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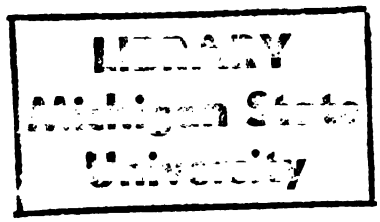


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Ilya Raskin

has been accepted towards fulfillment
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# PHYSIOLOGY OF GROWTH AND AERATION IN RICE

By

Ilya Raskin

A DISSERTATION

Submitted to
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# ABSTRACT

PHYSIOLOGY OF GROWTH AND AERATION IN RICE

By

Ilya Raskin

Isolated stem sections were used to study the rapid acceleration of internodal elongation in deep-water rice (Oryza sativa L. cv. Habiganj Aman II) caused by submergence. The effect of submergence on rice was, at least in part, mediated by $C_2H_4$, which accumulated in the air spaces of submerged sections. This accumulation resulted from slower diffusion of $C_2H_4$ from the tissue into the water and from increased $C_2H_4$ synthesis in the submerged internodes, triggered by reduced concentrations of $O_2$. Increased $C_2H_4$ levels accelerated internodal elongation and inhibited the growth of leaves. The enhancement of internodal elongation by $C_2H_4$ was particularly pronounced in an atmosphere rich in $CO_2$ and low in $O_2$. The effect of submergence on the growth of stem sections of deep-water rice could be mimicked by exposing non-submerged sections to a gas mixture which is similar to the gaseous atmosphere in the internodal lacunae of submerged sections, namely 3% $O_2$, 6% $CO_2$, 91% $N_2$ (by vol.) and 1 $\mu l\ l^{-1}$ $C_{2H4}$.

Ethylene probably causes internodal elongation in rice by increasing the activity of endogenous gibberellins. Stem sections excised from plants that had been watered with a solution of $10^{-6}$ M tetcyclacis, an inhibitor of gibberellin biosynthesis, did not elongate when submerged in the same solution nor when exposed to 1 $\mu l\ l^{-1}$ $C_2H_4$ in air. Applied gibberellic acid ($GA_3$) restored the rapid internodal elongation in submerged and $C_2H_4$-treated sections to the levels observed in control sections that had not been treated with tetcyclacis.

Submergence led to the rapid mobilization of starch from the older regions of rice internodes, which had been formed prior to submergence. Starch disappearance was accompanied by a 70-fold increase in α-amylase activity. A similar increase in α-amylase activity was detected in response to $C_2H_4$ and $GA_3$. Submergence also caused a 28-fold increase in the translocation of photosynthetic assimilates from the leaves to the internodes and younger regions of culms.

As in the adult rice plants, submergence altered the growth of different organs in rice seedlings. It markedly enhanced coleoptile and mesocotyl growth and inhibited leaf growth. Morphological changes similar to those observed in submerged seedlings were observed in seedlings grown in sealed containers in darkness. Ethylene, high levels of $CO_2$ and reduced levels of $O_2$ found in sealed containers contributed equally to the increase in coleoptile and

carbon fixation by enlarging the surface of the gas-liquid interface available for $CO_2$ uptake from the water. Deep-water rice plants without air layers did not grow in response to submergence and the submerged parts of the plant deteriorated as evidenced by a rapid loss of chlorophyll and protein.

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I sincerely thank my major professor, Dr. Hans Kende, for providing me with intelligent, sensitive and patient guidance and for teaching me not only how to receive a Ph.D. but how to be one. I extend my gratitude to the members of my guidance committee, Dr. Dave Dilley, Dr. Norman Good, Dr. Andrew Hanson and Dr. Jan Zeevaart for their constructive advice and encouragement. Last, but not least, I thank all other members of the faculty, post-docs, and graduate students of the D.O.E. Plant Research Laboratory for the wonderful time which I had in Michigan.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

# GENERAL INTRODUCTION

Rice occupies 11 percent of the world's arable land and is grown almost exclusively for human consumption. It constitutes half of the diet of 1.6 billion people living in many underdeveloped areas of the world where no other crop can be grown. Rice thrives in a wide variety of habitats ranging from the humid tropics of India, Thailand, Vietnam and the Philippines, to the hot and arid lands of Pakistan, Iran and Egypt, and the cool climates high in the mountains of Nepal and Northern China. Rice is grown either on dry land, in shallow paddies or in the flooded plains covered with several meters of water.

This dissertation is primarily concerned with deep-water or floating rice, grown predominantly in low-lying areas of Southeast Asia which are flooded every year during the rainy season. Seeds of deep-water rice are planted on flood plains or dry river beds at least a month before the rainy season starts. By the onset of the monsoon rains, deep-water rice has gained the ability to elongate as fast as 20 to 25 cm a day in order to keep some of its foliage above the rising water. Deep-water rice plants grow in water 1 to 6 m deep, usually forming a mat on the water surface. All deep-water rice varieties are photoperiodic

and are naturally selected in such a way that panicle emergence coincides with the end of the rainy season. Since no more internodes can be formed after the initiation of flowers, the end of vegetative development coincides with the loss of elongation ability. Harvesting is done after the flood waters have receded, although early maturing varieties are harvested from boats.

Deep-water rice has developed special adaptive mechanisms in order to survive the extreme conditions of its habitat. First, it possesses a regulatory mechanism enabling it to increase the growth rate in order to keep at least part of the foliage above the surface of rapidly rising waters during the first part of the monsoon season. Total submergence for more than 1 week results in plant death. Secondly, deep-water rice has an efficient aeration system which can transport $O_2$ all the way to the roots in order to maintain aerobic metabolism in the submerged organs. Roots and shoots of rice rapidly deteriorate without adequate supply of $O_2$. Of all rice varieties, deep-water rice has been studied least. No work has been done on the mechanism by which $O_2$ and $CO_2$ is supplied to the submerged organs of the plant. Practically nothing has been known about the physiological basis of internodal elongation induced by partial flooding, in spite of the possibility of increasing grain yields in flooded areas by increasing the elongation ability of high-yielding rice varieties which could not otherwise survive rapid flooding. There is also a great

potential for using deep-water rice as a model system in the study of regulation of plant growth.

This dissertation focusses on the physiological mechanisms involved in aeration and regulation of growth in partially flooded deep-water rice. The similarities of growth regulation in rice seedlings and deep-water rice plants are also discussed.

# Chapter 1

# Regulation of Growth in Stem Sections of Deep-Water Rice

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# Regulation of growth in stem sections of deep-water rice

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**Abstract.** Submergence in water greatly stimulates internodal elongation in excised stem sections of deep-water rice (*Oryza sativa* L. cv. "Habiganj Aman II") and inhibits growth of leaf blades and leaf sheaths. The highest rates of internodal growth have been observed in continuous light. Very little growth occurs in submerged sections kept in darkness or incubated under $N_2$ in the light. The effect of submergence on the growth of deep-water rice is, at least in part, mediated by $C_2H_4$, which accumulates in the air spaces of submerged sections. This accumulation results from increased $C_2H_4$ synthesis in the internodes of submerged sections and reduced diffusion of $C_2H_4$ from the tissue into the water. Increased $C_2H_4$ levels accelerate internodal elongation and inhibit the growth of leaves. Compounds capable of changing the rate of $C_2H_4$ synthesis, namely aminoethoxyvinylglycine, an inhibitor of $C_2H_4$ synthesis, and 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of $C_2H_4$, have opposite effects on growth of internodes and leaves. The enhancement of internodal elongation by $C_2H_4$ is particularly pronounced in an atmosphere of high $CO_2$ and low $O_2$. The increase in $C_2H_4$ synthesis in internodes of submerged sections is primarily triggered by reduced atmospheric concentrations of $O_2$. The rate of $C_2H_4$ evolution by internodes isolated from stem sections and incubated in an atmosphere of low $O_2$ is up to four times greater than that of isolated internodes incubated in air. In contrast, $C_2H_4$ evolution from the leaves is reduced under hypoxic conditions. The effect of submergence on growth of stem sections of deep-water rice can be mimicked by exposing non-submerged sections to a gas mixture which is similar to the gaseous atmosphere in the internodal lacunae of submerged sections, namely 3% $O_2$, 6% $CO_2$, 91% $N_2$ (by vol.) and 1 $\mu l\, l^{-1}$ $C_2H_4$. Our results indicate that growth responses obtained with isolated rice stem sections are similar to those of intact deep-water rice plants.

**Key words:** Carbon dioxide (ethylene, rice) – Deep-water rice – Ethylene (rice) – *Oryza* (growth regulation) – Oxygen (ethylene synthesis).

## Introduction

Floating or deep-water rice is mainly grown in the flood plains of Southeast Asia. Deep-water rice plants are capable of rapid elongation during the rainy season when the plants become partially submerged (see review in De Datta 1981). Growth rates of 20–25 cm a day have been recorded, with most of the elongation taking place in the submerged internodes (Vergara et al. 1976). In contrast to internodes, the lower leaves of deep-water rice stop elongating and eventually die when they become totally submerged.

Ethylene stimulates the growth of the internodes of non-submerged deep-water rice plants, and endogenous $C_2H_4$, which accumulates in the submerged internodes, is, at least in part, responsible for the stimulation of growth under water (Métraux and Kende 1983a). Internodal elongation in response to submergence is based on activation of cell division in the intercalary meristem and subsequent elongation of the newly formed cells (Métraux and Kende 1983b).

The use of isolated stem sections, described in this report, has provided us with a system of reduced complexity and increased versatility to study the submergence response in deep-water rice. We have evaluated the roles of $C_2H_4$, $O_2$ and $CO_2$ in controlling the growth of deep-water rice plants.

*Abbreviations:* ACC = 1-aminocyclopropane-1-carboxylic acid; AVG = aminoethoxyvinylglycine

The opposite effects of these gases on the growth of internodes and leaves have been examined and related to the growth habit of deep-water rice cultivars.

## Material and methods

*Chemicals.* 1-Aminocyclopropane-1-carboxylic acid (ACC) was purchased from Calbiochem-Behring Corp., La Jolla, Cal., USA; aminoethoxyvinylglycine (AVG) was a gift from Dr. M. Lieberman (U.S. Department of Agriculture, Beltsville, Md., USA).

*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 and grown as described in Métraux and Kende (1983a). Stem sections, 20 cm long and containing the highest two nodes and the top-most internode, were excised from the main stems and tillers of six- to ten-week-old plants with a sharp razor blade (Fig. 1A). The sections were cut in such a fashion that the lower node was 2 cm above the basal cut. We used only sections in which the expanding, youngest internode was 1 to 7 cm long. All leaf sheaths originating from nodes not included in the section were peeled from the latter. The initial length of each internode was measured by holding the section in front of a strong light which allowed localization of the two nodes included in each section. Following 3 d of experimental treatments, each section was cut open longitudinally to determine the final length of the internode or internodes if any new ones had developed during the incubation. Growth of the leaves was determined by subtracting the internodal growth from the total increase in section length. The term "leaf", as we use it, includes the leaf sheaths and the bases of the leaf blades that grow out of the original 20-cm-long stem section during the course of the experiment (Fig. 1B).

*Growth of submerged sections.* Ten to 15 sections were placed in an upright position in a 100-ml glass beaker which was then filled with glass beads to prevent the submerged sections from floating up. Each beaker containing the sections was lowered to the bottom of a 1-l volumetric cylinder, 42 cm deep, filled to the rim with distilled $H_2O$ or experimental solution. All experiments were performed at 27° C, either in darkness, or under a 13-h photoperiod, or in continuous light (cool-white fluorescent tubes; 70 μmol $m^{-2}$ $s^{-1}$).

*Growth in different gas mixtures.* Ten to 15 excised stem sections were placed upright in a 100-ml glass beaker containing 30 ml of distilled $H_2O$. Each beaker containing the sections was placed in a 2.5-l plastic cylinder, 60 cm deep, which was fitted with a gas-tight lid and with inlet and outlet tubings. The flow rate of air or gas mixtures through the cylinders was maintained at 80 ml $min^{-1}$. Experiments were carried out at 27° C either in continuous light (70 μmol $m^{-2}$ $s^{-1}$), or under a 13-h photoperiod (same photon flux as above), or in darkness. Nitrogen, $O_2$ and $CO_2$ were supplied from high-pressure gas cylinders. Gas mixtures were prepared with gas-pressure regulators and rotameters containing three calibrated flow-meter tubes equipped with high-accuracy valves and a mixing tube (Matheson Gas Products, Joliet, Ill., USA). Compressed laboratory air was used for all flow-through air treatments. Gas mixtures and air were humidified to 100% relative humidity by bubbling them through water. They were divided and dispersed to the incubation cylinders with flowmeter boards (Pratt et al. 1960). Ethylene was added to the gas stream with a $C_2H_4$-diffusion apparatus (Saltveit 1978). When stem sections were to be treated with $C_2H_4$-free air or gas mixtures, the gases were passed through a 25-cm-long column (7 cm inner diameter) packed with Purafil (Purafil, Atlanta, Ga., USA) to remove contaminating traces of $C_2H_4$.

*Analysis of internodal gases.* Each section was completely submerged in water in a large sink, and both ends of the internode were severed with a sharp razor blade. Gases from the internodal lacunae were allowed to escape into an inverted test tube filled with water. Internodal gases of sections used for the same treatment were collected in one test tube which was then stoppered with a serum-vial cap before being taken out of the water. A 2-ml sample was withdrawn from each test tube with a gas-tight syringe to determine the concentration of $O_2$ and $CO_2$ using a gas chromatograph equipped with a thermal conductivity detector (Model GC8700; Carle Instruments, Anaheim, Cal., USA). Ethylene was determined by gas chromatography of 1-ml gas samples (Kende and Hanson 1976). The gas volumes withdrawn for analyses were replaced with equivalent volumes of water.

*Measurement of $C_2H_4$ production.* The internodes were separated from most of the leaf tissue with a transverse cut across the upper node. Only the leaf tissues above the upper node were used for the determination of $C_2H_4$ synthesis. The single leaf sheath around the internode was carefully peeled away and discarded. A second transverse cut across the bottom node severed the internode from the 2-cm-long basal portion of the section. Excised internodes were weighed and placed in 30-ml test tubes (three internodes per test tube) containing 2 ml of distilled water or a solution of $10^{-5}$ M AVG. The leaf tissue from three sections was weighed and placed in one 60-ml test tube containing 3 ml of distilled water. All test tubes were stoppered with rubber serum-vial caps, each of which was fitted with a 3.5-inch, 16-gauge (9 cm long, 1.17 mm inner diameter) and a 1.5-inch, 16-gauge (4 cm long, 1.17 mm inner diameter) hypodermic needle to provide an inlet and an outlet for the gas stream. Each test tube was flushed for 1 h with the same gas mixture (40 ml $min^{-1}$) in which the stem sections had been incubated before separation of internodes and leaf sheaths. Internodes from sections that had been submerged in $H_2O$ were placed in test tubes and flushed with a gas mixture of 3% $O_2$, 6% $CO_2$ and 91% $N_2$ (by vol.). After 1 h, inlet and outlet needles were tightly stoppered, and the test tubes were placed on an orbital shaker operating at 20 cycles $min^{-1}$. They were kept in the light (photon flux 60 μmol $m^{-2}$ $s^{-1}$) at 24° C. One-ml gas samples were withdrawn every hour with a tuberculine syringe inserted through the serum-vial cap and were replaced with air; $C_2H_4$ was determined by gas chromatography (Kende and Hanson 1976).

*Determination of ACC and ACC-conjugate.* Excised internodes were ground in liquid $N_2$ in a mortar with a pestle. The resulting powder was extracted with 2 vols. of 70% (v/v) ethanol and centrifuged at 12,000 *g* for 15 min using a Sorvall RC-2B centrifuge and a SS-34 rotor (DuPont Instruments-Sorvall, Wilmington, Del., USA). The level of ACC-conjugate (presumed to be malonyl ACC) was determined by adding HCl to the supernatant to give a final concentration of 2 M HCl followed by hydrolysis at 100° C for 4 h. The hydrolyzate was neutralized with 2 M NaOH. The level of ACC was determined according to Lizada and Yang (1979). The amount of conjugated ACC was determined by subtracting the amount of ACC in the non-hydrolyzed sample from the amount of ACC in the hydrolyzed sample. Internal ACC standards were used to correct for losses during extraction and hydrolysis.

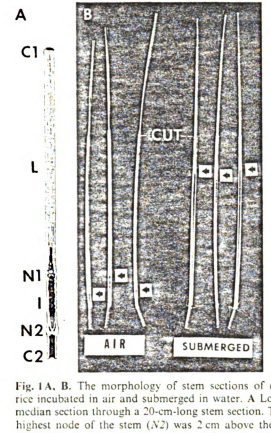


**Fig. 1A, B.** The morphology of stem sections of deep-water rice incubated in air and submerged in water. A Longitudinal median section through a 20-cm-long stem section. The second highest node of the stem (*N2*) was 2 cm above the basal cut (*C2*). This lower node was separated from the highest node (*N1*) by the youngest internode (*I*). The stem section between the highest node and the upper cut (*C1*) consisted of leaf sheaths and the developing youngest leaves (*L*). **B** Sections that had been incubated in a 2.5-l cylinder through which air was passed (80 ml $min^{-1}$) or were submerged in water under continuous light. After 3 d, the sections were cut open, and the length of the internode was measured. The position of the highest node is indicated by the *arrows*. The site of the original upper excision is also indicated (--CUT--)

## Results

Stem sections isolated from deep-water rice plants were incubated in a stream of air or were submerged in water under continuous light for 3 d (Fig. 1). The final length of the sections following both treatments was similar. However, when the stems were slit open, it became evident that the internodes of the non-submerged sections had elongated very little and that growth of the sections was based mainly on elongation of the leaf sheaths and leaf blades. In contrast, the internodes of the submerged sections had increased several fold in length while leaf growth was inhibited.

The levels of $CO_2$ and $C_2H_4$ increased up to 20- and 100-fold, respectively, and the level of $O_2$ declined by as much as tenfold in the internodal lacunae of submerged sections compared to sections incubated in air (Table 1). In submerged sections, the air spaces of leaf sheaths also contained about 1 $\mu l\,l^{-1}$ $C_2H_4$ as determined by vacuum evacuation of leaf sheaths under water (data not shown).

**Table 1.** Concentrations of $O_2$, $CO_2$ and $C_2H_4$ in the internodal lacunae of rice stem sections incubated in a stream of air (80 ml $min^{-1}$) or submerged in $H_2O$. Each value is the pooled average of 10 sections

| Gas | Submerged | | Air control | |
|---|---|---|---|---|
| | End of 2nd dark period | Middle of 3rd light period | End of 2nd dark period | Middle of 3rd light period |
| $O_2$ (%, v/v) | 2.1 | 7.4 | 19.7 | 22.1 |
| $CO_2$ (%, v/v) | 6.7 | 1.1 | 1.6 | 0.06 |
| $C_2H_4$ ($\mu l\,l^{-1}$) | 1.0 | 0.9 | 0.01 | 0.01 |

Air and six different mixtures of $O_2$, $CO_2$ and $C_2H_4$ in $N_2$ were used to evaluate the effect of high concentrations of $CO_2$ and $C_2H_4$ and low concentrations of $O_2$ on growth of internodes and leaves. The gas mixtures, which were passed through the chambers in which sections were incubated under a 13-h photoperiod, contained either 21% $O_2$, 0.03% $CO_2$ (both v/v) and no $C_2H_4$ or concentrations of these gases that were close to those in the internodal lacunae of submerged sections at the end of the dark period, namely 3% $O_2$, 6% $CO_2$ (both v/v) and 1 $\mu l\,l^{-1}$ $C_2H_4$. Ethylene at 1 $\mu l\,l^{-1}$ in air and 3% $O_2$ enhanced internodal growth by 6.9 and 4.4 times, respectively (Table 2). When 1 $\mu l\,l^{-1}$ $C_2H_4$ and 3% (v/v) $O_2$ were supplied in the same gas mixture, internodal growth was enhanced about tenfold. High concentrations of $CO_2$ (6%, v/v), in a gas mixture containing 21% (v/v) $O_2$ and no $C_2H_4$ had very little effect on internodal elongation in non-submerged sections. However, when 1 $\mu l\,l^{-1}$ of $C_2H_4$ was added to the above gas mixture, the growth of internodes was increased more than ninefold. While internodal elongation was strongly promoted by $C_2H_4$, leaf growth was inhibited when $C_2H_4$ was added to air or the other gas mixtures. For example, 1 $\mu l\,l^{-1}$ $C_2H_4$ added to the gas mixture containing 3% (v/v) $O_2$ inhibited growth of deep-water rice leaves by about 70% (Table 2). The effect of submergence on growth of internodes and leaves in deep-water rice stem sections was closely mimicked by passing a gas mixture of 3% (v/v) $O_2$, 6% (v/v) $CO_2$ and 1 $\mu l\,l^{-1}$ $C_2H_4$ in $N_2$ through the chambers containing rice stem sections.

**Table 2.** Effects of $O_2$, $CO_2$, $C_2H_4$ and submergence on the elongation of rice stem sections incubated under a 13-h photoperiod for 3 d. Air and gas mixtures (all v/v) were passed through the incubation cylinders at 80 ml $min^{-1}$. Each value is the average of 11 sections ± SE

| Treatment | Length increase (mm) | | | $C_2H_4$ concentration in internode ($\mu l\ l^{-1}$) |
|---|---|---|---|---|
| | Internode | Leaf | Total section | |
| Air | 9.5± 1.2 | 105.5± 9.9 | 115.0±10.4 | 0.02 |
| Air + 1 $\mu l\ l^{-1}$ $C_2H_4$ | 65.2± 7.8 | 62.9±11.9 | 128.1±17.8 | 0.9 |
| 21% $O_2$ + 6% $CO_2$ + 73% $N_2$ | 13.3± 2.8 | 118.3± 6.4 | 131.6± 6.8 | 0.02 |
| 21% $O_2$ + 6% $CO_2$ + 73% $N_2$ + 1 $\mu l\ l^{-1}$ $C_2H_4$ | 88.1±11.1 | 90.6±12.5 | 178.7±21.4 | 1.0 |
| 3% $O_2$ + 0.03% $CO_2$ + 97% $N_2$ | 41.4± 5.1 | 76.2± 7.7 | 117.6± 7.2 | 0.01 |
| 3% $O_2$ + 0.03% $CO_2$ + 97% $N_2$ + 1 $\mu l\ l^{-1}$ $C_2H_4$ | 95.9±11.7 | 35.1± 3.5 | 141.0±12.9 | 0.8 |
| 3% $O_2$ + 6% $CO_2$ + 91% $N_2$ | 54.7± 4.7 | 111.9± 6.9 | 166.6±10.4 | 0.02 |
| 3% $O_2$ + 6% $CO_2$ + 91% $N_2$ + 1 $\mu l\ l^{-1}$ $C_2H_4$ | 95.3± 9.6 | 52.8± 4.2 | 148.1± 8.1 | 1.1 |
| Submerged | 93.4±10.1 | 61.8± 7.8 | 155.2±14.3 | 1.0 |

**Table 3.** Effects of $O_2$, $CO_2$, $C_2H_4$ and submergence on the elongation of rice stem sections incubated in darkness for 3 d. Air and the gas mixtures (all v/v) were passed through the incubation cylinders at 80 ml $min^{-1}$. Each value is the average of 11 sections ± SE

| Treatment | Length increase (mm) | | | $C_2H_4$ concentration in internode ($\mu l\ l^{-1}$) |
|---|---|---|---|---|
| | Internode | Leaf | Total section | |
| Air | 5.1± 1.3 | 83.6± 7.4 | 88.7± 7.9 | 0.01 |
| Air + 1 $\mu l\ l^{-1}$ $C_2H_4$ | 25.4± 5.9 | 25.9± 2.7 | 51.3± 6.1 | 0.66 |
| 21% $O_2$ + 6% $CO_2$ + 73% $N_2$ | 16.8± 4.5 | 128.4±14.2 | 145.2±16.0 | 0.02 |
| 21% $O_2$ + 6% $CO_2$ + 73% $N_2$ + 1 $\mu l^{-1}$ $C_2H_4$ | 62.2±12.9 | 58.3±16.6 | 120.5±25.3 | 0.70 |
| 3% $O_2$ + 0.03% $CO_2$ + 97% $N_2$ | 17.1± 3.5 | 72.0± 2.9 | 89.1± 3.5 | 0.01 |
| 3% $O_2$ + 0.03% $CO_2$ + 97% $N_2$ + 1 $\mu l^{-1}$ $C_2H_4$ | 61.8±11.8 | 33.4±10.4 | 95.2±17.1 | 0.80 |
| 3% $O_2$ + 6% $CO_2$ + 91% $N_2$ | 27.1± 4.1 | 84.0± 8.8 | 111.1±10.6 | 0.02 |
| 3% $O_2$ + 6% $CO_2$ + 91% $N_2$ + 1 $\mu l\ l^{-1}$ $C_2H_4$ | 60.1±12.5 | 30.1± 3.4 | 90.2±12.6 | 0.64 |
| Submerged in $H_2O$ | 7.7± 0.9 | 22.0± 2.7 | 29.7± 3.0 | 0.10 |
| Submerged in 0.1 M sucrose | 9.4± 1.1 | 18.9± 2.0 | 28.3± 2.0 | 0.12 |

**Table 4.** Effects of $O_2$, $CO_2$, $C_2H_4$, submergence and anoxia on the elongation of rice stem sections incubated under continuous light for 3 d. The gases (v/v) were passed through the incubation cylinders at 80 ml $min^{-1}$. Each value is the average of 15 sections ± SE

| Treatment | Length increase (mm) | | | $C_2H_4$ concentration in internode ($\mu l\ l^{-1}$) |
|---|---|---|---|---|
| | Internode | Leaf | Total section | |
| Air | 10.9± 0.9 | 121.4±10.6 | 132.3±11.7 | 0.02 |
| 3% $O_2$ + 6% $CO_2$ + 91% $N_2$ + 1 $\mu l\ l^{-1}$ $C_2H_4$ | 123.4±11.5 | 38.7± 4.0 | 162.1±12.9 | 0.8 |
| Submerged | 118.0± 7.7 | 61.9± 7.6 | 179.9±11.8 | 1.1 |
| 100% $N_2$ | 5.1± 0.9 | 10.3± 1.2 | 15.4± 1.5 | <0.01 |

In darkness, the response of internodes and leaves to different concentrations of $O_2$, $CO_2$ and $C_2H_4$ was qualitatively similar to that obtained under a 13-h photoperiod (Table 3). However, growth in all gas mixtures was significantly smaller in the dark than under a 13-h photoperiod (compare with Table 2). Very little growth of internodes and leaves occurred when sections were submerged in distilled water or 0.1 M sucrose in continuous darkness. The highest rates of internodal growth in response to a mixture of 3% (v/v) $O_2$, 6% (v/v) $CO_2$ and 1 $\mu l\ l^{-1}$ of $C_2H_4$ or submergence were observed in stem sections of deep-water rice incubated under continuous light (Table 4). Very little growth was observed under continuous light if $N_2$ was passed through the incubation chambers.

