

Germination, Respiration, and Adenylate Energy Charge of Seeds at Various Oxygen Partial Pressures

Received for publication March 8, 1985 and in revised form June 17, 1985

ALI AL-ANI*, FRANÇOISE BRUZAU, PHILIPPE RAYMOND, VÉRONIQUE SAINT-GES, JEAN MARC LEBLANC, AND ALAIN PRADET

Station de Physiologie Végétale, Institut National de la Recherche Agronomique, Centre de Recherches de Bordeaux, B. P. 131, 33140 Pont de la Maye, France

ABSTRACT

The effect of O_2 partial pressure on the germination and the respiration of 12 cultivated species was studied. The reciprocal of the time necessary to observe rootlet emergence in 50% of the seeds was used to approach the germination rate. The maximum germination and respiration rates were reached in most seeds at O_2 pressures close to that of air. Decreasing the O_2 pressure produced a gradual decrease of the germination rate. The seeds could be classed in two groups according to their response to low O_2 pressures. Group I includes lettuce, sunflower, radish, turnip, cabbage, flax, and soybean: at O_2 pressures close to 2 kilopascals, the germination in this group was stopped and the adenylate energy charge was lower than 0.6. Group II includes rice, wheat, maize, sorghum, and pea. The germination rate of these seeds was also gradually decreased by lowering the O_2 partial pressure but germination still occurred, very slowly, at 0.1 kilopascal; the adenylate energy charge remained higher than 0.6. These differences in the germination rates and adenylate energy charge values could not be explained by differences in the sensitivity of respiration to O_2 .

The effect of pO_2 ¹ on seed germination has been studied by only a few authors (9, 15, 17, 28). In two graminiae, rice and *Echinochloa crus galli*, the coleoptile is able to grow under anaerobiosis (10, 26, 30) and the coleorhiza of *Echinochloa* is able to emerge (29). Apart from these two exceptions, seed germination, as well as other growth processes in higher plants, requires O_2 .

The respiration of seeds increases dramatically during the first hours of imbibition, which corresponds to germination phase I (11); afterwards, it stabilizes, or increases more slowly (germination phase II), until rootlet protrusion which completes the germination process (5). Heichel and Day (9) observed that the germination of monocots occurs at pO_2 as low as 2 kPa, whereas the germination of most dicots required higher pO_2 . They concluded that oxidation systems involved in germination require O_2 levels greater than 2 kPa and that these systems differ in monocots and dicots. The Cyt oxidase is the site of most respiratory oxidations (12, 19). Although the affinity of this enzyme for O_2 is extremely high (K_m 0.1 μM , *i.e.* 0.008 kPa), the respiration of many plant tissues and organs starts to be limited within a range of pO_2 several orders of magnitude higher, close to the pO_2 of air (*i.e.* 21 kPa). For instance, the critical pO_2 of the respiration of lettuce seeds during phase II of germination is

about 15 kPa (18). This has been explained by the presence of barriers to the diffusion of O_2 from the surface of the seed envelopes, to the internal membrane of the mitochondria (7, 20). Thus, it could be hypothesized that those seeds which are unable to grow below 2 kPa O_2 require higher pO_2 for respiration than the other seeds.

Oxidative phosphorylation is not the only mechanism that produces ATP in seeds. It has been shown that fermentation is very active during the initial phases of germination of some seeds (14). Nevertheless, it was shown recently that fermentation contributes very little to ATP regeneration in lettuce seeds (24). This observation led to a second hypothesis (1): the seeds which are unable to germinate at pO_2 lower than 2 kPa regenerate less ATP by fermentation than the others.

It has been established that the value of the ATP/ADP ratio, or the AEC, are correlated to the activity of the energy metabolism under hypoxia or anoxia (22). In rice embryos under anoxia, the rate of protein synthesis and the metabolism of mRNA (3, 21) are related to the AEC in the range 0.65 to 0.80. In lettuce seeds under anoxia, the low value of the AEC is correlated to a low metabolic activity (23, 24); the incorporation of amino acids into proteins is very low (21). The variation of these ratios could provide a mechanism for the control of growth at limiting pO_2 , or under anoxia.

We have undertaken studies to evaluate the above hypotheses and to obtain more quantitative information concerning the O_2 requirement for seed germination. In this paper, we show the effect of low pO_2 on the germination and respiration rates of 12 cultivated species. The results favor the hypothesis that germination at low pO_2 is related to an active fermentation.

MATERIALS AND METHODS

Plant Materials. The seeds of 12 cultivated species were used in this work: rice (*Oryza sativa* L., Cigalon); wheat (*Triticum sativum* L., Capitole); maize (*Zea mays* L., INRA 402); sorghum (*Sorghum bicolor* L., [Moench], Sg 4993); pea (*Pisum sativum* L., Kalife); soya (*Glycine max* [L.] Merr., Weber); lettuce (*Lactuca sativa* L., Reine de Mai); sunflower (*Helianthus annuus* L. 5H48); radish (*Raphanus sativus* L., Fakir); turnip (*Brassica napus* L., Croissy); cabbage (*Brassica oleracea*, Pasteur); flax (*Linum usitatissimum* L. Antares).

