Plant Physiol. (1985) 79, 879–884
0032-0889/85/79/0879/06$01.00/0

# ATP Production by Respiration and Fermentation, and Energy Charge during Aerobiosis and Anaerobiosis in Twelve Fatty and Starchy Germinating Seeds

Received for publication March 8, 1985 and in revised form June 19, 1985

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## ABSTRACT

**The respiration and fermentation rates were compared in germinating seeds of 12 different cultivated species from five families. In air, fermentation contributes significantly to the energy metabolism only in some species (pea, maize), but is generally negligible when compared to respiration. The fermentation rate under anoxia was related either to the metabolic activity under air or to the adenine nucleotide content of the seeds: it was generally higher in seeds which contain starchy reserves (rice, maize, sorghum, pea), than in seeds which do not contain starch (lettuce, sunflower, radish, turnip, cabbage, flax); however, it was similar in wheat, sorghum (starchy seeds), and soya (nonstarchy seeds). The value of the energy charge of all the seeds was lower under anoxia than in air: after 24 hours under anoxia, it was higher than 0.5 in the starchy seeds and in soya and it was around 0.25 in the other fatty seeds.**

When dry seeds are imbibed, the respiratory activity, characterized by $O_2$ uptake and $CO_2$ evolution, starts extremely rapidly (5). For a long time, it was thought that this oxidative process was not coupled to ATP production and some original mechanisms were recently proposed (17) to explain the origin of the ATP needed for the biosynthetic work done during germination. Even under well-aerated conditions, ethanol and lactate accumulation may occur. It is generally considered that, during the first hours of imbibition, seeds are under 'natural anaerobiosis' (15). However, oxidative phosphorylation was demonstrated in mitochondria of the freshly imbibed *Arachis hypogea* axis (28), and, using an *in vivo* method, in lettuce seeds after 15 min of imbibition (12). Hence, the respiration of seeds is probably an important source of ATP during the initial phases of germination (19).

In a recent study, we found that the fermentative pathways contributed very little to ATP regeneration in germinating lettuce seeds (21). Moreover, their respiratory activity is saturated by $pO_2$[1] below that of air (2, 22). Such characteristics are in contradiction with some of the general views reported above. Furthermore, most work concerning energy metabolism was done with a limited range of seeds, most of which were legumes or graminae. Until now, it seems that no experimental work has been done to quantify the importance of fermentative metabolism in the energy balance of seeds, or to evaluate the generality of natural anaerobiosis in seed germination.

The present work is part of a study in which we used a comparative, physiological approach with seeds of 12 different species belonging to five families. When studying the effect of the $pO_2$ on the rate of germination (2), we found that the seeds could be classed in two groups: group I included fatty seeds; in these seeds, radicle emergence did not occur below 1 kPa $O_2$. Group II included starchy species, which were able to germinate at $O_2$ partial pressures below 0.1 kPa. In the present work, the rate of $O_2$ uptake, the rate of ethanol and lactate synthesis, and the value of AdN ratios (ATP/ADP or AEC), were used as indicators of ATP regeneration.

We found that, in aerated conditions, respiration was the dominating ATP-regenerating pathway in all seeds, whereas fermentation contributed significantly only in some species. Starchy seeds appeared to maintain a higher energy metabolism under anoxia than the fatty seeds, although the two groups overlap to some extent.

## MATERIALS AND METHODS

**Seeds.** The seeds used are indicated in Table I. They were obtained from INRA and ORSTOM laboratories (Paris, France) and from Sté Clause (Brétigny sur Orge, France). They were stored at 4°C in closed vessels containing silica gel and used within 2 years.

**Soaking.** The seeds were agitated in water for the time indicated in Table 1 at 25°C for rice, wheat, maize, sorghum, pea, and soya, or at 20°C for lettuce, radish, turnip, cabbage, flax, and sunflower. The experiments were performed at the same temperatures.

**Respiratory Rates.** The $O_2$ uptake was estimated either polarographically as in (2) or by the manometric method of Warburg; each flask contained 5 to 100 seeds, depending on respiratory rate, and 2 ml of water. The center well contained 0.2 ml of 20% (w/v) KOH.

