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A Comparison of the Submergence Response of Deepwater and Non-Deepwater Rice[1]

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ABSTRACT

Twelve cultivars of rice (*Oryza sativa* L.), representing deepwater, short-statured, and semidwarf types, were tested for their response to submergence. The magnitude of the response varied between cultivars; however, all cultivars responded to submergence by rapid growth once internodal elongation had started. Three of these cultivars were tested for elongation capacity at four ages. The deepwater rice was capable of rapid internodal elongation in response to submergence at 4 weeks of age. Growth of the short-statured and semidwarf cultivars was not stimulated by submergence until about 10 weeks of age. In air, the internodes of deepwater rice grew slower than did those of the short-statured and semidwarf cultivars. We also investigated the elongation response of stem sections of all 12 cultivars to an atmosphere containing 3% O_2, 6% CO_2, 91% N_2 (all by volume), and 1 microliter per liter ethylene. We found that the response of each of the non-deepwater cultivars was qualitatively and quantitatively similar to that of the deepwater rice.

Deepwater rice is grown primarily as a subsistence crop in the flood plains of Southeast Asia where flood waters may reach a height of 6 m (1). It will elongate up to 25 cm/d in rapidly rising water (10). An important aspect of deepwater rice improvement programs involves breeding for high yielding cultivars which retain the deepwater or floating character, *i.e.* the ability to elongate rapidly when partially submerged (9). In experiments described in this report, we determined differences in the growth potential between deepwater and non-deepwater rice cultivars. We compared the growth habit of several cultivars of deepwater, short-statured, and semidwarf rice under conditions of partial flooding. Our data indicate that the deepwater character consists of more than the simple capacity to elongate rapidly under conditions of flooding. Deepwater rice appears to differ from non-deepwater rice by the timing of the phase during which rapid internodal elongation can take place.

MATERIALS AND METHODS

Plant Material. The growth of the following 12 cultivars of rice (*Oryza sativa* L.) was measured and compared under several environmental conditions: Kalar Harsall, Thavalu, Pin Gaew (PG[3]) 56, and Leb Mue Nahng (LMN) 111 (seeds provided by Dr. B. S. Vergara, International Rice Research Institute, Los Baños, Philippines); Labelle and Mars (seeds provided by Dr. T. Johnston, University of Arkansas, Stuttgart, AR); M9 and Calrose 76 (seeds provided by Dr. J. N. Rutger, University of California, Davis, CA); Habiganj Aman (HA) II, III, and VIII (seeds provided by Dr. S. M. H. Zaman, Bangladesh Rice Research Institute, Dacca, Bangladesh); IR8 (seeds provided by Dr. R. S. Bandurski, Michigan State University, East Lansing, MI). The rice plants were germinated in darkness on moist filter paper in Petri dishes at 30°C for 72 h. Seedlings were sown singly in 1-quart pots in a soil mixture previously described (6). Plants were grown in a glasshouse from March 14, 1983 through June 20, 1983 and from March 1, 1984 through June 14, 1984 under the following conditions: day temperature, 27 to 31°C; night temperature, 27°C; 16-h photoperiod. The natural photoperiod was extended to 16 h using sodium lamps. Plants were watered with half-strength Hoagland solution using a drip-irrigation system. The pots were placed in 6-cm-deep flats containing half-strength Hoagland solution to prevent complete drainage.

Submergence of Whole Plants. Whole plants were suspended in 300-L Nalgene tanks, which were filled with distilled H_2O. Approximately 10 to 20 cm of leaf material remained above the water level to create a condition of partial submergence. The tanks were placed in an environmental growth chamber as described by Métraux and Kende (6). Air-grown control plants were placed in the same environmental growth chamber for 5 d and were watered daily with half-strength Hoagland solution.

