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LIPID BIOCHEMISTRY OF *ECHINOCHLOA CRUS-GALLI* DURING ANAEROBIC GERMINATION*

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Key Word Index—*Echinochloa crus-galli*; Poaceae; watergrass; metabolism; lipids; anaerobiosis; germination.

Abstract—Seven day old seedlings of *Echinochloa crus-galli* var. *oryzicola* (Vasing) had a higher total lipid content when germinated under N_2 than in air, although ungerminated seeds contained more lipid than either seedling. The triacylglycerol pool was not depleted under anaerobiosis as it was in air and only air-grown seedlings showed a net increase in free fatty acids and polar lipids. Concentrations of most of the individual acids of the total fatty acid profile declined during germination in air and in the free acid and polar lipid fractions of these seedlings the relative proportion of polyunsaturated fatty acids increased. Compared to air-grown seedlings, ungerminated seeds and N_2-grown seedlings had a similar qualitative and quantitative lipid composition. Our results show that mobilization of storage lipids was apparently severely inhibited under anoxia. The importance of lipid metabolism to the germination and growth of *Echinochloa* during anoxia is discussed in terms of maintaining membrane integrity and serving (indirectly) to reoxidize pyridine nucleotides.

INTRODUCTION

The biochemistry of higher plants is reported to be substantially altered in anaerobiosis. Lipid synthesis occurs in the absence of O_2 [1–5] but the specific response to anoxia varied among the plants tested. [^{14}C]Acetate incorporation into lipid was depressed by anaerobic treatment of cotyledons [1], spinach chloroplasts [5] and of wheat and rice roots [3]. In contrast, anaerobic rice coleoptiles synthesized more lipid from labelled acetate [2] and accumulated more lipid on a dry wt basis than did those grown in air [4].

In considering plant lipids, much attention has been given to the relative quantities of saturated and unsaturated fatty acids (FA) during anaerobic metabolism. Since FA desaturases are O_2-requiring enzymes, in anoxia, unsaturated acids are not found labelled after [^{14}C]acetate incorporation studies [1, 2]. None the less, the relative share contributed by unsaturates in the total FA pool increased in rice roots [6], rice coleoptiles [2, 4] and *Iris* rhizomes [7]. Although various hypotheses exist, the significance of FA unsaturation to anaerobic tolerance has not been clearly defined. Most frequently, FA composition is thought to be related to membrane stability and/or function [6, 7].

Plant species adapted to low O_2 environments may have a unique lipid metabolism. Indeed, significant qualitative and quantitative differences in lipid composition have been found between plants differing in their sensitivity to low O_2 stress [6, 7]. Although rice has long been of interest because of its tolerance to anoxia, we have been investigating several rice weeds in the *Echinochloa* genus because of their even greater ability to germinate and grow in the absence of O_2 [8]. Since the metabolic adaptations to O_2 stress probably vary, it is necessary to examine as many tolerant species as possible. Previous microscopic [9, 10] and metabolic studies [11] have indicated to us that lipid synthesis not only occurs in *Echinochloa*, but that it may actually be enhanced by anaerobiosis. The present study of aerobically vs anaerobically germinated *E. crus-galli* var. *oryzicola* was undertaken to determine the effect of anoxia on lipid composition and further our understanding of the role of lipids in anaerobic metabolism.

RESULTS AND DISCUSSION

The dry wt of 7-day-old *E. crus-galli* seedlings was the same whether germinated aerobically or anaerobically, although in air, a greater proportion of the dry wt was attributed to shoot tissue (Table 1). In air, both coleoptile and radicle emerged and collectively are referred to as 'shoot' in this publication. It should be noted that under anoxia, only coleoptiles were produced. Previously reported data [8] showed shoot fr. wt of this species to be *ca* 80% lower at day 7 when germinated anaerobically vs aerobically. Due to these large differences in seedling development, lipid data revealed opposite trends when expressed on a per seed basis compared with a dry wt basis (Table 1). Seeds of both treatments suffered a net loss of total lipid during germination, however, air-grown seeds contained less lipid on an absolute basis, as well as on a concentration basis than N_2-grown seeds. Lipid concentration of anaerobic shoot tissue (65 mg/g dry wt) was greater than that of aerobic tissue (25 mg/g dry wt) despite the fact that each seedling accumulated nearly four times more lipid in shoot tissue when grown in air. Thus, aerobic seedlings appeared to be more actively catabolizing lipid, as well as exhibiting more vigorous growth. The data did not allow us to draw firm conclusions as to the relative rates of lipid synthesis in shoot tissue deprived of O_2 vs

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Table 1. Lipid content and dry weight of *Echinochloa crus-galli* var. *oryzicola* seeds and 7 day seedlings

	Total lipid		Dry wt		TAG*	FFA†	PL‡
	μg/seed	μg/shoot	mg/seed	mg/shoot	μg/seedling		
Ungerminated seed	319	—	5.21	—	171	45	40
Air, 7 days	191	35	3.73	1.01	78	68	50
N_2, 7 days	262	9	4.60	0.14	170	31	38
$LSD_{0.05}$	30	23	0.17	0.05	12	25	8

*Triacylglycerol.
†Free fatty acid.
‡Polar lipids.

that grown in air. Due to the high concentration of lipid in anaerobic coleoptiles, the ability of an O_2-stressed seedling to support shoot growth did not seem to be limited by the amount of lipid present.

