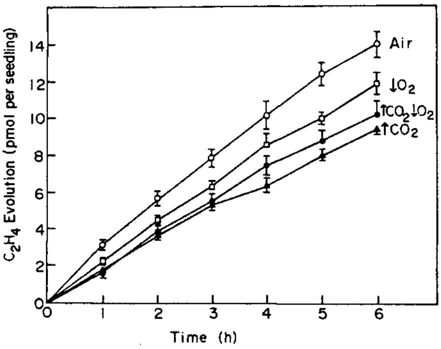


Fig. 2 (left). Growth of etiolated seedlings of different rice varieties in sealed and aerated containers. Twenty 2-day-old seedlings of each variety were incubated for 7 days in sealed (s) and aerated (a) 40-ml shell vials (10 seedlings per vial). The air flow was 30 ml min^{-1}. Total length of seedlings (T) and length of coleoptile (C), mesocotyl (M), and longest leaf (L) were measured after 7 days of incubation. Each point is the average value for 20 seedlings. Vertical bars denote S.E. **Fig. 3 (right).** Time course of C_2H_4 evolution from etiolated rice seedlings (cv. M-9) after 6 days of incubation in 40-ml shell vials: in a continuous flow of air (○); 0.03% CO_2, 3% O_2, 97% N_2 (□); 15% CO_2, 3% O_2, 82% N_2 (●), and 15% CO_2, 21% 0_2, 64% N_2 (▲). Prior to C_2H_4 measurements, the vials were sealed, and C_2H_4 accumulation in the vials was monitored for 6 h. All gas concentrations are given on a v/v basis; the gas flow was 30 ml min^{-1}. Each point is the average value of triplicate samples. Vertical bars denote S.E.

different organs of etiolated rice seedlings was markedly altered as a result of enclosure, the total length of the rice seedlings was only slightly affected.

The dose response of etiolated rice seedlings (cv. M-9) to C_2H_4 was determined by incubating seedlings for 7 days in containers through which air containing 1, 5, and 10 μl l^{-1} of C_2H_4 was passed continuously. The ability of C_2H_4 to promote the elongation of seedlings was close to saturation at 1 μl l^{-1} (Table 2). However, C_2H_4-induced acceleration of coleoptile and mesocotyl growth could account for only about 30% of the growth acceleration observed in sealed containers.

Since the enhancement of coleoptile and mesocotyl growth in high CO_2 and low O_2 could be caused by higher rates of C_2H_4 production, the evolution of C_2H_4 from etiolated rice seedlings (cv. M-9) incubated in mixtures of 15% CO_2

Table 1. Effect of different gas mixtures on the growth of etiolated rice seedings.

	Length (mm)			
Treatment	Total	Coleoptile	Mesocotyl	Longest leaf
Air (21% O_2 + 0.03% CO_2)	61 ± 2.9	23 ± 0.5	3 ± 0.3	57 ± 3.0
Air (21% O_2 + 0.03% CO_2) + 1 μl l^{-1} C_2H_4	71 ± 2.7	31 ± 0.3	4 ± 0.3	66 ± 2.7
21% O_2 + 15% CO_2	63 ± 2.7	32 ± 1.1	6 ± 0.6	55 ± 3.5
21% O_2 + 15% CO_2 + 1 μl l^{-1} C_2H_4	72 ± 2.0	44 ± 1.5	9 ± 0.7	61 ± 2.3
3% O_2 + 0.03% CO_2	77 ± 4.1	33 ± 1.2	5 ± 0.3	71 ± 4.4
3% O_2 + 0.03% CO_2 + 1 μl l^{-1} C_2H_4	64 ± 4.0	44 ± 1.5	6 ± 0.4	51 ± 5.8
3% O_2 + 15% CO_2	59 ± 4.0	40 ± 0.9	8 ± 0.5	47 ± 5.0
3% O_2 + 15% CO_2 + 1 μl l^{-1} C_2H_4	65 ± 2.2	55 ± 1.6	10 ± 0.6	39 ± 3.2
Sealed vial	69 ± 2.3	59 ± 1.4	9 ± 0.5	32 ± 2.6

Rice seedlings (cv. M-9) were treated as indicated for 7 days. All gas mixtures were made up in N_2 and were passed through the 40-ml incubation flasks at 30 ml min^{-1}. Concentrations of gases are given on a v/v basis. Each number is the average value for 30 seedlings incubated in three separate containers ± S.E.

