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Fatty Acids of Rice Coleoptiles in Air and Anoxia[1]

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ABSTRACT

The metabolism of lipids, like that of other components, was adversely and strongly affected when rice (*Oryza sativa* L.) coleoptiles were grown anaerobically. In aerobic coleoptiles, the amounts of total fatty acid, phospholipid, and total lipid per coleoptile increased by 2.5- to 3-fold between days three and seven, whereas under anoxia, the increases were all less than 60%. The total amount of lipid at day seven in anoxia was less than 30% of that in air. In air, the total fatty acid content at day three was 25 nanomoles per coleoptile and this increased to over 71 nanomoles per coleoptile at day seven. All acids except 18:0 showed substantial increases. In anoxia, the corresponding values for total fatty acids were 24 nanomoles and 27 nanomoles. The small increases were confined to the saturated fatty acids; no significant increase occurred in unsaturated fatty acids. A minor fatty acid constituent (16:1) increased from 0.09 to 1.99 nanomoles per coleoptile between days three and seven in air. This component was never observed in any fatty acid preparation from anaerobic coleoptiles. The major phospholipids under all conditions were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid. A small amount of unidentified phosphoester, not present on thin layer chromatography plates from aerobic coleoptiles, was seen in extracts of anaerobic coleoptiles. The fatty acyl substituents of each of the phospholipids were analyzed at days three and seven in coleoptiles grown aerobically and in anoxia. Each phospholipid had its own distinctive fatty acid composition which remained fairly constant under all treatments; 16:0 and 18:2 were the most abundant fatty acids in every phospholipid class. In air, the percentages of total fatty acids that were in the phospholipids were 86% on day three and 87% on day seven. In anoxia, the values at the corresponding ages were 47 and 57%. Since no net synthesis of unsaturated fatty acids occurred in anaerobic conditions, the small increase in total unsaturated acids in the phospholipids between days three and seven must have occurred at the expense of fatty acids preexisting in the neutral lipid. No unusual pathways of biosynthesis or unusual precursors are required to explain the presence of unsaturated fatty acids in the rice coleoptile. The present study and results of experiments where coleoptiles were fed [^{14}C]acetate (BB Vartapetian *et al.* 1978 Plant Sci Lett 13:321–328) clearly show that unsaturated fatty acid synthesis in rice coleoptiles requires O_2, as it does in other plants.

When completely deprived of O_2, the seeds of most plants fail to germinate. Rice (*Oryza sativa* L) seeds are a frequently-cited exception (2, 11, 16, 19). But even in rice the growth is quite limited—only the coleoptile elongates and, although it can surpass its aerobic counterpart in length, its weight is less and it is inferior in other respects (17). The underlying basis for this growth in anoxia remains obscure (1, 4).

In addition to its role as electron acceptor in aerobic respiration, O_2 is also specifically required in reactions leading to the generation of unsaturated fa[2] in all eucaryotic systems that have been studied (8). Thus, the findings by Vartapetian (20) that rice coleoptiles in anoxia showed a much higher fa content per mg dry weight and a higher percent of 18:1 and 18:2 than those in air came as a surprise since these results suggested that an anaerobic pathway of fa unsaturation previously known only in procaryotes may be present in rice (5, 7). Such a possibility, in turn, might indicate an underlying mechanism for the unusual success of rice coleoptiles in anoxia.

However, a subsequent study (21) showed that rice coleoptiles converted [^{14}C]acetate to unsaturated fa in air but completely failed to do so in anoxia, in accordance with pathways established in other plants. Thus to account for the higher content of unsaturated fa in anaerobic coleoptiles it was proposed that "an unusual metabolic pathway or unusual precursor for unsaturated fa biosynthesis" may be present (21).

The present work describes in some detail the fa and phospholipids of rice coleoptiles grown in air and in N_2. It shows that the rice coleoptile does not require an unusual system of unsaturated fa biosynthesis; there is no net synthesis of unsaturated fa in coleoptiles in anoxia.