In order to characterize lipid metabolism of germinating *Echinochloa* in more detail, major lipid classes from the seedlings were separated and recovered by prep. TLC. Triacylglycerols (TAG) comprised 54% of the total lipid in ungerminated seed (Table 1). During germination in air, a substantial amount was lost from this pool, while free FA and polar lipids (phospholipids and glycolipids) increased slightly. Under anaerobic conditions there were no significant changes in the amount of any fraction on a per seedling basis. Recently, however, we have found that [U-^{14}C]acetate is incorporated into each of these lipid pools during the first 5 days of germination under N_2 [unpublished data], although to a lesser extent than in air. Therefore, at least a small degree of lipid metabolism must be occurring even under total anaerobiosis. Taking this into account, the influence of anoxia was to limit the synthesis of polar lipids relative to neutral lipids (especially TAG). Similar results have been reported in various systems under anoxia, where neutral lipid synthesis was found to be either unaffected [1, 7] or enhanced [4] by the lack of O_2 while phospholipid synthesis was reduced. In previous ultrastructural studies [10] of *Echinochloa* seedlings grown without O_2, we observed large amounts of lipid bodies. The relatively high content of TAG could explain the occurrence of lipids seen in micrographs of anaerobic tissue.

The fatty acyl components of seed and shoot lipids were examined and quantitated on a per organ basis (Fig. 1). During 7 days growth in air, seeds showed decreased amounts of four out of five fatty acids, 18:3 being the only one to be maintained at its original level. These data reflect the overall loss of lipid from the aerobic seedling (Table 1). Shoots of air-grown seedlings contained higher concentrations of 16:0, 18:2 and 18:3 than did anaerobic tissue, although on a dry wt basis, all five acids would be more concentrated in the O_2-stressed shoots. The FA profile of the anaerobically-grown seedling as a whole was almost identical to the ungerminated seed, again indicating that there was an overall maintenance of lipid composition over a 7-day period. Consistent with published data from rice [2] the net incorporation of [^{14}C]acetate label into 18:3 occurred only in air.

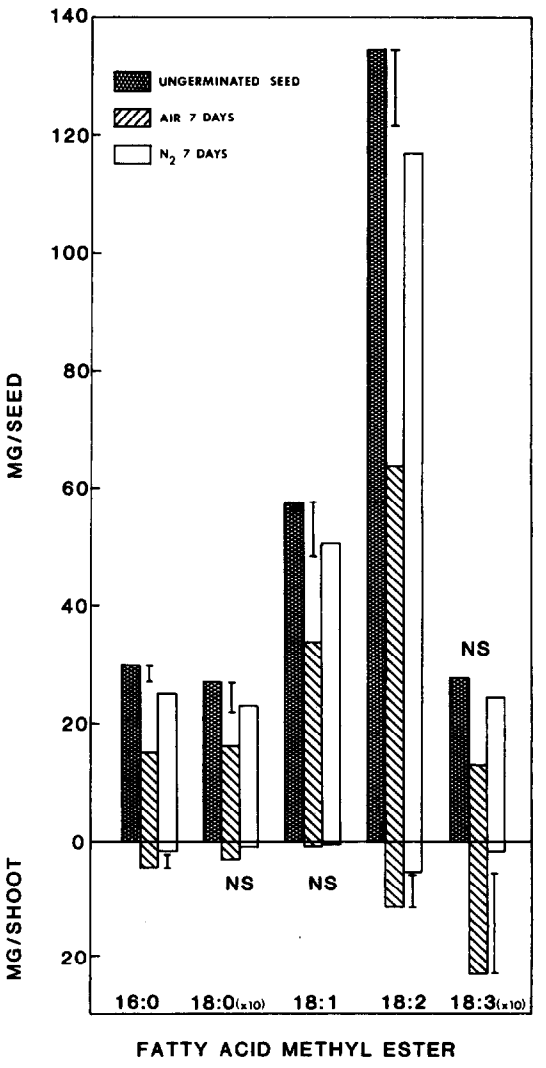

Fig. 1. Total fatty acids from seeds and 7-day-old seedlings of *Echinochloa crus-galli* var. *oryzicola* grown in air or N_2. Vertical bars indicate $LSD_{0.05}$. Where 'ns' appears treatment differences are not significant.