These seeds were kindly supplied by Sté Clause, Brétigny sur Orge, France and different research institutes: INRA, ORSTOM, IRHO, and CETIOM, Paris, France. They were stored at 2°C in closed vessels containing silica gel and used within 2 years.

The experiments were carried out at 25°C for rice, wheat, maize, sorghum, pea, and soya and at 20°C for lettuce, radish, turnip, cabbage, flax, and sunflower.

Seed Germination. The seeds were decontaminated by treat-

¹ Abbreviations: pO_2 , oxygen partial pressure; AEC, adenylate energy charge.

ment with NaOCl for 30 min, as described previously (21); a concentrated hypochlorite solution was used for rice, maize, pea, soya, and lettuce and a diluted, 10% solution for the other seeds. The seeds were then placed on a layer of filter paper in Petri dishes containing a layer of glass beads (diameter 6 mm) and enough water to moisten the filter paper. It was verified, for each species, that the surface sterilization treatment did not modify the rate of germination, nor the final percentage of germination in air. The seeds were submitted to either anoxia, or hypoxia, within 2 h after the beginning of the surface sterilization treatment.

High pressure mixtures of O₂ and N₂ were prepared in the laboratory and analyzed for O₂ as described previously (23).

The uncovered Petri dishes were placed in 1-L anaerobic jars; for each gas mixture, eight jars were connected in series. At the exit of the cylinder, the gas was sterilized through a Millipore membrane (0.2 μm) and moistened before entry into the first jar. In order to limit the time of equilibration, the jars were submitted, three times, to a partial vacuum (about 30 mm Hg) and filled with the gas mixture being studied. Afterwards, the seeds were left in the dark; a 100-ml/min gas stream was passed through the jars for the duration of the experimental period. After counting, the seeds were left under air to verify that germination could still occur. The criterion for germination was a 1-mm radicle emergence in dicot species and emergence of the radicle through the coleorhiza in the gramineae. Fifty to 100 seeds were counted for each experimental point; results are the mean value for two or more experiments except for wheat and sorghum.

Indices of Germination. The time required to obtain 50% of germination, t_{50} , was determined by interpolation from the curve of germination percentage *versus* time, at each pO₂. The t_{50} value corresponding to the maximum rate of germination, obtained in a nonlimiting pO₂, is $t_{50 \text{ max}}$ and the t_{50} values at limiting pO₂ = x are $t_{50 x}$.

The reciprocal of t_{50} was taken as the germination rate of the sample. The relative germination rate at a pO₂ = x is given by:

$$\text{Relative germination rate} = \frac{t_{50 \text{ max}}}{t_{50 x}} \cdot 100$$

The linear regression between the germination rates and the logarithm of the pO₂ was calculated. It accounted for 87 to 99% of the measured variability. Hence, it was justified to extrapolate to P100_{Germ}, the lowest pO₂ allowing a maximal germination rate, and P0_{Germ}, the low pO₂ limit, at which no germination could be observed. P80_{Germ}, P50_{Germ}, P20_{Germ}, are the pO₂ at which the relative germination rate is 80, 50, and 20% respectively. The ratio P80_{Germ}/P20_{Germ} was also calculated.

O₂ Uptake Measurements. The effect of pO₂ and the O₂ uptake by the seeds were studied with polarographic equipment (Beckman Field Lab). The glass cell was designed and checked to avoid any entry of O₂: the Clark type electrode (Beckman 802 BGA) was inserted into the cell with a ground joint; the cell was filled with wiped preimbibed seeds. After preliminary measurements, the initial pO₂ of the cell was adjusted, for each sample, to a value higher than the critical pO₂ (see below) and the cell was closed. The respiratory rates at various pO₂ were calculated from the decrease of pO₂ due to the respiratory activity of the seeds.

Indices of Respiration. The critical pO₂ of respiration (P100_{Resp}) is the highest pO₂ which limits the O₂ uptake (2). A theoretical respiration curve (respiration rate *versus* pO₂) was calculated according to Chevillotte and Ducet (6). The parameters of a cubic polynomial were calculated to fit with the experimental data (the r^2 coefficient ranged from 0.91–0.99). This equation allowed the calculation of P80_{Resp}, P50_{Resp}, and P20_{Resp}, the pO₂ that reduced the O₂ uptake to 80, 50, and 20%,

respectively, of its maximal rate. The P80_{Resp}/P20_{Resp} ratio was compared to the P80_{Germ}/P20_{Germ} ratio, in order to compare the effects of low pO₂ on respiration and germination.