**Determination of Fermentation Products.** Five to 50 seeds were placed in 20- to 100-ml vials containing 1 or 2 ml of water. To achieve anoxic conditions, the vials were stoppered and flushed for 5 min with $N_2$ (200 ml/min). The $O_2$ content of the gas was checked by GC or with an $O_2$ analyzer, as described by Raymond and Pradet (22). It was lower than 0.001% (v/v) at the exit of the vials. The vials were then sealed and incubated. At the end of the incubation period, they were rapidly frozen and stored at −80°C until assayed. Ethanol and lactate were assayed in $HClO_4$ extracts by enzymic methods as described in Saglio *et al.* (26). The extracts of sunflower and the cruciferae seeds were decolored with charcoal (Norit A from Sigma, 30 mg/sample). Known amounts of ethanol and lactate were added to some samples

[1] Abbreviations: $pO_2$, oxygen partial pressure; AEC, adenylate energy charge; AdN, adenine nucleotides.

before freezing in order to check the recovery of these products, as in Raymond *et al.* (21).

**Assay of Adenine Nucleotides.** The methods used for processing the tissues and determining the nucleotides by the luciferin-luciferase assay were as described in Raymond and Pradet (22).

## RESULTS

All the seed batches used in this work exhibited a percentage of germination in air higher than 95% except sunflower and turnip in which it was about 80%. In a preliminary study, the respiratory rate of the imbibing seeds was measured in order to determine the germination phases I and II (5). The seeds were imbibed aerobically for a time sufficient for the respiration to reach the plateau of respiration corresponding to phase II (Table I). The germination rates of the seeds have been presented in Al-Ani *et al.* (2).

**Energy Metabolism under Aerobic Conditions.** The amount of lactate detected at the end of the aerobic imbibition period was low in all species, except pea. Ethanol was found in large amounts in pea, maize, and soya, and in lower amounts in wheat, sunflower, and radish (Table II): these amounts represent the balance of ethanol production and losses by evaporation; in agreement with previous results (15) we observed that the ethanol content of the seeds was increased under conditions, such as excess water, which limit the gaseous exchanges, either $O_2$ input or ethanol evaporation.

The rate of ethanol accumulation in air was determined from the amount of ethanol accumulated during the 4 h following the period of aerobic imbibition: seeds were maintained in closed 20-ml vessels so as to avoid ethanol evaporation: according to the rate of $O_2$ uptake, the $pO_2$ did not descend below 19 kPa during this period: the observed rates (Table II) are representative of the rates of ethanol production in air. Significant ethanol production was found only in pea, maize, soya, wheat, and flax.

The $O_2$ uptake was measured at the end of the period of aerobic imbibition (Table I). From these values, assuming that 1 $O_2$ atom allows the synthesis of 3 molecules of ATP, we calculated the theoretical maximum rate of ATP regeneration at that time (Table II). From these data, it results that alcohol fermentation accounted for 43% of the ATP regeneration in pea, 24% in maize, 5% in soya, and 3% in wheat. In the other seeds, its contribution to energy metabolism was negligible (Table II).

The AdN and the AEC were measured in the aerobically imbibed seeds. Except for pea, the AEC in air ranged from 0.85 to 0.92 (Fig. 1) and the ATP/ADP ratios were higher than 5. Such high values indicate a normoxic metabolism. The AEC value of pea seeds was about 0.75, a value which may correspond to the well known limitation of their respiratory rate in air by hypoxia (6).

**Energy Metabolism under Anaerobic Conditions.** At the end of the aerobic imbibition period (Table I), the seeds were placed in closed vessels, in a $N_2$ atmosphere, for up to 48 h. An accumulation of ethanol was observed in all the seeds (Table III). During anoxia, the rate of ethanol accumulation increased in rice and remained more or less constant in maize and pea; it decreased in all the other seeds. The rate of lactate accumulation was nil, or 1 to 2 orders of magnitude lower than for ethanol, except in radish seeds. As previously shown for lettuce seeds (21), no accumulation of malate was observed under anoxia (not shown).

In pea and maize, the rate of ethanol accumulation was similar in air and in $N_2$. In the other seeds, it was increased several-fold after the transfer to anoxia.

In all the seeds, the rate of ATP regeneration under anoxia was lower than in air (Table IV). In pea, it reached 47 to 50% of the rate observed in air; during the first 4 h of anoxia, it was close to 20% in sorghum, soya, and maize, and close to 10% in rice and wheat. It increased in rice, reaching 21% of the rate in air during the 2nd d of anoxia. The rate of anaerobic ATP regeneration was below 7% the aerobic rate in radish, turnip, cabbage, sunflower, lettuce, and flax. As the experiments were performed at 20°C for most fatty seeds and at 25°C for starchy seeds, we performed an experiment with fatty seeds germinated and incubated at 25°C: the increase in the rate of ethanol production was not sufficient to modify the difference observed between the two groups.