An attempt was made to evaluate the role of endogenous $C_2H_4$ in the promotion of growth of

**Table 5.** Effects of AVG and ACC on the growth of submerged rice stem sections kept under a 13-h photoperiod for 2.5 d. Each value is the average of 12 sections ± SE

| Treatment | Length increase (mm) | | | $C_2H_4$ concentration in internode ($\mu l\ l^{-1}$) |
|---|---|---|---|---|
| | Internode | Leaf | Total section | |
| Air control | 6.5±0.9 | 69.5±4.6 | 76.0± 4.9 | 0.01 |
| Submerged in $H_2O$ | 66.4±7.3 | 51.5±6.1 | 117.9±14.7 | 1.0 |
| Submerged in $10^{-5}$ M AVG | 31.7±5.6 | 78.8±8.3 | 110.5±12.2 | 0.08 |
| Submerged in $10^{-5}$ M ACC | 72.8±7.6 | 36.5±7.0 | 109.3± 9.8 | 2.1 |
| Submerged in $10^{-5}$ M AVG + $10^{-5}$ M ACC | 52.6±3.6 | 41.7±5.3 | 94.3± 7.9 | 1.5 |

submerged stem sections using AVG, an inhibitor of $C_2H_4$ biosynthesis, and ACC, the immediate precursor of $C_2H_4$. Aminoethoxyvinylglycine ($10^{-5}$ M) inhibited internodal elongation in submerged sections by 52% and promoted leaf growth by 53% compared with sections submerged in distilled water (Table 5). 1-Aminocyclopropane-1-carboxylic acid ($10^{-5}$ M) increased internodal elongation by 10% and decreased leaf growth by 29% compared with sections submerged in water; it partially reversed the action of AVG on the submerged sections. However, even at concentrations above $10^{-5}$ M, ACC did not completely counteract the action of AVG (results not shown), probably because AVG inhibited some other important processes in addition to $C_2H_4$ synthesis.

The rise in the concentration of $C_2H_4$ in submerged rice stem sections may be caused by reduced diffusion of $C_2H_4$ from the tissue into the water while the rate of $C_2H_4$ synthesis remains unchanged. Alternatively, $C_2H_4$ accumulation in the tissues may be the combined result of enhanced $C_2H_4$ synthesis in and reduced $C_2H_4$ diffusion from submerged stem sections. Since it is not possible to compare $C_2H_4$ evolution in submerged and nonsubmerged sections directly, we transferred the internodal tissue from submerged stem sections into test tubes containing 3% $O_2$ and 6% $CO_2$ (both v/v), i.e. concentrations that were similar to those found in the internodal lacunae of submerged sections, and determined $C_2H_4$ evolution under these conditions. Internodal tissues from sections that had been incubated in air were transferred into test tubes containing air (Fig. 2). Internodal tissues from submerged sections evolved over five times more $C_2H_4$ than internodal tissue from air-incubated sections, and AVG ($10^{-5}$ M) inhibited $C_2H_4$ evolution by 80–90% in both instances.

We compared the effect of low $O_2$ and high $CO_2$ concentrations on $C_2H_4$ synthesis in internodal and leaf tissue from stem sections that had been incubated previously in different gas mixtures (Fig. 3). Ethylene evolution was measured in the same gas mixtures in which the sections had been incubated before the separation of the internodes from the leaf tissue. Internodal tissue excised from sections and incubated in a stream of gas containing 3% $O_2$ evolved up to four times more $C_2H_4$ than did internodes excised from sections incubated in a stream of air or 21% $O_2$, 6% $CO_2$ and 73% $N_2$ (by vol.) (Fig. 3). The reverse was true for leaves. These evolved the largest amounts of $C_2H_4$ in air or in a gas mixture of 21% $O_2$, 6% $CO_2$, 73% $N_2$ (by vol.). In contrast to internodal tissue, leaf tissue evolved the least $C_2H_4$ in

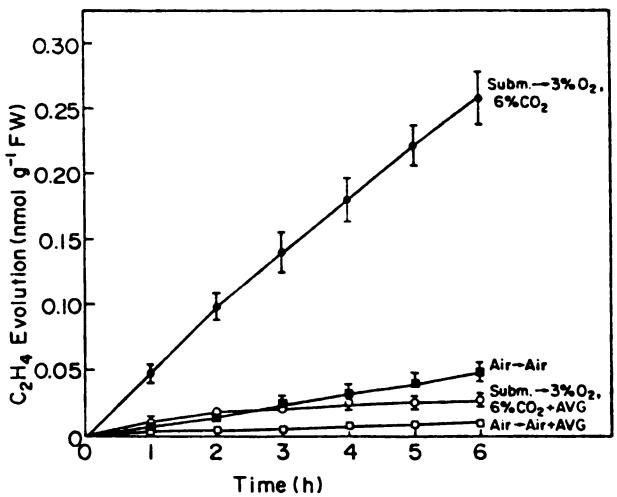


**Fig. 2.** Ethylene evolution from internodes excised from rice stem sections that had been submerged in $H_2O$ or incubated in a stream of air (80 ml $min^{-1}$) under continuous light. Internodes excised from submerged sections were sealed in 30-ml test tubes containing 3% $O_2$, 6% $CO_2$ and 91% $N_2$ (by vol.) and 2 ml of distilled $H_2O$ (•) or $10^{-5}$ M AVG solution (o). Internodes excised from the sections incubated in air were sealed in 30-ml test tubes containing air and 2 ml $H_2O$ (■) or $10^{-5}$ M AVG solution (□). Each point is the average of three replicate test tubes containing three internodes each. Vertical bars denote ± SE. When no bars are given, the SE is smaller than the symbol used. The lower or upper part of the bar is omitted when it would interfere with another SE bar

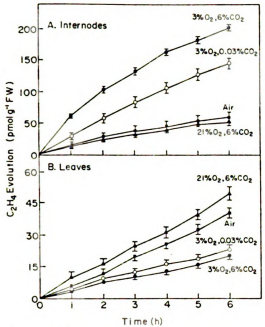


Fig. 3A, B. Ethylene production by internodes (A) and leaves (B) excised from rice stem sections that had been incubated in a stream of air (■), 3% $O_2$, 0.03% $CO_2$, 97% $N_2$ (○), 21% $O_2$, 6% $CO_2$, 73% $N_2$ (▲) or 3% $O_2$, 6% $CO_2$, 91% $N_2$ (●) (all by vol.) under continuous light. Air and the respective gas mixtures were passed through the incubation cylinders at 80 ml $min^{-1}$. For ethylene determinations, internodal and leaf tissues were isolated and incubated in 30- or 60-ml test tubes, respectively, containing 2 or 3 ml $H_2O$ and the same gas mixtures with which the whole stem sections had been treated earlier. Each point is the average of three replicate test tubes, each containing internodes or leaf tissue from three sections. The SE is given by the vertical bars (see legend Fig. 2)

Table 6. Effects of submergence and different gas mixtures on the levels of free and conjugated ACC in internodes of rice stem sections incubated under continuous light. Gases (v/v) were passed through the incubation cylinders at 80 ml $min^{-1}$. Each value is the pooled average from 10 internodes

| Treatment | ACC (nmol $g^{-1}$ FW) | Conjugated ACC (nmol $g^{-1}$ FW) |
|---|---|---|
| Air | 2.1 | 24.3 |
| 3% $O_2$, 0.03% $CO_2$, 97% $N_2$ | 2.5 | 28.5 |
| 21% $O_2$, 6% $CO_2$, 73% $N_2$ | 2.1 | 34.4 |
| 3% $O_2$, 6% $CO_2$, 91% $N_2$ | 2.3 | 28.6 |
| Submerged | 1.9 | 21.4 |

gas mixtures containing low levels of $O_2$. We verified the fact that the differences in $C_2H_4$ production in internodes and leaves isolated from stem sections were not the result of differences in the wound response following excision. When internodes and leaves from submerged and non-submerged sections incubated in different gas mixtures were cut longitudinally into two or four portions, $C_2H_4$ production was not further stimulated (results not shown).

Submergence or incubation of stem sections in different gas mixtures did not significantly affect the endogenous levels of free ACC or conjugated ACC, presumed to be malonyl ACC (Table 6).

All experiments described in this paper were repeated at least three times with similar results.

## Discussion

The growth response of submerged deep-water-rice stem sections is very similar to that of partially submerged, intact deep-water rice plants (Métraux and Kende 1983a, b). Elongation of internodes is greatly stimulated while leaf growth is inhibited. Our data indicate that stimulation of internodal growth and inhibition of leaf growth in submerged deep-water rice can be explained both by increased $C_2H_4$ synthesis in the internodal tissue and by accumulation of $C_2H_4$ in the internal air spaces of the submerged organs. The enhancement of $C_2H_4$ synthesis in the internodes and inhibition of $C_2H_4$ synthesis in the leaves of deep-water rice is brought about by low levels of $O_2$ (Fig. 3A) as found in the lacunae of submerged stem sections (Table 1). In this respect, internodes of deep-water rice differ from most other plant tissues where ethylene synthesis is inhibited at low $O_2$ levels (e.g. Raskin and Kende 1983). Stimulation of $C_2H_4$ synthesis under hypoxic conditions (5% $O_2$) has previously been reported for maize and barley roots (Jackson 1982). The opposite effects of AVG, an inhibitor, and ACC, a promoter of $C_2H_4$ synthesis on the growth of leaves and internodes agree well with the changes in the growth rates of internodes and leaves under water (Tables 2, 5). Accumulation of $C_2H_4$ in the submerged plant parts results from the almost $10^4$-fold slower rate of $C_2H_4$ diffusion through water when compared with $C_2H_4$ diffusion through air (Musgrave et al. 1972). Although the internodal tissue of non-submerged sections incubated under hypoxic conditions produces as much $C_2H_4$ as do internodes from sections that have been submerged (Figs. 2, 3A), no $C_2H_4$ accumulation in the internodal lacunae of non-submerged sections has been observed (Table 2) because of the high rate of $C_2H_4$ diffusion from the non-submerged tissue into the air.

Carbon dioxide at the high levels found in the lacunae of submerged sections promotes internodal elongation to a minor extent and does not stimulate $C_2H_4$ production (Table 2, Fig. 3A). This

is in contrast to etiolated rice seedlings in which $CO_2$ at high concentrations does promote growth of the coleoptiles and mesocotyls (Raskin and Kende 1983). However, $CO_2$ at high levels enhances the stimulatory effect of $C_2H_4$ on internodal growth in rice stem sections and decreases the inhibitory effect of $C_2H_4$ on leaf growth (Table 2). Therefore, the full effect of submergence on growth of stem sections of deep-water rice can be mimicked most closely by incubating non-submerged sections in a gas stream whose composition is similar to that of the internodal lacunae, namely 3% $O_2$, 6% $CO_2$, and 1 $\mu l\, l^{-1}$ $C_2H_4$ (Table 2).

Internodes and leaves do not elongate under anaerobic conditions (Table 4), and very little growth occurs in submerged sections kept in continuous darkness (Table 3). The $C_2H_4$ concentration in the internodal lacunae of submerged sections was about ten times lower in the dark, i.e. in the absence of photosynthetic $O_2$, than in the light (Tables 3, 4). This agrees with the previous observation that $C_2H_4$ synthesis is inhibited under anaerobic conditions (Hansen 1942).

In conclusion, $O_2$ at concentrations found in submerged internodes (approx. 3%) enhances $C_2H_4$ synthesis in internodes of deep-water rice. Ethylene, which accumulates in submerged stems, promotes growth of internodes and inhibits growth of leaves. The stimulatory effect of $C_2H_4$ on internodal growth is enhanced at the elevated levels of $CO_2$ which occur in submerged internodes (approx. 6%). Deep-water rice is adapted to grow very rapidly when partially submerged in water. This adaptation is based on the ability of deep-water rice to react to reduced $O_2$ and elevated $CO_2$ and $C_2H_4$ levels within the submerged stems.

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## References

De Datta, S.K. (1981) Principles and practices of rice production. Wiley, New York

Hansen, E. (1942) Quantitative study of ethylene production in relation to respiration of pears. Bot. Gaz. (Chicago) **103**, 543–558

Jackson, M.B. (1982) Ethylene as a growth promoting hormone under flooded conditions. In: Plant growth substances 1982, pp. 291–301, Wareing, P.F., ed. Academic Press, London New York

Kende, H., Hanson, A.D. (1976) Relationship between ethylene and senescence in morning-glory flower tissue. Plant Physiol. **57**, 523–527

Lizada, M.C.C., Yang, S.F. (1979) A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal. Biochem. **100**, 140–145

Métraux, J.-P., Kende, H. (1983a) The role of ethylene in the growth response of submerged deep water rice. Plant Physiol. **72**, 441–446

Métraux, J.-P., Kende, H. (1983b) The cellular basis of the elongation response in submerged deep-water rice. Planta **160**, 73–77

Musgrave, A., Jackson, M.B., Ling E. (1972) *Callitriche* stem elongation is controlled by ethylene and gibberellin. Nature (London) New Biol. **238**, 93–96

Pratt, H.K., Workman, M., Martin, F.W., Lyons, J.M. (1960) Simple method for continuous treatment of plant material with metered traces of ethylene or other gases. Plant Physiol. **35**, 609–611

Raskin, I., Kende, H. (1983) Regulation of growth in rice seedlings. J. Plant Growth Regul., in press

Saltveit, M.E., Jr. (1978) Simple apparatus for diluting and dispensing trace concentrations of ethylene in air. HortScience **13**, 249–151

Vergara, B.S., Jackson, B., De Datta, S.K. (1976) Deep-water rice and its response to deep-water stress. In: Climate and rice, pp. 301–319. International Rice Research Institute, Los Baños, Philippines

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# Chapter 2

## The Role of Gibberellin in the Growth Response of Submerged Deep-Water Rice

## ABSTRACT

We have shown previously that $C_2H_4$, 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 $10^{-6}$ M TCY for 7-10 d did not elongate when submerged in the same solution or when exposed to 1 $\mu$l $l^{-1}$ $C_2H_4$ in air. Gibberellic acid ($GA_3$) at $3 \times 10^{-7}$ M 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 $10^{-8}$ to $3 \times 10^{-7}$ M $GA_3$ on internodal elongation was enhanced two- to eight-fold when 1 $\mu$l $l^{-1}$ $C_2H_4$ was added to the air passing through the chamber in which the sections were incubated. $GA_3$ and $C_2H_4$ caused a similar increase in cell divisions in the intercalary meristem of rice internodes. Thus, ethylene may cause internodal elongation in rice by increasing the activity of endogenous GAs.

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 $C_2H_4$, the synthesis of which is stimulated in submerged internodes (3). The increase in $C_2H_4$ synthesis in submerged internodes is triggered by reduced concentrations of $O_2$ (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 $GA_3$ and light in the submergence response of rice using isolated stem sections. Tetcyclacis (TCY), 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 $C_2H_4$ on stem elongation in the semi-aquatic plant Callitriche platycarpa (5).

## MATERIAL AND METHODS

Chemicals. Gibberellic acid ($GA_3$) was a gift from Merck and Co., Inc. (Rahway, N.J., USA), TCY [5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,1,$0^{2,6}$,$0^{8,11}$-dodeca-3,9-diene] was a gift of BASF (Limburgerhof, F.R.G.). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo., USA).

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 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° C either in continuous light (cool-white fluorescent tubes; 70 μmole $m^{-2}s^{-1}$) or in darkness. Stem sections used for TCY treatments were excised from rice plants which had been watered daily for 7-10 d with $10^{-6}$ 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 for 2 d in air or in a gas mixture consisting of 3% $O_2$, 6% $CO_2$, 91% $N_2$ (by vol.) and 1 μl $l^{-1}$ $C_2H_4$. Stem sections kept in this gas mixture were standing in 40 ml of distilled water while the stem sections incubated in air were either standing in 40 ml of distilled water or 5 x $10^{-6}$ 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, free-hand longitudinal sections were cut from the surface of each internode

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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 6 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 $10^{-6}$ M TCY solution or distilled water, respectively, 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, expecially in TCY-treated sections. At about $3 \times 10^{-7}$ M, $GA_3$ restored the submergence response of TCY-treated internodes to the level of control (-TCY) internodes. Saturating concentrations of $GA_3$ ($10^{-4}$ M) 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 $GA_3$. In contrast to internodes, rice leaves were less inhibited in growth by TCY (Fig. 1B). Addition of $GA_3$ 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 non-submerged sections to a gas mixture which was similar to the gaseous atmosphere in the internodal lacunae of submerged sections, namely 3% $O_2$, 6% $CO_2$, 91% $N_2$ (by vol.) and 1 µl $l^{-1}$ $C_2H_4$ (7). Internodes of stem sections incubated in this gas mixture for 3 d responded similarly to $GA_3$ 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% $O_2$, 6% $CO_2$, 91% $N_2$ and 1 µl $l^{-1}$ $C_2H_4$ and treated with $10^{-4}$ M $GA_3$ was promoted by 23% while the internodal growth of sections incubated in air was enhanced 18-fold by the same concentration of $GA_3$. Leaf growth was two- to threefold inhibited by 3% $O_2$, 6% $CO_2$, 91% $N_2$ and 1 µl $l^{-1}$ $C_2H_4$ when compared to leaf growth in air (Fig. 2B). The amount of $GA_3$-induced leaf growth was similar in both

treatments. Ethylene did not enhance internodal elongation in sections standing in 40 ml of $10^{-5}$ M TCY solution for 3 d (Fig. 3A). When low concentrations of $GA_3$ 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, $C_2H_4$ inhibited leaf growth at all $GA_3$ concentrations, and only a small enhancement of leaf growth by $GA_3$ was observed in both treatments (Fig. 3B).

Cell divisions in the internodes of stem sections that had been submerged or incubated in 3% $O_2$, 6% $CO_2$, 91% $N_2$ and 1 $\mu$l $l^{-1}$ $C_2H_4$ for 3 d was greatly enhanced (4). We found that both 5 x $10^{-6}$ M $GA_3$ and a gas mixture of 3% $O_2$, 6% $CO_2$, 91% $N_2$ and 1 $\mu$l $l^{-1}$ $C_2H_4$ increased the number of cells in the growing region of rice internodes 17 fold with very little if any difference in the average cell length (Table I). Also, comparable amounts of [$^3$H]thymidine were incorporated into the DNA of the newly elongated region of internodes in sections treated with 5 x $10^{-6}$ M $GA_3$ 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, $10^{-5}$ M $GA_3$ and 3% $O_2$, 6% $CO_2$, 91% $N_2$ and 1 $\mu$l $l^{-1}$ $C_2H_4$ was severly inhibited (Table II). In darkness, the inhibition of growth of exposed internodes was much less pronounced or, in the case of $GA_3$-treated internodes, no inhibition was evident at all. Wrapping the lower 10 cm of submerged stem sections with aluminum foil to darken the exposed internode also increased growth of the exposed internodes compared to those not wrapped in foil (Table II). Thus, exposing internodes to light strongly inhibited the effects of submergence, $GA_3$ and $C_2H_4$ on internodal elongation.

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## DISCUSSION

Figures 1-3 indicate that $C_2H_4$ is likely to cause internodal elongaton 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 physiologically active GAs in the rice internode. This hypothesis is supported by the following results: (i) Stem sections excised from plants that had been watered with $10^{-6}$ M TCY did not elongate when submerged in the same solution or when exposed to 1 $\mu l\ l^{-1}$ $C_2H_4$ in air (Figs. 1,3). Addition of $GA_3$ at concentrations above $10^{-7}$ M restored rapid internodal growth in submerged and $C_2H_4$-treated internodes. (ii) Low $GA_3$ concentrations ($10^{-8}$ to $2x10^{-7}$ M) were much more effective in promoting internodal elongation when 1 $\mu l\ l^{-1}$ $C_2H_4$ was added to the air (Fig. 3). (iii) Saturating concentrations of $GA_3$ ($10^{-5}$ to $10^{-4}$ M) enhanced internodal elongation in the sections incubated in air to the levels observed in the gas mixture containing 3% $O_2$, 6% $CO_2$, 91% $N_2$ and 1 $\mu l\ l^{-1}$ $C_2H_4$ (Fig. 2). (iv) $GA_3$ stimulated cell divisions in the intercalary meristems of rice internodes to the same extent as did the gas mixture of 3% $O_2$, 6% $CO_2$, 91% $N_2$ and 1 $\mu l$ $C_2H_4$ (Table I).

The following chain of events appears to take place following submersion of rice stem sections. The level of $O_2$ in the tissue is greatly reduced as a result of submergence, and lowered $O_2$ concentrations stimulate ethylene synthesis (7). Ethylene accumulates 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).

Acknowledgements. We thank R. deZacks for help in preparing the plant material.

