Germination and seedling growth under anaerobic conditions in *Echinochloa crus-galli* (barnyard grass)*

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Abstract. Although rice has long been recognized to be uniquely adapted for growth in low oxygen environments of flooded rice fields, rice weeds of the *Echinochloa crus-galli* complex appear to be at least as well specialized for germination and growth under such unusual biological conditions.

Seeds of two varieties of *E. crus-galli* germinate and grow for prolonged periods in a totally oxygen-free environment. *E. crus-galli* germinates as well as rice (*Oryza sativa*) under a total nitrogen atmosphere and produces as large a seedling in spite of its much smaller seed size. Like rice, the seedlings of *E. crus-galli* are unpigmented, the primary leaves do not emerge from the coleoptile and no root growth occurs without oxygen. Of particular interest is the ultrastructure of mitochondria from anaerobically-grown seedlings. Mitochondrial profiles from the primary leaf of seedlings grown continuously in nitrogen are very similar to those grown aerobically. The size and shape of the mitochondria are similar and the cristae are numerous and normal in appearance. This is in sharp contrast to previous studies of other species which have reported that mitochondria were vesiculate and tended to lose their normal fine-structure after similar periods without oxygen.

Finally, based on ultrastructure and ^{14}C labeling studies, anaerobically-grown seedlings are highly active metabolically, which may explain, at least for *E. crus-galli* var. *oryzicola*, its ability to germinate and emerge from flooded rice fields.

Introduction

Higher plants require oxygen for growth and can tolerate low oxygen environments, as occurs naturally during root zone flooding, for only short periods of time before irreversible morphological and physiological damage occurs. The only well-known exception to this has been rice (*Oryza sativa*), often studied for its ability to germinate and grow for a limited time under anaerobic conditions. The rice coleoptile has been cited as the only plant organ which can grow in anoxia (Pradet & Bomsel, 1978). It is undoubtedly this unique and fundamental difference in the physiology of rice which allows it to grow in its typical, hydrophytic or flooded environment.

Varieties of the *E. crus-galli* complex are among the world's most serious agricultural weeds (Holm *et al.*, 1977). They are particularly abundant in flooded rice fields where they reduce yields by up to 40% (Kasasian, 1971; Smith, Flinchum & Seaman 1977). Despite several decades of annual herbicide applications all rice growing regions of North America are infested with *E. crus-galli*. Our interest in the physiology and ecology of *E. crus-galli* was stimulated by field observations of the germination ability of var. *oryzicola* in California rice fields. Seedlings emerge from up to 30 cm of water and, unlike most rice weeds, populations cannot be controlled by flooding alone (Jones, 1933; Smith & Fox, 1973). This report describes patterns of germination, and the seedling growth and ultrastructure of *E. crus-galli* varieties grown under anaerobic conditions.

Materials and methods

Seeds of *Echinochloa crus-galli* var. *oryzicola*, *E. crus-galli* var. *crus-galli*, (hereafter *oryzicola* and *crus-galli*, respectively) *Pisum sativum* var. *alaska* and *Oryza sativa* (comercial var. *colusa*) were germinated in 250-cm³ Erlenmeyer flasks. Seeds of *crus-galli* were collected from rice field populations of the two varieties at Biggs, Butte Co., California. Rice seeds were obtained from the Rice Experiment Station, Biggs, California. One hundred seeds were imbibed on two layers of filter paper with 10 cm³ of de-gassed, glass re-distilled water in each flask and ten flasks per treatment. Seeds were germinated in controlled temperature growth chambers with 30/20°C temperature and a 16 h photoperiod of 300 μmol m⁻² s⁻¹ photon flux

density. Dark-grown plants were grown similarly except flasks were wrapped in double aluminum foil. Humidified air or nitrogen (99.995% N_2) was passed continuously through the flasks, with five flasks in series. For anaerobic experiments, the same results were obtained when N_2 gas was further purified by passing it through alkaline pyrogallol or vacuum infiltrating the seeds to remove O_2 trapped within the seed.