Determination of the Adenine Nucleotides. Samples of 5 to 50 seeds, after 3 to 5 h of imbibition in air were submitted to controlled pO₂ atmospheres for 12 h, and then processed for the determination of the AEC as described in Raymond and Pradet (23).

RESULTS

Percentage of Germination in Air. For all the species, in air (21 kPa O₂), the protrusion of the first rootlet occurred after 12 to 24 h of imbibition. The maximal percentage of germination was higher than 95% in all the seeds, except sunflower and turnip, in which it was about 80%. The maximal percentage of germination was reached within 24 h in sorghum and lettuce, within 48 h in wheat, pea, turnip, cabbage, sunflower and within 72 h in rice, maize, and flax.

Germination rate. The variation of the germination percentage with time, at different pO₂, in radish and rice is presented in Figures 1 and 2. These seeds exhibit very different sensitivities to the pO₂. The germination of radish seeds was stopped below 3 kPa O₂, whereas that of rice still occurred at 0.01 kPa. At this very low pO₂, the time of germination was greatly increased: the first rootlets appeared after 7 to 9 d; the germination percentage then increased slowly, reaching 80% after 21 d. Similar curves were drawn for all the other seeds, except that pO₂ below 0.1 kPa were not studied (results not shown). The $t_{50 \text{ max}}$ are given in table I and the linear regressions between the germination

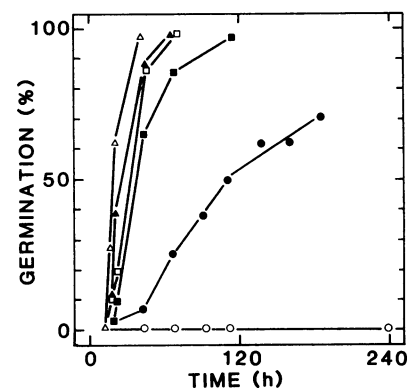


FIG. 1. Time-course of the cumulative germination of radish seeds at various pO₂. The values of the pO₂, expressed in kPa, are: (Δ), 21 (air); (▲), 13; (□), 10; (■), 7; (●), 4; (○), 1 kPa.

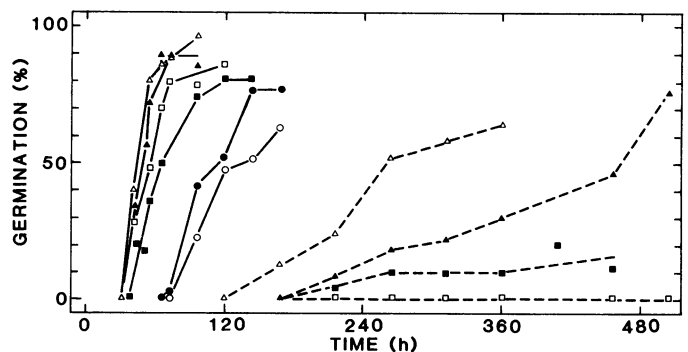


FIG. 2. Time-course of the cumulative germination of rice seeds at various pO₂. The values of the pO₂, expressed in kPa are: (Δ—Δ), 21 (air); (▲—▲), 10; (□—□), 3; (■—■), 1; (●—●), 0.3; (○—○), 0.1; (Δ—Δ), 0.03; (▲—▲), 0.01; (■—■), 0.005; (□—□) < 0.001.

Table I. Indices of Germination and Respiration in Various Seeds at Limiting pO_2

t_{50} max is the time required to observe radicle emergence in 50% of the seeds under nonlimiting pO_2 ; it was determined from the curve of percentage of germination versus time, in air ($pO_2 = 21$ kPa) for all seeds except rice and maize ($pO_2 = 50$ kPa). P100, P50, and P0 are the pO_2 at which the germination (Germ) and respiration (Resp) rates are 100, 50, and 0, respectively, of their maximum value; they were determined from the curves of Figs. 3 and 4.

| Species | t_{50} max | P100 | | P50 | | PO | P80/P20 | | |
|-----------|--------------|------------|------|------|------|-------|---------|--------------|--|
| | | Germ | Resp | Germ | Resp | Germ | Germ | Resp | |
| | <i>h</i> | <i>kPa</i> | | | | | | <i>ratio</i> | |
| Radish | 19.5 | 21 | 26 | 7.5 | 9 | 3 | 3.5 | 5.8 | |
| Turnip | 30.7 | 18 | 16 | 8 | 8 | 3 | 2.7 | 4.9 | |
| Cabbage | 23.6 | 17 | 16 | 7 | 7 | 3 | 2.9 | 4.6 | |
| Sunflower | 15.6 | 19 | 14 | 4 | 4 | 1 | 5.5 | 6.7 | |
| Lettuce | 17.2 | 14 | 16 | 5.5 | 6 | 2 | 3.1 | 5.1 | |
| Flax | 26.7 | 20 | 32 | 8 | 10 | 3 | 3.2 | 6 | |
| Soya | 28.2 | 15 | 19 | 6 | 7 | 2 | 3.1 | 5.8 | |
| Rice | 46.3 | 21 | 15 | 0.22 | 2.5 | 0.002 | 234 | 14.3 | |
| Wheat | 37 | 3.5 | 7 | 0.5 | 1.7 | 0.07 | 10.7 | 5.3 | |
| Sorghum | 15.5 | 15 | 12 | 0.5 | 2.7 | 0.015 | 62 | 6.4 | |
| Maize | 26.1 | 33 | 56 | 0.9 | 6.4 | 0.02 | 78 | 9.7 | |
| Pea | 43.7 | 36 | 69 | 0.9 | 20.3 | 0.02 | 80.3 | 5.5 | |