Table 2. Effect of different concentrations of C_2H_4 on the growth of etiolated rice seedlings.

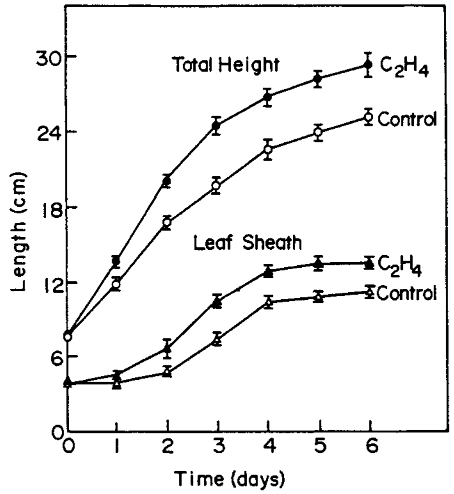
	Length (mm)			
Treatment	Total	Coleoptile	Mesocotyl	Longest leaf
Air	66 ± 2.5	25 ± 0.8	2 ± 0.2	64 ± 2.5
Air + C_2H_4 (1 μl l^{-1})	77 ± 2.4	32 ± 0.7	4 ± 0.2	73 ± 2.6
Air + C_2H_4 (5 μl l^{-1})	80 ± 3.6	35 ± 1.2	4 ± 0.3	75 ± 3.8
Air + C_2H_4 (10 μl l^{-1})	82 ± 3.4	36 ± 0.8	5 ± 0.3	77 ± 3.5
Sealed vial	68 ± 2.1	56 ± 1.9	8 ± 1.1	37 ± 3.7

Rice seedlings (cv. M-9) were treated as indicated for 7 days. Ethylene was continuously added to the air stream passing at 30 ml min^{-1} through the 40-ml incubation vials. Each number is the average value for 30 seedlings incubated in three containers ± S.E.

and 3% O_2 was measured (Fig. 3). The highest rates of C_2H_4 release were observed in seedlings grown in air rather than in high CO_2 or low O_2.

C_2H_4 at a concentration of 5 μl l^{-1} supplied for 6 days in the air stream stimulated elongation of 8-day-old rice seedlings (cv. M-9) grown under a 16-h photoperiod (Fig. 4). Total height of the seedlings and length of the highest leaf sheath were increased by C_2H_4. Similar results were obtained with the deep-water rice varieties Habiganj Aman III and VII (data not shown).

Nine-day-old rice seedlings (cv. M-9) continued to grow even when completely submerged in distilled water and kept under a 16-h photoperiod (Fig. 5). The slight initial increase in the growth rate caused by submergence was lost after 4 days of flooding. Similar results were obtained with the deep-water rice varieties Habiganj Aman III and VII (data not shown). To evaluate the relative contributions of continuous light (intensity 70 μE m^{-2} s^{-1}) and restricted gas exchange on the growth of rice seedlings, 2-day-old seedlings were submerged under different depths of water in light and in darkness. In dark-



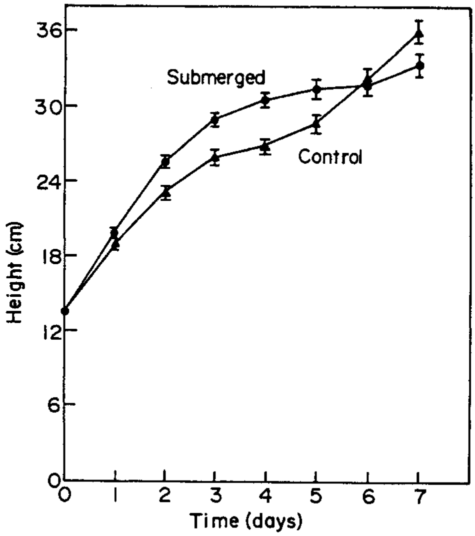


Fig. 4 (left). The effect of C_2H_4 on the growth of rice seedlings (cv. M-9) under a 16-h photoperiod. C_2H_4 (5 μl l^{-1}) was added to the stream of water-saturated air passing through the 8-l incubation chamber at a flow rate of 400 ml min^{-1}. The seedlings were 8 days old at the start of the C_2H_4 treatment. Each point is the average value of total height (○) and longest leaf sheath (△) of 6 seedlings. Vertical bars denote S.E. **Fig. 5 (right).** Time course of elongation of completely submerged (●) and air-grown (▲) rice seedlings (cv. M-9) under a 16-h photoperiod. The seedlings were 9 days old at the start of the experiment. Each point is the average value of five seedlings. Vertical bars denote S.E.