## LITERATURE CITED


1. DE DATTA SK 1981 Principles and practices of rice production. Wiley, New York
2. KAUFMAN PB, P DAYANANDAN 1983 Gibberellin-induced growth in Avena internodes. In A Crozier, ed, The biochemistry and physiology of gibberellins, Vol 2, Praeger Publishers, New York, pp 129-157
3. MÉTRAUX J-P, H KENDE 1983 The role of ethylene in the growth response of submerged deep water rice. Plant Physiol. 72:441-446
4. MÉTRAUX J-P, H KENDE 1984 The cellular basis of the elongation response in submerged deep-water rice. Planta 160:73-77
5. MUSGRAVE A, MB JACKSON, E LING 1972 Callitriche stem elongation is controlled by ethylene and gibberellin. Nature New Biol. 238:93-96
6. RADEMACHER W, J JUNG, E HILDEBRANDT, JE GRAEBE 1983 Influence of the bioregulator tetcyclacis (BAS 106..W) on gibberellin biosynthesis and the hormonal status of plants. In AR Cooke, ed, Proceedings, 10th Annual Meeting, Plant Growth Regulator Society of America, Plant Growth Regulator Soc. of America, Lake Alfred, Florida, pp 36-41
7. RASKIN I, H KENDE 1984 Regulation of growth in stem sections of deep-water rice. Planta 160:66-72
8. VERGARA BS, B JACKSON, SK DE DATTA 1976 Deep water rice and its response to deep water stress. In Climate and rice, International Rice Research Institute, Los Baños, Philippines, pp 301-319

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% $O_2$, 6% $CO_2$, 91% $N_2$ (by vol.) and 1 μl $l^{-1}$ $C_2H_4$ were passed at 80 ml $min^{-1}$. The sections kept in air had their cut ends 2 cm deep in 40 ml of water or 5 x $10^{-6}$ M $GA_3$ solution while sections kept in the above gas mixture were standing in 40 ml of water. Cells were counted in the regions that had elongated during last 20 h of incubation.

| Treatment | Increase in internodal length (mm) | Total number of cells in one file of the newly elongated region | Average cell length (μm) |
|---|---|---|---|
| Air + $H_2O$[a] | 1.1 ± 0.4 | 18.7 ± 5.7 | 57.3 ± 2.6 |
| Air + 5x$10^{-6}$ M $GA_3$[b] | 41.3 ± 2.9 | 308.4 ± 25.1 | 135.0 ± 3.8 |
| 3% $O_2$, 6% $CO_2$, 91% $N_2$ + 1 μl $l^{-1}$ $C_2H_4$[b] | 39.0 ± 1.8 | 316.5 ± 11.4 | 123.3 ± 3.7 |

[a] n = 3 ± SE

[b] n = 6 ± SE

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

The air and gas mixtures (all by vol.) were passed through the 2.5-l incubation cylinders at 80 ml $min^{-1}$.

| Treatment | Leaf sheath | Conditions | Increase in internodal length (mm) |
|---|---|---|---|
| Submerged | + | light | 95.4 ± 5.8 |
| " " | - | light | 2.9 ± 0.5 |
| Submerged, wrapped[a] | + | light | 95.1 ± 6.3 |
| " " " " | - | light | 48.3 ± 4.2 |
| $10^{-5}$ M $GA_3$, air | + | darkness | 81.5 ± 9.5 |
| " " | - | darkness | 86.6 ± 9.4 |
| " " | + | light | 121.8 ± 6.5 |
| " " | - | light | 17.5 ± 5.0 |
| 3%$O_3$ +6%$CO_2$ +91%$N_2$ +1 µl $l^{-1}$ $C_2H_4$ | + | darkness | 66.6 ± 5.7 |
| " " | - | darkness | 46.0 ± 5.9 |
| " " | + | light | 99.8 ± 9.1 |
| " " | - | light | 12.0 ± 2.1 |

[a]The lower 10 cm of the stem sections were wrapped in a 10 x 4 cm piece of aluminum foil.

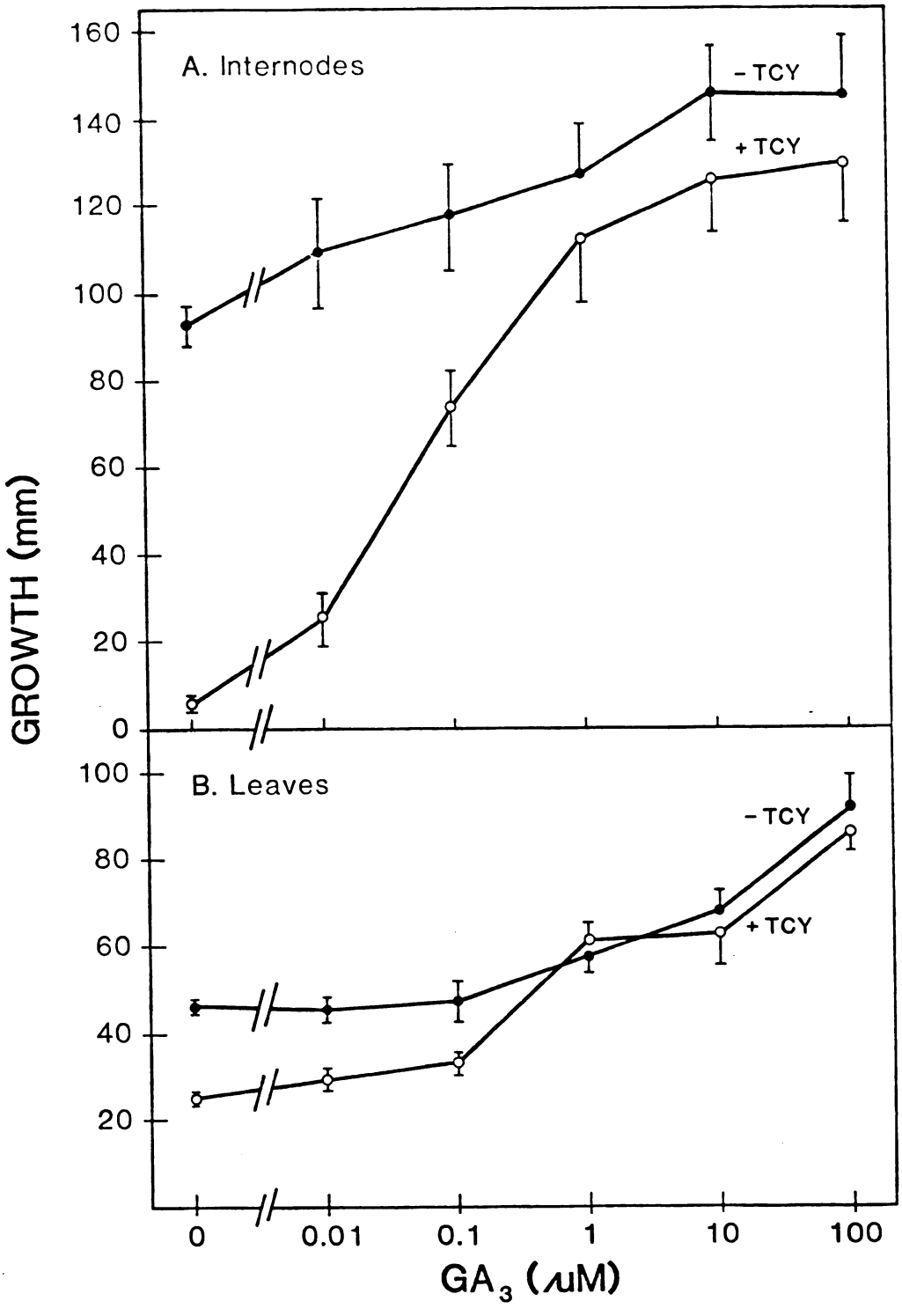
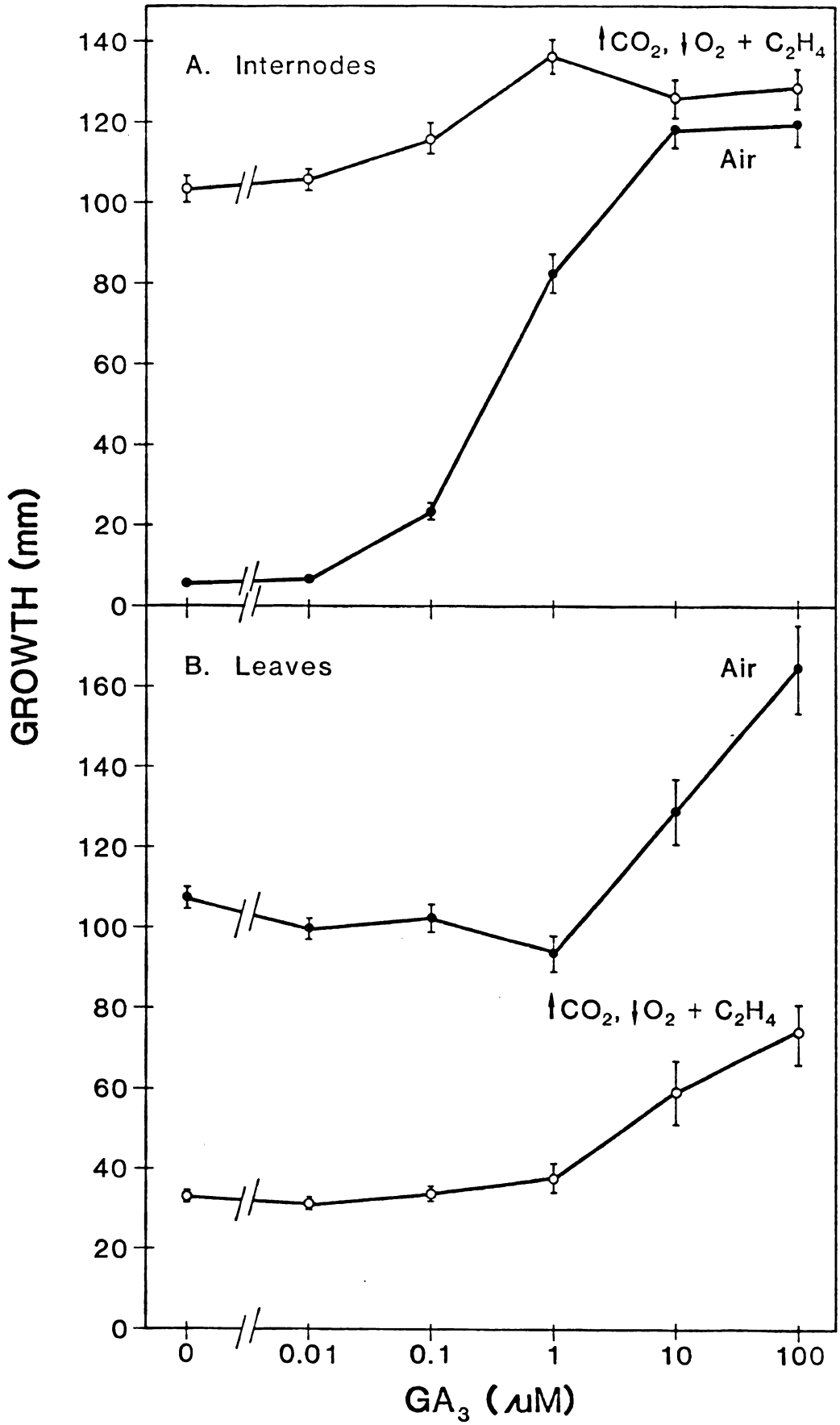



Fig. 1. A,B Effect of $GA_3$ on the growth of internodes (A) and leaves (B) of rice stem sections submerged in $10^{-6}$ M TCY solution (o) or distilled water (•) for 3 d in continous light. Each point is the average of 14 sections. Vertical bars denote ± SE. The lower or upper part of the bar is omitted when it would interfere with another SE bar.

Fig. 2. A,B 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 vol.) and 1 µl $l^{-1}$ $C_2H_4$ ($+O_2$,$+CO_2$ + $C_2H_4$) (o) 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 ml $min^{-1}$. Each point is the average of 25 sections ± SE (see legend Fig. 1). When no bars are given, the SE is smaller than the symbol used.


A. Internodes
↑CO2, ↓O2 + C2H4
Air
B. Leaves
Air
↑CO2, ↓O2 + C2H4
GROWTH (mm)
GA3 (μM)
0
20
40
60
80
100
120
140
160
0
0.01
0.1
1
10
100

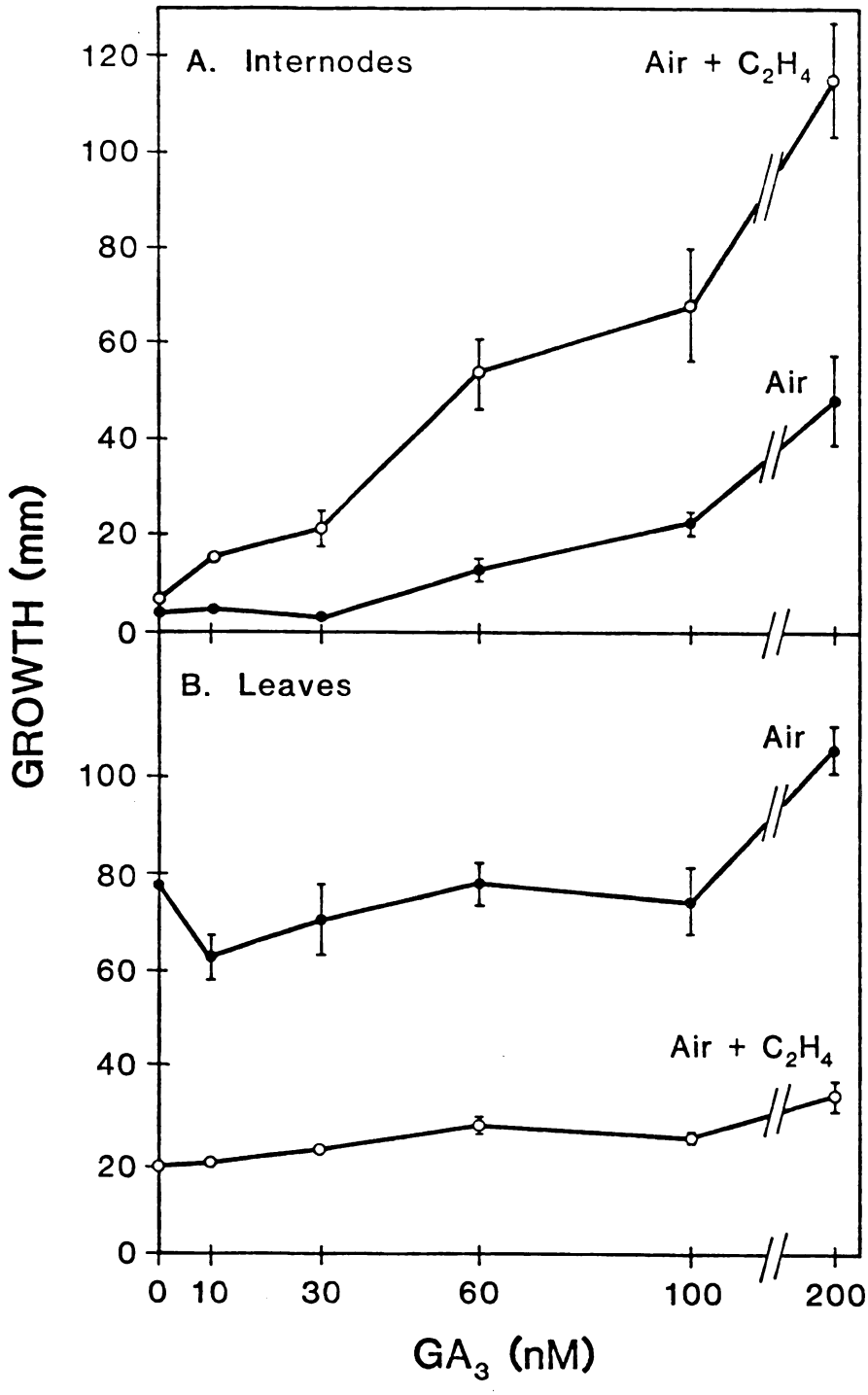



Fig. 3. A,B Effect of low $GA_3$ concentrations on the growth of internodes (A) and leaves (B) of rice stem sections treated with $10^{-6}$ M TCY and incubated in a stream of air (•) or air containing 1 µl $l^{-1}$ $C_2H_4$ (o). The sections, standing upright in 100-ml glass beakers containing 40 ml of $10^{-6}$ 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 ml $min^{-1}$ in continuous light for 3 d. Each point is the average of 14 sections ± SE (see legend Fig. 2).

# Chapter 3

## Effect of Submergence on Translocation, Starch Content and α-Amylase Activity in Deep-Water Rice

Abstract.

Rice seed

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16-fold in

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elongation

Key words:

Partitioni

Abstract. Submergence induces rapid internodal elongation in deep-water rice (Oryza sativa L. cv. "Habiganj Aman II"). We investigated the metabolic activities which help to support such fast growth. Three days of submergence in water under continuous light led to the mobilization of 65% of the starch from those regions of rice internodes which had been formed prior to submergence. Disappearance of starch was accompanied by a 70-fold enhancement of α-amylase activity. Similar increases in α-amylase were detected in response to ethylene and gibberellic acid ($GA_3$). Submergence also caused a 26-fold increase in the translocation of photosynthetic assimilates from the leaves to the internodes and younger regions of the culms. These physiological processes are likely to provide the metabolic energy required for internodal elongation in response to submergence.

Key words: α-Amylase - Deep-water rice - Oryza (growth regulation) - Partitioning - Starch.

## Introduction

Internodes of deep-water rice elongate rapidly, up to 25 cm a day, in response to submergence (Vergara et al. 1976; Métraux and Kende 1983). Internodal elongation is primarily caused by $C_2H_4$ which accumulates in the submerged culms (Métraux and Kende 1983). Growth of internodes in response to submergence or $C_2H_4$ can be reproduced in excised stem sections of deep water rice (Raskin and Kende 1984a). Ethylene appears to promote growth by increasing the activity of endogenous gibberellins (GAs) (Raskin and Kende 1984b). The submergence response in whole deep-water rice plants and stem sections is based on the activation of cell division in the intercalary meristem and subsequent elongaton of the newly formed cells (Métraux and Kende 1984). It has been shown earlier that the "tops" of air-grown rice contain significantly larger amounts of starch than those of submerged rice plants (Yamaguchi 1973) and that amylase activity in rice leaves and internodes increases during submergence (Yamaguchi and Sato 1963). We have examined in greater detail the sources of energy which help sustain the large increase in cell division activity and elongation in the internodes of submerged stem sections of deep-water rice. We have also investigated the effects of $GA_3$ and ethylene, both of which enhance growth of stem sections, on α-amylase activity.

Mate

## Materials and methods

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 and grown as described by Métraux and Kende (1983). Twenty-cm-long stem sections containing the top-most internode were excised, submerged, treated with air or $C_2H_4$ and measured as described by Raskin and Kende (1984a). All experiments were performed at 27° C in continuous light (cool-white fluorescent tubes; 70 μmole $m^{-2}s^{-1}$).

Chemicals. $NaH^{14}CO_3$ solution (2.1 GBq $mmol^{-1}$) was purchased from ICN, Chemical and Radioisotype Div. (Irvine, Cal., USA). Gibberellic acid ($GA_3$) was a gift from Merck and Co. (Rahway, N.J., USA). All other chemicals and enzymatic reagents were purchased from Sigma Chemical Co. (St. Louis, Mo., USA).

α-Amylase determination. Internodes of 5 or 6 stem sections were measured to determine their length increase during the experimental treatment, excised with a razor blade and separated into the old regions formed before the experimental treatment and the new regions formed during the experimental treatment. The internodal tissue was sliced into small pieces, weighed, and 1 g was ground at 1-3° C in a mortar with a pestle in 3 ml of 25 mM sodium acetate buffer (pH 5.4) containing 10 mM $CaCl_2$, 0.1% (v/v) Triton X-100 and 20 mg polyvinylpolypyrrolidone (PVP). The slurry was centrifuged at 12,000 g for 15 min using a Sorvall RC-2B centrifuge and a SS-34 rotor (DuPont Instruments-Sorvall, Wilmington, Del., USA). α-Amylase in the supernatant was assayed according to Jones and Varner (1967). The pellet was saved for starch determination. One enzyme unit (E.U.) of α-amylase activity is the amount of enzyme that causes a change in absorbance of 1 at 620 nm during 10 min.

Starch determination. The pellet left after α-amylase extraction was resuspended in 10 ml hot 80% ethanol and centrifuged at 12,000 g for 15 min. Extraction with hot 80% ethanol and centrifugation was repeated once more to remove residual chlorophyll and soluble sugars. The resulting pellet was extracted with 10 ml of boiling 0.02 M KOH for 15 min, cooled to room temperature and centrifuged at 8,000 g for 15 min. One ml aliquots of the supernatant were added to 2 ml of 50 mM sodium acetate buffer (pH 4.5) in which 50 mg of amyloglucosidase (1,4-α-D-glucan glucohydrolase from Rhizopus mold) were dissolved. The reaction mixture was incubated at 46° C for 90 min, boiled for 2 min and centrifuged at 13,000 g for 15 min in a Micro-Centrifuge Model 235B (Fisher Scientific, Pittsburg, Pa., USA) for 15 min. Glucose in the supernatant was analyzed enzymatically using the hexokinase and glucose-6-phosphate dehydrogenase diagnostic kit.

Carbon translocation. For the translocation experiments, so-called modified stem sections were used. They consisted of stem sections as described by Raskin and Kende (1984a) except that the outer leaf originating from the lower of the two nodes remained attached. It was trimmed to a size of 70 cm above, the basal end of the section (see Fig. 3). Twelve such modified stem sections were placed in each of two 2.5-l cylinders, 60 cm deep, so that 10 cm of the leaf blade protruded above the cylinder rim. One of the cylinders was filled to the rim with distilled water while the other, containing only 50 ml of distilled water in the bottom, was continously flushed with air at the rate of 80 ml $min^{-1}$. Glass beads were placed on the bottom of the submergence cylinder to prevent sections from floating up. After 3 d, six sections were selected from each treatment. The top 7 cm of each leaf were introduced into a 1.1-l transparent plastic chamber through which air containing $^{14}CO_2$ (2.4 MBq $^{14}C$ $l^{-1}$) was passed for 15 min with a circulating pump. $^{14}CO_2$ was obtained by injecting 50 µl of a $Na_2{}^{14}CO_3$ solution into a 250-ml glass flask containing

100 ml of 30% (w/v) $H_3PO_4$. The flask was connected with Tygon tubing to the pump and the leaf chamber. Supplementary illumination was provided with 150 W incandescent bulbs during exposure to $^{14}CO_2$ to give a total light intensity in the leaf chamber of 200 μmol $m^{-2}$ $s^{-1}$. Following $^{14}CO_2$ exposure, the stem sections remained in the incubation cylinders for 210 min under the same conditions as those preceding labeling. Thereafter, the modified stem sections were dissected into 6 different regions: the 7 cm tip of the leaf blade which had been exposed to $^{14}CO_2$, the remainder of the leaf blade, the leaf sheath, the lower half of the internode containing the intercalary meristem, the upper half of the internode, the remainder of the section containing the apical meristem and younger leaf sheaths. Each region was weighed and ground in liquid $N_2$ in a mortar with a pestle. The resulting powder was extracted with 4 volumes of 75% ethanol and centrifuged at 12,000 g for 20 min. One ml aliquots of the supernatant were dried overnight at 70° C, resuspended in 200 μl of 75% ethanol and combusted in a Tri-Carb Sample Oxidizer (Model B 306, Packard Instrument Co., Downers Grove, Ill., USA). The pellets were resuspended in 10 ml 75% ethanol and centrifuged at 12,000 g for 15 min. Resuspension and centrifugation were repeated once more. The pellets were dried overnight at 70° C and combusted in the sample oxidizer. Radioactivity was determined in a Tri-Carb Liquid Scintillation Spectrometer (Model 3255).

Measurement of photosynthetic rates. The net rate of photosynthetic carbon fixation was measured with a Type 225-MK II Infra-Red Gas Analyzer (Analytical Development Co., Hertfordshire, U.K.).

## Results and discussion

Internodes of air-grown rice plants contain large amounts of starch (about 10% of their dry weight). Submergence under continuous light led to rapid disappearance of starch from the older regions of the internodes, which were already present before the start of submergence (Fig. 1). Those portions of the internodes which were formed during submergence, contained 18 to 41 times less starch than the internodes of control sections incubated in air. Decrease of starch content in the older part of the internodes coincided with a marked increase in α-amylase activity. (Fig. 2). No increase in α-amylase activity was detected in the young internodal tissue formed during submergence of rice stem sections and in the sections incubated in air. We showed earlier that acceleration of internodal growth in response to submergence was mediated by ethylene and that accumulation of ethylene in the lacunae of submerged internodes increased the activity of endogenous gibberellins (Raskin and Kende 1984b). An increase in α-amylase activity comparable to that of submerged sections was also detected in the older regions of internodes of non-submerged sections treated with 1 μl $l^{-1}$ $C_2H_4$ or $5x10^{-7}$ M $GA_3$ (Table 1). Submergence, $C_2H_4$ and $GA_3$ also caused a 6- to 12-fold increase in internodal elongation compared to sections incubated in air.

