Legume embryos develop in a hypoxic environment

Hardy Rolletschek¹, Ljudmilla Borisjuk¹, Matthias Koschorreck², Ulrich Wobus¹ and Hans Weber¹,³

¹ Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), D-06466 Gatersleben, Germany
² Umweltforschungszentrum Leipzig-Halle, Aussenstelle Magdeburg, Brückstr. 3a, D-39114 Magdeburg, Germany

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Abstract
Specific morphological and biochemical characteristics of seeds can cause oxygen deficiency within maternal and embryonic tissues. In this study, optical sensors were used to measure O₂ profiles across developing seeds of Vicia faba and Pisum sativum and developmental and environmental modulations of internal O₂ levels were studied. In addition, the metabolic state of developing embryos was analysed by monitoring adenylate energy charge, adenylate nucleotides and the levels of nucleotide sugars. Within the seed coat O₂ concentration decreased sharply to ~3% towards the inner border. Lowest O₂ levels were detected within the endospermal cavity between the seed coat and embryo. It is probable that low seed coat permeability provides an hypoxic environment for legume embryo development. The O₂ concentration in embryonic tissue changed during development with the lowest levels in the early stages. Measured in darkness, the levels were below 3%, but increased upon illumination indicating that photosynthesis significantly contributes to internal O₂ levels. Only in very young embryos were ATP levels and energy charge low. Otherwise they were maintained at a constant higher value. ADP-glucose and UDP-glucose did not show large fluctuations. Throughout embryo development fermentative activity did not play a major role. Obviously, specific mechanisms prevent seed tissues from becoming anoxic during development. The possible role of low oxygen on seed metabolism and on the control of seed development in legumes is discussed.

Key words: Energy charge, hypoxia, optical oxygen sensors, photosynthesis, seed development.

Introduction
Seeds possess morphological and biochemical characteristics which lead to O₂ deficiency within the organ. Seeds are covered with thickly cutinized cell layers which strongly restrict gas exchange (Pisum: Wager, 1974a; Brassica: Sheoran et al., 1991; wheat and barley: Nutbeam and Duffus, 1978; Cochrane and Duffus, 1979). In addition, the pod wall of legumes has a low stomatal frequency compared with leaves, which further diminishes gas exchange capability. Seeds are bulky organs with high metabolic and biosynthetic activity. However, specialized systems for O₂ delivery are lacking and this lowers oxygen levels within tissues. Photosynthesis as a principal source of O₂ supply is regarded to be of minor importance, at least in terms of CO₂ fixation (Harvey et al., 1976; Atkins and Flinn, 1978; Flinn, 1985). However, detailed analysis of O₂ budgets, especially on the importance of photosynthetic O₂ production, is lacking.

Recent literature provides mostly indirect evidence for O₂-depleted zones inside seed structures. These include in vitro germination behaviour (Hess and Carman, 1993), the induction of lactate dehydrogenase and alcohol dehydrogenase (Boyle and Yeung, 1983; Yeung and Blackman, 1987) as well as ethanol production (Wager, 1974b) and the γ-aminobutyrate shunt (Shelp et al., 1995). From the decline in the adenylate energy charge during certain stages of seed development an O₂ concentration of 5–10% was suggested for soybean seeds (Shelp et al., 1995). The internal O₂ concentration in siliques of Arabidopsis and Brassica were measured using small-diameter glass electrodes (Porterfield et al., 1999). Mean O₂ levels were between 12.2 and 16.2 kPa, respectively, when measured in light, and 6.1 and 12.2 kPa in darkness. Although these values were relatively high, the authors postulated that hypoxia is an important factor for controlling seed development. Considerably lower values
of less than 5% O₂ occurred in growing potato tubers (Geigenberger et al., 2000). The potential importance of hypoxia is further supported by the observation that seed production and seed size depend on atmospheric (external) O₂ level (Quebedeaux and Hardy, 1975; Musgrave and Strain, 1988; Kuang et al., 1998; Porterfield et al., 1999). It was postulated that substantial concentration gradients of O₂ are necessary to drive diffusion into seeds. Otherwise, diffusive influx is too low to meet O₂ demand followed by disturbed seed development or even seed abortion.

The effects of O₂ deficiency on plant growth are well described and include changes in metabolism and gene expression pattern (Crawford and Brändle, 1996; Drew, 1997). Both the extent and kinetics of changes in the adenylate energy charge in response to low O₂ are used as indicators of tolerance to hypoxia and/or anoxia. At a metabolic level, respiration, protein, as well as starch synthesis, are inhibited (Geigenberger et al., 2000). In general, metabolism is shifted towards energy-saving adaptations, thereby conserving ATP and redox charge of the cells. Gene expression also provides an indication of anaerobic responses including fermentative and glycolytic pathways (Sachs et al., 1996). The repression of invertases but induction of sucrose synthase gene expression by low O₂ is considered as part of this shift (Zeng et al., 1999).