For electron microscopy, sections taken from the primary leaf prior to its emergence from the coleoptile were fixed and vacuum infiltrated in 2% glutaraldehyde, 0.1 mol dm^{-3} phosphate buffer (pH 6.8) and post-fixed in 2% OsO_4 for 2 h. Tissues were then rinsed three times in buffer, dehydrated in a graded ethanol series and stained *en bloc* with 1% uranyl acetate in 60% ethanol. Propylene oxide was used prior to embedding in Spurr's plastic (Spurr, 1969). Sections were cut with a diamond knife, post-stained with lead citrate and observed with a Zeiss EM-9 electron microscope. For scanning electron microscopy (SEM), tissues were fixed and treated similarly except for *en bloc* staining. After dehydration with two changes of ethanol, the material was critical point dried, sputter-coated with gold and examined with a Joel SEM.

For ^{14}C-labeling studies, seeds were germinated as above, with the addition of ^{14}C-labeled glucose, and the seeds killed and radioactivity analysed as given before (Kennedy & Laetsch, 1973).

Results and discussion

Germination. The comparative ability of *Echinochloa* and rice to germinate under anaerobic conditions is illustrated in Fig. 1. *Oryzicola, crus-galli* and rice all germinate at approximately 90% or better and are virtually unaffected by light conditions (light versus dark) or oxygen concentration (21% O_2 versus N_2) (Fig. 2). Compared to rice, the germination percentage of *crus-galli* and *oryzicola* is as high under N_2 conditions as under control conditions (air), and relative to the original seed size, a much larger seedling results, especially for the larger-seeded variety, *oryzicola*. Although seeds of *oryzicola* are less than one-quarter the weight of those of rice (fresh wt of 5.05 mg/seed compared to 24.2 mg/seed) the seedlings are nearly identical in size (Fig. 2) and dry weight (Fig. 3) after 7 days germination under N_2 conditions.

Seedling growth and ultrastructure. Although light or O_2 conditions did not affect the germination percentage much in any of the three taxa, they did affect seedling growth. All seeds produced seedlings greater than 1 cm after 7 days of growth (Fig. 2). The seedlings were generally taller when grown in air, and the tallest seedlings (etiolated) were produced under air in the dark. Seedling length was very similar in the dark or light under anaerobic conditions, perhaps indicating a much lower or negligible phytochrome-mediated etiolation under anaerobic conditions.

Changes in seed weight and seedling growth (fresh or dry weight per seedling) also varied among taxa. Seed fresh weight or dry weight in the light or dark for *crus-galli, oryzicola* or rice was not significantly different in air-grown seeds and N_2-grown seeds (Fig. 3). In
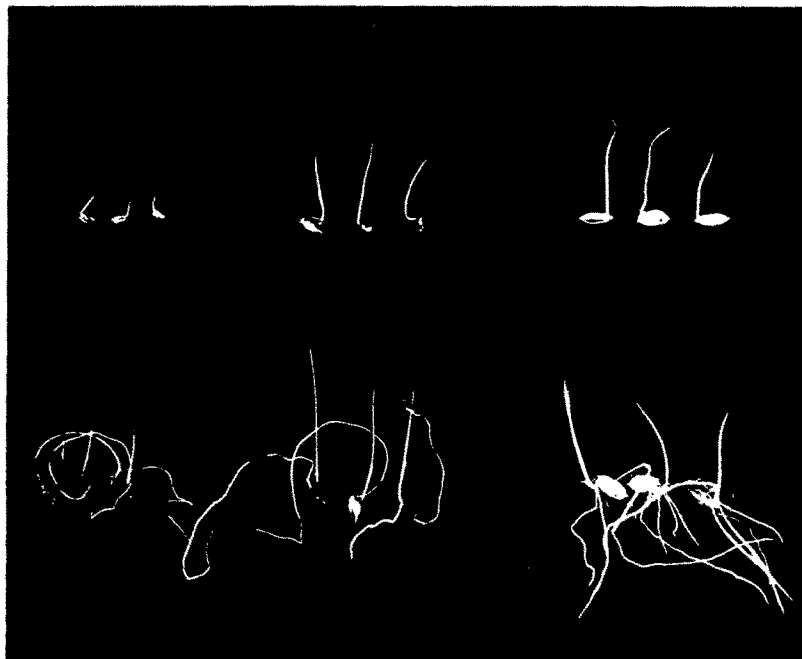