MATERIALS AND METHODS

Plant Materials. Dehulled rice seeds (*Oryza sativa* L. cvs S-6 and S-201) were obtained from the University of California Rice Experiment Station, Biggs, CA. Seeds sterilized in 2.5% NaOCl were rinsed three times in sterile distilled H_2O and grown under aseptic conditions in air or anoxia. For aerobic growth, seeds were sown in 100 × 80 mm culture dishes lined with three disks of Whatman No. 1 filter paper and wetted with 20 ml of water; the dishes were loosely capped with 100 × 15 mm Petri dishes. For anoxic growth, seeds were placed in screw cap jars (250 ml) lined with moistened filter papers, as above. The jars were sealed and flushed continuously with moist N_2 gas (99.998% purity) which entered and left the jars through disposable syringe needles at flow rates of 30 to 40 ml/min. All containers were placed in the dark at 25°C for up to 7 d. At the end of the incubation period the seedlings were removed and coleoptile growth was examined.

Lipid Analyses. Shoots were cut from seedlings at the node between the coleoptile and mesocotyl. Leaves were removed and discarded and coleoptiles were held in aerated or N_2-equilibrated water at 0°C. When 2 to 3 g of coleoptiles had been collected they were counted and weighed. Ten to 15 were sampled and used to determine dry weight. They were weighed and dried in an oven at 70°C until they reached a constant weight. The remainder of the coleoptiles were steam killed to inactivate

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[2] Abbreviations: fa, fatty acid, PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI phosphatidylinositol; PA, phosphatidic acid; PS, phosphatidylserine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; Px, undentified phospholipid.

phospholipases. These were placed on a 10 × 10 cm screen held 2 cm above a boiling water bath for 2 min.

Lipids were extracted from killed coleoptiles by homogenizing them for 3 min in 19 volumes of chloroform:methanol (2:1, v/v). The homogenate was filtered through chloroform-washed Whatman No. 1 filter paper and the filtrate was collected in a suction flask. The filter cake was scraped from the filter paper and reextracted by homogenizing it with another 19 volumes of chloroform:methanol (2:1,v/v) for 3 min. The homogenate was filtered and the filter cake was reextracted with another 19 volumes of solvent. The combined extracts were filtered, given a Folch wash (6), and taken to dryness at reduced pressure under N_2. The dried lipids were dissolved in chloroform and stored at −20°C.

Phospholipids were separated and identified on thin layer chromatograms (Silica Gel 60, 20 × 20 cm, 0.25 mm layer thickness, E. M. Merck) (12). About 1.0 mg of lipid in chloroform was applied to the plates in the lower left corner, 2 cm from either edge. Plates were developed to a height of 15 cm in two dimensions—first with chloroform:methanol:7 N NH_4OH (65:30:4, v/v) and then chloroform:methanol:acetic acid:water (170:25:25:6, v/v). To locate lipids, dried plates were placed in a tank with iodine vapors or sprayed with concentrated H_2SO_4 and heated at 120°C for 1 to 2 h. Vaskovsky and Kostetsky phospholipid stain (22) and authentic standards were used to identify the phospholipids. Phospholipids were quantified by the Bartlett method of phosphate microanalysis (3) on samples scraped from the TLC plates.

Fatty acids were identified by GC of their methyl esters. A lipid sample containing 1.0 to 5.0 mg of lipid was transferred to a 13 × 100 mm screw top test tube. Solvent was evaporated with a stream of N_2, the residue was dissolved in 0.5 ml benzene, and 1.0 ml of methylating reagent was added. This reagent was prepared fresh for each experiment by slowly adding 1.0 ml of acetyl chloride to 10 ml of cold, dry methanol. The tube was sealed with a teflon-lined cap and held at 75°C for 2 to 3 h. After methylation, the tube was cooled to room temperature and 2.0 ml 5% NaCl was added. The fa methyl esters were collected in hexane by twice partitioning the aqueous methanol solution against 2.0 ml of the solvent. Hexane portions were combined, partitioned against 2.0 ml $KHCO_3$ and taken to dryness under N_2. The fa methyl esters were dissolved in chloroform and stored at −20°C.

Fatty acid methyl esters were separated and identified on a Beckman GC-65 (2 m × 2 mm, 10% Silar-10C on 100/120 Gas-Chrom Q glass column at 180°C; He carrier; Flame Ionization Detector [FID]), or a Perkin-Elmer 3920 (2 m × 2 mm, SP 2340 glass column; temperature program, initial temperature = 130°C, final temperature = 230°C, rate = 4°C/min; N_2 carrier; FID) equipped with an integrator. They were quantified by comparing sample peak areas with an internal standard of methyl heptadecanoic acid.

For the analysis of fatty acids in the major phospholipid classes, lipids were first separated on a TLC plate with the solvent system described above. Lipid spots were located by spraying the plate with 2′,7′-dichlorofluorescein and viewing it under UV light. Phospholipid spots were scraped from the plate, collected in test tubes, and then methylated and analyzed as above.