rates and the logarithms of the pO_2 are presented in Figures 3 and 4. The P100_{Germ} of most seeds range from 15 to 21 kPa. This means that the germination rate of these seeds was maximum at pO_2 slightly lower than, or equal to, the pO_2 of air. The germination rate of two seeds, pea and maize, was slightly stimulated by pO_2 higher than the values in air: the P100_{Germ} of these seeds is about 36 and 33 kPa, respectively.

The P50_{Germ} values of the various seeds were very different (Table I). Two groups of seeds could be distinguished: group I includes one legume (soybean), the three cruciferae (turnip, cabbage, and radish), the two compositae (lettuce and sunflower), and the linaceae (flax). In these seeds, the P50_{Germ} values range from 4 to 8 kPa. Group II contains all the graminiae studied here and the other legume, pea. The P50_{Germ} in group II range from 0.2 to 0.8 kPa. The slope of the straight line representing the relative germination rate versus the log of pO_2 is different in the two groups defined above. The calculation of the P80_{Germ}/P20_{Germ} ratio gives an estimation of this difference. The values of this ratio in group I range between about 3 and 5. In group II, they are all higher than 30. The value of P0_{Germ}, the pO_2 at which

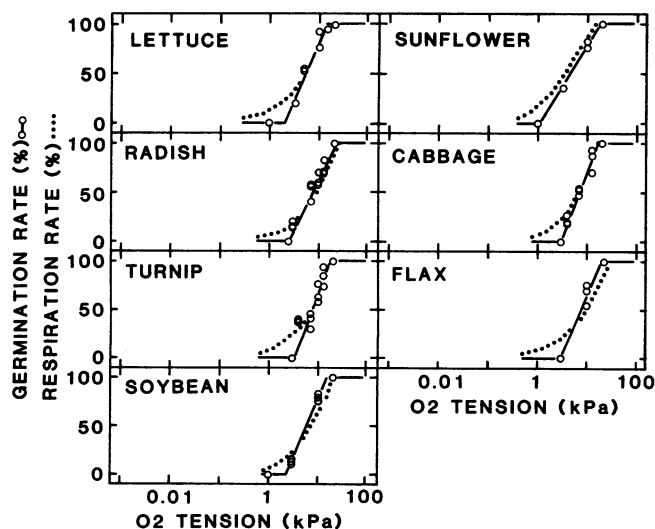


FIG. 3. Effect of pO_2 on germination and respiration, expressed as a percentage of their maximum rates in fatty seeds.

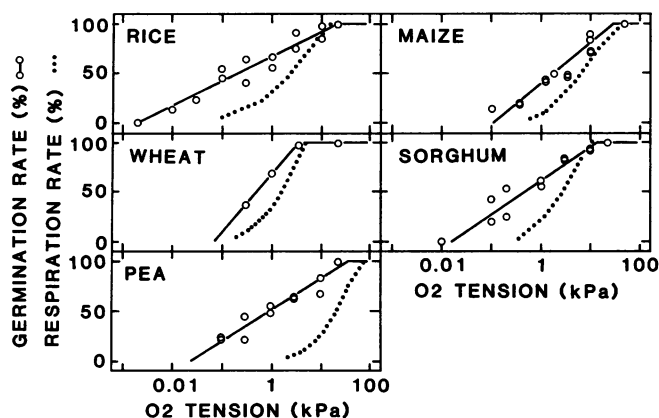


FIG. 4. Effect of pO_2 on germination and respiration, expressed as a percentage of their maximum rates in starchy seeds.

the germination rate is nil, can be determined by extrapolation in Figures 3 and 4. P0_{Germ} is around 2 kPa in group I and below 0.1 kPa in group II. When the seeds were returned to air, the percentage of radicle emergence was at least 90% of the control in air.