Figure 1. Seven day old seedlings. Left to right: var. *crus-galli,* var. *oryzicola,* and rice germinated in nitrogen (top row) or air (bottom row) in the light. (x0.65)
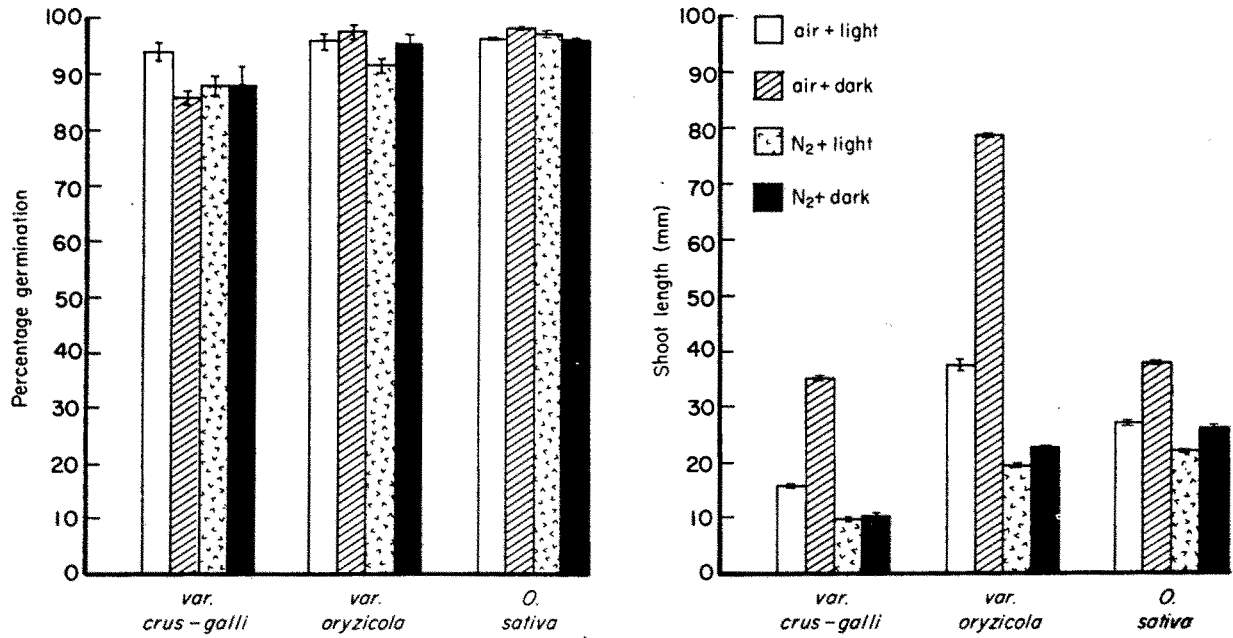
Figure 2. Percentage germination (left) and seedling height (right) after 7 days growth, $\pm$ SE. Germination conditions are given in inset.
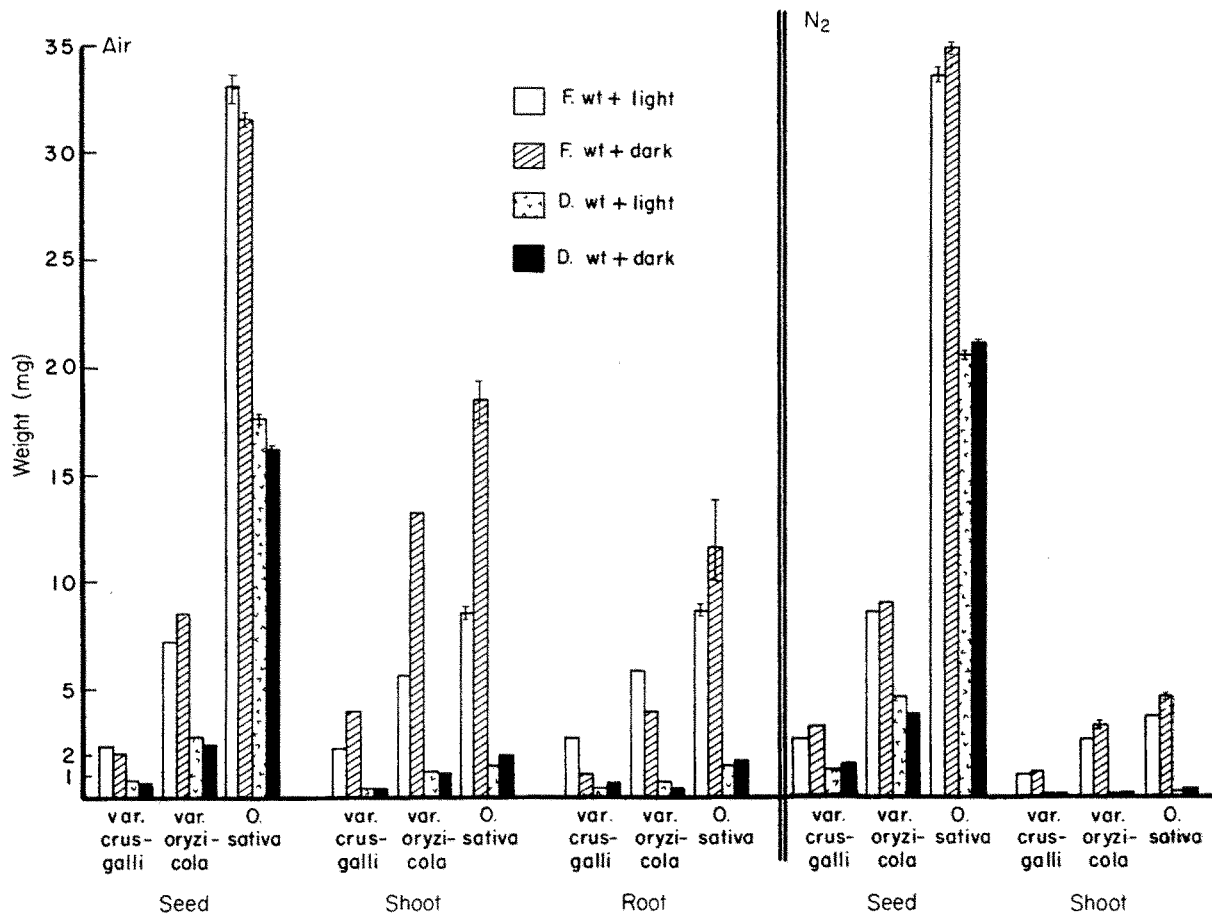