Measurements of Internodal Length in Whole Plants. Prior to treatment, the length of each internode in each tiller of every plant to be tested was determined. Nodes in whole plant stems were detected by holding the culms in front of a high intensity lamp in an otherwise darkened room and were marked with water-insoluble ink. The length of each internode was then recorded and designated as initial internodal length. Following treatment, each culm was dissected longitudinally, and the length of each internode was recorded and designated final internodal length. For each culm, the sum of the initial internodal lengths was subtracted from the sum of the final internodal lengths to give the internodal elongation. In experiments conducted in 1983, the mean internodal length (initial or final) and the mean internodal elongation were taken as the average of 7 to 10 tillers for a given cultivar randomly selected from all tillers measured. In experiments conducted in 1984, the mean internodal length and internodal elongation were taken as the average of 3 tillers per plant and of 3 or 4 plants per cultivar. In some cultivars and at some ages, the internodes were nested at the base of the culm making it difficult to determine the initial internodal length using a high intensity lamp. In these cultivars, 10 or more culms were dissected and measured in order to obtain a mean initial internodal length.

Treatment of Stem Sections. Twenty-cm-long stem sections containing the two uppermost nodes and the highest internode were excised from well developed tillers of 12- to 13-week-old plants as described by Raskin and Kende (7). Initial and final internodal lengths were determined after excision, using a high

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[3] Abbreviations: PG, Pin Gaew; LMN, Leb Mue Nahng; HA, Habiganj Aman; GA, gibberellin; TCY, tetcyclacis; AOV, analysis of variance.

intensity lamp, as described above. Final measurements were made after 3 d of experimental treatment. Mean internodal lengths (initial and final) and mean internodal elongation were determined for each cultivar. Seven to 10 sections from each variety were subjected to one of the following three treatments: submergence, flow-through gas mixture, or flow-through air (control). Sections to be submerged were placed upright in a 100-ml glass beaker filled with glass beads to anchor the sections. The beaker was then lowered into a 1-L graduated cylinder, 42 cm deep, which was filled to the rim with distilled H_2O. Sections to be treated with air or with the gas mixture were placed in a 100-ml glass beaker containing 20 to 30 ml of distilled H_2O. The beaker was then placed in a 2.5-L Plexiglas cylinder, 60 cm deep, which had inlet and outlet ports and was fitted with a gas-tight lid. Either air or a gas mixture containing 3% O_2, 6% CO_2, 91% N_2 (all by volume), and 1 $\mu l\ L^{-1}$ C_2H_4 was passed through the cylinder at a flow rate of 80 ml h^{-1} as described by Raskin and Kende (7). All experiments with stem sections were performed at 27°C in continuous light (photosynthetic photon flux density, 70 $\mu mol\ s^{-1}\ m^{-2}$). After 3 d, the sections were removed from the experimental conditions, dissected longitudinally, and the final internodal length was measured.

Statistical Analysis. Experiments in 1983 were performed with whole plants and stem sections with 7 to 10 replicates using a completely randomized design. The effect of air, submergence, and gas treatment (the latter in sections only) was tested by least square difference (LSD). Experiments in 1984 were performed with whole plants in a split-split plot design. The main plot was age, the subplot was treatment (air or submergence), the sub-subplot was cultivar. Data were analyzed by AOV. To determine whether there were differences in initial internodal length, data from the two treatments, air and submergence, were pooled and analyzed by AOV.

RESULTS

Response to Twelve Rice Cultivars to Partial Submergence. Whole plants of 12 cultivars representing three rice types (deepwater, semidwarf, and short-statured) were partially submerged in deionized H_2O or left to grow in air for 5 d. The average internodal length of well developed tillers prior to and following treatment as well as the mean internodal elongation is reported for each cultivar (Table I). Each value represents the average of 7 to 10 tillers randomly selected from all tillers measured. The internodal elongation of partially submerged plants was significantly greater than the internodal elongation of air-grown plants in all cultivars tested. The elongation rates of partially submerged plants fell in the range of 12 to 87 mm d^{-1} (assuming that elongation was constant over the entire treatment period). PG 56 exhibited the highest rate of elongation, while IR8 internodes grew slower than those of any other cultivar tested. However, M9, a semidwarf cultivar, had a higher elongation rate than several deepwater cultivars such as HA II, HA VIII, and LMN 111. In addition, Thavalu, a tall, flood-tolerant cultivar, and Kalar Harsall, a deepwater rice, both grew slower than all cultivars tested except IR8. The final total internodal lengths after 5 d of submergence ranged from 90 cm in HA III to 7.1 cm in IR8. The data demonstrate that all rice cultivars tested are capable of responding to partial submergence with rapid internodal elongation at age 12 to 13 weeks.