RESULTS

In agreement with previous studies (1), growth of rice seedlings was greatly affected by anoxia. After 7 d the primary leaf was less than 1 mm in length and no roots developed. Only the coleoptiles elongated—to about 3 cm in 7 d, a length comparable to that of aerobic seedlings. However, the coleoptiles of seedlings in anoxia were fragile and often misshapen. As shown in Table I the dry weight of coleoptiles in air increased 7-fold between d 3 and 7 whereas in anoxia the increase was less than 2-fold; the fresh weight of aerobic coleoptiles at d 7 was more than 4-fold that of anaerobic coleoptiles.

Coleoptiles did not reach an acceptable size for sampling and analysis before d 3. To document in detail the changes in phospholipids and fatty acids during growth in anoxia and in air data were collected from d 3 and d 7 seedlings and expressed initially on a per coleoptile basis.

As shown in Table I, the imposition of anoxia strongly affects the lipid metabolism of the coleoptile. The total lipid content of anaerobic coleoptiles at d 3 was less than half that of aerobic controls, and during the subsequent 4 d period the gain in total lipid was less than one-fifth that shown in air. After 7 d, anoxic coleoptiles contained 30% of the total lipids, 40% of the total fatty acids, and 25% of the phospholipids that were present in aerobic coleoptiles. In a similar analysis of the lipids of *Echinochloa crus-galli* var *oryzicola* (an aquatic grass which, like rice, grows in the absence of O_2), Knowles and Kennedy (10) showed that the lipid metabolism of this plant is also restricted by anoxia. It is clear that in anoxia elongation is achieved without a proportionate increase in lipids.

Detailed data on the fa of aerobic and anaerobic rice coleoptiles are presented in Table II. In both air and N_2, the dominant fa were 16:0, 18:1, and 18:2; together they accounted for >90% of the total fa throughout the growth period. Between d 3 and 7 in air there was an almost 3-fold increase in total fa per coleoptile, and all fa, except 18:0, contributed to this increase. The largest relative increases were those of 18:1 (9-fold) and of 16:1, although a minor component (20-fold). A very different picture emerges from the coleoptiles in anoxia. There was only a minor increase in total fa content between d 3 and 7 and, of greater importance, there was no significant increase in the amount of any of the unsaturated fa; 16:1 was never found in any coleoptile from anoxia.

Phospholipids contained a greater proportion of the total fa in air than in N_2. As calculated from data in Tables I and VI, 85% of the total fa in air were substituents of phospholipids whereas, in N_2, only about 50% of the total fa were in phospholipids. Thus, a larger proportion of the fa in N_2 are present in components other than phospholipids; this results from the fact that neutral lipid, originally present in the embryonic coleoptile, is utilized less rapidly in N_2 than in air.

The phospholipid content of aerobic and anoxic coleoptiles are given in Table III. In all treatments, PC, PE, PI, and PA were the most abundant classes. In air, total phospholipid content increased 3-fold between d 3 and 7 and the relative proportions of each class were nearly unchanged. In anoxia, phospholipid content increased by only 40% and there were changes in the relative abundance of some classes. The proportion of PC decreased while that of PA increased. In addition, a small amount of phospholipid (PX in Table III) appeared in the extract which cochromatographed with phosphatidylethanol, a phosphoester produced by phospholipase D in the presence of ethanol (23).

The most abundant fa substituents of each phospholipid class are presented in Table IV. Each phospholipid had a distinct fa complement. The proportions of fa in PC, PE, and PS resembled the overall fa content of the cell but the composition of other classes varied. DPG, a major component of mitochondrial membranes (9), contained more than 70% 18:2 while in PG, PI, and PA 16:0 was most abundant. In air, the proportion of 18:1 increased in all phospholipid classes between d 3 and 7. This change was not seen in anoxic coleoptiles where the relative abundance of the fa remained constant. Except for PS on d 3, each of the phospholipids from aerobic coleoptiles contained some 16:1 at each sampling period. This fatty acid was never found in any phospholipid from anaerobic coleoptiles.