Figure 3. Fresh weight and dry weight of the seeds, shoots and roots for var. *crus-galli*, var. *oryzicola* and rice after 7 days growth in air (left) or N_2 (right) in the light and dark, $\pm$ SE. Where no SE is given, it was less than 0.1.

general, seedling growth (seedling length and fresh and dry weight) was much reduced in all taxa under anaerobic conditions.

One of the most obvious effects of anaerobiosis on seedling growth in *Echinochloa* was on root emergence. Under anaerobic germination, no radical emergence was observed, as reported before in rice (Vartapetian, Andreeva & Nuritdina, 1978; Kordan, 1972).

Another striking feature of anaerobically-grown *oryzicola* is that even after 134 h under N_2 the mitochondria are virtually indistinguishable in size and shape from those grown aerobically (Fig. 4). Under these conditions, the mitochondrial matrix is electron dense, the cristae are numerous and well developed, and the mitochondrial envelope is clear. In contrast to these findings, earlier reports of mitochondrial ultrastructure in a variety of species grown under low or no oxygen showed very elongate mitochondria with a complex cristae arrangement (Vartapetian, *et al.*, 1977), vesiculated cristae (Ueda & Tsuji, 1971; Oliveira, 1977; Vartapetian *et al.*, 1978), or large changes in mitochondrial frequency and size (Oliveira, 1977).