ness, 2-day-old rice seedlings planted at water depths below 40 mm developed coleoptiles and mesocotyls of similar lengths, as did etiolated, enclosed seedlings (Table 3). Characteristically, leaf growth was inhibited in seedlings planted at depths below 40 mm. Even in continuous light, seedlings planted 40 mm below the water surface responded with a greater than 4-fold increase in coleoptile length as compared with air-grown seedlings exposed to light of the same intensity. Coleoptiles of rice seedlings submerged to depths below 40 mm in light were actually 70% larger than coleoptiles of aerated seedlings grown in darkness under 5 mm of water.

The effect of restricted gas exchange on seedling growth could also be demonstrated when seedlings were planted at different depths of vermiculite. The length of etiolated rice coleoptiles showed a strong positive correlation to the depth of planting (Table 4). In complete darkness, the coleoptiles of rice seedlings planted at a depth of 80 mm were about 3 times longer than the coleoptiles of seedlings planted at a depth of 10 mm. The depth of planting had no effect on the length of the coleoptiles of etiolated wheat, barley, and oat seedlings.

Discussion

Based on our results, it is difficult to single out any one gas (CO_2, O_2, or C_2H_4) as being most important for the stimulation of growth of rice seedlings. Enclo-

Table 3. Effect of darkness and light on the growth of rice seedlings submerged at different depths of water.

Treatment	Depth of submergence (mm)	Length (mm)			
		Total	Coleoptile	Mesocotyl	Longest leaf
Darkness	5	77 ± 3.7	24 ± 1.0	2 ± 0.3	75 ± 3.8
Darkness	20	74 ± 4.0	34 ± 1.6	7 ± 0.6	67 ± 4.4
Darkness	40	86 ± 4.4	49 ± 1.2	9 ± 0.5	78 ± 4.7
Darkness	60	76 ± 2.8	62 ± 1.1	10 ± 0.7	50 ± 5.7
Darkness	80	79 ± 2.1	70 ± 2.1	10 ± 0.7	19 ± 2.1
Darkness, sealed	5	78 ± 2.3	64 ± 2.7	12 ± 1.6	35 ± 3.2
Light	5	50 ± 0.9	9 ± 0.7	0	50 ± 0.9
Light	20	55 ± 2.2	23 ± 0.7	0	55 ± 2.2
Light	40	50 ± 2.6	40 ± 1.2	0	44 ± 4.1
Light	60	50 ± 2.0	40 ± 1.3	1 ± 0.1	46 ± 3.0
Light	80	47 ± 3.3	39 ± 1.3	2 ± 0.2	42 ± 4.1
Light, sealed	5	53 ± 2.3	14 ± 0.7	0	53 ± 2.3

Rice seedlings (cv. M-9) were treated as indicated for 7 days. Each number is the average value for 20 seedlings from duplicate treatments ± S.E.

Table 4. Length of coleoptiles of selected cereals as a function of the depth of planting in Vermiculite.

Plant	Depth of planting (mm)					
	10	20	40	60	80	Length of incubation (days)
Rice (cv. M-9)	16 ± 0.6[a]	25 ± 0.8	35 ± 1.4	43 ± 2.4	49 ± 1.3	6
Oats (cv. Korwood)	62 ± 0.9	58 ± 1.5	60 ± 1.0	59 ± 1.8	59 ± 1.2	5
Wheat (cv. Ionia)	69 ± 1.7	73 ± 1.7	69 ± 1.9	70 ± 1.8	65 ± 2.4	5
Barley (cv. Lakeland)	46 ± 1.7	43 ± 1.2	44 ± 1.3	43 ± 2.4	46 ± 1.3	5