Respiratory rate. The critical O_2 pressure, P100_{Resp}, of the different seeds is given in Table I. Two species, pea and maize, exhibited high P100_{Resp} values: 69 and 56 kPa, respectively. The P100_{Resp} of flax seeds (32 kPa) and radish (26 kPa) were also higher than the pO_2 in air. The P100_{Resp} of the other seeds ranged from 7 kPa (in wheat), to 19 kPa (in soybean). The pO_2 at which the respiratory rates are 50% of the maximum values, P50_{Resp}, ranged from 20 kPa (in pea), to about 2 kPa (in rice, sorghum, and wheat). In most seeds, the value of the P80_{Resp}/P20_{Resp} ratio was about 6. The lowest value observed was 4.6 (in cabbage) and the highest was 14.3 (in rice). These data show that the sensitivity of respiration to O_2 is similar in the various seeds and cannot explain the differences observed in the sensitivity of the germination rates to the pO_2 .

Relationship between Respiration and Germination. The relationship established from our results for germination and respiration (Figs. 5 and 6) is only an approximation of the true relationship between these two processes: the respiration rate was measured using seeds imbibed aerobically for a period which was about half of the t_{50} in air, whereas the germination rate depends

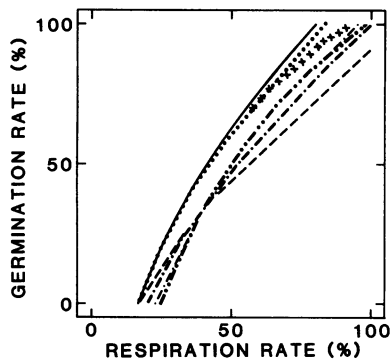


FIG. 5. Relationship between the relative germination and respiration rates in fatty seeds. (xx), radish; (—••), turnip; (—•—), cabbage; (— —), sunflower; (—•—), lettuce; (— —), flax; (•••), soya.

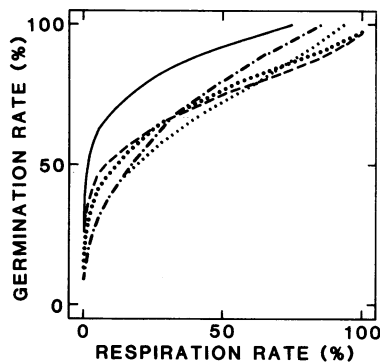


FIG. 6. Relationship between the relative germination and respiration rates in starchy seeds. (■), rice; (—•—), wheat; (— —), sorghum; (•••), maize; (— —), pea.

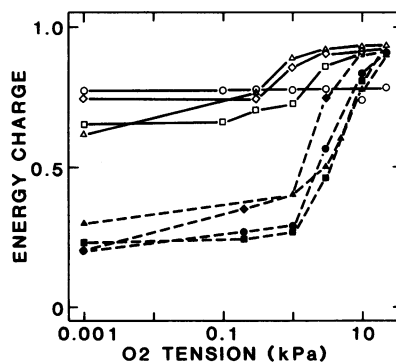


FIG. 7. Effect of pO_2 on the AEC in starchy and fatty seeds: seeds in germination phase II were transferred from air to the pO_2 indicated for 12 h. (◇), rice; (Δ), sorghum; (○), pea; (□), maize; (◆), radish; (●), turnip; (■), cabbage; (▲), lettuce.

on the metabolic activity from the beginning of imbibition until the occurrence of the rootlet protrusion. Nevertheless, the metabolic activity during phase II of germination is probably close to the mean of the metabolic activities in phases I and II; preliminary results showed that during phase II, the sensitivity of respiration to PO_2 varies little (data not shown).

In maize, sorghum, rice, lettuce, cabbage, and radish, the values of $P100_{Germ}$ and $P100_{Resp}$ were similar. In some seeds, the maximum germination rate was observed at a pO_2 ($P100_{Germ}$) lower than the $P100_{Resp}$: the relative respiration rate at $P100_{Germ}$ was 75% in pea, and about 80% in wheat, flax, and soybean. In all seeds, the rate of germination decreased gradually when the respiratory activity decreased. In seeds of group I (Fig. 5), in the

range of pO_2 between $P100_{Resp}$ and $P50_{Resp}$, the germination rate decreased roughly linearly with respiration. An extrapolation of this part of the curve would yield a germination rate close to zero when respiration is nil; at lower pO_2 values, the decrease of the germination rate is sharper than that of the respiratory rate; at $P20_{Resp}$, the relative germination rate was close to zero. In group II seeds, at pO_2 above $P50_{Resp}$, germination was much less affected than respiration by decreasing pO_2 . At $P20_{Resp}$, the relative germination rate was 80% in pea, 60% in sorghum and rice, and about 50% in maize and wheat. At $P5_{Resp}$, it still ranged from 30 to 60% for all the seeds of group II.