The submergence response was associated with a 26-fold increase in the translocation of photosynthetic assimilates from the above-water parts of the leaves to the rapidly growing internodes and younger regions of the culms (Fig. 3). When rice stem sections, to which a 70-cm-long leaf remained attached at the lower of two nodes, were submerged with the tip of the leaf protruding above the water, 18.1% of the total $^{14}C$ fixed in the uppermost 7 cm of the leaf were transported to the internode during 210 min following labeling. In control sections incubated in air, only 0.7% of the total labeled photosynthetic

assimilates (ethanol soluble and insoluble) were translocated to the internode. The total amount of $^{14}C$ incorporated into submerged sections was 3.2 times greater than that incorporated into sections kept in air. Gas exchange studies showed that under similar light conditions, the net rate of photosynthetic carbon fixation in the above-water leaf tips of submerged sections was 14.8 mg $CO_2$ $dm^{-2}$ $h^{-1}$ while in the tips of air-incubated sections it was 4.8 mg $CO_2$ $dm^{-2}$ $h^{-1}$. At least part of this difference appeared to be caused by wider opening of the stomata on the above-water parts of the leaves of submerged sections when compared to the stomata on the leaves of sections kept in air (data not shown).

Our results indicate that two physiological processes help to satisfy the increased demand for energy, substrates and osmotica in rapidly dividing and elongating cells of the sumberged internodes of deep-water rice. First, flooding causes a marked increase in $\alpha$-amylase activity in rice internodes. This is accompanied by hydrolysis of internodal starch. Second, submergence greatly enhances the rate of carbon export from the leaves to the stem, indicating an increased sink strength in the growing internodes.

We gratefully acknowledge the technical assistance of R. deZacks. This work was supported by the National Science Foundation through Grant No. PCM 81-09764 and by the U.S. Department of Energy under Contract No. DE-AC02-76ER01338.

References

Jones, R.L., Varner, J.E. (1967) The bioassay of gibberellins. Planta 72, 53-59

Mētraux, J.-P., Kende, H. (1983) The role of ethylene in the growth response of submerged deep water rice. Plant Physiol. 72, 441-446

Mētraux, J.-P., Kende, H. (1984) The cellular basis of the elongation response in submerged deep-water rice. Planta 160, 73-77

Raskin, I., Kende, H. (1984a) Regulation of growth in stem sections of deep-water rice. Planta 160, 66-72

Raskin, I., Kende, H. (1984b) The role of gibberellin in the growth response of submerged deep-water rice. Manuscript in preparation.

Vergara, B.S., Jackson, B., De Datta, S.K. (1976) Deep-water rice and its response to deep-water stress. In: Climate and rice. pp. 301-319, International Rice Research Institute, Los Baños, Philippines

Yamaguchi, T., Sato, T. (1963) Studies on floating rice. (2) The effect of water level treatment upon carbohydrate content and the amylase and invertase activities. Proc. Crop Sci. Soc. Japan 31, 357-361

Yamaguchi, T. (1973) Studies on the floating rice III. Effects of raising water level on the growth and carbohydrate contents of the tops. Proc. Crop Sci. Soc. Japan 42, 29-34

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Table 1. Effect of $C_2H_4$ and $GA_3$ on internodal elongation (± SE) and α-amylase activity in the older regions of the internodes formed before isolation of rice stem sections. Air and air containing 1 μl $l^{-1}$ $C_2H_4$ were passed at 80 ml $min^{-1}$ through the incubation cylinders in which sections were standing upright in 100 ml glass beakers containing 40 ml of distilled water or $5x10^{-7}$ M $GA_3$ solution. Each number is the average of 5 or 6 sections incubated for 3 d in continuous light.

| Treatment | Increase in internodal length (mm) | α-Amylase activity (E.U.) |
|---|---|---|
| Air | 8.6 ± 1.9 | 1.3 |
| Submerged | 98.4 ± 13.1 | 19.2 |
| Air + 1 μl $l^{-1}$ $C_2H_4$ | 53.9 ± 6.6 | 15.2 |
| $5x10^{-7}$M $GA_3$ | 99.8 ± 9.5 | 14.6 |

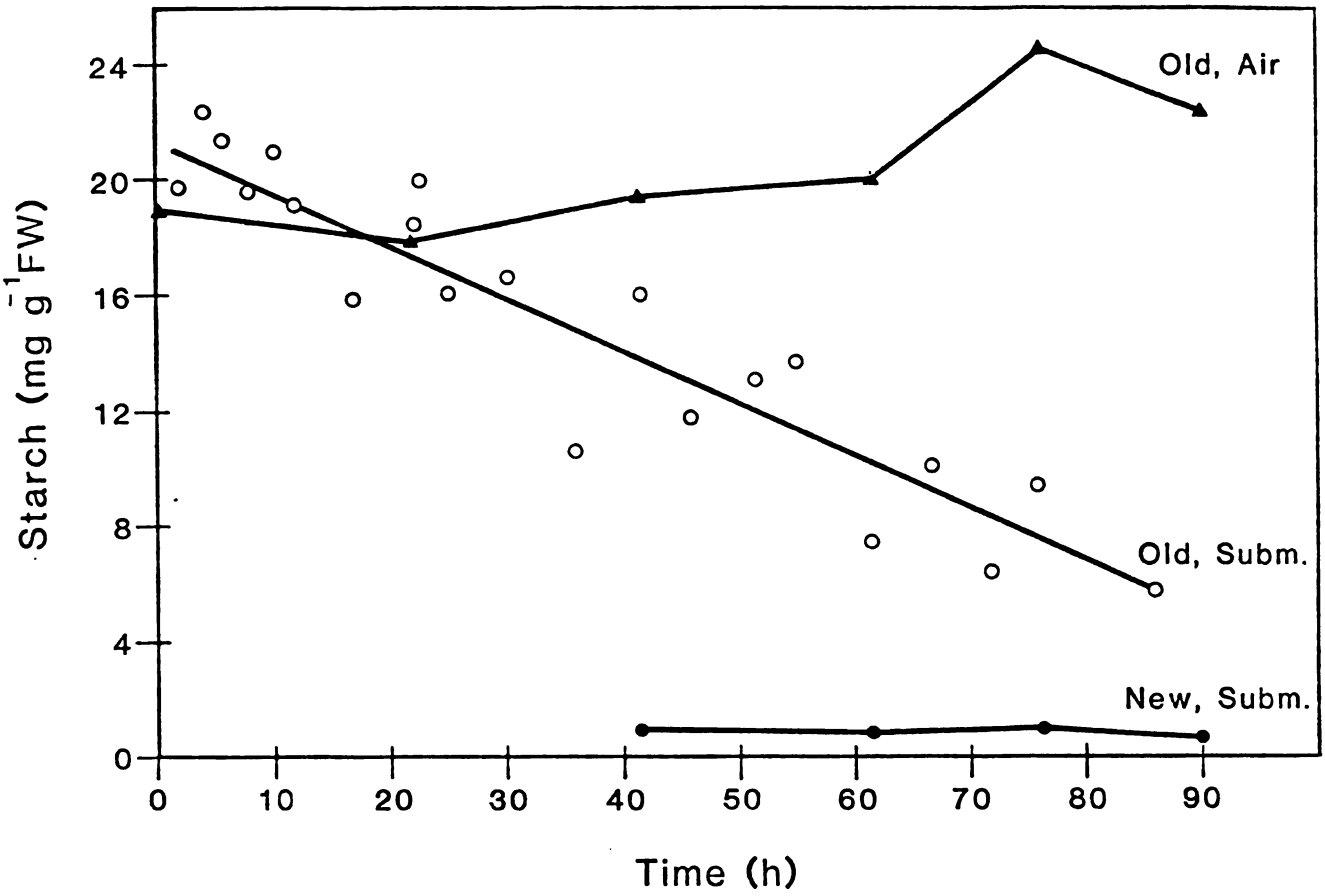


Fig. 1. Starch content of different regions of internodes excised from submerged or air-incubated rice stem sections kept in continuous light. ▲—▲ Older internodal region of stem sections formed prior to isolation of the sections from the plant and incubated in air. o—o Older internodal region of submerged sections formed prior to isolation of the section. ●—● Younger internodal region formed after the stem sections had been submerged. Each point is the pooled average of 5 or 6 sections.

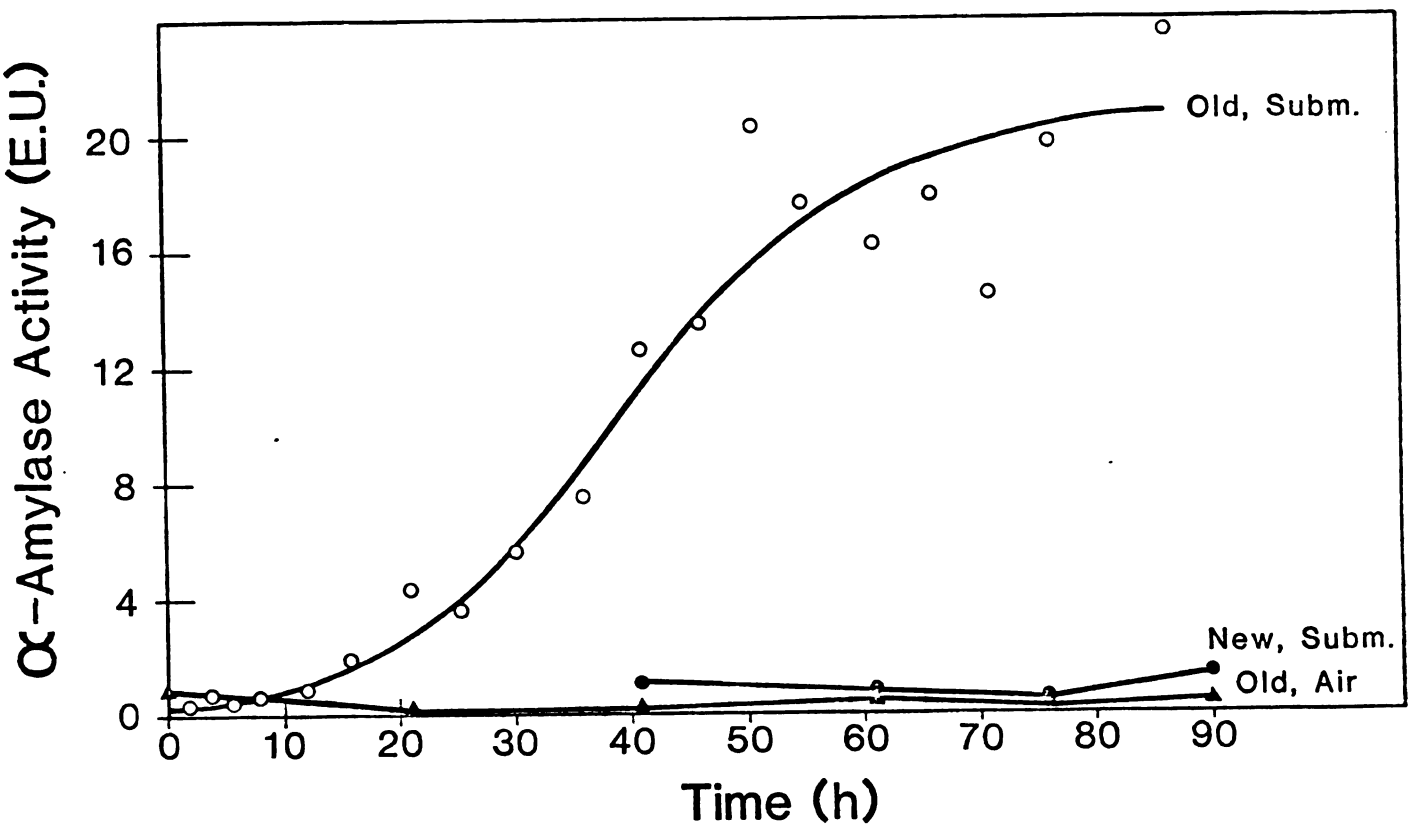


Fig. 2. α-Amylase activity in different regions of internodes excised from submerged or air-incubated rice stem sections kept in continuous light. ▲—▲ Older internodal region of stem sections incubated in air that had been formed prior to isolation of these sections. o—o Older internodal region of submerged sections formed prior to isolation of the sections. ●—● Younger internodal region formed after the stem sections had been submerged. Each point is the pooled average of 5 or 6 sections.

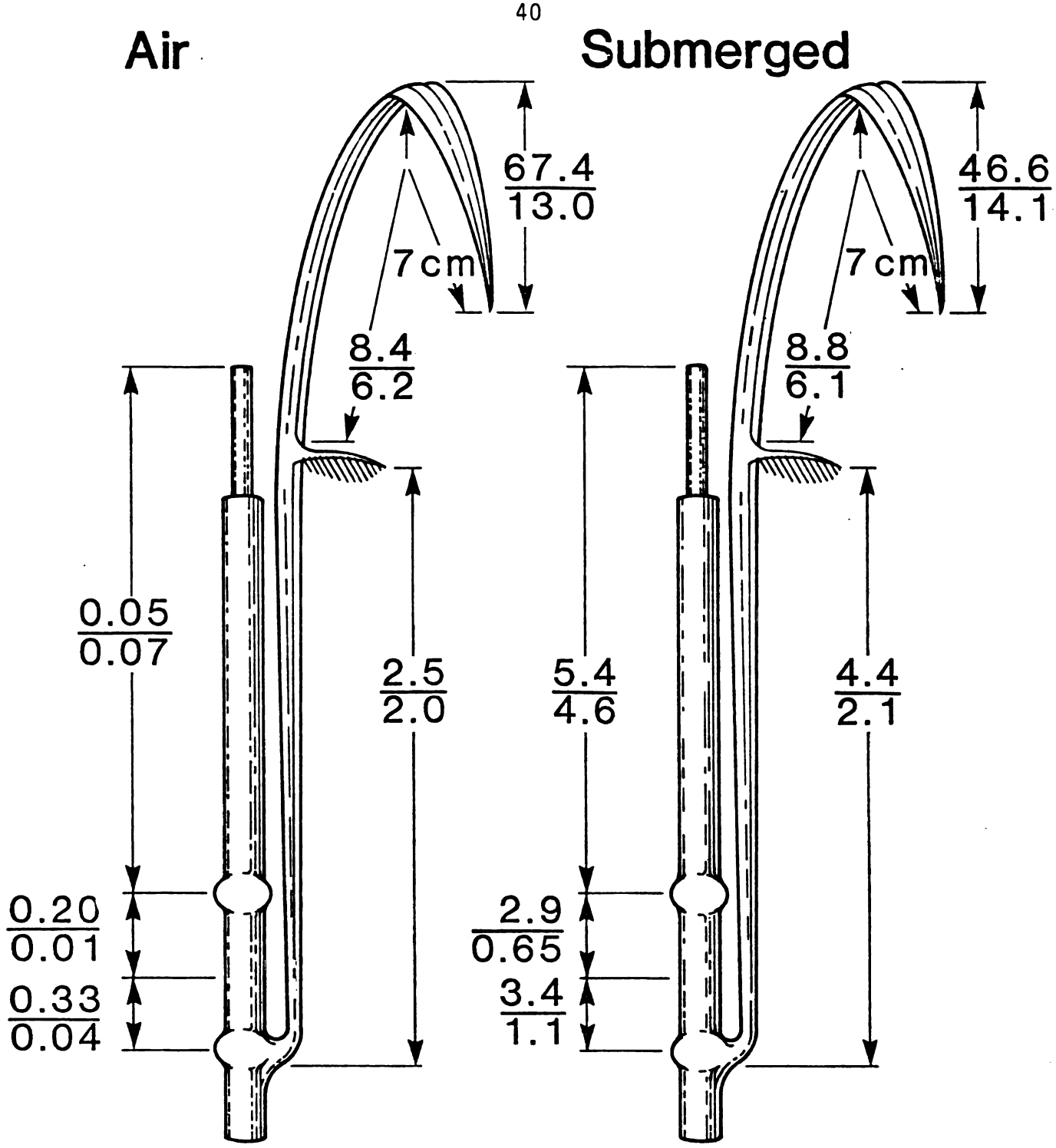


Fig. 3. Effect of submergence and incubation in air on the partitioning of $^{14}C$-labeled assimilates in modified rice stem sections kept in continuous light (% incorporation, ethanol soluble/ethanol insoluble fractions). $^{14}CO_2$ was supplied to 7-cm-long tips of the leaf blades. Sections were separated into different regions 210 min after the end of the exposure to $^{14}CO_2$. Total $^{14}C$ incorporation in submerged stem sections was 37.6 KBq per section while in air-incubated sections total incorporation was 11.7 KBq per section.

# Chapter 4

# Regulation of Growth in Rice Seedlings

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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 $\mu l\ l^{-1}$ 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 $\mu E\ m^{-2}\ s^{-1}$). 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 $\mu l\ l^{-1}$ $C_2H_4$ (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

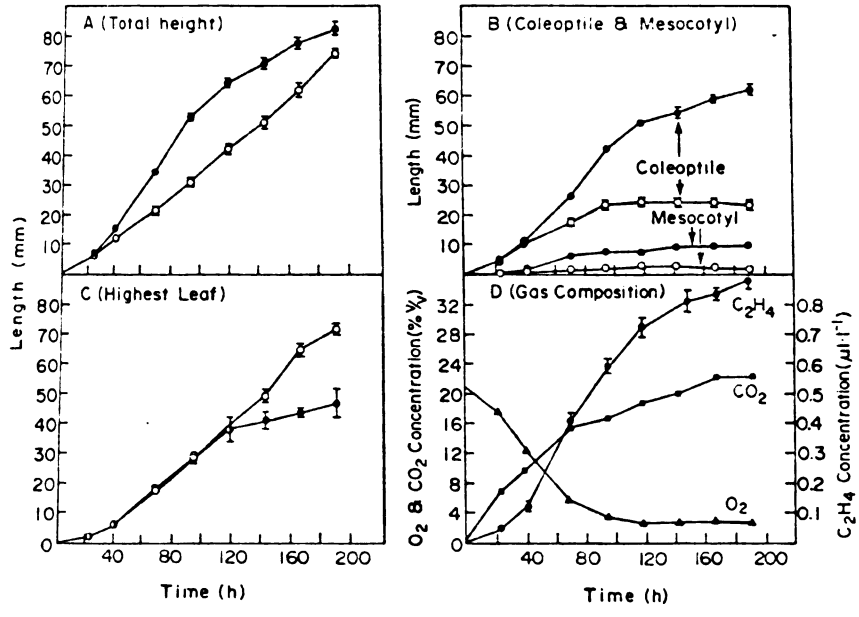
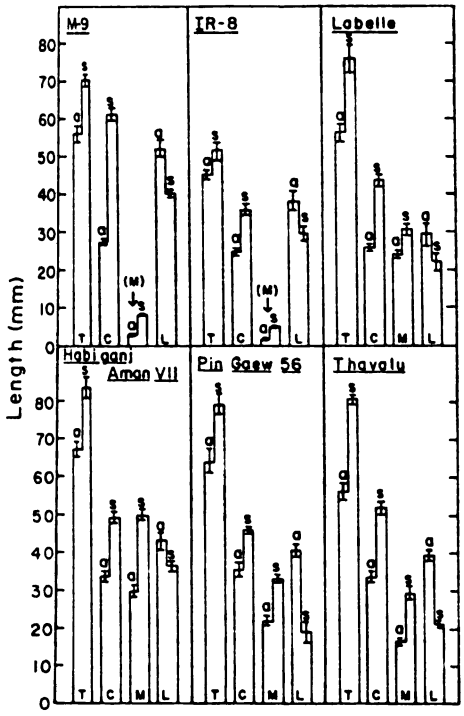



Fig. 1. A–C: The time course of growth of etiolated rice seedlings (cv. M-9) in sealed (●) and aerated (○) containers. D: The time course of changes in $O_2$, $CO_2$, and $C_2H_4$ concentrations inside the containers. Twenty-four 40-ml shell vials, each containing 10 2-day-old seedlings, were sealed, 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 $\mu l\ l^{-1}$ 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 $\mu l\ l^{-1}$) 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



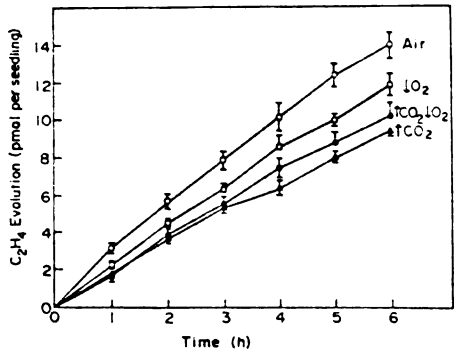




**Fig. 2 (left).** Growth of etiolated seedlings of different rice varieties in sealed and aerated containers. Twenty 2-day-old seedlings of each variety were incubated for 7 days in sealed (s) and aerated (a) 40-ml shell vials (10 seedlings per vial). The air flow was 30 ml $min^{-1}$. Total length 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% $O_2$, 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.

| Treatment | Length (mm) | | | |
|---|---|---|---|---|
| | 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.

| Treatment | Length (mm) | | | |
|---|---|---|---|---|
| | 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-

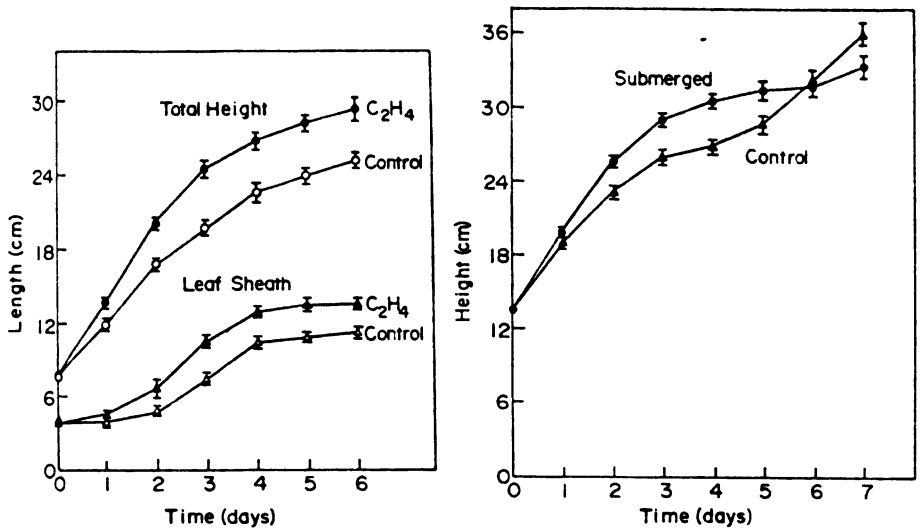



**Fig. 4 (left).** The effect of $C_2H_4$ on the growth of rice seedlings (cv. M-9) under a 16-h photoperiod. $C_2H_4$ (5 μl $l^{-1}$) was added to the stream of water-saturated air passing through the 8-l incubation chamber at a flow rate of 400 ml $min^{-1}$. The seedlings were 8 days old at the start 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) | | | | | Length of incubation (days) |
|---|---|---|---|---|---|---|
| | 10 | 20 | 40 | 60 | 80 | |
| 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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## References

Atwell BJ, Waters I, Greenway H (1982) The effect of oxygen and turbulence on elongation of coleoptiles of submergence-tolerant and -intolerant rice cultivars. J Exp Bot 33:1030–1044

Evans ML, Ray PM, Reinhold L (1971) Induction of coleoptile elongation by carbon dioxide. Plant Physiol 47:335–341

Grodzinski B, Boesel I, Horton RF (1982) Ethylene release from leaves of *Xanthium strumarium* L. and *Zea mays* L. J Exp Bot 33:344–354

Hochachka PW, Mommsen TP (1983) Protons and anaerobiosis. Science 219:1391–1397

Hoson T, Wada S (1980) Role of hydroxyproline-rich cell wall protein in growth regulation of rice coleoptiles grown on or under water. Plant Cell Physiol 21:511–524

Imaseki H, Pjon CJ, Furuya M (1971) Phytochrome action in *Oryza sativa* L. IV. Red and far red reversible effect on the production of ethylene in excised coleoptiles. Plant Physiol 48:241–244

Jackson MB (1982) Ethylene as a growth promoting hormone under flooded conditions. In: Wareing PF (ed) Plant growth substances 1982. Academic Press, London, New York, pp 291–301

Kao CH, Yang SF (1982) Light inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in leaves is mediated through carbon dioxide. Planta 155:261–266

Kefford NP (1962) Auxin-gibberellin interaction in rice coleoptile elongation. Plant Physiol 37:380–386