Metabolism. Although *oryzicola, crus-galli* and rice were all active metabolically under anaerobic conditions, their rate of $^{14}CO_2$ evolution during ^{14}C-glucose feeding experiments was lower in N_2 than air, especially for the first 24–48 h. These results contrast sharply with the pattern of $^{14}CO_2$ evolution in peas, a flooding and anaerobic intolerant plant species (Crawford, 1977). In peas, similar rates of $^{14}CO_2$ evolution were observed in N_2 and O_2 (Fig. 5), indicating a Pasteur effect (CO_2 produced under N_2/CO_2 produced under $O_2 > 0.3$) (Effer & Ranson, 1967) and the intolerance of the species to anaerobiosis. The lower rate of $^{14}CO_2$ evolution in N_2 compared to O_2 for rice, *crus-galli*, and *oryzicola* may agree with the mechanism for flooding tolerance proposed by McManmon & Crawford (1971). He has suggested that many plants such as rice which can tolerate anoxia or low oxygen concentrations during flooding do so by having a depressed respiration rate and show little or no Pasteur effect. This results in a lower accumulation of toxic respiratory end-products under N_2, such as ethanol. While our results are consistent with that hypothesis, the
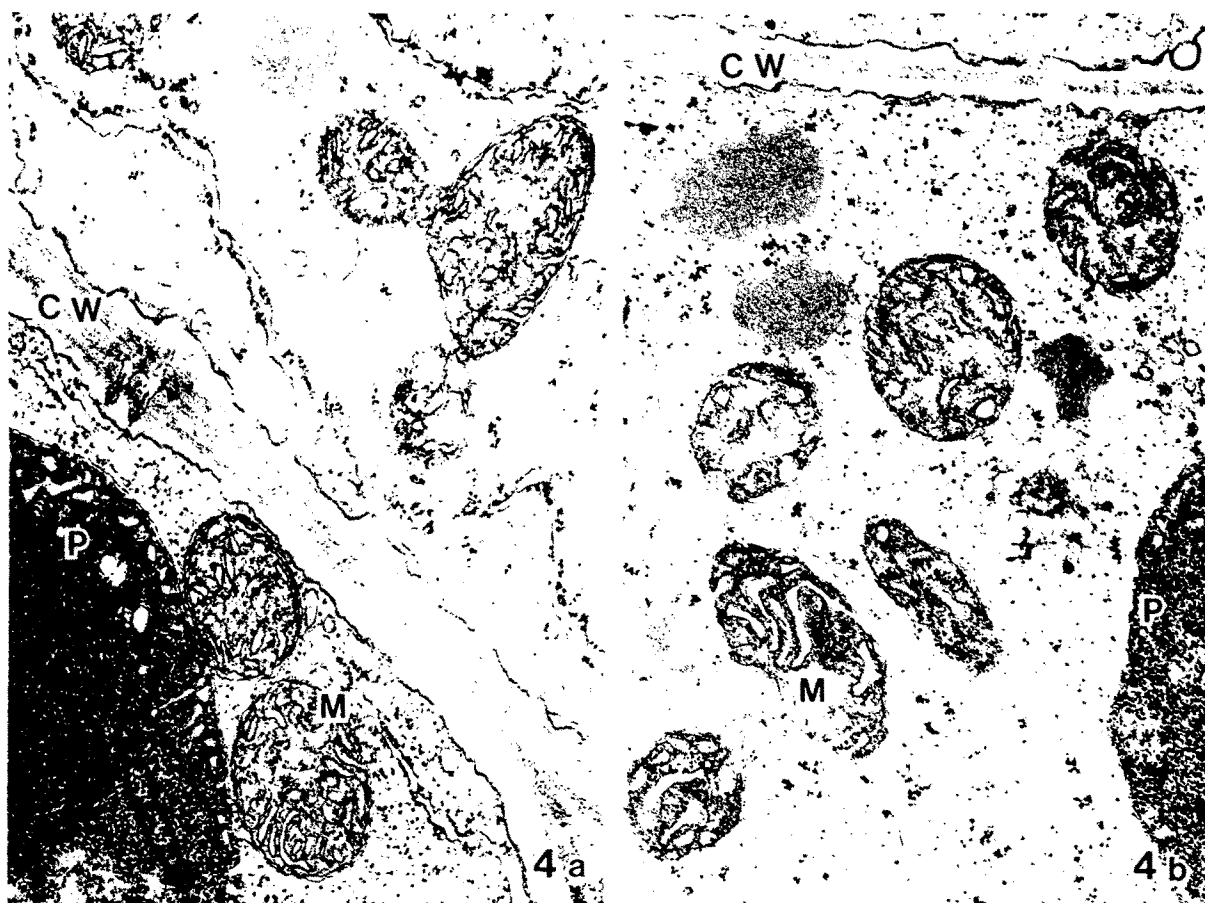