The AEC values were determined after 30 min, 3 h, and 24 h of anoxic treatment (Fig. 1). In all the seeds, the AEC was lower after 30 min of anoxia than it was in air. In the seeds of rice, wheat, sorghum, maize, and pea, which all contain starch, the AEC values after 3 h of anoxia were higher than 0.60. After 24 h, a high value of 0.80 was observed in rice, and values between 0.60 and 0.70 in the other starchy seeds. In the other seeds (fatty seeds), the AEC was significantly lower: after 24 h, it was 0.15 in turnip and close to 0.25 in the other fatty seeds except soya (0.55).

## DISCUSSION

In order to evaluate the contribution of aerobic fermentation to energy metabolism, it was necessary to estimate the production

Table I. *Some Data Concerning the Seeds and Experimental Conditions*

Seed dry weight was determined from the weight of a sample of 50 seeds. The AdN were measured on triplicate samples at the end of the period of aerobic imbibition indicated in the next column: Coefficients of variation between different experiments were within 10%.

| Common Name | Botanical Name | Variety | Dry Wt | AdN | Imbibition | |
|---|---|---|---|---|---|---|
| | | | | | Time | Temp. |
| | | | *mg/seed* | *nmol/seed* | *h* | *°C* |
| Lettuce | *Latuca sativa* L. | Reine de Mai | 1 | 0.55 | 6 | 20 |
| Turnip | *Brassica napus* L. | Croissy | 3.2 | 1 | 6 | 20 |
| Sorghum | *Sorghum bicolor* (L.) Moench | S9 4993 | 15 | 1.4 | 12 | 25 |
| Rice | *Oryza sativa* L. | Cigalon | 23 | 1.4 | 12 | 25 |
| Cabbage | *Brassica oleracea* L. | Pasteur | 3.7 | 1.9 | 6 | 20 |
| Flax | *Linum usitatissimum* L. | Antares | 10.5 | 1.9 | 8 | 20 |
| Radish | *Raphanus sativus* L. | Fakir | 9.5 | 5.5 | 6 | 20 |
| Wheat | *Triticum sativum* L. | Capitole | 50 | 6 | 12 | 25 |
| Sunflower | *Helianthus annuus* L. | 5648 | 96 | 17 | 6 | 20 |
| Maize | *Zea mays* L. | INRA 402 | 260 | 25.3 | 12 | 25 |
| Soya | *Glycine max* (L.) Merr. | Weber | 125 | 73 | 6 | 25 |
| Pea | *Pisum sativum* L. | Kalife | 240 | 240 | 12 | 25 |

Table II. *Respiration and Fermentation of Seeds Germinating in Air*

Ethanol and lactate (columns 1 and 2) were measured at the end of the period of aerobic imbibition indicated in Table I. The rate of ethanol and lactate accumulation (column 3) was calculated from the accumulation in air, in stoppered vials, during the 4 h following the period of aerobic imbibition. Results are the mean of determinations on triplicate samples in a representative experiment. The rate of $O_2$ uptake is the average of the values measured on triplicate samples, every 30 min for 4 h after the time of imbibition and at the temperature shown in Table I. The coefficient of variation in the determination of the $O_2$ uptake rate were lower than 10%. The rate of ATP regeneration (ATP [total]) is the sum: (ATP from fermentation + ATP from respiration); it was calculated assuming that the ATP yield of fermentation is 1 ATP per lactate or ethanol and that of respiration is 3 ATP per $O_2$ atom consumed (see "Discussion"). ATP (ferm) is the rate of ATP regeneration from fermentation.