Kende H, Hanson AD (1976) Relationship between ethylene evolution and senescence in morning-glory flower tissue. Plant Physiol 57:523–527

Kordan HA (1976a) Adventitious root initiation and growth in relation to oxygen supply in germinating rice seedlings. New Phytol 76:81–86

Kordan HA (1976b) Oxygen as an environmental factor influencing normal morphogenetic development in germinating rice seedlings. J Exp Bot 27:947–952

Kordan HA (1977) Coleoptile emergence in rice seedlings in different oxygen environments. Ann Bot 41:1205–1209

Ku HS, Suge H, Rappaport L, Pratt HK (1970) Stimulation of rice coleoptile growth by ethylene. Planta 90:333–339

Métraux J-P, Kende H (1983) The role of ethylene in the growth response of submerged deep water rice. Plant Physiol 72:441–446

Miller JH, Miller PM (1974) Ethylene and the responses to light of rice seedlings. Physiol Plant 30:206–211

Ohwaki Y (1967) Growth of rice coleoptiles in relation to oxygen concentrations. Sci Rep Tôhoku Univ Ser IV 33:1–5

Pratt HK, Workman M, Martin FW, Lyons JM (1960) Simple method for continuous treatment of plant material with metered traces of ethylene or other gases. Plant Physiol 35:609–611

Ranson SL, Parija B (1955) Experiments on growth in length of plant organs. II. Some effects of depressed oxygen concentrations. J Exp Bot 6:80–93

Saltveit ME, Jr (1978) Simple apparatus for diluting and dispensing trace concentrations of ethylene in air. HortSci 13:249–251

Schneider EA, Wightman F (1974) Metabolism of auxin in higher plants. Ann Rev Plant Physiol 25:487–513

Suge H (1971) Stimulation of oat and rice mesocotyl growth by ethylene. Plant Cell Physiol 12:831–837

Taylor DL (1942) Influence of oxygen tension on respiration, fermentation, and growth in wheat and rice. Am J Bot 29:721–738

Turner FT, Chen C-C, McCauley GN (1981) Morphological development of rice seedlings in water of controlled oxygen levels. Agronomy J 73:566–570

Vlamis J, Davis AR (1943) Germination, growth, and respiration of rice and barley seedlings at low oxygen pressures. Plant Physiol 18:685–692

Yamada N (1954) Auxin relationships of the rice coleoptile. Plant Physiol 29:92–96

# Chapter 5

# The Role of Air Layers in the Aeration of Deep-Water Rice

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# How Does Deep Water Rice Solve Its Aeration Problem[1]

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## ABSTRACT

**In partially flooded deep water rice (*Oryza sativa* L. cv Habiganj Aman II), continuous air layers trapped between the hydrophobic, corrugated surface of the leaf blades and the surrounding water constitute the major path of aeration. The conduction of gases through the internal air spaces of the leaf is negligible compared to the conduction of gases through the external air layers. The total volume of the air layers on both sides of a leaf blade is about 45% of the volume of the leaf blade itself. The size of the air layers around submerged leaf blades of cereals not adapted to conditions of partial flooding, *e.g.* of oats, barley, and wheat, is considerably smaller than that of rice. Gases move through the air layers not only by diffusion but also by mass flow. In darkness, air is drawn down from the atmosphere through the air layers along a pressure gradient created by solubilization of respiratory $CO_2$ in the surrounding water. In light, photosynthetic $O_2$ is expelled through the air layers to the atmosphere because the solubility of $O_2$ in water is much lower than that of $CO_2$. Air layers greatly increase the rate of photosynthetic carbon fixation by enlarging the surface of the gas-liquid interface available for $CO_2$ uptake from the water. Air layers are vital for the survival of the partially submerged rice plant. When leaves are washed with a dilute solution of a surfactant (Triton X-100), no air layers are formed under water. Plants without air layers do not grow in response to submergence, and the submerged parts of the plant deteriorate as evident by rapid loss of chlorophyll and protein. Air layers provide a significant survival advantage even to completely submerged rice plants.**

Floating or deep water rice is mainly grown in the floodplains of Southeast Asia where the water can rise up to 6 m during the rainy season (6). Floating rice has great agronomic importance because it is the subsistence crop in many densely populated areas where no other crop can be grown. The distinguishing characteristic of this rice is its ability to elongate with rising waters. Growth rates of 20 to 25 cm/d have been recorded in response to submergence, with the total plant height reaching up to 7 m (15). Survival of deep water rice depends on its ability to keep part of its foliage above the water surface. Completely submerged plants cease to elongate and eventually die (14). Flooding imposes a severe stress on rice plants as $O_2$ and $CO_2$ supplies become limiting under water. The slow diffusion of gases in water (10,000 times slower than in air) greatly curtails the gas exchange between the plant and the surrounding water. This problem would be further aggravated if the gas exchange were limited primarily to the relatively small area of stomata on the rice leaf.

It is commonly believed that the aeration requirements of the submerged organs of rice and other plants tolerant to partial flooding are met by $O_2$ entering the above-water parts of the leaves through the stomata and diffusing to the submerged organs via the internal air spaces loosely termed aerenchyma (1, 2, 4, 7, 9). This view fails to explain the aeration mechanism in deep water rice. In rice, the aerenchyma occupies a significant volume of the roots, internodes, and leaf sheaths. However, aerenchyma is poorly developed in the lower half of the leaf blade and is completely absent in the upper half of the blade (10). Thus, the aerenchyma cannot provide the necessary air connection between the atmosphere and the underwater organs of deep water rice plants which can survive prolonged flooding with only their leaf tips above the water.

This paper presents evidence for the existence of air layers between the corrugated, hydrophobic surface of a submerged rice leaf and the surrounding water. These air layers provide an aeration path which is vital for the partially flooded plant. The mechanism of gas movement through the air layers and the function of air layers in the gas exchange between plant and water are also described.

## MATERIALS AND METHODS

**Plant Material and Growth Conditions.** Seeds of a Bangladesh floating rice variety (*Oryza sativa* L. cv Habiganj Aman II) were obtained from the Bangladesh Rice Research Institute (Dacca, Bangladesh). Rice plants were grown as described previously (12).

Seeds of wheat (cv Ionia), barley (cv Lakeland), and oats (cv Korwood) were sown singly in one-quart plastic pots in potting soil of the same composition as described before (12). These plants were grown in an environmental chamber under the following conditions: day temperature, 24°C; night temperature, 21°C, RH, 60%; 16-h photoperiod with a light intensity of 350 $\mu$E $m^{-2}$ $s^{-1}$ at soil level. Plants were watered twice daily with half-strength Hoagland solution. All experiments were performed with 33- to 55-d-old plants. In the case of rice, this age corresponds to the time of internode elongation. Only fully expanded, healthy looking blades from the upper leaves, 9 to 12 mm in width, were used in our experiments. Leaf sections were excised from the midportions of the leaf blades with a sharp razor blade.

**Chemicals.** Ethane was purchased from Matheson Gas Products (Joliet, IL), $NaH^{14}CO_3$ (7.9 mCi/mmol) was from New England Nuclear, and platinum black was from Fisher Scientific Co. All other chemicals were purchased from Sigma Chemical Co.

**Triton X-100 Treatment.** Formation of air layers on the submerged parts of leaves was prevented by wetting the leaves or leaf sections with a 0.05% (v/v) Triton X-100 solution, followed by a thorough rinse with distilled $H_2O$.

**Ethane Diffusion Measurements.** The experimental set-up for ethane diffusion measurements is shown in Figure 2. The diffusion experiments were performed in light (100 $\mu$E $m^{-2}$ $s^{-1}$) at 24°C. The blade of an attached leaf was introduced into a U-shaped tube which contained water in the bottom. The tip and the lower part of the leaf protruded into the head spaces in the right and left arm of the U-tube, respectively, while 15 cm of the midportion of the blade became submerged in the water. Ethane was injected into the closed head space in the right arm of the tube to yield a

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final concentration of 4.5% (v/v). The atmospheric pressure in the right arm of the U-tube was maintained by periodic insertion of a hypodermic needle through the ethane injection port. Ethane was assayed in the sampling compartment of the left arm through which air was passed at a rate of 2 ml $min^{-1}$. One-ml gas samples were withdrawn through the sampling port with a gas-tight syringe, and the ethane content was determined by GC using the conditions of Kende and Hanson (11). Samples were withdrawn slowly to keep constant pressure in the sampling compartment. The open outlet tubing was sufficiently long to prevent drawing of air from the outside into the sampling compartment.

**Application of Archimedes' Principle for the Determination of the Volumes of Air Layers and Leaves.** The fresh weight of three 7-cm-long leaf sections ($P_1$), excised from the midportion of the leaf blades, was measured, and the sections were attached to a metal clamp of sufficient weight to keep the sections submerged in $H_2O$. The clamp with the leaf sections was suspended with a wire from the pan hook of an analytical balance and submerged in distilled $H_2O$. The weight of the submerged clamp with the sections ($P_2$) was measured. Thereafter, the leaf sections were treated with Triton X-100 to eliminate the air layers, and the weight of the submerged clamp with sections ($P_3$) was measured again. Elimination of air layers led to a decrease in buoyant force ($B$), whereby $B = P_3 - P_2$. According to Archimedes' principle, the buoyant force exerted on the air layers is equal to the weight of water displaced by the air layers. The volume of air layers ($V_{air}$) can then be calculated from:

$$V_{air} = \frac{B}{\rho_{H_2O}} \quad (1)$$

where $\rho_{H_2O}$ is the density of water.

The volume of air layers on each side of a leaf section was determined after wetting each side of the section separately with Triton X-100 solution. The volume of the leaf sections was also calculated by applying Archimedes' principle:

$$V_{leaf} = \frac{B_{leaf}}{\rho_{H_2O}} \quad (2)$$

where $B_{leaf}$ is the buoyant force acting on the submerged sections without air layers and equals

$$B_{leaf} = P_1 - (P_3 - P_4) \quad (3)$$

where $P_4$ is the weight of the submerged clamp. The density of the leaf section ($\rho_{leaf}$) was determined as:

$$\rho_{leaf} = \frac{P_1}{V_{leaf}} \quad (4)$$

The area of the leaf sections was measured with a portable area meter, model LI-3000 (Lambda Instruments Co., Lincoln, NE), equipped with a transparent belt conveyer accessory.

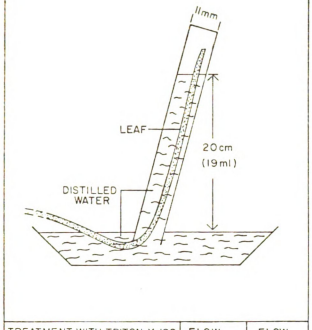
**Oxygen Movement in the Air Layers.** A glass cylinder (40 cm deep, 4.2 cm i.d.) was filled with 450 ml of a 15 mg $L^{-1}$ methylene blue solution, leaving a head space of 65 ml. Forty mg of Pt-black powder was added to the solution as a catalyst. Reduction of methylene blue was accomplished by bubbling gases through the solution in the following order: $N_2$ for 4 min, $H_2$ until the solution became colorless, and $N_2$ for 10 min to remove $H_2$ from the solution and fill the head space with $N_2$. After the reduction was completed, the cylinder was tightly stoppered. All experiments were performed at 28°C in darkness with a green safe light turned on only during measurements. Plants were kept in darkness for 1 h before the experiment. The opposite ends of an excised leaf blade were clamped, adaxial surface outward, to a thin glass rod. At time zero, the stopper was removed from the cylinder, and the leaf blade fixed to the glass rod was lowered into the solution through the head space filled with $N_2$. One cm of the leaf blade was left above the surface of the solution, and the cylinder remained open so that air could diffuse into the head space. The $O_2$ movement along the adaxial surface was followed by measuring the distance between the color front and the surface of the solution every 5 min.

**Manometric Measurement of Gas Flow into and out of Air Layers.** The manometric set-up depicted on the inset of Figure 4 was used to measure the mass flow of gases from the atmosphere into the air layers and vice versa. The set-up consisted of a 60-cm-long glass tube, containing 70 ml of distilled $H_2O$. A leaf blade was placed into the tube such that the leaf tip protruded into the small head space. The tube was closed at both ends with rubber stoppers. Two air-exchange needles and a 50-$\mu$l glass micropipet (manometric tube), which connected the atmosphere to the head space, were fitted through the top rubber stopper. When the air exchange needles were closed, the movement of a water bubble in the manometric tube reflected the volume change in the head space. Eleven manometric set-ups, 10 with excised leaf blades (5 control, 5 Triton X-100 treated) and one without leaf blade (thermobarometer) were immersed in a constant temperature bath at 30°C. Volume changes shown by the thermobarometer were subtracted from the volume changes in the other manometric set-ups to correct for fluctuations in the atmospheric pressure or slight changes of temperature in the water bath.

**Measurement of $^{14}CO_2$ Fixation.** Twelve randomly selected 6-cm-long leaf sections (six Triton X-100 treated and six control) were preincubated in 6 mM $NaHCO_3$ in 0.15 mM Tricine buffer, pH 7.86, in the light (250 $\mu$E $m^{-2}$ $s^{-1}$). After 15 min, the sections were quickly transferred to the same medium containing $NaH^{14}CO_3$ (0.5 $\mu$Ci/ml) under the same light conditions. After 5, 10, and 15 min, two Triton X-100-treated and two control sections were removed from the incubation medium and frozen in liquid $N_2$. Frozen sections were ground in liquid $N_2$ in a mortar with a pestle. The resulting powder was transferred to small test tubes, and 2 ml of 1 M HCl in 80% ethanol was added to the powder to liberate $^{14}CO_2$ from residual, unmetabolized $NaH^{14}CO_3$. The test tubes were dried overnight at 70°C, and their contents were combusted in a Tri-Carb sample oxidizer, model B 306 (Packard Instrument Co., Downers Grove, IL). Radioactivity was determined in a Tri-Carb liquid scintillation spectrometer, model 3255.

**Submergence Tests.** Plants were randomly divided into three groups, and half of the plants in each group was treated with Triton X-100 (treatments 3, 4, and 6, Fig. 8) while the other half was left as nontreated control (treatments 1, 2, and 5). Plants of the first group, referred to as 'partially submerged' (treatments 1 and 3) were lowered with plastic lines into a 300-L Nalgene tank (Nalge, Rochester, NY) filled to the rim with deionized $H_2O$ so that only 15 cm of the foliage remained above the water. The tanks were located in the same environmental chamber where the plants had been grown. Plants of the second group, referred to as 'completely submerged' (treatments 2 and 4) were lowered to the bottom of the tank and left completely submerged for the whole duration of the experiment. Plants of the third group (treatments 5 and 6), referred to as 'not-submerged,' remained under the same growing conditions as before. Plant elongation and protein and Chl contents were measured after 90 h of experimental treatments.

**Protein and Chl Determinations.** Leaf discs, 7 mm in diameter, were cut with a cork borer from the midportions of the leaf blades. In partially submerged plants, protein and Chl contents were determined only in the underwater midportions of those leaf blades which had tips above the water. The leaf discs from each treatment were randomized and floated, adaxial surface up, on water in a Petri dish. Seven randomly selected leaf discs were homogenized in a glass homogenizer with a motor-driven glass plunger in 3 ml of 150 mM Tris-HCl buffer, pH 7.9, containing 0.1% (v/v) Triton X-100. The homogenate was centrifuged at 12,000$g$ for 15 min using a Sorvall RC-2B centrifuge and an SS-




| TREATMENT WITH TRITON X-100 | | FLOW | FLOW |
|---|---|---|---|
| ABAXIAL SIDE | ADAXIAL SIDE | (ml·min⁻¹) | (%) |
| − | − | 0.24±0.023 | |
| + | − | 0.20±0.017 | 80% |
| − | + | 0.05±0.008 | 20% |
| + | + | 0.00±0.00 | |

FIG. 1. Mass flow of air along a submerged rice leaf blade. To eliminate air layers, either the adaxial, abaxial, or both sides of the blade were washed with a 0.05% (v/v) solution of Triton X-100. Each value is the mean of measurements using 20 randomly selected leaf blades ±SE.

34 rotor (DuPont Instruments-Sorvall, Wilmington, DE). Protein in the supernatant was determined according to Bradford (5) using the Bio-Rad protein assay mixture (Bio-Rad Laboratories). For Chl determinations, seven randomly selected leaf discs were homogenized in a glass homogenizer with motor-driven glass plunger in 10 ml 80% acetone (v/v). The homogenate was centrifuged at 6,000*g* for 10 min. Total Chl was determined according to Arnon (3).

## RESULTS

**Conduction of Gases through Air Layers along Leaves.** Conductance of air through the air layers on both sides of the leaf was demonstrated using the experimental set-up shown in Figure 1. A detached leaf blade of rice, with the tip cut off, was introduced through water into an inverted cylinder which had a small head space of air. The slight reduction of pressure in the head space created by the weight of the water column caused a mass flow of air, 0.24 ml $min^{-1}$, along the leaf from the outside into the head space. When both sides of the leaf had been washed with a Triton X-100 solution, no detectable air flow occurred (Fig. 1). Similar results (not shown) were obtained when a 15-mm wide ring of clear nail polish had been applied around the basal portion of the submerged leaf blade to disrupt the continuity of the air layers. These results indicate that the mass flow of gases through the internal air spaces of the submerged leaf or through the water is negligible compared to the mass flow through the external air layers. Treating either side of the leaf with Triton X-100 demonstrated that about 80% of the air flow was conducted through the air layer on the adaxial and 20% through the air layer on the abaxial surface of the blade (Fig. 1).

While the results shown in Figure 1 demonstrate the possibility of a mass flow of gases through the air layers along a pressure gradient, Figure 2 shows the diffusion of gases through the air layers along a concentration gradient. Ethane diffused from the right side of the U-tube through the air layers along the submerged part of the leaf to the sampling compartment on the left side of the tube. After 50 min, the ethane concentration in the air at the exit port of the sampling compartment reached a constant average level of 83 $\mu l\ L^{-1}$. Only traces of ethane appeared in the sampling compartment when the air layers were eliminated by treatment with Triton X-100 or interrupted by applying a 1-cm wide ring of paraffin oil around the leaf. Ethane has been chosen as gas for this experiment because its diffusion coefficient and solubility in water are close to those of $O_2$ and because its concentration can be easily determined by gas chromatography.

The data from Figure 2 can be used to calculate the combined cross-sectional area $A$ ($cm^2$) of the air layers on both sides of a leaf blade provided that the lateral loss of ethane from the air layers is small. The steady state equation of one-dimensional diffusion according to Armstrong (1) is:

$$J = \frac{DA\ (C_0 - C_1)}{L} \tag{5}$$

where $J$ is the rate of diffusion of ethane through the air layers (g $s^{-1}$), $D$ the diffusion coefficient of ethane (0.128 $cm^2\ s^{-1}$, see Ref. 13), $C_0$ (g $cm^{-3}$) the concentration of the ethane in the right arm, $C_1$ the concentration of ethane in the sampling compartment (g $cm^{-3}$), and $L$ (15 cm) the length of the diffusion path which is equal to the length of the submerged portion of the leaf blade.

$J$ can be calculated from:

$$J = C_1 F \tag{6}$$

where $C_1$ is the steady state concentration of ethane in the sampling compartment after about 50 min (83 $\mu l\ L^{-1}$ or 0.11 $\mu g\ cm^{-3}$) and $F$ is the flow rate of air in the sampling compartment ($cm^3\ s^{-1}$). The value of $A$ can be calculated after substitution of Equation 6 for $J$ in Equation 5. From the above equations, the cross-sectional area $A$ of the air layers around an 1.1-cm wide leaf was calculated to be 0.0070 $cm^2$.

A theoretical curve for the diffusion of ethane into the sampling compartment as a function of time (Fig. 2) could be derived from the equation for one-dimensional diffusion modified from Jacobs (8),

$$C_1 = \frac{ADC_0}{LF}\left(1 + 2\sum_{n=1}^{n=\infty}(-1)^n\, e^{-\frac{n^2\pi^2 Dt}{L^2}}\right) \tag{7}$$

where $t$ is the time (s) from the start of the experiment. The combined cross-sectional area of the air layers on both sides of a 1.1-cm wide leaf blade $A$ used to solve Equation 7 was determined by applying Archimedes' principle (see below). Based on these measurements, $A$ was found to be 0.0066 $cm^2$ (Table I), which was very close to the value of 0.0070 $cm^2$ obtained in the diffusion experiments. The time needed to attain a constant concentration of ethane in the sampling compartment was longer than predicted on the basis of Equation 7 (Fig. 2). This discrepancy very likely arose because of initial losses of ethane to the water and the leaf. Such lateral losses are not taken into account in Equation 7.

**Measurement of the Volumes of Air Layers and Leaves.** The volumes of air layers and leaves as well as leaf density and thickness were determined by applying Archimedes' principle (Table I). The ability to trap air along a submerged leaf was also present in cereals other than rice, e.g. in oats, barley, and wheat

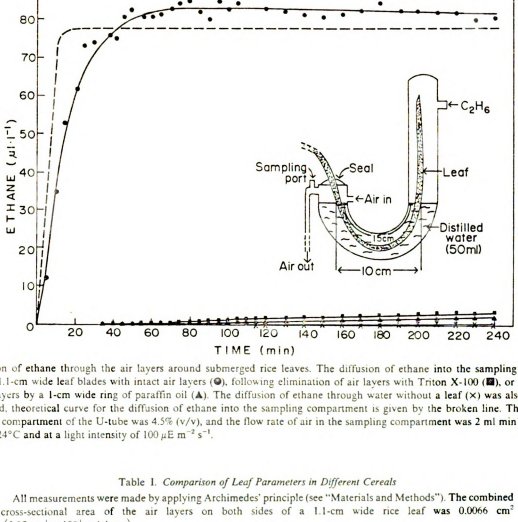



FIG. 2. Diffusion of ethane through the air layers around submerged rice leaves. The diffusion of ethane into the sampling compartment was determined using 1.1-cm wide leaf blades with intact air layers (●), following elimination of air layers with Triton X-100 (■), or interruption of the continuity of air layers by a 1-cm wide ring of paraffin oil (▲). The diffusion of ethane through water without a leaf (×) was also determined. The computer-generated, theoretical curve for the diffusion of ethane into the sampling compartment is given by the broken line. The concentration of ethane in the right compartment of the U-tube was 4.5% (v/v), and the flow rate of air in the sampling compartment was 2 ml $min^{-1}$. The experiment was performed at 24°C and at a light intensity of 100 μE $m^{-2}$ $s^{-1}$.

Table I. *Comparison of Leaf Parameters in Different Cereals*

All measurements were made by applying Archimedes' principle (see "Materials and Methods"). The combined cross-sectional area of the air layers on both sides of a 1.1-cm wide rice leaf was 0.0066 $cm^2$ $\left(\frac{5.97\ cm^3 \times 10^{-1} \times 1.1\ cm}{1\ cm^2}\right)$.

| Parameter | Rice | Wheat[a] | Barley[a] | Oats[a] |
|---|---|---|---|---|
| Leaf density (g $cm^{-3}$) | 0.79 ± 0.02[b] | 0.83 ± 0.004 | 0.80 ± 0.005 | 0.79 ± 0.007 |
| Average leaf thickness (mm) | 0.14 ± 0.003[c] | 0.21 ± 0.004 | 0.27 ± 0.01 | 0.28 ± 0.004 |
| Volume of air layers over 1 $cm^2$ of the leaf ($cm^3$ × $10^{-1}$) | 5.97 ± 0.3[c] | 1.85 ± 0.15 | 2.27 ± 0.22 | 1.19 ± 0.11 |
| Volume of air/volume leaf | 0.44 ± 0.02[c] | 0.09 ± 0.007 | 0.08 ± 0.009 | 0.04 ± 0.004 |
| Volume of air layers over abaxial side (%) | 24.8[d] | | | |
| Volume of air layers over adaxial side (%) | 75.2[d] | | | |

[a] $n = 10 \pm$ SE.
[b] $n = 17 \pm$ SE.
[c] $n = 27 \pm$ SE.
[d] $n = 10$.

(Table I). However, the ratio of the volume of the air layers to the volume of the leaf in rice was five times larger than that in wheat, close to six times larger than that in barley, and 11 times larger than that in oats. It can be calculated that the leaves of a completely submerged 65-d-old rice plant of 145 cm height with a total leaf blade area of 34 $dm^2$ trap about 20 $cm^3$ of air in their air layers.