Table I. *Growth Measurements and Lipid Content of Rice Coleoptiles*

	Air		Anoxia	
	Day 3	Day 7	Day 3	Day 7
Fresh wt (mg)	3.1 ± 0.8[a]	27.7 ± 6.0	2.4 ± 0.1	6.6 ± 0.8
Dry wt (mg)	0.22 ± 0.08	1.53 ± 0.23	0.12 ± 0.02	0.22 ± 0.05
Total fatty acid (nmol)	25.0 ± 3.5	71.4 ± 14.0	24.1 ± 6.3	27.5 ± 4.6
Phospholipid (nmol)	10.7 ± 1.9	31.0 ± 1.6	5.7 ± 0.9	7.9 ± 0.7
Total lipid (μg)	24.0 ± 1.0	59.3 ± 12.7	10.7 ± 2.3	17.0 ± 1.0
Length (cm)	0.5–1.0	3.0–4.0	0.5–1.5	1.5–3.0

[a] The data for weight and lipid content are per coleoptile and show the average of three replicates (200–400 coleoptiles each) ± SE. The data for length are the upper and lower limits of coleoptile lengths that were used at each age.

Table II. *Total Fatty Acid Content of Rice Coleoptiles*

Fatty Acid	Content per Coleoptile			
	Air		Anoxia	
	Day 3	Day 7	Day 3	Day 7
	nmol			
14:0	0.2 ± 0.1[a]	0.4 ± 0.1	0.1 ± <0.1	0.2 ± 0.1
16:0	8.3 ± 1.6	20.0 ± 3.7	5.9 ± 0.9	7.8 ± 0.2
16:1	0.1 ± 0.1	2.0 ± 0.4	0 ± 0	0 ± 0
18:0	0.2 ± 0.4	0.1 ± <0.1	0.1 ± <0.1	0.1 ± 0.1
18:1	2.3 ± 0.3	18.0 ± 3.8	4.9 ± 1.2	5.4 ± 1.2
18:2	13.0 ± 1.3	28.8 ± 5.9	12.8 ± 4.2	13.7 ± 3.7
18:3	0.9 ± 0.2	2.0 ± 0.5	0.3 ± 0.1	0.3 ± 0.1
Total	25.0 ± 3.5	71.4 ± 14.0	24.1 ± 6.3	27.5 ± 4.9

[a] Data show the average of three replicates (200–400 coleoptiles each) ± SE.

Table III. *Phospholipid Content of Rice Coleoptiles*

Phospholipid Class	Content per Coleoptile			
	Air		Anoxia	
	Day 3	Day 7	Day 3	Day 7
PC	4.8 ± 0.6[a] (46)[b]	15.6 ± 1.0 (50)	2.8 ± 0.5 (49)	3.2 ± 0.5 (40)
PE	2.4 ± 0.4 (23)	8.0 ± 0.1 (26)	1.4 ± 0.3 (25)	2.1 ± 0.1 (27)
PI	1.0 ± 0.3 (10)	2.3 ± 0.2 (7)	0.5 ± 0.1 (8)	0.7 ± 0.2 (8)
PA	1.1 ± 0.6 (10)	1.7 ± 0.2 (6)	0.5 ± 0.2 (9)	1.2 ± 0.3 (15)
PG	0.7 ± 0.1 (7)	1.6 ± 0.3 (5)	0.3 ± 0.1 (6)	0.3 ± <0.1 (4)
DPG	1.0 ± 0.1 (1)	0.3 ± <0.1 (1)	0.03 ± 0.01 (1)	0.1 ± <0.1 (1)
PS	0.2 ± 0.1 (2)	0.8 ± 0.2 (3)	0.1 ± 0.1 (2)	0.3 ± <0.1 (4)
PX	0 (0)	0 (0)	0.01 ± 0.01 (<1)	0.03 ± 0.01 (<1)
Other	0.3 ± 0.3 (3)	0.7 ± 0.3 (2)	0.04 ± 0.04 (1)	0.1 ± 0.1 (1)
Total	10.7 ± 1.9	31.0 ± 1.6	5.7 ± 0.9	7.9 ± 0.7

[a] Data show the mean of three replicates (200–400 coleoptiles each) ± SE. [b] Data in parentheses show the percent of total lipid phosphorus represented by each phospholipid class.