| Seeds | Fermentation Products | | Metabolic Activity | | | $\frac{\text{ATP (ferm)} \cdot 100}{\text{ATP (total)}}$ |
|---|---|---|---|---|---|---|
| | Lactate | Ethanol | Ethanol Production | $O_2$ uptake | ATP (total) | |
| | *nmol/seed* | | *nmol/h·seed* | | | *ratio* |
| Lettuce | <2 | <2 | <2 | 27 | 162 | <2 |
| Turnip | <5 | <5 | <2 | 18 | 108 | <2 |
| Sorghum | 9 | <10 | <10 | 60 | 360 | <3 |
| Rice | 21 | <10 | <10 | 82 | 494 | <2 |
| Cabbage | <5 | <5 | <2 | 33 | 198 | <1 |
| Flax | <5 | <5 | 11 ± 0.5 | 140 | 840 | <2 |
| Radish | 15 | 13 ± 1 | <5 | 140 | 845 | <1 |
| Wheat | 20 | 39 ± 7 | 35 ± 4 | 160 | 995 | 3.5 |
| Sunflower | <20 | 70 ± 5 | <10 | 160 | 960 | <1 |
| Maize | 58 | 1200 ± 100 | 700 ± 90 | 370 | 2920 | 24 |
| Soya | 47 | 1100 ± 120 | 215 ± 30 | 660 | 4175 | 5 |
| Pea | 500 | 4000 ± 300 | 2500 ± 300 | 550 | 5800 | 43 |

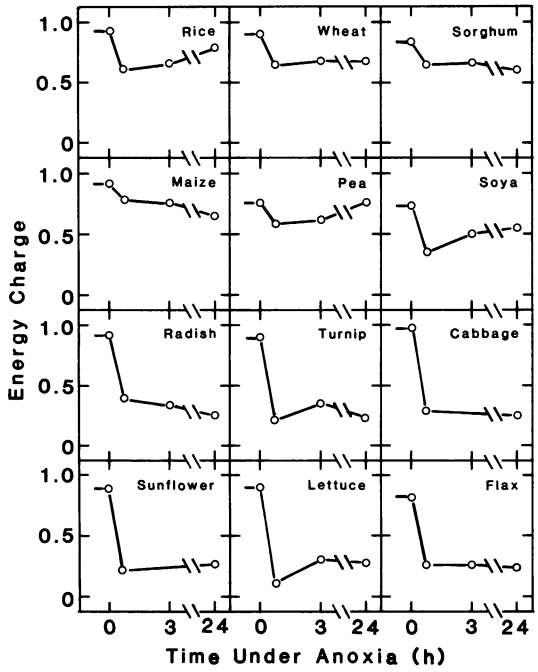


FIG. 1. AEC in germinating seeds submitted to anoxia during phase II of germination. The germinating seeds were transferred to anoxia after a time of aerobic imbibition indicated in Table I. The nucleotides were determined as indicated in "Materials and Methods".

Table III. *Rate of Accumulation of Lactate and Ethanol in Seeds under Anoxia*

Seeds in phase II of germination (Table I) were placed under anoxia. Ethanol and lactate were determined as indicated in "Materials and Methods" and their rates of accumulation were calculated from the amounts determined after 0, 4, 24, and 48 h. Values are mean ± SD; $n = 3$.

| Seeds | Time under Anoxia (h) | | | | | |
|---|---|---|---|---|---|---|
| | 0–4 | | 4–24 | | 24–48 | |
| | Lactate | Ethanol | Lactate | Ethanol | Lactate | Ethanol |
| | *nmol/seed·h* | | | | | |
| Lettuce | <1 | 3.5 ± 0.2 | 0.1 | 2.7 ± 0.2 | <0.1 | 2 ± 1 |
| Turnip | <2 | 5 ± 0.5 | <0.4 | 5 ± 0.5 | <0.4 | 3 ± 0.1 |
| Sorghum | <5 | 70 ± 5 | <1 | 68 ± 2 | <1 | 23 ± 2 |
| Rice | <5 | 52 ± 3 | <1 | 72 ± 5 | <1 | 102 ± 7 |
| Cabbage | 3 | 9 ± 2 | 1 | 7 ± 2 | 0.2 | 4 ± 2 |
| Flax | 1 | 20 ± 1 | 1 | 15 ± 1 | <0.1 | 9 ± 0.5 |
| Radish | 15 | 21 ± 0.1 | 3 | 6 ± 0.2 | 1 | 2 ± 1 |
| Wheat | 5 | 90 ± 20 | <1 | 85 ± 20 | <1 | 50 ± 10 |
| Sunflower | 9 | 40 ± 10 | 0.5 | 20 ± 8 | 0.4 | 10 ± 5 |
| Maize | <5 | 775 ± 90 | <1 | 685 ± 50 | <1 | 820 ± 100 |
| Soya | 2 | 825 ± 100 | 1 | 680 ± 90 | <1 | 580 ± 40 |
| Pea | <5 | 2750 ± 100 | <1 | 2400 ± 400 | <1 | 3000 ± 300 |

of ATP by respiration and fermentation.