Adenylate Energy Charge. The AEC in seeds at various pO_2 is shown in Figure 7. In all the seeds, except pea, the AEC values in air were close to 0.90: such high values characterize tissues in which respiration is not limited by O_2 (22). In lettuce, radish, turnip, and cabbage, the AEC decreased sharply at pO_2 below 10 kPa, reaching values lower than 0.5 at 1 kPa. In rice, maize, and sorghum, the AEC at 10 kPa O_2 was similar to that in air. Even under N_2 ($pO_2 < 0.001$ kPa), the AEC remained above 0.6. In pea, the AEC in air was 0.75 to 0.80, a low value which may be correlated to the well known natural anaerobiosis in the early germination stages of this seed (14; Fig 4). The AEC was increased to higher values when pea seeds were submitted to 50 kPa O_2 (not shown); at lower pO_2 , the AEC did not decrease below the level in air.

DISCUSSION

Technical Difficulties. When germination is slowed down by hypoxia, the contamination of seeds by microorganisms becomes a very important problem. The hypochlorite treatment reduced these contaminations. In spite of this, some fungi and bacteria still contaminated the seeds. In order to shorten the length of the experiments, we chose seeds for which the t_{50} in air was 12 to 48 h. This is the case for most cultivated annual species. However, the t_{50} of rice seeds was 22 d at $pO_2 = 0.01$ kPa (Fig. 2). At the end of the experiments, more than 90% of the nongerminated seeds, as compared to the control, were still alive: they germinated when returned to air.

The surface sterilization treatment was done in air: it corresponded to the initial phase of imbibition. During the 2-h period before the seeds were placed under a controlled atmosphere, the metabolic activity is initiated gradually. Many syntheses occur during this period (5, 8) and may modify the behavior of the seeds under limiting pO_2 . Nevertheless, in control experiments with nonsterilized seeds imbibed under hypoxia, we found that the pO_2 value during the initial period of imbibition did not significantly affect the results.

In their study of germination under hypoxia, Siegel and Rosen (28) returned the seeds to air every day for counting and then replaced them under hypoxia: the germination rate could be drastically altered by the transient periods of normoxic metabolism. To avoid this difficulty, Heichel and Day (9) counted the germinated seeds only after a long period: they determined the final percentage of germination, not the germination rate. In the study presented here, the seeds were submitted to the same gaseous environment until counting and the use of a series of eight jars allowed the determination of germination rates. Using GC, we verified that the O_2 pressure was not significantly decreased at the exit of the series of jars; in order to avoid any entry of air into the system, we used the last jar of the series for each germination count; the gradient of CO_2 or other volatile products of seed metabolism along the line may have affected the germination curve of some seeds but this effect was probably small: we verified for lettuce and rice that the germination curve was similar in the jar series blown with air or in covered Petri dishes left in air; the growth of rice at pO_2 below 0.02 kPa was similar in the jar series or in individual vials.

pO₂ and Respiration. The estimation of the affinity of cells and tissues for O₂ by the polarographic method is usually done in a liquid medium. During the course of this study, we observed that, even with strong shaking, the method yields values of P100_{Resp} and P50_{Resp} higher than those determined in gaseous medium (results not shown). This phenomenon is probably due to the layer of water immobilized around or under the seed envelopes (27). Consequently, the respiration was estimated in gaseous media, as was the germination rate. The cell was filled with wiped seeds in order to obtain respiratory rates high enough to carry out the measurements before the end of germination phase II.

Some years ago, Armstrong and Gaynard (2) asserted that the high P100_{Resp} reported for plant tissues were due to the excision of organs, or blocks of tissues. However, Pradet and Bomsel (20) underlined that the high values of P100_{Resp} and P50_{Resp} (15 and 5 kPa, respectively) observed with lettuce seeds (18), could not be attributed to this phenomenon. More recently, Saglio *et al.* (27) demonstrated that the P100_{Resp} of maize root tips was also high. The results presented here support this view, since the P100_{Resp} of most seeds was close to the pO₂ of air.

It has been reported that, before radicle protrusion, germinating seeds are under natural anaerobiosis (14). Our results question the generality of this observation, since the respiratory activity of 9 of the 12 studied species was saturated in air. On the basis of these data, natural anaerobiosis would be observed only in pea, maize, and flax. However, in *Phaseolus mungo* seeds, fermentation during the early germination phases was attributed to an excess of the flux of glycolysis over that of respiration (16). A study of fermentation in the seeds studied here, showed that fermentation in air was significantly active only in pea and maize (25).

pO₂ and Germination. The cultivated seeds studied here present a common picture: the highest germination rate is observed at pO₂ slightly lower, or higher (pea and maize), than the pO₂ of air. This result is of agricultural importance, since it means that in the soil, pea and maize seeds are necessarily submitted to suboptimal pO₂. It also means that soil aeration may become a critical factor during the initial phases of seed germination for most, if not all, cultivated species.