DISCUSSION

It is clear from the results that lipid metabolism in the rice coleoptile grown in N_2 is grossly impaired; the amounts of total lipid, total fa, and phospholipids per coleoptile are strikingly lower than in aerobic coleoptiles (Table I). In addition, a clear qualitative difference is revealed in the data for unsaturated fa. The characteristic unsaturated fa substituents of all lipid classes in air increased during d 3 to 7. In coleoptiles in N_2, although there were small increases in phospholipids and total fa (Table I), there was no net increase in any of the unsaturated fa during this period (Table II). Furthermore, 16:1, a (minor) substituent of all phospholipids in air, was never observed in samples grown in N_2. We conclude that the rice coleoptile is unable to generate unsaturated fa in anoxia.

From previous work, which was indeed a stimulus to the present analysis, it had been concluded that rice coleoptiles must have the capacity for this unusual synthesis. The reason for these apparently opposing conclusions is evident from Table V, which includes data on total fa/coleoptile and dry weights. From these data it is clear that, since the dry weight of aerobic coleoptiles is up to 7-fold higher than those in N_2, the amount of fa per mg dry weight is considerably greater in anaerobic coleoptiles although the amount of fa per coleoptile is actually lower. When expressed in the same way, the data from our experiments (final 3 columns of Table V) agree completely with those of Vartapetian *et al.* (20). Their finding that [^{14}C]acetate was not incorporated into unsaturated fa in coleoptiles in N_2 (21) is, of course, fully consistent with our conclusion that no unsaturated fa synthesis occurs under these conditions.

Table IV. *Fatty Acids of the Phospholipid Classes*

Fatty Acid	Content			
	Air		Anoxia	
	Day 3	Day 7	Day 3	Day 7
	mol %			
A—Phosphatidylcholine				
16:0	27.0 ± 5.3[a]	25.7 ± 2.1	25.0 ± 5.2	26.0 ± 2.0
16:1	1.1 ± 0.8	5.3 ± 1.2	0 ± 0	0 ± 0
18:0	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.2
18:1	11.7 ± 1.5	30.0 ± 1.0	24.0 ± 2.6	23.0 ± 1.7
18:2	58.0 ± 2.6	38.0 ± 0.0	50.0 ± 2.6	50.0 ± 2.6
18:3	2.0 ± 1.0	0.9 ± 0.1	0.4 ± 0.6	0.7 ± 0.3
B—Phosphatidylethanolamine				
16:0	39.0 ± 6.2	34.3 ± 4.7	37.3 ± 6.5	35.3 ± 3.5
16:1	0.3 ± 0.3	2.0 ± 0.0	0 ± 0	0 ± 0
18:0	0.2 ± 0.2	0.2 ± 0.2	0 ± 0	0.3 ± 0.3
18:1	4.0 ± 1.0	13.0 ± 1.0	10.0 ± 1.0	12.0 ± 1.0
18:2	55.0 ± 4.4	49.7 ± 4.0	52.0 ± 6.0	52.3 ± 2.9
18:3	1.1 ± 0.8	0.7 ± 0.3	0 ± 0	0.3 ± 0.6
C—Phosphatidylinositol				
16:0	61.0 ± 9.8	56.0 ± 13.7	51.3 ± 11.7	44.7 ± 9.3
16:1	0.2 ± 0.3	1.7 ± 0.6	0 ± 0	0 ± 0
18:0	0.3 ± 0.6	0.7 ± 0.3	0.5 ± 0.5	0.7 ± 0.6
18:1	5.0 ± 4.0	21.7 ± 6.7	12.7 ± 3.8	12.7 ± 4.0
18:2	32.7 ± 4.7	19.3 ± 5.5	35.3 ± 7.8	41.7 ± 5.5
18:3	0.9 ± 1.0	1.0 ± 0.8	0.3 ± 0.6	0.3 ± 0.6
D—Phosphatidic Acid				
16:0	53.0 ± 11.5	41.0 ± 10.5	32.7 ± 4.6	34.7 ± 4.0
16:1	0.2 ± 0.3	2.0 ± 1.0	0 ± 0	0 ± 0
18:0	0.8 ± 0.3	0.5 ± 0.5	0.1 ± 0.2	0.5 ± 0.5
18:1	9.0 ± 3.0	22.7 ± 4.5	21.0 ± 1.7	18.3 ± 2.3
18:2	36.7 ± 7.6	33.7 ± 6.4	46.0 ± 2.6	46.0 ± 1.0
18:3	1.0 ± 0.1	0.5 ± 0.5	0.1 ± 0.2	0.5 ± 0.5
E—Phosphatidylglycerol				
16:0	64.7 ± 11.6	52.3 ± 6.0	60.7 ± 9.0	65.0 ± 9.5
16:1	0.3 ± 0.5	0.5 ± 0.5	0 ± 0	0 ± 0
18:0	0.8 ± 0.2	0.6 ± 0.3	0.3 ± 0.5	1.3 ± 0.6
18:1	5.3 ± 2.5	24.7 ± 4.0	9.0 ± 2.0	9.0 ± 3.0
18:2	28.7 ± 7.8	21.3 ± 1.2	30.3 ± 7.0	25.0 ± 6.9
18:3	1.5 ± 1.3	0.5 ± 0.5	0.3 ± 0.5	0 ± 0
F—Diphosphatidylglycerol				
16:0	13.0 ± 6.1	9.3 ± 8.7	15.3 ± 6.4	15.0 ± 8.7
16:1	0.2 ± 0.3	0.7 ± 1.2	0 ± 0	0 ± 0
18:0	2.3 ± 1.5	1.3 ± 1.5	2.0 ± 1.0	2.7 ± 3.1
18:1	8.0 ± 2.0	15.7 ± 3.5	10.0 ± 3.0	8.3 ± 2.1
18:2	74.3 ± 4.2	71.7 ± 9.3	72.0 ± 4.4	73.7 ± 9.3
18:3	2.3 ± 1.5	1.7 ± 2.1	1.0 ± 1.0	0.3 ± 0.6
G—Phosphatidylserine				
16:0	38.7 ± 5.1	30.7 ± 3.2	27.7 ± 11.5	31.0 ± 12.1
16:1	0 ± 0	0.7 ± 1.2	0 ± 0	0 ± 0
18:0	3.0 ± 1.0	4.3 ± 3.8	7.7 ± 8.1	2.3 ± 1.5
18:1	5.7 ± 1.5	11.0 ± 10.1	19.3 ± 9.6	12.3 ± 5.0
18:2	50.3 ± 6.7	56.7 ± 10.3	37.0 ± 21.7	53.3 ± 6.7
18:3	2.3 ± 0.6	0.2 ± 0.4	9.0 ± 14.7	0.7 ± 0.6