Effect of Partial Submergence and Flow-Through Gas Treatment on Stem Sections. Ten stem sections containing the uppermost internode of tillers from each of 12 cultivars were excised from 12- to 13-week-old plants and were exposed to the following treatments for 3 d: (a) complete submergence in distilled H_2O; (b) a gas mixture composed of 3% O_2, 6% CO_2, 91% N_2 (all by volume), and 1 $\mu l\ L^{-1}$ C_2H_4; or (c) air; treatments b and c were adminstered in a flow-through system (7). The average internodal length of all sections from each cultivar prior to and following treatment as well as the internodal elongation is reported (Table II). The enhancement of internodal growth observed in whole plants was also seen in stem sections which were submerged or placed in an atmosphere composed of 3% O_2, 6% CO_2, 91% N_2, and 1 $\mu l\ L^{-1}$ C_2H_4; this was true for all cultivars tested except Calrose 76. (We observed a significant difference in internodal elongation between Calrose 76 internode sections placed in the above gas composition and in air. Therefore, the lack of significance between the elongation of Calrose internodes which were submerged or placed in a flow-through-air system is probably due to high variability of the plant material and too low a number of replicates.) Sections from short-statured lowland rice cultivars, such as Mars, or semidwarf cultivars, such as M9, were capable of responding to submergence or to gas treatment in the same

Table I. *Effect of Partial Submergence on Internodal Elongation in Whole Plants of 12 Rice Cultivars*

The experiments were conducted in 1983 with 12- to 13-week-old plants. The plants were treated for 5 d.

Cultivar	Classification	Air			Partial Submergence		
		Mean internodal length		Mean internodal elongation	Mean internodal length		Mean internodal elongation
		Initial	Final		Initial	Final	
					mm		
Kalar Harsall	Deepwater	198	230	32[a]	152	272	120[b]
Thavalu	Tall	162	186	24[a]	207	291	84[b]
PG 56	Deepwater	249	276	27[a]	134	570	436[b]
LMN 111	Deepwater	243	294	51[a]	290	550	260[b]
HA II	Deepwater	375	390	15[a]	423	685	262[b]
HA III	Deepwater	509	534	25[a]	617	898	281[b]
HA VIII	Deepwater	632	654	22[a]	289	545	256[b]
IR8[c]	Semidwarf	11	13	2[a]	11	71	60[b]
Calrose 76[c]	Semidwarf	29	36	6[a]	29	204	175[b]
M9[c]	Semidwarf	147	204	57[a]	180	451	271[b]
Mars[c]	Short-statured	186	209	23[a]	258	467	209[b]
Labelle	Short-statured	103	142	39[a]	101	295	194[b]

[a,b] Means across treatments followed by different letters are significantly different at the 0.05 level, using LSD.

[c] Cultivars in which panicle initiation had begun by the time experiments were conducted.

Table II. *Effect of Submergence and Gas Treatment on Internodal Elongation in Excised Stem Sections*

The experiments were conducted in 1983 with sections excised from 12- to 13-week-old plants. The sections were treated for 3 d.