**ATP Regeneration by Respiration.** The estimation of the ATP regeneration rate by respiration is a difficult problem, because the regulation of the respiratory cyanide-sensitive and -insensitive chains, *in vivo*, is practically unknown, as is the contribution of nonrespiratory oxidases to the balance of the $O_2$ utilization in the cells. The P/O ratios measured in mitochondria depends on the substrate used. With succinate and NADH, a maximum value of 2 is observed, whereas with malate and oxoglutarate, the maximum value is 3. However, the proportion of the different organic acids and cytosolic NADH that are oxidized *in vivo*, is not known. The P/O ratio for the cyanide-insensitive pathway is 0 or 1, depending on the substrate (14). In germinating seeds, the cyanide-insensitive pathway was sometimes considered to be active (19), but it seems now that, during early germination, the pathway involved is the cyanide-sensitive, energy-conserving one (12, 14, 19).

The residual respiration after inhibition by cyanide and hy-

Table IV. *Anaerobic Versus Aerobic ATP Regeneration Rate*

The rates of anaerobic ATP regeneration were calculated from the data in Table III. The rates of aerobic ATP regeneration are from Table II.

| Species | Time under Anoxia (h) | |
|---|---|---|
| | 0–4 | 24–48 |
| | *ratio* | |
| Lettuce | 3[a] | 1 |
| Turnip | 6 | 3 |
| Sorghum | 19 | 6 |
| Rice | 12 | 21 |
| Cabbage | 6 | 2 |
| Flax | 3 | 1 |
| Radish | 4 | 0.3 |
| Wheat | 10 | 5 |
| Sunflower | 5 | 1 |
| Maize | 27 | 28 |
| Soya | 19 | 14 |
| Pea | 47 | 50 |

[a] Rate of anaerobic ATP regeneration · 100/rate of aerobic ATP regeneration.

droxamic acids, is 5 to 15% of the control, depending on the tissues (19). This value is probably an indication of the amount of the nonrespiratory (*i.e.* nonphosphorylating) $O_2$ uptake due to oxygenases and oxidases. In the calculation of the respiratory ATP regeneration, we have neglected the ATP produced by substrate-level phosphorylation in glycolysis and the TCA cycle (10% of the respiratory ATP). This underestimation was counterbalanced by considering that all the $O_2$ consumed was engaged in oxidative phosphorylation. As we considered that three molecules of ATP were regenerated for each oxygen atom consumed, we overestimated the respiratory ATP regeneration: the values calculated are theoretical maximum values.

**ATP Regeneration by Fermentation.** If sucrose is hydrolyzed to hexoses by an invertase, the conversion of sucrose to ethanol, or lactate, is considered to allow the regeneration of 1 mol of ATP per mole of fermentative end-product accumulated. In a recent review, Feingold and Avigad (11) examined other pathways of sucrose catabolism. In many plant tissues, including seed tissues, sucrose is utilized to a large extent via the sucrose synthase reaction; they proposed that sucrose could be a source of nucleoside diphosphate glucose. The UDP-glucose pyrophosphorylase has sometimes been implicated in the degradation of UDP-glucose: this reaction uses PPi and produces UTP and glucose-1-phosphate. Feingold and Avigad (11) consider that the role of this enzyme is predominantly to catalyze the synthesis of UDP-glucose, because PPi is generally considered to be actively degraded by pyrophosphatases. However, Edwards *et al.* (10) showed that PPi is present at relatively high concentrations in pea tissues and can act as phosphoryl donor. If PPi was freely available, the ATP yield of fermentation from sucrose would be 1.5 ATP per ethanol, or lactate. The phosphorylation of fructose-6-P by a pyrophosphate: fructose-6-P 1-phosphotransferase, instead of by phosphofructokinase, would increase the energy yield of the pathway by 1 ATP per hexose. On the other hand, when starch is consumed as the energy reserve, the synthesis of sucrose from glucose, for transport to the axis, costs 3 ATP per sucrose (1). As there is, at present, no indication of the true energy yield by fermentation, we considered that the value was 1 ATP per ethanol, or lactate, accumulated.