[a] Data show the mean of three replicates (200–400 coleoptiles each) ± SE.

Lipid shortages are known to limit the growth of yeast in anoxia and a medium supplemented with unsaturated fa and sterol improves their growth (15). The ungerminated seeds of rice and another aquatic grass, oryzicola (*Echinochloa crus-galli* var *oryzicola*), contain numerous lipid bodies (13, 18). Opik (14) has suggested that for rice, these lipids may provide an endogenous supply of lipid that would help seedlings avoid similar lipid shortages during anaerobic growth. The phospholipid content of anoxic rice coleoptiles increased between d 3 and 7 (Table III). As shown in Table VI the fa required for this net increase were distributed among all classes of fa, both saturated and unsaturated. Since no unsaturated fa were synthesized in anoxia from d 3 to 7 (Table II) their increase in the phospholipids requires that some were derived from storage lipid present in the embryonic coleoptiles.

The present study also revealed that the major lipid components of membranes—the phospholipids—are altered in content and composition. In air, all phospholipids, with the exception of

Table V. *Total Fatty Acids of Rice Coleoptiles*

Treatment	Fatty Acids (nmol) per Coleoptile		Dry wt (mg) per Coleoptile		Fatty Acids (nmol) per Dry Wt (mg)		
	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7	Day 5[a]
Air	25	71	0.22	1.53	114	47	95
Anoxia	24	28	0.12	0.22	200	125	135

[a] Data derived from Vartapetian *et al.* (20).

Table VI. *Fatty Acids of the Phospholipids of Rice Coleoptiles*

Fatty Acid	Content per Coleoptile[a]			
	Air		Anoxia	
	Day 3	Day 7	Day 3	Day 7
	nmol			
16:0	8.02 (38)	19.80 (32)	3.72 (33)	5.20 (33)
16:1	0.14 (1)	2.16 (4)	0 (0)	0 (0)
18:0	0.16 (1)	0.22 (<1)	0.04 (<1)	0.08 (<1)
18:1	1.76 (8)	14.36 (23)	2.06 (18)	2.62 (17)
18:2	10.60 (50)	24.24 (40)	5.38 (48)	7.56 (49)
18:3	0.34 (2)	0.50 (1)	0.04 (<1)	0.12 (<1)
Total	21.02	61.28	11.24	15.58

[a] Calculated from mol % fatty acid × nmol lipid phosphorus (as presented in Table III). Figures in parentheses are the percent of fa of the phospholipids represented by each fa.