Cultivar	Air			Submergence			Gas[a]		
	Internodal length		Internodal elongation	Internodal length		Internodal elongation	Internodal length		Internodal elongation
	Initial	Final		Initial	Final		Initial	Final	
					mm				
Kalar Harsall	55	64	9^b	60	133	73^c	62	127	65^c
Thavalu	55	61	6^b	49	70	21^c	54	68	14^c
PG 56	46	48	2^b	42	92	50^c	39	89	50^c
LMN 111	70	92	22^b	59	126	67^c	70	140	70^c
HA II	76	81	5^b	83	152	69^c	73	135	62^c
HA III	47	65	18^b	47	132	85^c	50	145	95^c
HA VIII	44	56	12^b	39	115	76^c	38	153	115^c
IR8[d]	30	34	4^b	12	25	13^c	12	24	12^c
Calrose 76[d]	25	28	3^b	26	31	5^b	25	33	8^c
M9[d]				42	88	46^c	47	112	65^c
Mars[d]	73	88	15^b	66	114	48^c	71	117	46^c
Labelle				25	67	42^c	26	49	23^c

[a] Gas treatments consisted of 3% O_2, 6% CO_2, 91% N_2 (all by volume), and 1 μl L^{-1} C_2H_4 being passed through a chamber containing sections. [b,c] Means across treatments labeled with different letters differ significantly at the 0.05 level, using LSD. [d] Cultivars in which panicle initiation had begun by the time experiments were conducted.

fashion as did deepwater rice. Our results also demonstrate that treatment of the excised internodes of all cultivars with 3% O_2, 6% CO_2, and 1 μl L^{-1} C_2H_4 in a flow-through incubation chamber mimicked the response to submergence.

Effect of Age on Submergence Response of Three Rice Cultivars. Since we found no qualitative differences between classes of cultivars in their elongation response to partial submergence, we decided to investigate the ability of each class of cultivar to elongate rapidly at several ages. For these experiments, we chose three cultivars, one representing each class of cultivar used in the 1983 experiments. Whole plants of HA II, a deepwater cultivar, Mars, a short-statured cultivar, and M9, a semidwarf cultivar were analyzed at four ages (5, 7, 10, and 13 weeks) for their ability to respond to partial submergence. Figure 1 shows the internodal length per tiller of each cultivar prior to treatment and after 5 d of partial submergence. The same measurements were also made with control plants kept in air. Internodal elongation in HA II plants (Fig. 1A) which had been partially submerged for 5 d was 5 to 6 times greater than internodal elongation of identical plants grown in air (Fig. 1A). This was true at all ages tested. In Mars, there was little or no internodal elongation in either partially submerged or control plants at ages 5 and 7 weeks (Fig. 1B). However, at both 10 and 13 weeks, internodal elongation in partially submerged plants was twice that of air controls. Like Mars, M9 did not elongate at 5 and 7 weeks of age, but at 10 and 13 weeks, internodal elongation of partially submerged plants increased 2-fold over that of control plants. In air, elongation of HA II was slow at all ages tested (about 0.5 cm d^{-1}). In contrast, internodal growth in Mars and M9 was quite fast in air at 10 and 13 weeks of age (up to 4 cm d^{-1}). The difference in the age at which internodal elongation occurs in various cultivars is confirmed by a highly significant interaction between age and cultivar (AOV; P = 0.01).

Age of Panicle Initiation. Following dissection of internodes for final growth measurement, panicle development was observed in Mars at 13 weeks and in M9 at both 10 and 13 weeks. No panicle development was seen in HA II up to 13 weeks of age. Rapid internodal elongation in Mars and M9 is, therefore, correlated with panicle development (Fig. 1, B and C).

DISCUSSION

To identify the components of the so-called floating character in deepwater rice, we compared the elongation capacity of 12 rice cultivars in response to partial submergence. These cultivars included several types of rice with different growth habits, namely deepwater, tall, short-statured, and semidwarf rices. We found that whole plants of all cultivars tested were capable of elongating rapidly when partially submerged at 12 to 13 weeks of age (Table I). Raskin and Kende (7) established that the gas composition in the internodal lacunae of stem sections of deepwater rice was approximately 3% O_2, 6% CO_2, and 1 μl L^{-1} C_2H_4 after 3 d of submergence. These workers and Métraux and Kende (6) also demonstrated that the growth response of deepwater rice to submergence was mediated by C_2H_4 and that the submergence response could be reproduced by exposing non-submerged stem sections to the above gas mixture in a flow-through system (7). We showed that the enhancement of internodal growth observed in whole plants was also seen in stem sections of all rice types which had been submerged or treated with 3% O_2, 6% CO_2, and 1 μl L^{-1} C_2H_4 (Table II). These data indicate that the elongation response of each of these cultivars to partial submergence is mediated by C_2H_4 and that the physiological response of each of these cultivars to partial submergence is similar. Both whole plants and stem sections of all cultivars tested elongated rapidly in response to submergence or gas treatment when compared to controls.