There is increasing evidence indicating that ethanol is the preponderant end product of fermentation in plants (21, 27). Many other products, such as alanine, succinate, shikimate, malate, and lactate, have also been reported to accumulate under anoxia (7) but, except for lactate, the amount of these substances is usually negligible, compared to that of ethanol. In the seeds studied here, only radish accumulated lactate in significant amounts, as compared to ethanol, and the accumulation continued for the 2 d of anoxia (Table III). In pea, maize, sorghum, and rice, the level of lactate in air was relatively high (Table II) and no further accumulation was observed under anoxia. This, as well as the sharp decrease in the rate of accumulation observed in the other seeds, is in agreement with the view of Davies et al. (8) that lactate dehydrogenase and pyruvate decarboxylase are regulated by the pH so as to limit the decrease in pH caused by the accumulation of lactate.

Under aerobic conditions, the rate of ethanol accumulation is probably an underestimated value of the rate of ethanol synthesis for two main reasons. First, under aerobic conditions, plant tissues are able to metabolize ethanol (for a review, see Cossins [6]). In terms of the energy balance, this ethanol is used as a respiratory substrate and, consequently, it is taken into account when calculating ATP from the $O_2$ uptake. Second, ethanol has been shown to be transformed into ethyl glucose in bean seeds and seedlings (9). This synthesis necessarily requires a high energy intermediate such as UDPG and, consequently, there is no net regeneration of ATP by this pathway. Hence, these underestimations of ethanol production do not affect the calculation of the rate of ATP production by fermentation.

**Occurrence of Natural Anaerobiosis.** From many old results and from her own data, Leblova (15) asserted that, during the initial phases of germination, seeds are under natural anaerobiosis. It should be noticed that most published studies concerning fermentative and respiratory metabolism in seeds were carried out with seeds of legumes. Pea and maize were included among the six seeds studied by Leblova: the others were lentil, bean, broad bean, and soybean. From our results, it is clear that the phenomenon of natural anaerobiosis is not general. We found indications of active fermentative metabolism in air only in pea and maize; in most other seeds, this activity is very low.

The relative contribution of the two pathways to ATP regeneration may vary for each species, depending on the seed batch. We have noticed that in lettuce seeds imbibed for 6 h, published values of $O_2$ uptake vary from 15 to 33 $\mu$mol/h·g (23). In works related to seed conservation, it has been observed that the RQ ($CO_2/O_2$) and aerobic ethanol production increases with aging, and the $O_2$ uptake decreases (18 and references therein). The question therefore arises as to whether the differences we observed between species were due to variations in seed quality. This does not seem to be the case for several reasons: (a) the differences caused by aging are smaller than those that we found between species; (b) over the past years, using different seed batches or varieties, we constantly found similar results for each species, particularly for lettuce (21) and rice (16); (c) our results concerning natural anaerobiosis, or fermentation activity, in rice and pea are similar to those found by other workers. Hence, it seems likely that the differences reported here represent intrinsic metabolic properties of different species, rather than variations between seed samples.

As discussed above, the contribution of respiration to ATP regeneration has been overestimated: the ATP yield of fermentation may be higher, and that of respiration slightly lower, than those used for the calculations; however, it seems likely that fermentation has a secondary role in the energy metabolism of most aerated, germinating seeds.

**Comparison of the Metabolic Activities in Seeds of Various Species.** The ATP regeneration rate, expressed on a 'per seed' basis, may be misleading for comparing the metabolic activities of seeds of different species, because the amount of living matter per seed varies greatly according to species. For the same reason, the amount of protein, and the dry weight are not good refer-

ences; we have therefore chosen to relate the metabolic activity under anoxia of the various seeds to (a) their metabolic activity under air, (b) their adenine nucleotide content.

*Anaerobic versus Aerobic Metabolic Activity.* The expression of the rate of ATP regeneration under anoxia as a percentage of that under air indicates to what extent the stopping of respiration under anoxia is compensated for by fermentation. The results are shown in Table IV. No relation is apparent between the values of this ratio and either the size or the AdN content of the seeds. The highest values in the first 4 h of anoxia were found in starchy seeds (pea, maize, sorghum, rice, wheat) and in soya: they are comprised between 47 and 10%. In the other seeds, the rate of ATP regeneration under anoxia is less than 6% of the rate in air. During the second day of anoxia, this ratio is lower except for pea and rice, but the highest values (ranging from 50 to 5%) still correspond to the starchy seeds and soya.