Figure 4. Electron micrographs of mitochondria from primary leaves of var. *oryzicola* grown in aerobic (a) or anaerobic (b) conditions for 96 and 134 h, respectively. (M) mitochondria; (CW) cell wall; (P) plastid. Note dense appearance of mitochondrial profiles in both micrographs, similar size, and normal appearance of the cristae. (× 36,000)
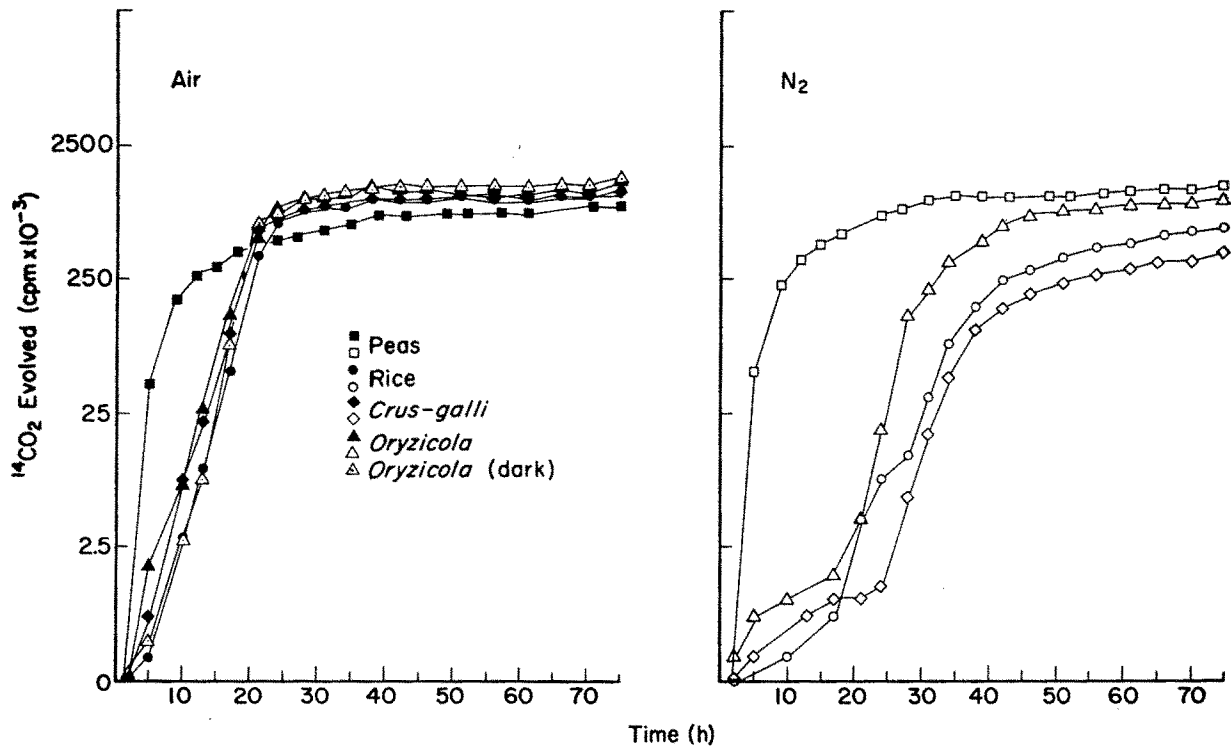
Figure 5. Evolution of $^{14}CO_2$ during germination of peas (intolerant), rice (tolerant), var. *crus-galli* and var. *oryzicola* grown in presence of U-^{14}C-glucose in air (left) or in N_2 (right). Amount of plant material was: peas, 1.3 g dry wt; rice, 0.7 g dry wt.; *crus-galli*, 0.25 g dry wt; *oryzicola*, 0.25 g dry wt. Roughly equal amounts of labeled glucose were taken up by the seeds in N_2 or air, with approximately one half the radioactivity (15×10^6 cpm) added per flask evolved as $^{14}CO_2$.
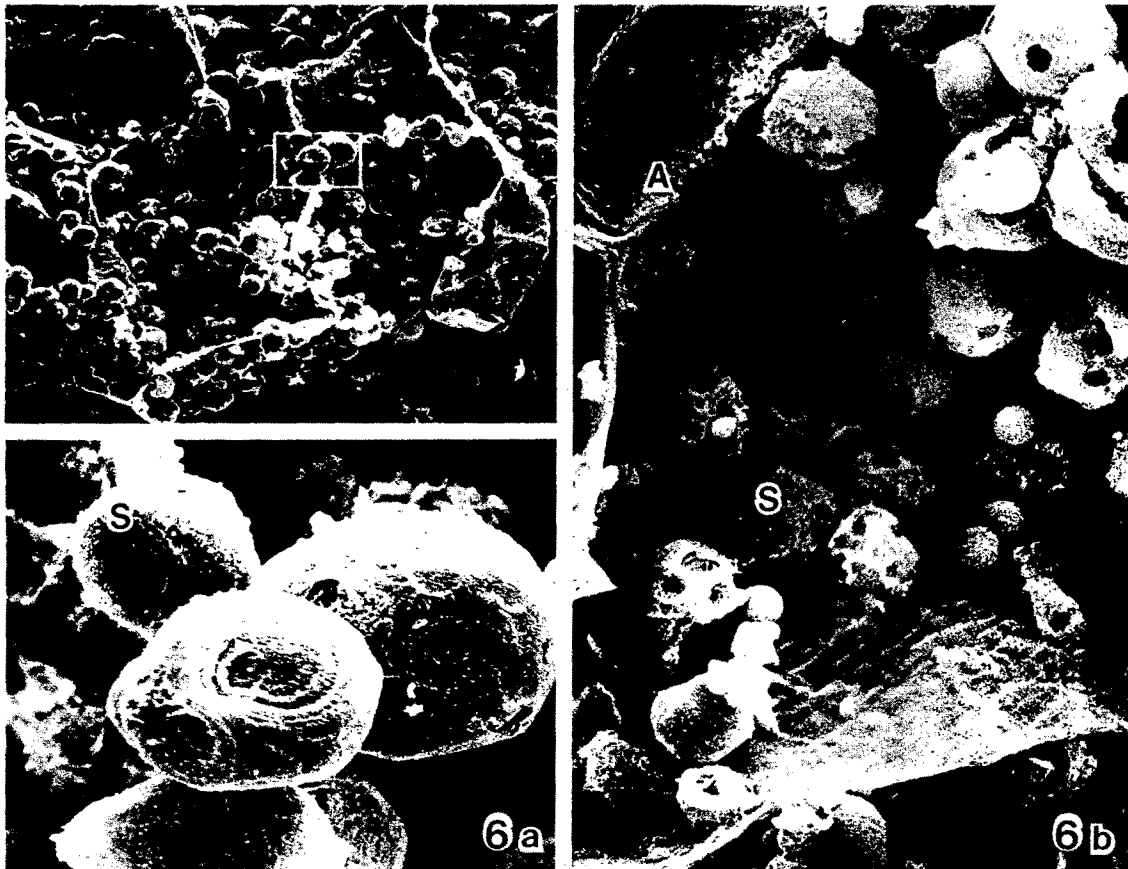