We investigated the development of the capacity for rapid internodal growth in different rice types by determining the ability of each of these cultivars to respond with enhanced growth to partial submergence at four ages (Fig. 1). Elongation of internodes begins at 4 weeks of age in HA II as does the growth response to partial submergence (6). Short-statured and semidwarf cultivars did not begin internodal expansion and did not elongate in response to partial submergence at 5 and 7 weeks of age. However, by 10 weeks, internodal expansion had begun, and both Mars and M9 were capable of rapid internodal elongation when submerged (Fig. 1, B and C). Therefore, we conclude that rapid internodal elongation in response to submergence (and also a gas mixture containing C_2H_4) will occur in any rice cultivar

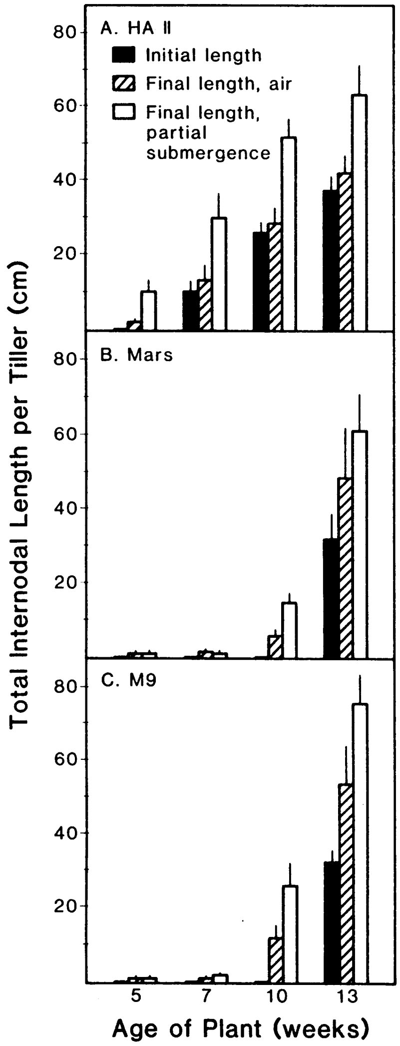

FIG. 1. Effect of partial submergence on the average internodal length per tiller in three rice cultivars, HA II (A) Mars (B) M9 (C). Internodal lengths were measured prior to treatment (solid bars), following 5 d of submergence during which the top 20 cm of the foliage was above the water surface (open bars), or after 5 d of growth in air (striped bars). The plants were 5, 7, 10, or 13 weeks of age on the 1st d of treatment. Each bar represents the average internodal length of 3 tillers per plant and 4 plants per cultivar for each age and treatment (±SD). All treatment effects (age, cultivar, and submergence) and first order interactions (age by cultivar, age by submergence, and cultivars by submergence) were highly significant (AOV; P = 0.01).

provided that internodal growth in air has already begun. Similar conclusions can be drawn from results of Kihara *et al.* (4), who compared growth of pronounced and weak floaters, if one analyzes them in light of our data.