As noted previously (9) the sensitivity of germination to pO₂ differs greatly depending on the species. At low pO₂, two opposite behaviors were observed. The germination of some of the seeds (classed in group I) was stopped below 3 to 1 kPa O₂, whereas the germination of others (group II) still occurred at 0.1 kPa. The seeds of *Echinochloa* are similar to those of group II (26). We found no species exhibiting an intermediate behavior, but it may be expected that such seeds exist among other cultivated, or wild species.

In a study which included 12 monocots, Heichel and Day (9) found that the monocots were able to germinate below 2 kPa O₂. The 4 monocots studied here belong to the gramineae and their germination occurs at very low pO₂. The seeds of *E. crus galli*, which is also a gramineae, exhibit similar properties (26).

However, radicle emergence in two dicots (*Celosia* and cucumber) is also possible at low pO₂ (28), and we show here that pea is able to complete its germination below 0.1 kPa O₂. Consequently, the ability to germinate at low pO₂ is not a property that is restricted to monocots. It seems to be related to the nature of the seed reserves: the seeds exhibiting germination at low pO₂ contain starch, while most seeds unable to grow at low pO₂ do not. However, we found recently that in the starchy seeds of lentil the radicle did not emerge below 1 kPa. The growth of rice coleoptiles is still possible at pO₂ lower than 0.1 Pa (13). This growth is certainly O₂ independent. The protrusion of some rice rootlets was observed after a long delay at 3 Pa, but not below. We know of no report of biological effects of O₂ at such low

partial pressures.

Controlling Factors in Germination. Our results show that the hypothesis that the differences in the sensitivity of seed germination to O₂ could be explained by differences in the sensitivity of respiration to O₂ has to be discarded. None of the seeds exhibited a high affinity for O₂ and the two seeds having the lowest affinity for O₂ (pea and maize) belong to the group of seeds that are able to complete germination at very low pO₂.

In seeds of group I, the relative rates of respiration and germination correlate well between 100 and 50% of their maximum values (Fig 6). Within this range of values, germination seems to be under the direct control of respiration. At lower pO₂, the germination rates decreased more rapidly than the respiration rates, showing that a factor other than the rate of ATP regeneration becomes limiting. That the controlling factor is related to the value of the adenine nucleotide ratios, such as ATP/ADP, is suggested by the fact that, in all the seeds studied, the AEC reached values close to 0.6 when the pO₂ was close to the PO_{Germ}: the ATP/ADP ratio is related to the value of the AEC, because the reaction catalyzed by the adenylate kinase is in near-equilibrium (4, 22). Such a control could be in agreement with Atkinson's hypothesis that the flux in ATP-consuming pathways is decreased when the AEC is decreased, although this control would occur at AEC values much lower than those considered by Atkinson (4). In seeds of group II, the relative germination rate was still close to 50% when the respiratory rate was only about 10%. This shows that the respiratory rate exerts little control on the germination rates in these seeds and suggests that, under hypoxia, fermentation yields enough energy to compensate for the lower respiratory rate. The block of germination at low pO₂ in seeds of group II does not result from a decrease of the AEC (Figs. 3, 4, and 7); in this case, O₂ may become limiting at the level of some biosynthetic pathways.

LITERATURE CITED

- AL-ANI A, JM LEBLANC, P RAYMOND, A PRADET 1982 Effet de la pression partielle d'oxygène sur la vitesse de germination des semences à réserves lipidiques et amylacées: rôle du métabolisme fermentaire. C R Acad Sci 295: 271-274
- ARMSTRONG W, TJ GAYNARD 1976 The critical oxygen pressures for respiration in intact plants. *Physiol Plant* 37: 200-206
- ASPART L, A GOT, M DELSENY, B MOCQUOT, A PRADET 1983 Adaptation of ribonucleic acid metabolism to anoxia in rice embryos. *Plant Physiol* 72: 115-121
- ATKINSON DE 1977 Cellular Energy Metabolism and its Regulation. Academic Press, New York, pp 85-107
- BEWLEY JD, M BLACK 1978 Physiology and Biochemistry of Seeds in Relation to germination, Vol 1. Springer-Verlag, Berlin
- CHEVILLOTTE P, G DUCET 1969 Respiration du tubercule de pomme de terre. Influence de la tension d'oxygène sur des disques minces. *Physiol Vég* 7: 305-323
- COLLIS-GEORGE N, MD MELVILLE 1974 Models of oxygen diffusion in respiring seed. *J Exp Bot* 25: 1053-1069
- DELSENY M, L ASPART, A GOT, R COOKE, Y GUITTON 1977 Early synthesis of polyadenylic acid, polyadenylated and ribosomal nucleic acids in germinating radish embryo axes. *Physiol Vég* 15: 413-428
- HEICHEL GH, PR DAY 1972 Dark germination and seedling growth in monocots and dicots of different photosynthetic efficiencies in 2% and 20.9% oxygen. *Plant Physiol* 49: 280-283
- KENNEDY RA, D BARRETT, D VAN DER ZEE, ME RUMPHO 1980 Germination and seedling growth under anaerobic conditions in *Echinochloa crus galli* (Barnyard grass). *Plant Cell Environ* 3: 243-248
- KOLLOFEL C 1967 Respiration rate and mitochondrial activity in the cotyledons of *Pisum sativum* L. during germination. *Acta Bot Néer* 16: 111-122
- LATIES GG 1982 The cyanide-resistant, alternative path in higher plant respiration. *Annu Rev Plant Physiol* 33: 519-555
- LEBLANC JM, M RANCILLAC, A PRADET 1983 Germination de la semence d'*Oryza sativa* L. variété "Cigalon" en stricte anoxie; généralisation aux *Oryza* cultivés de ce caractère adaptatif. *Agronomie* 3: 259-264
- LEBLOVA S 1978 Pyruvate conversions in higher plant during natural anaerobiosis. In DD Hook, RMM Crawford, eds, *Plant Life in Anaerobic Environments*. Ann Arbor Science, Ann Arbor, MI
- MORINAGA T 1926 The favorable effect of reduced oxygen tension upon the germination of certain seeds. *Am J Bot* 13: 159-166
- MOROHASHI Y, M SHIMOKORIYAMA 1975 Development of glycolytic and