Figure 6. Scanning electron micrographs of endosperm starch grains in var. *oryzicola* grown in air (a, left) or N_2 (b, right) after 42 and 66 h, respectively. (S) starch grains; (A) aleurone layer. Note the holes and layered appearance of the starch granules, indicating starch hydrolysis. a: $\times 500$ top, $\times 3940$ bottom. b: $\times 3000$.

lower rates of respiration in *oryzicola, crus-galli* and rice may not be a causative factor for their tolerance or ability to grow in anaerobiosis, as suggested.

Active metabolism under anaerobic conditions was also shown by scanning electron micrographs of *oryzicola* endosperm starch granules. Ultrastructural evidence of starch breakdown and utilization can be seen by the layered, partially digested appearance of the starch (Fig. 6), as reported to occur during starch hydrolysis (Kessel & Shih, 1974).

Recent experiments (Kennedy, unpublished) with $^{14}C_6$- and $^{14}C_1$-glucose indicate the early involvement of the oxidative pentose phosphate pathway during anaerobic germination of *oryzicola* seeds, as commonly reported during the early stages of seed germination under aerobic conditions. The C_6/C_1 ratio of peas did not suggest operation of the pentose pathway. Additional results using SHAM, KCN and azide, inhibitors of the alternate and conventional mitochondrial electron transport respectively, in a non-flow through system, show that germination of *oryzicola* under N_2 is blocked by cyanide and azide, but not by SHAM (Kennedy, unpublished data). This suggests that the normal, cyanide-sensitive electron transport chain is operative under anaerobic conditions, but that a terminal electron acceptor other than O_2 may be responsible for re-oxidation of the pyridine nucleotides. This is speculative and more experiments on these aspects of anaerobic germination are underway.

In summary, our present results show that *Echinochloa crus-galli* has an unusual ability to germinate under anaerobic conditions and that germination and early growth are as successful, or in some respects more vigorous than that of rice, the only other plant species known to be able to withstand germination under nitrogen environments. The germination physiology of *E. crus-galli* is thus highly adapted to the flooded rice field habitat. It is likely that this is one of the major factors responsible for the success of the *E. crus-galli* complex as world-wide weeds of rice.

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