The rice leaf is about half as thick as the leaves of the other cereals (Table I). In thinner leaves, the diffusion of gases to the mesophyll cells is more rapid because of the shorter diffusion path. Although the density of rice leaves is not significantly different from that of wheat, barley, and oat leaves, the density of the submerged rice leaves is lower because of the larger air layers. The size of air layers of nonfloating rice varieties, such as M 9, is similar to that of Habiganj Aman II (data not shown).

**Movement of Oxygen in the Air Layers.** In darkness, air layers

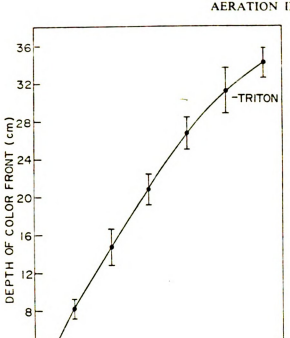



FIG. 3. $O_2$ movement through the air layers in darkness determined by the oxidation of reduced methylene blue (15 mg $L^{-1}$). $O_2$ movement from the atmosphere through the air layers along the adaxial surface of a partially submerged rice leaf was followed by measuring the position of the blue color front relative to the surface of the solution at 28°C. Every 5 min, a green safe light was turned on for about 5 s to take each measurement. (●), Leaves with intact air layers; (■), Triton X-100-treated leaves without air layers. Each point represents the mean of measurements using five leaf blades; the vertical bars denote SE.

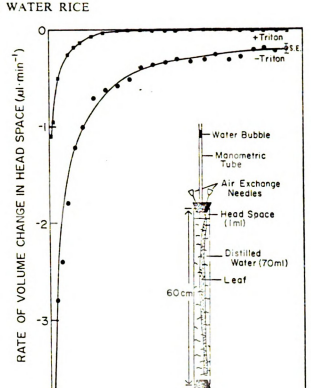



FIG. 4. Mass flow of air into the air layers in darkness measured as the rate of volume change in the head space of the manometric set-up containing an excised leaf blade with intact air layers (●), and following elimination of air layers by Triton X-100 treatment (■). The experiment was performed at 30°C. After each measurement of volume change, the head space was flushed with 10 ml of air through the air exchange needles which were then tightly closed again. Each point represents the mean of measurements using five leaf blades and is adjusted for the fluctuation in the atmospheric pressure recorded by the thermobarometer.

conducted atmospheric $O_2$ to the lower portions of the submerged leaf blades (Fig. 3). The downward movement of $O_2$ through the air layers was much faster than liquid phase diffusion of $O_2$ down from the water surface. The lateral loss of $O_2$ from the air layers due to the respiratory demand of the leaf and solubility of $O_2$ in water did not seem to prevent longitudinal movement of $O_2$ along the air layers.

**Mass Flow of Gases through Air Layers: the 'Snorkel' Function.** Figures 4 to 6 demonstrate that there is directional mass flow of gases in and out of the air layers. In darkness, flow of air into the air layers caused the volume of the head space to decrease continuously. The rate of this decrease diminished monotonically with time (Fig. 4). Even after 12 h of dark incubation, the untreated blades withdrew air from the head space at the rate of 0.2 $\mu l\ min^{-1}$. Elimination of air layers with Triton X-100 greatly diminished the initial rate of volume reduction and brought it to zero within 3 h. Mass flow of gases from the head space into the air layers was also inhibited when the air in the head space was exchanged with $N_2$ (Fig. 5). The withdrawal of gas into the air layers totally ceased within 2 h after the introduction of $N_2$, while it continued for the duration of the experiment in the air controls. When the air in the head space was exchanged with $O_2$, the high rate of $O_2$ uptake from the head space was maintained for the duration of the experiment (Fig. 5).

Figure 6 shows the changes in the volume of the head space during the following schedule of light-dark treatments: 5 h darkness → 75 min light (200 $\mu E\ m^{-2}\ s^{-1}$) → 5 h darkness. In the case of untreated leaf blades, illumination led to immediate reversal in the direction of gas movement. Gases were expelled from the air layers into the head space at an average rate of 6.7 $\mu l\ min^{-1}$. When the light was turned off, the direction of gas movement was immediately reversed again. Air from the head space was drawn back into the air layers, causing a continuous decrease in the head space volume. The similar rates of volume changes at the beginning of the first and second dark period indicated that light fully restored the high rate of gas withdrawal from the head space. During the second dark period, the high initial rate of mass flow decreased again with kinetics similar to those observed during the first dark period. Partially or completely submerged, illuminated leaf blades without air layers slowly formed small bubbles of photosynthetic $O_2$ along the leaf surface. The formation of these bubbles increased the volume inside the manometric set-up (Fig. 6).

**$CO_2$ Uptake through Air Layers: the 'Gill' Function.** Submerged plants use $CO_2$ dissolved in water for photosynthesis. In herbaceous plants, $CO_2$ is primarily taken up through the stomata. Therefore, the surface area through which $CO_2$ is absorbed by submerged leaves without air layers is largely limited to the combined area of the stomatal pores. Air layers greatly increase

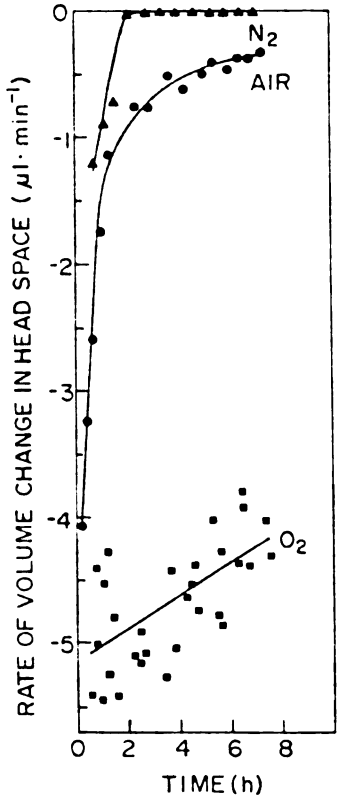



FIG. 5. Mass flow of gases into the air layers in darkness measured as the rate of volume change in the head space containing air (●), $N_2$ (▲), or $O_2$ (□). Nitrogen or $O_2$ was bubbled through the water of the manometric set-ups for at least 2 h. Thereafter, the leaf blades were introduced into the cylinders (time 0), the manometers were stoppered, and the head spaces of the manometric set-ups were flushed with $N_2$ or $O_2$ through the air exchange needles for 20 min at a flow rate of 30 ml $min^{-1}$. After flushing, the air exchange needles were tightly stoppered again. The thermobarometers were prepared like the manometric set-ups, except that no leaf was inserted into them. In the case of the $O_2$ atmosphere, the head space was flushed with 10 ml $O_2$ after every fourth measurement of volume change. The experiment was performed at 30°C. Each point is the mean of measurements using five leaf blades and is adjusted for the fluctuation in the atmospheric pressure recorded by the thermobarometer.

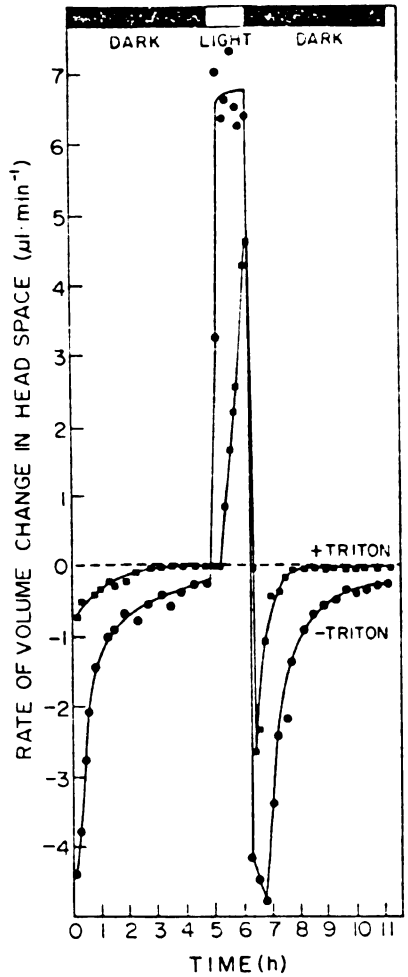



FIG. 6. Mass flow of gases in and out of the air layers measured as the rate of volume change in the head space of the manometric set-up containing the excised leaf blade with intact air layers (●), and following elimination of air layers by Triton X-100 treatment (■). Leaf blades were illuminated with white light for 75 min (light intensity, 200 μE $m^{-2}$ $s^{-1}$) after 5 h of dark incubation. The experiment was performed at 30°C. Each point is the mean of measurements using five leaf blades and is adjusted for the fluctuation in the atmospheric pressure recorded by the thermobarometer.

the surface area for $CO_2$ absorption from the surrounding water, thus increasing the amount of $CO_2$ available for photosynthesis. The results shown in Figure 7 demonstrate this gill function of air layers. Leaf sections submerged in a solution of $NaH^{14}CO_3$ incorporated $^{14}C$ into photosynthetic products at a rate that was 9- to 10-fold higher with air layers than without.

**Physiological Significance of Air Layers.** Elimination of air layers led to loss of Chl and protein in the underwater parts of partially submerged leaf blades (compare treatments 1 and 3, Fig. 8, A and B) and severely reduced the elongation response to flooding (compare treatments 1 and 3, Fig. 8, C and D). Completely submerged plants also lost protein and Chl, and no elongation response to flooding was observed. The most severe manifestation of these symptoms was seen in completely submerged plants without air layers (compare treatments 2 and 4, Fig. 8, A–D). Therefore, even under conditions of total submergence, air layers conferred some adaptive advantage to plants. Treatments 5 and 6 (Fig. 8, A–D) showed that Triton X-100 by itself did not cause loss of protein and Chl and did not inhibit growth. This experiment also confirmed that partial submergence stimulated growth and internode elongation of plants with intact air layers (compare treatments 1 and 5, Fig. 8, C and D).

## DISCUSSION

Floating or deep water rice plants remain submerged for a significant period of the growing season with only part of their foliage above the water. Because of the poorly developed internal air spaces, the interior of the leaf blade does not provide an aeration path from the atmosphere to the aerenchyma of the submerged culms and roots. Our results demonstrate that rice leaves have a unique external system of gas exchange which insures continuous supply of $O_2$ and $CO_2$ to the submerged parts of the plant. The corrugated surface of rice leaves is covered with hydrophobic waxes which prevent water from entering the longitudinal grooves on the leaf blade. These grooves are particularly pronounced on the adaxial surface of the leaves. The air trapped between the water-repellant surface of the leaf and the surrounding water gives a characteristic silvery appearance to the submerged part of the leaf blade. The existence of air layers on the surface of submerged leaf blades and the ability of these layers to conduct gases has been demonstrated in Figures 1 to 3. Aeration of a partially submerged leaf blade of rice proceeds almost exclusively through these air layers which provide an uninterrupted, low resistance, and linear path for gas movement. In partially

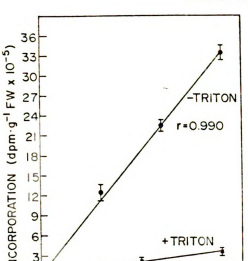



FIG. 7. Photosynthetic carbon fixation in submerged leaf blades of rice with intact air layers (●) and following elimination of air layers by Triton X-100 treatment (■), measured as the incorporation of $^{14}C$ from a $NaH^{14}CO_2$ solution (0.5 $\mu$Ci/ml) at 22°C and a light intensity of 250 $\mu E\ m^{-2}\ s^{-1}$. Each point is the average of three replicates with two leaf sections each; the vertical bars denote SE.

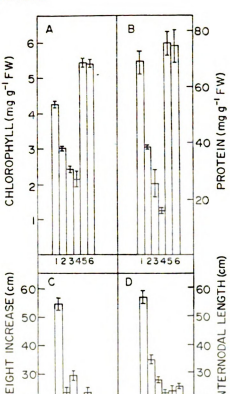
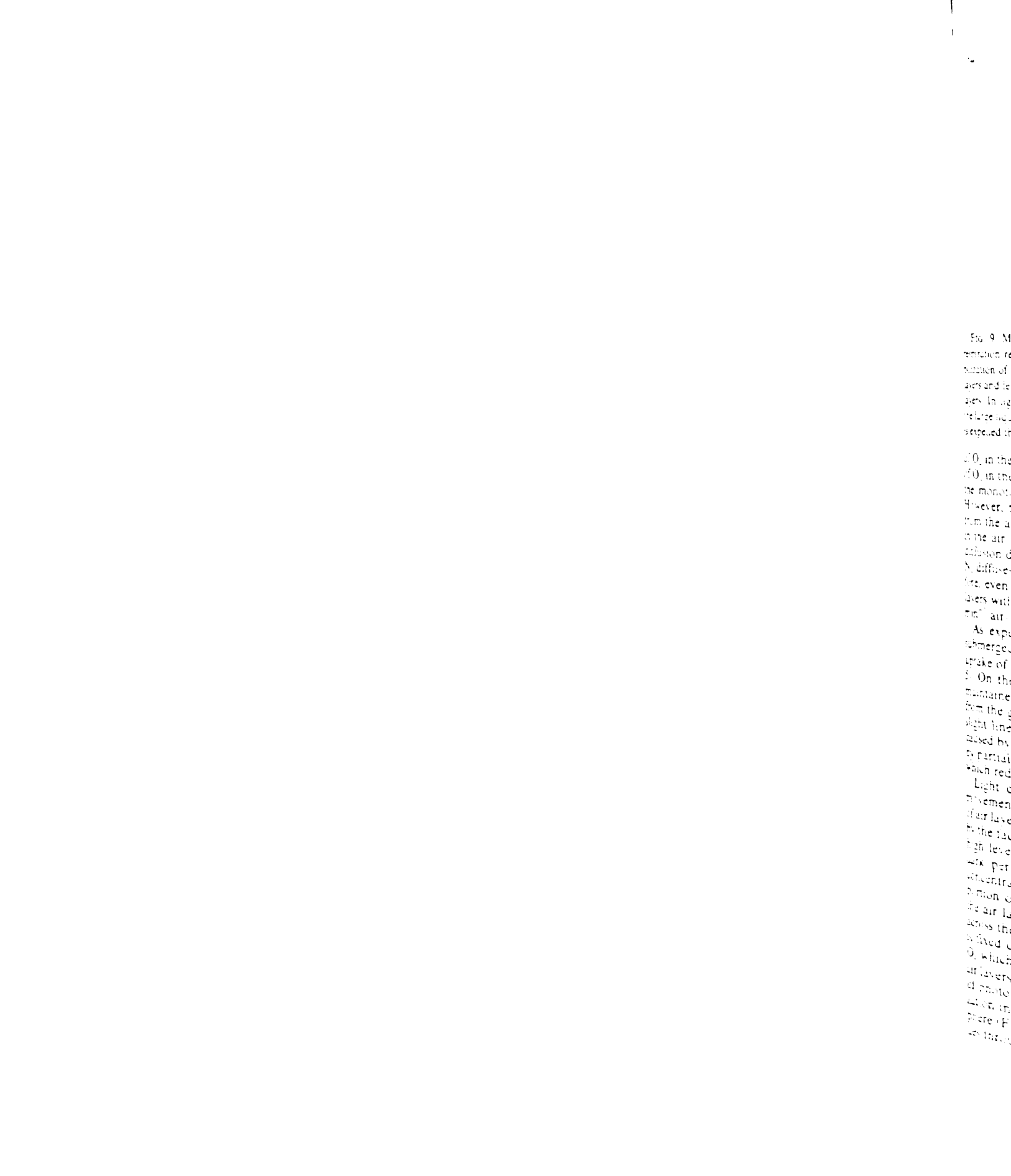



FIG. 8. A to D, physiological importance of air layers for the elongation response and for the maintenance of Chl and protein levels. Plants were divided into six groups for the following treatments: partially submerged plants with intact air layers (treatment 1); totally submerged plants with intact air layers (treatment 2); partially submerged plants with air layers eliminated by Triton X-100 (treatment 3); totally submerged plants with air layers eliminated by Triton X-100 (treatment 4); nonsubmerged plants (treatment 5); nonsubmerged plants treated with Triton X-100 (treatment 6). Total height, internodal length, and protein and Chl contents were measured after 90 h of experimental treatments. The experiment was performed in the same growth chamber where the rice plants were grown. Each value is the mean derived from three plants; the vertical bars denote SE.

submerged floating rice plants, only the younger, upper leaves protrude from the water. The older, lower leaves die after they become completely submerged so that the basal part of the submerged floating rice plant consists of elongated internodes only. $O_2$ from the air layers must eventually be taken up into the internal gas-conducting lacunae of the leaf sheaths and internodes. The diffusion of gases from the air layers into the internal air spaces of the leaves occurs, presumably, through the stomata in the basal regions of the leaf blades. In that region of the leaf, the air lacunae become more prominent in size and number and have direct connections with the lacunae of the leaf sheaths. The uptake of gases is probably completed in the less cuticularized leaf sheath where the air layers become trapped between the concentric sheaths of the different leaves.

The volume of the air layers on both surfaces of the leaf was determined by applying Archimedes' principle and was found to be 44% of the volume of the leaf blade. About 20% of the air was trapped along the abaxial side of the leaf and about 80% along the adaxial leaf surface (Table 1) on which most of the stomata are located (10). The calculation of the size of air layers from the diffusion equation using the data from Figure 2 and the determination of mass flow of gases along each surface of the leaf (Fig. 1) confirmed the above numbers. Other cereals, *e.g.* oats, barley, and wheat, also form air layers around submerged leaves. However, the size of the air layers in rice is much larger than that in the other cereals not adapted to life under flooded conditions (Table 1).

The kinetics of $O_2$ movement in the air layers (Fig. 3) do not conform to the diffusion equation according to which the time required for a gas to diffuse across a given distance is directly proportional to the square of that distance. The relative linearity of $O_2$ movement through air layers (Fig. 3) indicates that this process is based on mass flow in addition to diffusion. The experiments of Figures 4 to 6 confirm that gases not only diffuse through the air layers of partially submerged leaf blades but are actively moved into and out of these layers. This process greatly increases the effectiveness of air layers in the aeration of the plant. In darkness, air from the above-water atmosphere was drawn to the submerged parts of the leaf for at least 12 h, the approximate duration of the natural night (Fig. 4). The rate of air uptake decreased monotonically with time but, even after 12 h, it remained as high as 0.2 $\mu l\ min^{-1}$ for the average leaf blade. We propose that the movement of gases into and out of the air layers is based on the different solubilities of $O_2$ and $CO_2$ in water (Fig. 9). At 30°C, the solubility of $CO_2$ in water is about 28 times greater than that of $O_2$. At the beginning of the night, the air layers are rich in photosynthetic $O_2$, which has accumulated during the preceding photoperiod. The submerged parts of leaves use $O_2$ from the air layers for respiration and produce $CO_2$ which diffuses back into the air layers and rapidly dissolves in the water across the large gas-liquid interface. The consumption of $O_2$ in darkness and solubilization of respiratory $CO_2$ in the water leads to a decrease in pressure inside the air layers and results in mass flow of air from the atmosphere into the air layers (Fig. 9). The air which moves into the air layers to replace the consumed $O_2$ contains only 21% of $O_2$, which is eventually also consumed and replaced by the same amount of air. This will reduce the amount



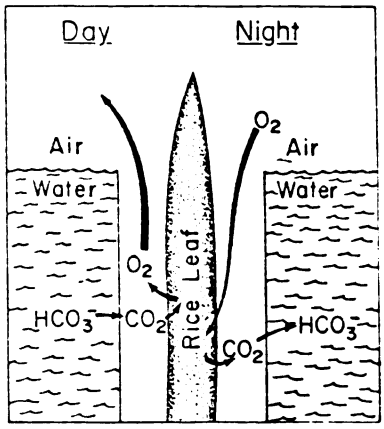



FIG. 9. Model for the mass flow of gases through air layers. In darkness, respiration replaces relatively water-insoluble $O_2$ with $CO_2$. Rapid solubilization of $CO_2$ in water causes a decrease in the pressure inside the air layers and leads to the movement of air from the atmosphere into the air layers. In light, photosynthetic $CO_2$ is absorbed from the water through the large liquid-gas interface provided by the air layers. Photosynthetic $O_2$ is expelled through the air layers into the above-water atmosphere.

of $O_2$ in the air layers by another factor of 0.21. Thus, the amount of $O_2$ in the air layers decreases continuously which is reflected in the monotonical decrease in the rate of air uptake (Figs. 4–6). However, this process does not lead to complete removal of $O_2$ from the air layers because the decrease in the $O_2$ concentration in the air layers inevitably causes an increase in the rate of $O_2$ diffusion down the air layers. At the same time, the accumulated $N_2$ diffuses from the air layers back into the atmosphere. Therefore, even at the end of a 12-h night, diffusion supplied the air layers with sufficient $O_2$ to maintain a mass flow of about 0.2 $\mu$l $min^{-1}$ air/leaf blade.

As expected, a $N_2$ atmosphere around the tips of partially submerged rice leaves stopped respiration and eliminated the uptake of gas as soon as all internal $O_2$ had been consumed (Fig. 5). On the other hand, an $O_2$ atmosphere around the leaf tips maintained a high rate of mass flow because $O_2$ was not depleted from the gas layer around the submerged leaf blade (Fig. 5). The slight linear decrease in the rate of $O_2$ uptake might have been caused by decreased respiration in the detached leaf blade and/or by partial saturation of the water around the leaf blade with $CO_2$ which reduced the rate of further $CO_2$ solubilization in water.

Light caused an immediate reversal in the direction of gas movement through the air layers. In light, gases began to flow out of air layers at a rate of 6.7 $\mu$l $min^{-1}$ (Fig. 6). This can be explained by the fact that the water around the submerged rice leaf contains high levels of $CO_2$ which has accumulated during the previous dark period. Light initiates photosynthesis which reduces the concentration of $CO_2$ in the air layers around the submerged portion of rice leaves. $CO_2$ dissolved in water then diffuses into the air layers to maintain the equilibrium concentration of $CO_2$ across the gas-liquid interface. The $CO_2$ absorbed from the water is fixed during photosynthesis and is replaced by photosynthetic $O_2$ which, because of its low water solubility, accumulates in the air layers and increases the pressure in them. Inasmuch as the rate of photosynthetic $O_2$ production exceeds the rate of its solubilization in water, $O_2$ is expelled from the air layers into the atmosphere (Fig. 9). The movement of $O_2$ through the air layers continues throughout the light period and leads to enrichment of the air layers with $O_2$. At the start of the next dark period, the high initial rate of gas uptake into the air layers is restored, and the cycle of gas movement in and out of air layers can continue.

The fact that the volume of gases in the air layers increases in the light indicates that air layers help to absorb $CO_2$ from the water surrounding the rice leaf. Figure 7 demonstrates that the air layers indeed enhance the rate of photosynthetic carbon fixation in submerged rice leaves by about 9-fold. Air layers increase the amount of $CO_2$ available for photosynthesis by creating a much larger surface area for $CO_2$ absorption from the water. In leaves where the air layers have been eliminated by treatment with Triton X-100, the surface area for $CO_2$ absorption from the water is limited primarily to the combined area of the stomatal pores.

The present study shows that a continuous air connection between the atmosphere and the submerged plant organs via the air layers is vital for the survival of the partially submerged rice plant (Fig. 8). The contribution of air layers to the gas exchange of the submerged parts of a rice plant is based on two functional components. First, the air layers serve as snorkels through which gases like $O_2$ not only diffuse but are moved by mass flow to and from the submerged organs. Second, the air layers serve as gills which facilitate $CO_2$ absorption from the water and thereby increase the rate of photosynthesis in submerged leaves. Even in totally submerged rice plants, the gill function of air layers provides a significant advantage to the plant and probably enhances its chances for survival during short periods of complete submergence.