Table 2. Fatty acid composition of lipid fractions from *Echinochloa crus-galli* var. *oryzicola* seeds and 7-day-old seedlings (% of total acids)

		16:0	18:0	18:1	18:2	18:3
Triacylglycerol	Ungerminated seed	11	1	24	62	2
	Air, 7 days	11	1	25	61	2
	N_2, 7 days	11	1	24	62	2
Free fatty acid	Ungerminated seed	21	3	38	38	*
	Air, 7 days	13	1	28	56	2
	N_2, 7 days	20	5	40	35	—
Polar lipid	Ungerminated seed	23	1	24	51	1
	Air, 7 days	25	1	13	52	9
	N_2, 7 days	27	1	20	52	—

*Less than 1%.

Individual lipid pools were examined to determine if qualitative FA changes occurred within specific fractions of the lipid extract of whole seedlings. The TAG pool was qualitatively similar in all treatments (Table 2). The free FA of aerobic seedlings contained a relatively larger proportion of 18:2 compared to ungerminated seed and anaerobic seedlings. Polar lipids accumulated a larger proportion of 18:3 in air. In no case, however, did there appear to be significant qualitative changes in fatty acyl content of lipids during germination in N_2.

The lipid composition of *E. crus-galli* seedlings germinated under anoxia resembled that of ungerminated seed in three respects: (1) the amount of various lipid classes/seed; (2) the concentration of individual acyl moieties and (3) the qualitative acyl composition of individual lipid classes. Air-grown seedlings, on the other hand, differed from dry seed in all of these respects. Although the lipid content of anaerobic seeds and shoots was higher than in aerobic tissue on a dry wt basis, it is evident that this phenomenon is not due to an enhanced synthesis of lipid, but rather a maintenance of the original lipid levels in ungerminated seed. Such a finding is unusual since in a germinating seed, lipid metabolism is normally characterized by the net catabolism of storage pools (e.g. TAG) and transport of the by-products into developing organs.

It is interesting to note that although desaturation reactions are presumably inhibited, a high degree of unsaturation was preserved in the total FA profile under anoxia (Fig. 1). The maintenance of unsaturated acids may compensate for the inability of anaerobic seedlings to synthesize and accumulate 18:3 into polar lipids.

Two features of anaerobic lipid metabolism in *Echinochloa* have suggested to us different lipid functions in the survival of the species under low O_2: (1) the synthesis of FA and their incorporation into neutral lipids may fulfil a metabolic demand by reoxidizing pyridine nucleotides, and (2) the presence of stable amounts of unsaturated FA may allow preservation of membrane integrity.

EXPERIMENTAL

Seeds of *E. crus-galli* (L.) Beauv. var oryzicola (Vasing) were surface sterilized for 15 min with 2.5% NaOCl soln prior to germination. Flasks containing 100 seeds were flushed continuously for 7 days with H_2O-satd air in darkness or H_2O-satd N_2 in light. Dry seeds were used as controls and treatments were replicated × 5 in each of two separate expts. Lyophilized seeds and shoots of 7-day-old seedlings were sepd. Seed tissue (*ca* 0.5 g) was ground initially in 8 ml boiling *iso*-PrOH then homogenized for 2 min after addition of 16 ml $CHCl_3$. At this point, 1 mg heptadecanoic acid was added to serve as an int. standard. A second homogenization with 10 ml $CHCl_3$–MeOH (2:1) followed and the combined extracts were washed with 0.25 vols of 1% KCl then 0.25 vols of 50% MeOH. Shoot tissue (*ca* 50 mg) was extracted with a proportionately smaller vol. of solvent. Aliquots of the lipid extracts were sampled for gravimetric determination of total lipid. For analysis of FA components a portion of the crude extract (2–4 mg) was transesterified with a soln of MeOH–C_6H_6–H_2SO_4 (19:4:1).

Intact seedlings were extracted as described and separated into major lipid classes. Prep. TLC was performed on 0.5 mm silica gel G (20 mg lipid/plate) with hexane–Et_2O–HOAc (40:10:1) as developing solvent. Bands were visualized under UV light after spraying with 0.1% 2′,7′-dichlorofluorescein in EtOH. Commercial tripalmitin, heptadecanoic acid and phosphatidyl choline were spotted to identifiy TAG, free FA and polar lipid, respectively. Lipids were eluted from the adsorbent with $CHCl_3$–MeOH (2:1), weighed and methylated as before.

GC of all FA Me esters was performed on a 1.8 m × 2 mm (i.d.) column packed with 10% Silar 10 C. Operating conditions were 170° with an N_2 flow rate of 20 ml/min. Identification and quantitation were accomplished with aid of authentic compounds.

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