PA, increased 2- to 3-fold between d 3 and 7 and the proportions of each phospholipid class were nearly constant. In anoxia, increases were smaller for all classes except PA. In addition, these increases were unequal and they altered the proportional composition of the phospholipids (Table III). The net effect was an increase in the proportion of PA and a decrease in PC.

LITERATURE CITED

1. ALPI A, H BEEVERS 1983 Effects of O_2 concentration on rice seedlings. Plant Physiol 71: 30–34
2. ATWELL BJ, I WATERS, H GREENWAY 1982 The effect of oxygen and turbulence on elongation of coleoptiles of submergence-tolerant and -intolerant rice cultivars. J Exp Bot 33: 1030–1044
3. BARTLETT GR 1959 Phosphorus assay in column chromatography. J Biol Chem 234: 466–468
4. BERTANI A, I BRAMBILLA, F MENEGUS 1980 Effect of anaerobiosis on rice seedlings: growth, metabolic rate, and fate of fermentation products. J Exp Bot 31: 325–331
5. BLOCH K 1969 Enzymatic synthesis of monounsaturated fatty acids. Accts Chem Res 2:18–27
6. FOLCH J, M LEES, GH SLOANE-STANLEY 1957 A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509
7. GURR MI 1974 The biosynthesis of unsaturated fatty acids. *In* TW Goodwin, ed, Biochemistry of Lipids. University Park Press, Baltimore, pp 181–235
8. GURR MI, AT JAMES 1975 Lipid Biochemistry. Halstead, New York, p 42
9. HARWOOD JL 1980 Plant acyl lipids: structure, distribution, and analysis. *In* PK Stumpf, ed, Lipids: Structure and Function, The Biochemistry of Plants, a Comprehensive Treatise, Vol. 4. Academic Press, New York, pp 177–204
10. KNOWLES LD, RA KENNEDY 1984 Lipid biochemistry of *Echinochloa crus-galli* during anaerobic germination. Phytochemistry 23: 529–532
11. KORDAN HA 1972 Rice seedlings germinated in water with normal and impeded environmental gas exchange. J Appl Ecol 9: 527–533
12. NICHOLS BW 1964 TLC of plant polar lipids. *In* AT James, LJ Morris, eds, New Biochemical Separations. Van Nostrand, New York, pp 321–337
13. OPIK H 1972 Some observations on coleoptile cell ultrastructure in germinated grains of rice (*Oryza sativa* L.) Planta 102: 61–71
14. OPIK H 1973 Effects of anaerobiosis on respiratory rate, cytochrome oxidase activity and mitochondrial structures in coleoptiles of rice (*Oryza sativa* L.) J Cell Sci 12: 725–739
15. RATTRAY JBM, A SCHIBECI, DK KIDBY 1975 Lipids of yeast. Bacteriol Rev 39: 197–231
16. TAYLOR DL 1942 Influence of oxygen tension on respiration, fermentation, and growth in wheat and rice. Am J Bot 29:721–738
17. TSUJI H 1976 Growth and metabolism in plants under anaerobic conditions. Environ Control Biol 11: 79–84
18. VANDERZEE D, RA KENNEDY 1982 Plastid development in seedlings of *Echinochloa crus-galli* var. *oryzicola* under anoxic germination conditions. Planta 155: 1–7
19. VARTAPETIAN BB, IN ANDREEVA, N NURITDIVOV 1978 Plant cells under oxygen stress. *In* DD Hook, RMM Crawford, eds, Plant Life in Anaerobic Environments. Ann Arbor, MI, pp 13–88
20. VARTAPETIAN BB, R BAZIER, C COSTES 1978 Lipids in rice seedlings grown under anaerobic and aerobic conditions. *In* DD Hook, RMM Crawford, eds, Plant Life in Anaerobic Environments. Ann Arbor, MI, pp 539–548
21. VARTAPETIAN BB, P MAZLIAK, C LANCE 1978 Lipid biosynthesis in rice coleoptiles grown in the presence or in the absence of oxygen. Plant Sci Lett 13: 321–328
22. VASKOVSKY VE, EY KOSTETSKY 1968 Modified spray for the detection of phospholipids on thin-layer chromatograms. J Lipid Res 9: 369
23. YANG SF, S FREER, AA BENSON 1967 Transphosphatylidation by phospholipase D. J Biol Chem 242: 477–484