- mitochondrial activities in the early phase of germination of *Phaseolus mungo* seeds. *J Exp Bot* 26: 932-938
17. MOYSE A 1952 La germination du blé aux basses tensions d'oxygène. I. Tension minimum d'oxygène et pouvoir germinatif. *C R Acad Sci* 234: 874-876
 18. PRADET A 1969 Etude des adénosine-5'-mono, di et triphosphates dans les tissus végétaux. V. Effet *in vivo* sur le niveau de la charge énergétique d'un déséquilibre induit entre fourniture et utilisation de l'énergie. *Physiol Vég* 7: 261-275
 19. PRADET A 1982 Oxidative phosphorylation in seeds during initial phases of germination. In AA Khan, ed, *The Physiology and Biochemistry of Seed Dormancy and Germination*. Elsevier/North-Holland Biomedical Press, Amsterdam, pp 347-369
 20. PRADET A, JL BOMSEL 1978 Energy metabolism in plants under hypoxia and anoxia. In DD Hook, RMM Crawford, eds, *Plant Life in Anaerobic Environments*. Ann Arbor Science, Ann Arbor, MI pp 89-118
 21. PRADET A, B MOCQUOT, P RAYMOND, C MORISSET, L ASPART, M DELSENY 1984 Energy metabolism and synthesis of nucleic acids and proteins under anoxic stress. In JL Key, T Kosuge, eds, *Cellular and Molecular Biology of Plant Stress*, UCLA Symposia on Molecular and Cellular Biology, New Series, Vol 22. A. R. Liss, New York, pp 227-245
 22. PRADET A, P RAYMOND 1983 Adenine nucleotide ratios and adenylate energy charge in energy metabolism. *Annu Rev Plant Physiol* 34: 199-224
 23. RAYMOND P, A PRADET 1980 Stabilization of adenine nucleotide ratios at various values by an oxygen limitation of respiration in germinating lettuce (*Lactuca sativa*) seeds. *Biochem J* 190: 39-44
 24. RAYMOND P, A AL-ANI, A PRADET 1983 Low contribution of nonrespiratory pathways in ATP regeneration during early germination of lettuce seeds. *Physiol Vég* 21: 677-687
 25. RAYMOND P, A AL-ANI, A PRADET 1985 ATP production by respiration and fermentation, and energy charge during aerobiosis and anaerobiosis in twelve fatty and starchy germinating seeds. *Plant Physiol* 79: 879-884
 26. RUMPHO ME, A PRADET, A KHALIK, RA KENNEDY 1984 Energy charge and emergence of the coleoptile and radicle at varying oxygen levels in *Echinochloa crus galli*. *Physiol Plant* 62: 133-138
 27. SAGLIO PH, M RANCILLAC, F BRUZAU, A PRADET 1984 The critical oxygen pressure for growth and respiration of excised and intact roots. *Plant Physiol* 76: 151-154
 28. SIEGEL SM, LA ROSEN 1962 Effects of reduced oxygen tension on germination and seedling growth. *Physiol Plant* 15: 437-444
 29. VAN DER ZEE D, RA KENNEDY 1981 Germination and seedling growth in *Echinochloa crus galli* var. *Oryzicola* under anoxic conditions: structural aspects. *Am J Bot* 68: 1269-1277
 30. VARTAPETIAN BB, IN ANDREEVA, NURITDINOV 1978 Plant cells under oxygen stress. In DD Hook, RMM Crawford, eds, *Plant Life in Anaerobic Environments*. Ann Arbor Science, Ann Arbor, MI pp 13-18