In comparing and analyzing the elongation capacity of non-deepwater and deepwater rice cultivars, many researchers refer to the ability of internodes to elongate rapidly as an indispensable character of deepwater rice (2). Others have referred to elongation genes which may be bred from a deepwater cultivar into higher yielding semidwarf cultivars (9). Our data indicate that the deepwater character is comprised of more factors than the ability to elongate rapidly under flood conditions. What, then, is the difference between deepwater and other rice cultivars? Based on our results, we do not believe that there is a major difference in the short-term elongation capacity of different rice cultivars. We rather think that the duration of the period over which rapid elongation can occur differs between deepwater and non-deepwater rices. Internode elongation in deepwater rices begins very early, in many cultivars as early as 3 to 4 weeks after planting. Flowering, which terminates vegetative growth, is photoperiodically controlled and occurs late in the season. In most non-deepwater rices, internode elongation begins late in the growing season, *i.e.* either shortly before or at the time of panicle development. In the improved rice cultivars, flowering is not under photoperiodic control and occurs at a genetically determined stage of the plant's development. Therefore, the time period during which internodes elongate is much shorter in photoperiodically insensitive plants than in deepwater rices.

It has been shown that submerged or C_2H_4-treated deepwater rice does not elongate if the level of endogenous GA is reduced using TCY, a specific inhibitor of GA biosynthesis. Supplying the plant with exongenous GA_3 in the presence of TCY restores the elongation response to submergence (8). GA is also known to be directly involved in the induction of bolting and flowering in a number of rosette plants (3, 5). These results have led to a hypothesis on the regulation of internodal growth in rice plants. We believe that deepwater rice is able to respond to submergence (or C_2H_4) at an early stage because GA biosynthesis commences early in the vegetative growth phase of the plant. This is, of course, manifested by early internodal elongation in air (Fig. 1). By contrast, other rice types behave more like rosette plants. For much or all of their vegetative growth phase, internodes are telescoped at the base of the plant. Internodal elongation or bolting occurs usually at or just preceding panicle development. We suspect that GA biosynthetic activity is very low at the rosette stage, but increases prior to internodal elongation or bolting. Therefore, the time at which internodal elongation can be stimulated by submergence or C_2H_4 may be determined by the ability of the plant to produce GA.

In conclusion, the deepwater character is more complex than appeared at the onset of our studies comparing the growth potential of deepwater and non-deepwater rices. Transfer of the so-called floating trait from the relatively low-yielding deepwater rice cultivars to high yielding nondeepwater cultivars entails much more than transferring elongation genes. All rice cultivars tested were found to respond to submergence by rapid growth, once internodal elongation had begun. Our results indicate that deepwater rice differs from non-deepwater rice primarily by the timing of three distinct growth phases: the rosette stage during which internodal growth cannot be stimulated; the phase of internodal elongation during which growth is promoted by submergence; the reproductive phase which sets a limit to the final size of the plant.

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LITERATURE CITED

1. De Datta SK 1981 Principles and Practices of Rice Production. John Wiley & Sons, New York
2. Hanada K 1983 Growth of internodes, leaves and tillers of floating and non-floating rice varieties under deepwater conditions. Jpn J Trop Agric 27: 221–236
3. Jones MG, JAD Zeevaart 1980 Gibberellins and the photoperiodic control of stem elongation in the long day plant *Agrostemma githago* L. Planta 149: 269–273
4. Kihara H, TC Katayama, K Tsunewaki 1962 Floating habit of 10 strains of wild and cultivated rice. Jpn J Genet 37: 1–9
5. Koorneef M, JH Van Der Veen 1980 Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* L. Heynh. Theor Appl Genet 58: 257–263
6. Métraux J-P, H Kende 1983 The role of ethylene in the growth response of submerged deep water rice. Plant Physiol 72: 441–446
7. Raskin I, H Kende 1984 Regulation of growth in stem sections of deep-water rice. Planta 160: 66–72
8. Raskin I, H Kende 1984 Role of gibberellin in the growth response of submerged deep water rice. Plant Physiol 76: 947–950
9. Swaminathan MS 1984 Rice. Sci Am 250(1): 81–93
10. Vergara BS, B Jackson, SK De Datta 1976 Deep water rice and its response to deep water stress. Climate and Rice. International Rice Research Institute, Los Baños, Philippines, pp 301–319