[a] Average coleoptile length (mm) of 16 plants ± S.E.

sure of 2-day-old rice seedlings in sealed containers produced what we call the "enclosure syndrome." The development of the enclosure syndrome could be mimicked by passing a mixture of 3% O_2, 82% N_2, 15% CO_2, and 1 $\mu l\ l^{-1}$ C_2H_4 through the vials containing rice seedlings. Therefore, the morphological changes observed during enclosure are caused by the combined effects of increased CO_2 and C_2H_4 and decreased O_2 levels in the ambient atmosphere. The effect of each of these gases appeared to be additive for the stimulation of coleoptile and mesocotyl growth in sealed containers (Table 1).

The use of the flow-through system with eight different gas mixtures allowed us to differentiate among the effects of CO_2, O_2, and C_2H_4 on the growth of rice seedlings. Because of the involvement of respiratory gases (CO_2 and O_2) in the regulation of the morphogenesis of rice seedlings, an independent evaluation of the role of C_2H_4 can only be accomplished when the atmosphere around the plant is continuously renewed. The use of sealed containers in which C_2H_4 was injected (Ku et al. 1970, Miller and Miller 1974) led to an overestimation of the C_2H_4 effect on growth. Similarly, the marked enhancement of coleoptile elongation under water may not be ascribed only to low O_2 supply, as suggested by Kordan (1976b). Our results contradict those of Atwell et al. (1982), who found coleoptile elongation to be largely unaffected by low O_2 supply and who suggest that growth of the coleoptile is inhibited by CO_2.

CO_2 was found to stimulate C_2H_4 synthesis and release in the leaves of corn, *Xanthium,* and rice (Grodzinski et al. 1982, Kao and Yang 1982). Similarly, 5% O_2 was found to enhance C_2H_4 production in apical segments of maize roots (Jackson 1982). However, in etiolated rice seedlings, C_2H_4 evolution was inhibited by 15% CO_2 or 3% O_2 (Fig. 3). Thus, it is unlikely that high CO_2 and/or low O_2 levels stimulate the growth of rice coleoptiles and mesocotyls through the enhancement of C_2H_4 production. However, high CO_2 and/or low O_2 concentrations may sensitize seedlings to C_2H_4. Alternatively, these three gases may stimulate growth independently of each other. For example, high concentrations of CO_2 could enhance growth of etiolated rice coleoptiles through cell-wall acidification, as has been described for oat coleoptiles (Evans et al. 1971). The stimulatory effect of low O_2 on coleoptile and mesocotyl growth may be based on either reduced accumulation of hydroxyproline-rich protein in the cell walls (Hoson and Wada 1980), or on acidification of the cell wall as a result of acid production during fermentation (Hochachka and Mommsen 1983), or on inhibition of IAA-oxidase activity (Schneider and Wightman 1974, Yamada 1954).

An enclosure syndrome of comparable magnitude was observed in traditional lowland, semi-dwarf, flood-tolerant, and deep-water rices. Thus, the magnitude of the enclosure syndrome cannot be used to distinguish between different varietal groups, e.g. between deep-water and regular rice cultivars.

The enclosure syndrome could also be observed when the gas exchange between the seedling and the environment was restricted, either in seedlings submerged in water or in seedlings planted in water-saturated vermiculite. Under both conditions, the length of the rice coleoptile was positively correlated to the depth of planting (Tables 3 and 4). In the case of submerged seedlings, this correlation has already been observed by Kefford (1962) and Yamada (1954). We have shown that restricted gas exchange overrode the photoinhibition of coleoptile elongation in rice. Therefore, elevated CO_2 and ethylene levels and reduced O_2 tensions are more important factors in regulating growth of rice coleoptiles than is light. In contrast, the growth of wheat, barley, and oat coleoptiles was not affected by the depth of planting.

In rice, the stimulation of coleoptile and mesocotyl growth by increased concentrations of CO_2 and ethylene and decreased levels of O_2 is a unique adaptive feature that permits rice seedlings to grow rapidly in shallow waters and waterlogged soils. While coleoptile and mesocotyl growth are promoted under hypoxic conditions, leaf growth is strongly inhibited. Once the tip of the coleoptile emerges into the air, rapid growth of the leaf commences.

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