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## LITERATURE CITED

1. ARMSTRONG W 1978 Root aeration in the wetland condition. *In* DD Hook, RMM Crawford, eds, Plant Life in Anaerobic Environments. Ann Arbor Science Publishers Inc., Ann Arbor, MI, pp 269–297
2. ARMSTRONG W 1979 Aeration in higher plants. Adv Bot Res 7: 225–332
3. ARNON DI 1949 Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
4. BARBER DA, M EBERT, NTS EVANS 1962 The movement of $^{15}O$ through barley and rice plants. J Exp Bot 13: 397–403
5. BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
6. DE DATTA SK 1981 Principles and Practices of Rice Production. John Wiley and Sons, New York
7. GREENWOOD DJ 1967 Studies on the transport of oxygen through the stems and roots of vegetable seedlings. New Phytol 66: 337–347
8. JACOBS MH 1967 Diffusion Processes. Springer-Verlag, New York
9. JOHN CD, V LIMPINUNTANA, H GREENWAY 1974 Adaptation of rice to anaerobiosis. Aust J Plant Physiol 1: 513–520
10. KAUFMAN PB 1959 Development of the shoot of *Oryza sativa* L. II. Leaf histogenesis. Phytomorphology 9: 277–311
11. KENDE H, AD HANSON 1976 Relationship between ethylene evolution and senescence in morning-glory flower tissue. Plant Physiol 57: 523–527
12. MÉTRAUX J-P, H KENDE 1983 The role of ethylene in the growth response of submerged deep water rice. Plant Physiol 72: 441–446
13. PRITCHARD DT, JA CURRIE 1982 Diffusion coefficients of carbon dioxide, nitrous oxide, ethylene and ethane in air and their measurement. J Soil Sci 33: 175–184
14. SUGAWARA T, T HORIKAWA 1971 Studies on the elongation of internodes in floating rice plants: morphological and histological observations. Bull Coll Agricultsunomiya Univ 8: 25–46
15. VERGARA BS, B JACKSON, SK DE DATTA 1976 Deep water rice and its response to deep water stress. *In* Climate and Rice, International Rice Research Institute, Los Baños, Philippines, pp 301–319

# Chapter 6

# Mechanism of Aeration in Rice

# The mechanism of aeration in rice

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Mass flow of air to the submerged parts of the plant constitutes the major mechanism of aeration in partially submerged rice. The flow of air results from the reduction of pressure in the air-conducting system of the plant caused by $O_2$ consumption and by solubilization of respiratory $CO_2$ in the surrounding water.

Most of the world's rice is grown under partially flooded conditions which impose severe limitations on the supply of $O_2$ and $CO_2$ to the submerged parts of the plant[1]. Some rice cultivars, the deep-water or floating rices, can grow in water as deep as 6 m, with the plant height reaching up to 7 m (ref. 2). It has been postulated that the aeration requirements of the submerged organs of rice and other plants tolerant to partial flooding are met by $O_2$ entering the above-water parts of the leaves and diffusing to the submerged organs through internal air spaces[3-6]. In rice, these are particularly well-developed in the culms and roots[7,8]. Using excised leaves, we have demonstrated that continuous air layers trapped between the hydrophobic surfaces of rice leaves and the

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surrounding water form a low-resistance pathway for gas movement. We have also shown that gases in these air layers move not only by diffusion but also by mass flow[9]. We know of only one other instance where aeration in an aquatic plant, the water lily, has been shown to proceed via mass flow[10]. In that case, the flow of air is thought to be caused by thermal transpiration and hygrometric pressurization. This system of aeration, however, cannot operate in darkness when $O_2$ supply is particularly restricted.

In this article, we report on the mass flow of gases to the roots of partially submerged rice plants via the external air layers and the internal air spaces. We also provide evidence that this mass flow is driven by solubilization of respiratory $CO_2$ in water and that it constitutes the major mechanism of aeration in rice. The method of monitoring the mass flow of gases in partially submerged rice plants is described in the legends of Figures 1 and 4. Rice plants (Oryza sativa L., cv. Habiganj Aman II) were grown as described previously[11]. All experiments were performed with 35- to 55-day-old plants.

## Mass flow of gases

The leaf tips of a submerged rice plant were placed in a small glass chamber (= leaf chamber) which was inverted into the submergence tank and connected to a manometer. The mass flow of air into the plant was monitored by recording the disappearance of air from the head space of the leaf chamber using an angular transducer (Fig. 1, inset). In the experiment of Figure 1, the rate of gas intake was 4.9 ml $h^{-1}$ during the first light period. When the lights were turned off after 3 h, the rate of gas intake increased abruptly fourfold but

gradually fell over a period of 3 h to about 6 ml $h^{-1}$. In the subsequent second light period, the rate of air intake returned to that observed during the first light period. Rates of air intake up to 20.3 ml $h^{-1}$ in light were observed for particularly large and vigorous plants of the same age. No significant differences in the magnitude of air flow were found in rice cultivars belonging to different varietal groups. When the shoot of a submerged rice plant kept in light was severed from the root, the rate of gas intake was reduced tenfold (Fig. 2). Isotope experiments showed that gases were rapidly transported from the above-water atmosphere to the submerged roots. Twenty min after the air in the leaf chamber was replaced with 80% $N_2$ and 20% $^{18}O_2$, the gas sampled from the lowest internodal lacuna 1 m below the water level contained 0.13% $^{18}O_2$ (v/v) as detected by mass spectrometry.

The rate and direction of gas flow depended on the amount of $CO_2$ dissolved in the solution surrounding the plant. When $CO_2$ was bubbled through the submergence tank filled with 90 mM phosphate buffer at pH 6.91 gas flow into the rice plant was gradually inhibited and then reversed, the gases being expelled from the plant into the head space of the leaf chamber (Fig. 3A). The degree of saturation of the phosphate buffer with $CO_2$ was monitored as the decrease in the pH of the solution (Fig. 3B). The volume change in the head space was not significantly affected by direct release of $CO_2$ from the solution since removal of the leaves from the leaf chamber virtually stopped the flow of gases (Fig. 3A). We verified that the pH decrease by itself did not alter the mass flow of gases. When rice plants were submerged in 90 mM phosphate buffer not enriched in $CO_2$ at pH 6.9 and 6.2, no differences in the rate of gas intake were observed. Changes in the direction of mass flow of gases were completely reversible (Fig. 4). A single culm of a rice plant immersed in 90 mM phosphate buffer at pH 7 drew in gases at the rate of 20 µl $min^{-1}$, while a single culm of a second plant submerged in 0.23 M KOH solution

saturated with $CO_2$ at pH 7 expelled gases at the rate of 20-25 µl $min^{-1}$. After 95 min, the plants were interchanged between the two solutions. This reversed the direction of mass flow in each plant, indicating that the abnormally high levels of dissolved $CO_2$ did not affect the flow of gases in an irreversible manner.

Role of air layers

Both surfaces of the submerged rice leaf are separated from the water by continuous air layers, the volumes of which can be as large as 44% of the volume of the leaf[9]. The role of air layers in the transport of gases from the atmosphere into the internal air passages is documented in Figure 5. A single rice leaf, protruding 6 cm from the water into the leaf chamber, drew air from the atmosphere at a rate of 7.2 ml $h^{-1}$ (Fig. 5A). After 60 min, both sides of this leaf were covered with transparent nail polish from the tip down to a point 1 cm above the water level (Fig. 5B). This treatment blocked the stomatal pores over five-sixths of the leaf surface above the water. Another 5-mm-wide ring of nail polish was painted on the leaf blade 14 cm below the water surface to block the continuity of air layers and to demonstrate that nail polish did not interfere with air conductance inside the leaf. Since the width of the leaf used in this experiment was 1.4 cm, 1.4 $cm^2$ of uncovered leaf blade was left above the water connected by the air layers to 19.6 $cm^2$ of leaf area below the water. For 55 min, the rate of air flow into the plant was not affected by these treatments (Fig. 5B). Owing to the design of the manometric set-up, the withdrawal of air by the leaf caused the water level in the leaf chamber to rise. After about 125 min from the start of the experiment, the water level reached the area of the leaf blade which was covered with nail polish, thereby closing the entrance to the air layers. This resulted in an immediate and complete cessation of gas intake (Fig. 5C).

After the water level in the leaf chamber was lowered to open the entrance to the air layers, the original rate of gas intake was restored (Fig. 5D). When another 5-mm-wide ring of nail polish was painted on the leaf just below the water surface so that the entrance to the air layers was blocked and only 1.4 $cm^2$ of uncovered leaf remained above the water, the rate of gas intake was reduced 12-fold (Fig. 5E). These results indicate that, regardless of the size of the leaf area above the water, large amounts of air can be drawn through the air layers and be moved to the submerged organs via mass flow, as long as the entrance to the air layers remains open.

## Discussion

To survive under conditions of partial flooding, rice has developed an efficient system of aeration that insures continuous supply of $O_2$ and $CO_2$ to the submerged organs of the plant. This system of aeration consists of two elements, a network of internal air spaces throughout the plant and air layers along the surface of the submerged leaf blades[9]. Air layers conduct gases along the underwater parts of the leaves where the internal air passages are much less developed than in the culms and roots. They also increase the rate of gas exchange between the plant and the water by greatly expanding the surface of the gas-liquid interface which, otherwise, would be restricted to the area above the stomatal pores[9].

Air is moved from the above-water atmosphere to the submerged organs of the plant by mass flow. We suggest that this continous uptake of air is driven by the difference in the solubility of $O_2$ and $CO_2$ in water. At 25 $^oC$ and pH 7, $CO_2$ ($CO_2$, $H_2CO_3$ and $HCO_3^-$ combined) is 140 times more soluble in water than is $O_2$. The consumption of $O_2$ in the submerged organs and the solubilization

of respiratory $CO_2$ reduce the pressure in the extensive network of internal air spaces and in the external air layers of the rice plant. The resulting pressure gradient causes a mass flow of gases from the atmosphere into the internal air spaces through the stomata of the above-water foliage and of the submerged parts of the leaves which are surrounded by air layers. The rate of air intake is lower in light than in darkness because photosynthesis decreases the $CO_2$ concentration inside the air spaces of the shoot. This reduces the amount of $CO_2$ that dissolves in the surrounding water. Thus, in light, the main "pull" for mass flow is created by the roots whose well developed internal air passages are separated from the soil water only by a thin layer of cells[7]. This notion has been confirmed by the observation that the rate of air movement into an illuminated plant is greatly reduced when the root is severed from the shoot (Fig. 2). In darkness, the solubilization of respiratory $CO_2$ from the submerged parts of the shoot, facilitated by the air layers, increases the rate of gas intake. The average dry weight of the shoot of a rice plant employed in our experiments has been 6.4 g and that of the root 1.0 g. Using previously determined respiration rates of rice roots and leaves[12], we have calculated that the root of an average rice plant respires ca. 3.8 ml $O_2$ per hour and the shoot ca. 14.7 ml $O_2$ per hour. The initial rate of air intake in darkness has been 17.7 ml $h^{-1}$ (Fig. 1), which is very close to the respiratory rate of a whole rice plant.

The rate of gas intake in darkness decreases with time (Fig. 1) because $O_2$, which is consumed during respiration, is replaced by an equal volume of air, i.e. by only one-fifth of the amount of $O_2$ used. Thus, the concentration of $O_2$ in the submerged organs decreases continously while the concentration of $N_2$ increases. This leads to the steady decrease in the rate of air intake in darkness. An analogous but much faster decline in the rate of gas intake has also been observed with isolated leaves in darkness[9]. In

whole plants, this decrease is slowed by the buffering effect of the large reservoir of $O_2$-rich gas in the internal air spaces of the plant at the end of the light period, by the increased rate of $O_2$ diffusion to the root and the diffusion of $N_2$ back into the atmosphere and into the surrounding water.

The dependence of air flow on the solubilization of $CO_2$ is demonstrated in Figures 3 and 4. The gas intake from the atmosphere continues as long as the $CO_2$ activity gradient favors net movement of $CO_2$ from the internal air spaces into the solution around the plant. The direction of the gas flow is reversed when $CO_2$ activity in the solution becomes greater than that in the internal air passages and air layers. This causes net $CO_2$ movement from the solution into the plant and expulsion of gas into the above-water atmosphere.

Air layers significantly decrease the leaf area which must be kept above the water to replace $O_2$ consumed in respiration. Even a tiny area of an intact leaf above the water (smaller that 1.4 $cm^2$) can maintain a normal level of air intake as long as the connection between the air layers and the atmosphere remains open (Fig. 5). Under these conditions, a single leaf is drawing at least as much air as the 5-cm-long tips of 6 leaves (compare Fig. 5B to Figs. 1 and 2). As long as the tips of rice leaves protrude from the water, air from the atmosphere is drawn into the air layers and through the stomata into the internal air passages where it moves all the way to the base of the plant as demonstrated by the $^{18}O_2$ experiments. When the air layers are blocked, a much larger area of the leaf has to be kept above the water to maintain unimpeded gas flow (Fig. 5E). If less than 15 $cm^2$ of the leaf with blocked air layers is kept above the water in light, the mass flow of air into the plant is reduced. If such a situation persists for several hours, the air layers decrease in size until water is drawn into the plant and all internal air spaces become infiltrated (results not shown).

We propose a mechanism of aeration in partially flooded rice which involves mass flow of gases along a pressure gradient created by solubilization of respiratory $CO_2$ in the surrounding water. A large part of this air flow takes place in the continous air layers trapped along the submerged portions of rice leaves. Similar aerational mechanisms may also exist in other semi-aquatic plants.

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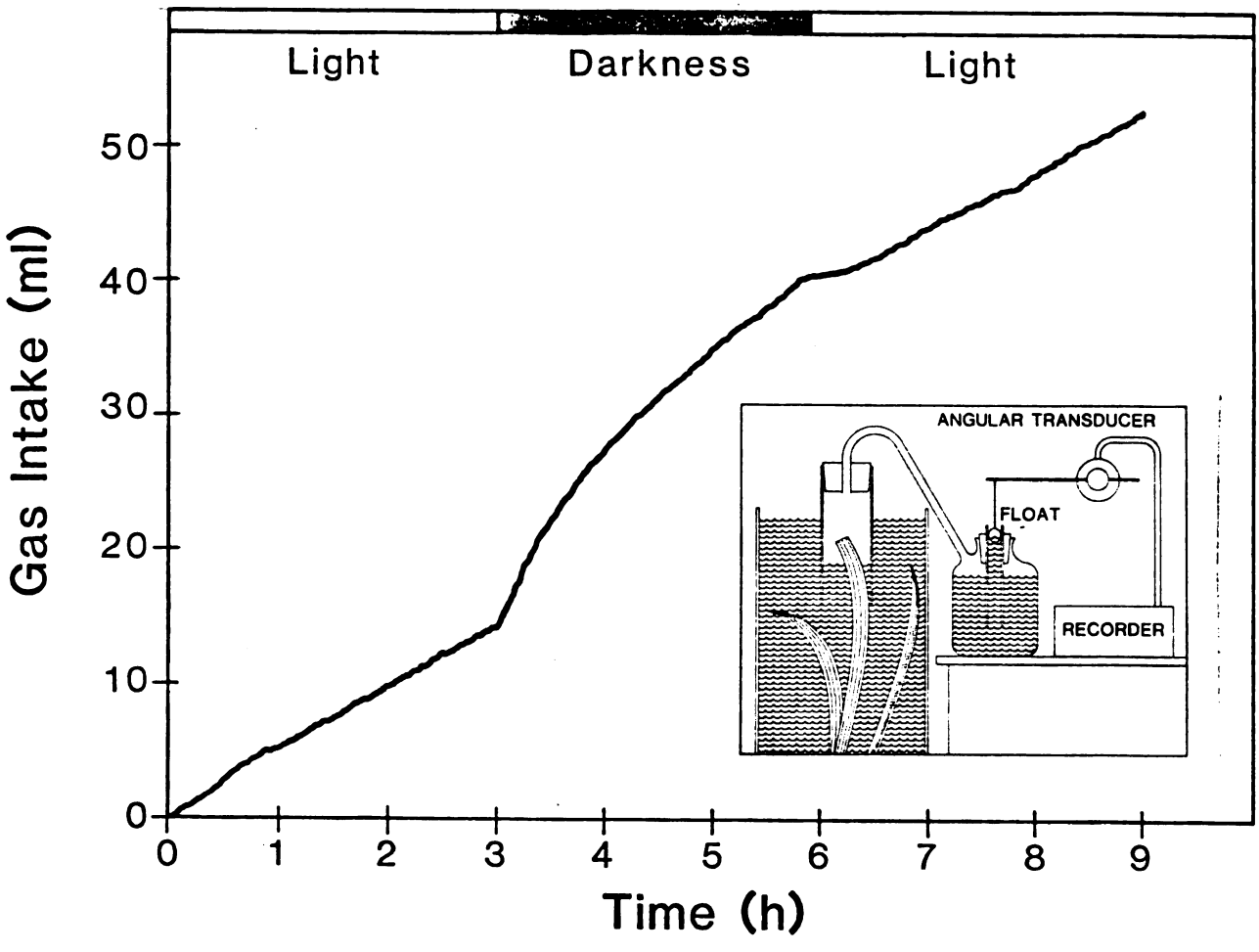
1. De Datta, S.K. Principles and Practices of Rice Production (John Wiley and Sons, New York, 1981).
2. Vergara, B.S., Jackson, B. & De Datta, S.K. in Climate and Rice, 301-319 (International Rice Research Institute, Los Baños, Philippines, 1976).
3. Armstrong, W. Adv. Bot. Res. 7, 225-332 (1979).
4. Armstrong, W. In Plant Life in Anaerobic Environments (eds. Hook, D.D. & Crawford, R.M.M.) 269-297 (Ann Arbor Science Publishers, Ann Arbor, MI, 1978).
5. Barber, D.A., Ebert, M. & Evans, N.T.S. J. Exp. Bot. 13, 397-403 (1962).
6. Greenwood, D.J. New Phytol. 66, 337-347 (1967).
7. John, C.D. Plant and Soil 47, 269-274 (1977).
8. Kaufman, P.B. Phytomorphology 9, 277-311 (1959).
9. Raskin, I. & Kende, H. Plant Physiol. 72, 447-454 (1983).
10. Dacey, J.W.H. Science 210, 1017-1019 (1980).
11. Métraux, J.-P. & Kende, H. Plant Physiol. 72, 441-446 (1983).
12. Tajima, K. Proc. Crop Sci. Soc. Jpn. 33, 371-378 (1965).

Fig. 1 Mass flow of gases into the underwater parts of a partially submerged rice plant. The plant was lowered into a 300-l plastic tank filled with deionized water. The light intensity at water level was 350 $\mu E\ m^{-2}\ s^{-1}$ and the temperature 24 $^{o}C$. To monitor the mass flow of gases into the rice plant, the top six leaves were enclosed in a glass leaf chamber (head space volume 50 ml) attached to the manometric set-up depicted in the inset. Prior to the start of the experiment, the tips of all 6 leaves were cut at the same height so that only 5 cm of each leaf blade was above the water level in the leaf chamber. All other parts of the plant were kept submerged. The leaf chamber was connected to the manometer with Tygon tubing so that the height of the water column in the glass tube with the styrofoam float was always equal to the difference between the water level of the submergence tank and of the leaf chamber. Movements of the float, therefore, reflected volume changes in the head space of the leaf chamber. The float was linked with a rigid steel rod to the lever of an angular transducer (Model R30D, Schaevitz, Pennsauken, N.J.) to amplify the movement of the float. The transducer was connected to a chart recorder via a power source and an amplifier. The displacement of the recorder pen was directly proportional to the changes in the head space volume of the leaf chamber and was calibrated in milliliters of gas withdrawn from the head space or expelled into it. The graph shows a direct tracing of the chart recorder. Every 1 or 2 h, the leaf chamber was lifted from the submergence tank for 1 min to replace the gases in the head space with air and to readjust the height of the water column in the manometer. We verified that the rate of gas movement into the rice plant was not affected by the small increase in pressure in the leaf chamber of the manometric set-up. The results were essentially the same with atmospheric of slightly negative pressure inside the leaf chamber.


Light
Darkness
Light
Gas Intake (ml)
50
40
30
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0
1
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Time (h)
ANGULAR TRANSDUCER
FLOAT
RECORDER

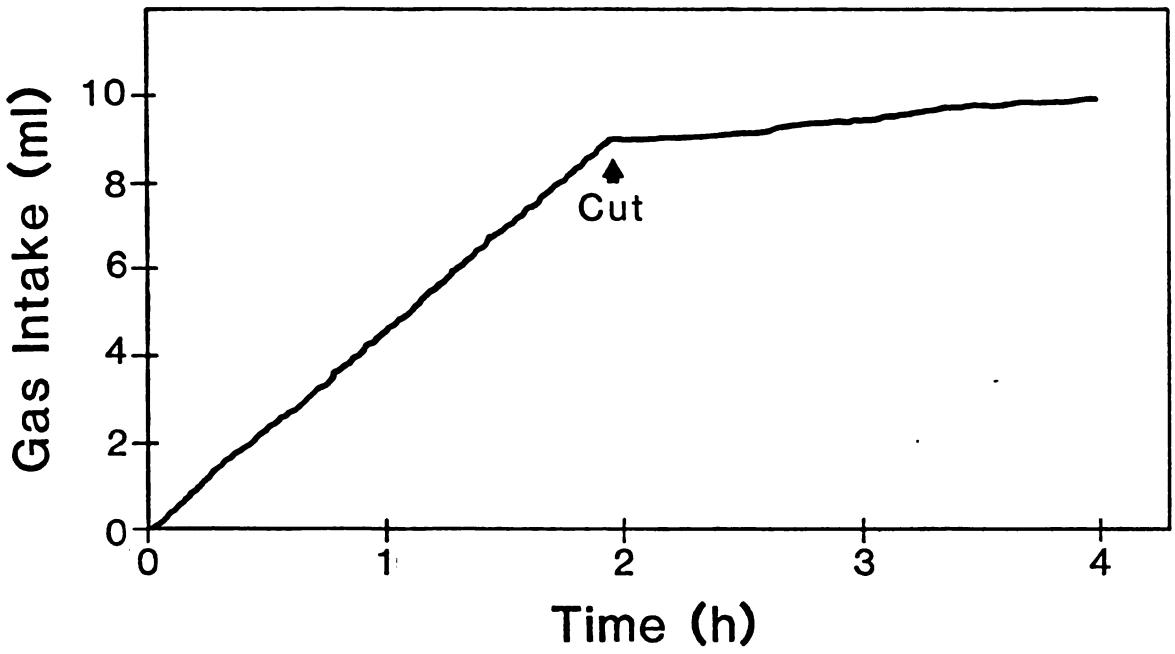



Fig. 2 The role of roots in the mass flow of gases into a rice plant. The experimental procedure was the same as outlined for Fig. 1. The plant was kept in the light, and the roots were severed from the shoot with a razor blade under water at the time indicated by the arrow.