*Metabolic Activity versus AdN Content.* The ATP content and the AdN content are often used to obtain an approximation of the biomass (13). Although the amount of AdN often decreases when the cells or tissues are transferred to anaerobiosis (16, 21), this parameter is more stable than the level of ATP; we therefore used the amount of AdN per seed as a basis for comparing the rate of ATP-regeneration in the various seeds (Table V).

In aerated seeds, the rate of ATP regeneration varies from 24 to 442 nmol ATP/h·nmol AdN, with low and high values in both starchy and fatty seeds; the lowest values are associated with the highest AdN content per seed.

With the exception of pea and maize, which already exhibited a high rate of fermentation in air, the rate of fermentation was increased dramatically after transfer to nitrogen in all the seeds. However, the rate of ATP regeneration was always lower under anoxia than in air. During the first 4 h of anoxia, it varies from 3 to 50 nmol ATP/h·nmol AdN. Higher values were found in the starchy seeds sorghum, rice, maize, and wheat than in the fatty seeds lettuce, radish, turnip, cabbage, sunflower, and flax, but similar intermediate values were found in pea, soya, and flax, during the first 4 h, and in wheat and soya during the second day of anoxia.

The results of Crawford (7), showing that both lettuce and rice seeds had a low fermentation, were expressed on a seed dry weight basis. Our results do not support Crawford's proposal that survival during flooding is correlated to low ethanol production because seeds with active fermentation, such as rice and pea, did survive under anoxia for 2 weeks (2), and the fermentation activity in rice is higher than that in lettuce by 5 to 18 times according to the length of anoxia.

The highest metabolic activity and an increasing fermentation rate were found in rice. These characteristics seem to be in accord with the ability of rice coleoptile to grow under anoxia (3). In *Echinochloa crus galli*, a high fermentation rate, but no increase of this rate, has been described (24).

**Adenylate Energy Charge.** It has been established that, under energy-limiting conditions such as anoxia, the value of the AEC is an indicator of the rate of ATP regeneration (16, 21, 26). Here, we show that this indicator also allows us to distinguish the seeds according to their metabolic activity under anoxia. The higher value of the energy charge in starchy seeds is correlated to their higher fermentation activity. At present, we have very little information concerning the metabolic work in plant tissues under anoxia. Active syntheses of protein, RNA, and DNA in rice seeds under anoxia, are correlated with high values of the energy charge (4, 16). Similarly, under anoxia, *E. crus galli* exhibits shoot growth, is able to synthesize lipids and adenine nucleotides, and its AEC is close to 0.80 (25). In contrast, in lettuce seeds under anoxia, where the energy charge is low, we found little incorporation of radioactive precursors into proteins (20).

This work constitutes part of a study of the energy metabolism and the $O_2$ requirements of seeds of cultivated species during germination. We showed recently (2) that the germination processes of fatty and starchy seeds exhibit very different sensitivities to low $pO_2$: fatty seeds are unable to complete germination at $pO_2$ which hardly affect the germination of starchy seeds, although the respiration may be affected similarly in the two groups. Here, we show that these two groups of seeds can also be distinguished according to metabolic criteria: in fatty seeds (group I) except soya, the reduction of the metabolic activity by transfer from air to anoxia is stronger than in starchy seeds (group II) and, under anoxia, the fermentation rate as related to the AdN content and the AEC are lower. The present results support the hypothesis that the high fermentative activity of starchy seeds could explain their ability to complete germination at very low $pO_2$ as well as the growth of rice coleoptiles in the absence of $O_2$.

Table V. *Aerobic and Anaerobic Metabolic Activity of Seeds in Relation to Their AdN Content*

The data are the rates of ATP regeneration indicated in Tables II and III, divided by the amount of AdN per seed indicated in Table I.

| Species | ATP Regeneration Rate | | |
|---|---|---|---|
| | Aerobic | Anaerobic | |
| | | 0–4 | 24–48 |
| | *nmol/h/nmol AdN* | | |
| Lettuce | 294 | 8 | 4 |
| Turnip | 112 | 7 | 3 |
| Sorghum | 257 | 50 | 16 |
| Rice | 353 | 41 | 73 |
| Cabbage | 104 | 6 | 2 |
| Flax | 442 | 11 | 5 |
| Radish | 153 | 7 | 0.5 |
| Wheat | 166 | 16 | 8 |
| Sunflower | 56 | 3 | 0.6 |
| Maize | 115 | 30 | 32 |
| Soya | 57 | 11 | 8 |
| Pea | 24 | 11 | 12 |

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