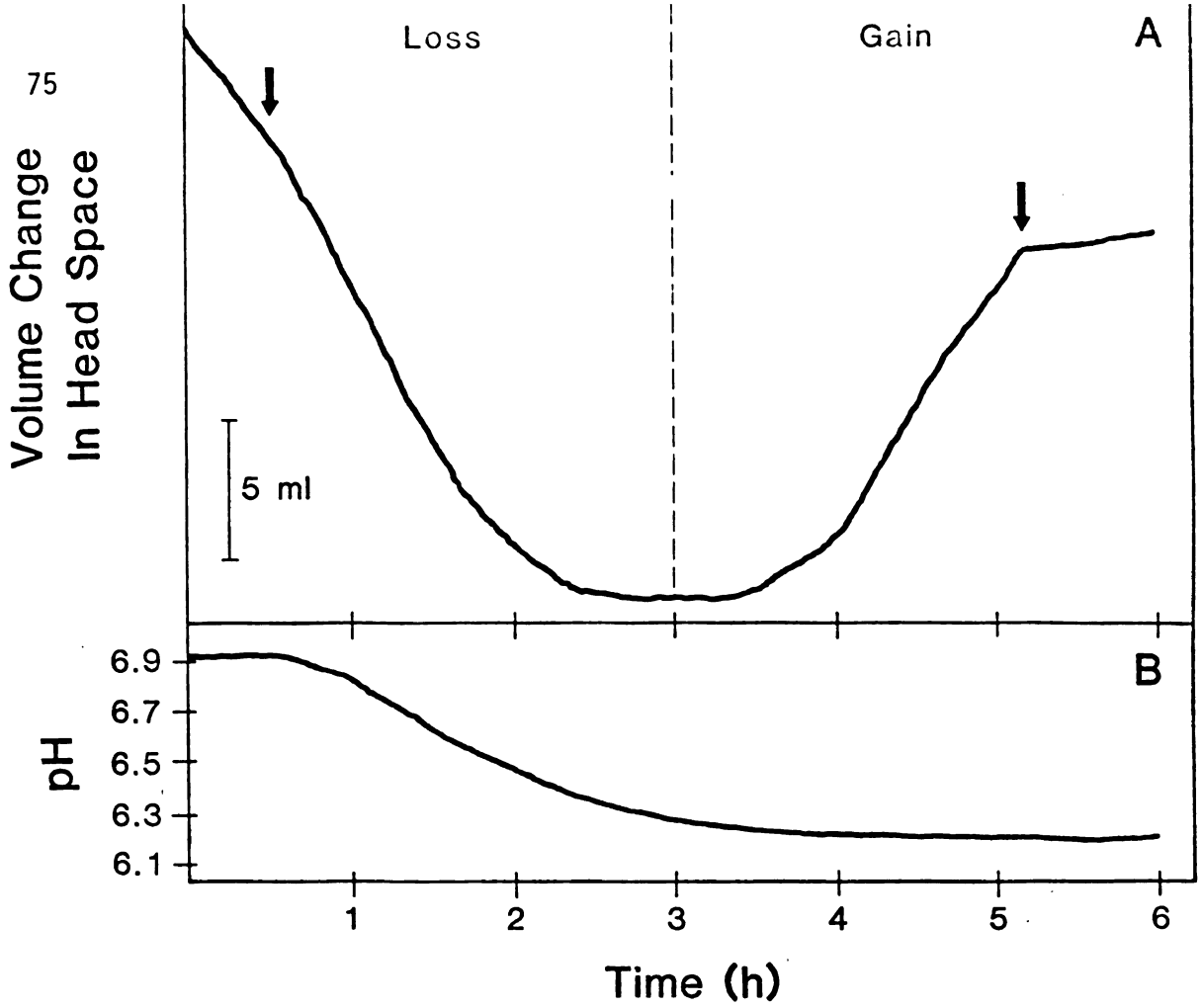
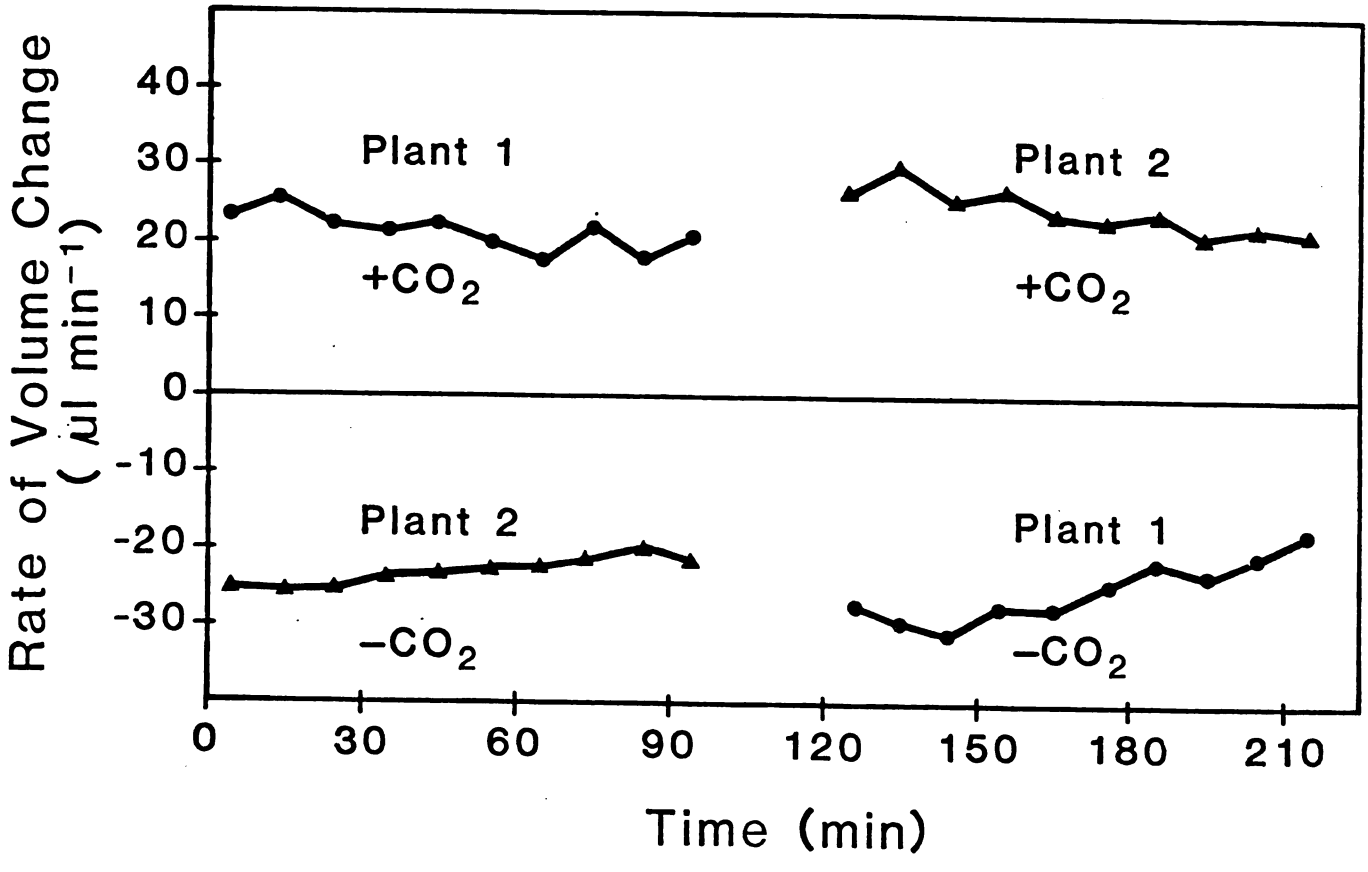



Fig. 3 A The effect of the $CO_2$ concentration in the solution around the plant on the rate and direction of the gas flow. A rice plant was placed in a 71-cm-tall plastic tank filled with 14 l of 90 mM phosphate buffer (pH 6.91). Mass flow of gases was monitored as described for Fig. 1 except that 4 leaves were used for the measurements, and the experiment was performed in light of 100 $\mu E\ m^{-2}s^{-1}$ intensity at 24 $^{o}C$. After the initial rate of gas intake was determined for 30 min, $CO_2$ from a high-pressure gas cylinder was bubbled through the solution using an air stone placed at the bottom of the submergence tank (first arrow). After 5 h 10 min, the leaves were removed from the leaf chamber (second arrow), and changes in the head space volume were monitored for another 50 min. The recorder tracing on the left side of the graph represents loss of gas from the head space of the leaf chamber, i.e. flow of gas into the plant. The tracing on the right side represents gain of head space volume, i.e. release of gas into the head space.

B The pH change in the submergence tank was monitored with a pH meter (Model PHM 26, Radiometer, Copenhagen, Denmark) equipped with a combination electrode (Model GK 23215) and connected to a chart recorder.

Fig. 4 Reversibility of the effect of $CO_2$ on mass flow of gases. Two rice plants were placed in two 52-cm-tall plastic tanks filled either with 10 l of 90 mM phosphate buffer (pH 7), or 0.23 M KOH solution saturated with $CO_2$ (pH 7). Saturation with $CO_2$ was achieved by bubbling $CO_2$ through the KOH solution until pH 7 was reached. The five largest culms were cut with a razor blade 2 cm above the water surface. The other culms and leaves were cut below the water surface. The above-water end of a single culm was introduced into a 7-cm-long glass chamber of 2.5 ml volume, which was then partially immersed in water so that the head space volume was 1.5 ml. A chamber was connected with thin Tygon tubing to one end of a horizontally placed glass tube, i.d. 2 mm, into which a bubble of 40% ethanol was introduced from the other end. The glass tube was calibrated so that the rate of the liquid bubble movement, recorded every 10 min, represented the rate of volume change in the head space caused by the flow of gases into and out of the culm. All measurements were performed in light (100 $\mu E\ m^{-2}s^{-1}$) at 24° C. Since mass flow of gases in only one out of five culms was measured, the total flow for each plant was about 5 times larger than shown here. After 95 min, the plants were interchanged between the two solutions and equilbrated for 30 min before measurements of gas flow were resumed.


Rate of Volume Change
( µl min⁻¹)
40
30
20
10
0
-10
-20
-30
Plant 1
+CO2
Plant 2
+CO2
Plant 2
-CO2
Plant 1
-CO2
0
30
60
90
120
150
180
210
Time (min)

Gas Intake (ml)

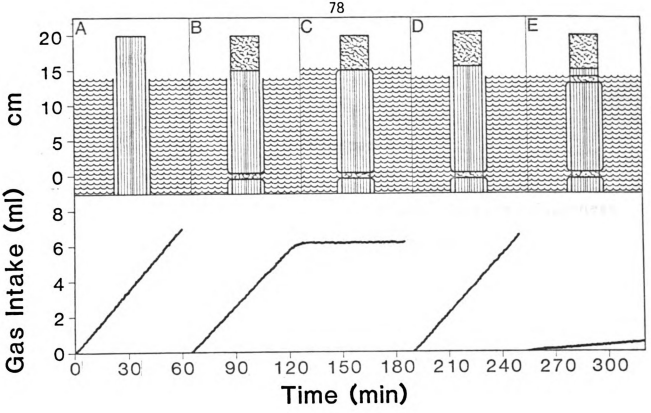



Fig. 5 The role of air layers in the conductance of gases. The experimental procedure was the same as outlined for Fig. 1 except that only one leaf was kept above the water for the gas intake measurements. Prior to the beginning of the experiment, the top 6 cm of the leaf tip were cut off, and the cut surface was sealed with transparent nail polish. Of the remaining leaf, 6 cm protruded from the water into the leaf chamber. The shaded areas represent the regions of the leaf that were covered with nail polish.

# Chapter 7

# A Method for Measuring Leaf Volume, Density, Thickness and Internal Gas Volume

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# A Method for Measuring Leaf Volume, Density, Thickness, and Internal Gas Volume

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***Abstract.*** **A fast, simple, and accurate method is presented for the determination of volume, thickness, and density of leaves and the volume of internal air spaces of leaves and other plant organs. The method is based on the application of Archimedes' principle and involves the determination of the buoyant force acting on plant organs submerged in water.**

There is a noticeable lack of reliable methods for measuring volume, thickness, and density of leaves and size of the internal air spaces of leaves despite the importance of these parameters in understanding leaf morphogenesis and photosynthetic productivity (1, 10). Microscopes (4), micrometers (11), and linear variable transducers (8) have been used to determine leaf thickness. Leaf volume has been measured volumetrically (3), and superficial leaf density (mg $cm^{-2}$) has been measured with a β-gauge (2). The volume of intercellular air spaces has been determined by direct microscopic measurements of sectioned tissue (7) or by weighing the tissue in air before and after infiltration with water or isoosmotic solutions (6).

This paper describes a fast and accurate method for determining many physical parameters of the leaf by applying Archimedes' principle, the derivation of which can be found in any standard physics textbook. The method requires simple equipment which should be available to every experimenter; it is easily performed and can be used on leaves of any size and shape. This method can be applied equally well to the determination of physical parameters of other plant organs and tissues; its accuracy is limited only by the precision of the analytical balance used in weighing the plant material.

Archimedes' principle states that the buoyant force acting upon a body immersed in a fluid is equal to the weight of the fluid displaced by the body:

$$B = \rho V \qquad [1]$$

where B is the buoyant force, ρ is the density of the fluid (weight/unit volume), and V is the volume of the immersed body. Thus, a close approximation of B acting on the body completely submerged in water can be obtained from the difference in the weight of the body in air ($W_{air}$) and its weight in water ($W_{water}$):

$$B = W_{air} - W_{water} \qquad [2]$$

Combining equations 1 and 2:

$$W_{air} - W_{water} = \rho V \qquad [3]$$

*Determination of leaf volume.* It is clear from equation 3, that only 2 values are required for the determination of leaf volume—namely, the weight of the leaf in air ($W_{air}$) and its weight in water ($W_{water}$). A simple assembly can be used to measure the weight of the leaf in water (Fig. 1). A holder, consisting of a Hoffman clamp of sufficient weight to keep the plant material submerged in water, is clipped to 2 hypodermic needles, which pierce the leaf. Larger leaves may be cut into several sections if necessary. The holder with the whole leaf or leaf sections is then suspended from the pan hook of an analytical balance and submerged in distilled water. The weight of the leaf in water can be calculated by subtracting the weight of the submerged holder with the leaf ($W_{leaf + holder}$) from the weight of the submerged holder ($W_{holder}$). The weight of the leaf in water is usually negative because of the positive buoyancy of leaves.

Thus, the volume of the leaf ($V_{leaf}$) from equation 3 is:

$$V_{leaf} = \frac{W_{air} - (W_{holder} - W_{leaf + holder})}{\rho H_2O} \qquad [4]$$

where $\rho H_2O$ is the density of water.

If the weight is measured in milligrams, the volume of the leaf, in cubic millimeters, is numerically equal to $W_{air} - (W_{holder} - W_{leaf + holder})$ because $\rho H_2O$ at room temperature can be approximated as 1 mg $mm^{-3}$. An experimental error may be introduced in the determination of $V_{leaf}$ by the presence of small air bubbles trapped on the surface of the submerged leaf. Air bubbles increase the buoyancy of the sample and lead to an overestimation of leaf volume. This problem can be overcome by wetting the leaf with a surfactant solution: e.g., a 0.05% (v/v) solution of Triton X-100, prior to determining $W_{leaf + holder}$. Surfactant treatment effectively eliminated all air bubbles from the leaf surface.

Column 3 in Table 1 gives the average volumes of 12 fully developed trifoliate leaves of *Phaseolus vulgaris* L. cv. Sacramento and fully expanded leaf blades of *Oryza sativa* L., cv. Habiganj Aman II collected randomly from 12 eight-week-old plants grown in the greenhouse. Columns 1 and 2 in Table 1 contain the average fresh weights of bean and rice leaves ($W_{leaf}$) and their weight in water ($W_{holder} - W_{leaf + holder}$), respectively.

*Determination of leaf density.* Leaf density ($\rho_{leaf}$) can be calculated from the equation:

$$\rho_{leaf} = W_{leaf}/V_{leaf} \qquad [5]$$

Column 4 in Table 1 contains the average densities ± SD of 12 bean and rice leaves calculated from equation 5.

*Determination of the volume of the internal air spaces of leaves.* Gases in the internal spaces in the leaf can be replaced by vacuum infiltration with a 0.05% Triton X-100 solution. The plant material fixed to the holder is placed in the bottom of the desiccator or vacuum flask partially filled with surfactant solution so that the tissue is submerged totally. Agitation of the desiccator and sectioning of the leaves assist the infiltration process.

The vacuum, about 500 mm $H_g$, should be applied and released at least 4 times, with every evacuation lasting about 40 sec. The infiltrated tissue attached to the holder is then weighed in water as described above. The buoyant force exerted on the intercellular air spaces ($B_{air\ spaces}$) is:

$$B_{air\ spaces} = W_{inf.\ leaf + holder} - W_{leaf + holder} \qquad [6]$$

where $W_{int.\ leaf + holder}$ is the weight of the infiltrated leaf with the holder in water and $W_{leaf + holder}$ is the weight of the same leaf and a holder in water before infiltration. For all practical purposes, the density of the 0.05% Triton X-100 solution inside the leaf can be considered equal to the density of water. Therefore, the internal gas volume of the leaf

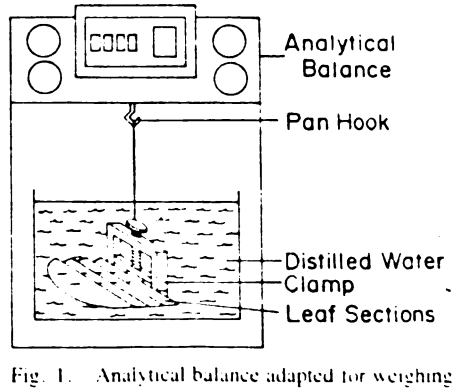


Fig. 1. Analytical balance adapted for weighing leaves underwater.

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Table 1. Average volume, weight density, volume of the internal air spaces, and density of the nongaseous leaf content of leaves of *Phaseolus vulgaris*[a] and *Oryza sativa*[b] collected from 8-week-old plants.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Plant | $W_{air}$ Leaf fresh wt (mg) | $W_{water}$ Underwater wt (mg) | $V_{leaf}$ Leaf vol ($mm^3$) | $\rho_{leaf}$ Leaf density (mg $mm^{-3}$) | $V_{air\ spaces}$ Vol internal air spaces ($mm^3$) | % leaf vol occupied by air spaces | $V_{nongas}$ Vol nongaseous leaf content ($mm^3$) | $\rho_{nongas}$ Density of nongaseous leaf content (mg $mm^{-3}$) |
| Bean | 2501 ± 605 | −714 ± 234 | 3215 ± 827 | 0.78 ± 0.02 | 754 ± 234 | 23.5 ± 1.9 | 2461 ± 602 | 1.02 ± 0.01 |
| Rice | 850 ± 138 | −634 ± 104 | 1483 ± 239 | 0.57 ± 0.01 | 585 ± 96 | 39.5 ± 1.0 | 898 ± 145 | 0.95 ± 0.01 |

[a]Each leaflet of the trifoliate was cut into 2 pieces, and the resulting 6 sections were used for all measurements. Each number is the average obtained from 12 leaves ± SD.
[b]Each leaf blade was cut transversely into 6 or 7 pieces, which were used for all measurements. Each number is the average obtained from 12 leaves ± SD.

($V_{air\ spaces}$) is given by:

$$V_{air} = \frac{B_{air\ spaces}}{\rho H_2O} = \frac{W_{inf.\ leaf\ +\ holder} - W_{leaf\ +\ holder}}{\rho H_2O} \quad [7]$$

No differences in electrical conductivity of Triton X-100 solution before and after infiltration of 4 rice leaves were detected, indicating that infiltration does not cause any detectable leakage of ionic solutes from the cells.

The possible swelling of tissue infiltrated with hypotonic Triton X-100 solution will not affect the value of the leaf thickness calculated from Archimedes' principle. Swelling, however, would pose a serious problem if the infiltrated tissue is blotted and weighed in air according to other methods of determination of the intercellular air volume (6).

Columns 5 and 6 in Table 1 contain the average volume of the internal air spaces of 12 bean and rice leaves and the average percentage of the total leaf volume occupied by the air spaces ± SD.

*Determination of the density of the nongaseous leaf content.* The volume of the liquid and solid components of the leaf ($V_{nongas}$), which comprise the nongaseous leaf content, can be calculated by subtracting the volume of the internal air spaces ($V_{air\ spaces}$) from the volume of the leaf ($V_{leaf}$). The density of the nongaseous leaf content, $\rho_{nongas}$, is:

$$\rho_{nongas} = W_{leaf}/V_{nongas} \quad [8]$$

Column 8 in Table 1 contains the average density of the nongaseous leaf content of 12 bean and rice leaves ± SD.

*Determination of average leaf thickness.* The average leaf thickness ($T_{leaf}$) can be determined from the volume of a leaf section of known area ($A_{leaf}$):

$$T_{leaf} = V_{leaf}/A_{leaf} \quad [9]$$

The surface area of cut-out or punched-out leaf sections (using, for example, a large cork borer) with square, rectangular, or circular shapes can be determined easily from the linear dimensions. The most accurate values of the leaf thickness can be obtained from large sections, the average density of which equals the density of the whole leaf. For example, using small sections containing disproportionately large or small amounts of vascular tissue can cause errors in determination of average leaf thickness. Sectioning can be avoided by determining the area of the whole leaf using a variety of methods (5, 9). Column 6 in Table 2 contains the average thickness ± SD of 8 randomly selected, fully developed bean trifoliates and mid-laminar regions of rice leaves collected from 8-week-old plants grown in the greenhouse.

The thickness of 20 randomly selected bean leaflets from the same bean plants was measured with a micrometer for comparison with the results of Table 2. Care has been taken not to position the micrometer on ridges on the leaf surface. The average thickness obtained with the micrometer was 0.29 mm ± 0.06 SD. The average thickness calculated from Table 2 was 0.26 mm ± 0.01. The thickness of the mid-laminar region of 20 randomly selected rice leaves measured with the micrometer was 0.25 mm ± 0.03, compared to 0.16 mm ± 0.01 as calculated from equation 9 (Table 2). Larger values of leaf thickness are obtained with the micrometer because the micrometer measures the maximum distance between the leaf surfaces which are not even (11). In highly corrugated leaves (e.g., those of rice), the average leaf thickness determined with the buoyancy method and maximum leaf thickness determined with the micrometer are significantly different. The values obtained by the buoyancy method seem to represent the best approximation for average thickness of the leaf.

Table 2. Average thickness of *Phaseolus vulgaris*[a] trifoliates and mid-laminar region of *Oryza sativa*[b] leaves collected from 8-week-old plants.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Plant | No. sections | $A_{leaf}$ Combined area of sections ($mm^2$) | $W_{air}$ Fresh wt of sections (mg) | $W_{water}$ Underwater wt of sections (mg) | $V_{leaf}$ Combined vol of sections ($mm^3$) | $T_{leaf}$ Avg thickness of leaf ± SD (mm) |
| Bean | 6 | 3435 | 727 ± 38 | −180 ± 17 | 907 ± 46 | 0.26 ± 0.01 |
| Rice | 2 or 3 | 2825 ± 546 | 315 ± 51 | −122 ± 25 | 437 ± 72 | 0.16 ± 0.01 |

[a]Two leaf discs, 27 mm in diameter, were punched out from each leaflet of the trifoliate with a cork borer. The average thickness of the resulting 6 discs was measured as described in the text. Each number is the average obtained from 8 leaves.
[b]A mid-laminar region of a rice leaf was cut transversely into 2 rectangular pieces (about 7 cm long). The area of the sections was calculated as a product of length and width. Each number is the average obtained from 8 leaves.

## Literature Cited


1. Beakbane, A.B. 1967. A relationship between leaf structure and growth potential in apple. Ann. Appl. Biol. 60:67–76.
2. Jones, H.G. 1973. Estimation of plant water status with the beta-gauge. Agr. Meteorol. 11:540–546.
3. Huxley, P.A. 1971. Leaf volume: a simple method for measurement and some notes on its use in studies of leaf growth. J. Appl. Ecol. 8:147–153.
4. Maksymowych, R. 1973. Thickness of the lamina, p. 25–26. In: M. Abercrombie, D.R. Newth, and J.G. Torrey (eds.). Analysis of leaf development. Cambridge Univ. Press, Cambridge, U.K.
5. Obraztsov, A.S. and V.M. Kovalev. 1976. Volumetric method for determining area of the leaf surface in plants and plantings. Soviet Plant Physiol. 23:911–914.
6. Smith, J.A.C. and S. Heuer. 1981. Determination of volume of intercellular spaces in leaves and some values for CAM plants. Ann. Bot. 48:915–917.
7. Turrel, F.M. 1936. The area of the internal exposed surface of dicotyledon leaves. Amer. J. Bot. 23:255–264.
8. Tyree, M.T. and S.I. Cameron. 1977. A new technique for measuring oscillatory and diurnal changes in leaf thickness. Can. J. For. Res. 7:540–546.
9. Watson, D.J. 1952. The physiological basis of variation in yield. Adv. Agron. 4:101–145.
10. Wilson, D. and J.P. Cooper. 1967. Assimilation of *Lolium* in relation to leaf mesophyll. Nature 214:989–992.
11. Woodward, F.I. 1979. The differential temperature responses of the growth of certain plant species from different altitudes: II. Analysis of the control and morphology of leaf extension and specific leaf area of *Phleum bertolonii* D.C. and *P. alpinum* L. New Phytol. 82:397–405.

# CONCLUSIONS

1. The growth responses of deep-water and non-deep-water rice varieties to flooding can be reproduced in excised stem sections.
2. Submergence reduces oxygen levels in the internodal lacunae. Lower oxygen concentrations, in turn, stimulate ethylene synthesis in the internodal tissue.
3. Ethylene accumulation in the underwater parts of rice causes rapid internodal elongation and inhibition of leaf growth.
4. Ethylene action is probably mediated by an increase in the activity of endogenous gibberellins.
5. α-Amylase activity in submerged and ethylene-treated internodes increases greatly. Simultaneously, submergence increases the translocation of photosynthetic assimilates from the leaves to the internodal region.
6. Submerged parts of rice leaves are surrounded by continuous air layers which provide the major aeration path in the rice plant.
7. Gases move in the rice plant not only by diffusion but, primarily, by mass flow down a pressure gradient created by the solubilization of respiratory $CO_2$ in water.