Seed development of V. faba has been described at both morphological and biochemical levels (for reviews see Borisjuk et al., 1995; Weber et al., 1998), and there is evidence that legume seed development including storage activity is under metabolic control. During the early development when mitotic activity is high, an invertase-mediated pathway of sucrose breakdown operates in the seed (Weber et al., 1995). Following the loss of invertase storage/maturation phase is initiated. In parallel, the sugars change characteristically from a high ratio of hexoses to sucrose to high sucrose levels (Weber et al., 1995). Although there is some evidence for hypoxic zones within legume seeds and its role in seed development, the detailed time and spatial resolution of oxygen distribution within seeds is unknown.

In this study, O₂ levels were measured in developing Vicia and pea seeds and developmental and environmental modulations of internal O₂ levels were studied. In addition the metabolic state of developing embryos was analysed by monitoring energy status, adenylate nucleotides and the levels of key metabolic precursors. Optical sensors were used to measure O₂ profiles across developing seeds and to show that oxygen drastically decreases across the seed coat resulting in a hypoxic environment for the growing legume embryo and that photosynthesis significantly contributes to internal O₂ levels. Embryo tissue becomes hypoxic but never anoxic during development. The possible role of low oxygen effects on seed metabolism and on the control of seed development in legumes is discussed.

Materials and methods

Plant material

Vicia faba L. and Pisum sativum L. were grown in growth chambers under a light-dark regime of 16/8 h dark at 20 °C. Seeds were harvested at distinct developmental stages in the mid-light phase. Immediately after harvest, embryos were isolated and frozen in liquid nitrogen. Frozen embryos were weighed (fresh weight), and used for enzyme assays and determination of metabolic intermediates.

Determination of oxygen concentration and photosynthesis in seeds using optical sensors

Oxygen concentration inside seeds was determined using O₂-sensitive optical glass-sensors (microsensor, Presens, Neuburg, Germany) connected to a fibre optic oxygen meter (Microx TX, Presens). The tip of the microsensor has a diameter of approximately 30 μm and, unlike Clark-type electrodes, the microsensor does not consume oxygen, thereby preventing the establishment of an artificial oxygen sink. Microsensors were calibrated with ambient air (21% O₂ and 100% N₂, respectively. The electrode signal was stable for at least 4 h. First, the pod was placed in a horizontal plane and fixed. Subsequently, a small window was made in the upper half of the pod wall. The microsensor was observed for correct positioning on the seed surface by a microscope (Zeiss, Jena, Germany), and driven into the seed by a micromanipulator at 50–100 μm intervals. Just after tip insertion the microsensor sealed the small hole of its entry point. For additional sealing the entry point was covered with a drop of silicone oil to prevent oxygen diffusion into the seed along micro-channels. At each position the sensor was paused for approximately 10 s to allow equilibration and to obtain measurement. The mean of ten measurements with a standard error of less than 5% represents one data point in the figures. During measurements, the light intensity at the seed surface was adjusted to 400–450 μmol m⁻² s⁻¹ using the lighting system (Schott, Germany) of the microscope. This value corresponds to those measured inside the pod when plants are exposed to sunlight, and mimics the shading effect of intact pods as determined in preliminary studies. Light intensity was measured by a quantum sensor (model LI 185 A, Li-Cor, USA). After measurement, seeds were dissected at the measured transect to identify the exact position of the sensor tip within distinct zones of the seed (seed coat, vacuole, embryo).

To determine the effect of light, comparative measurements were done in darkness. After measuring oxygen profiles of illuminated seeds, the light was switched off and the plant was allowed to re-establish a steady-state oxygen profile within 15 min. Subsequently, the profiles were measured. There was no significant difference when the measurement was first done in light and afterwards in darkness or vice versa.

Using the microsensor technique, gross photosynthetic rates can be estimated as the rate of decrease in oxygen concentration during the first few s following extinction of light (Glud et al., 1992). This method is based on the following assumptions: (1) a steady-state oxygen profile before darkening, (2) a constant rate of respiration before and during the first few s of darkening and (3) identical diffusive fluxes during this time. Theoretical considerations have been discussed in detail (Revsbech et al., 1981; Glud et al., 1992).
Enzyme assays
For the preparation of crude extracts cotyledons were homogenized on ice in a precooled pestle and mortar with 5 vols of cold extraction buffer (125 mM MES, 100 mM NaCl, 2.5 mM MgCl₂, 0.5 mM thiamine pyrophosphate (TPP), 1 mM EDTA, 2 mM DTT, pH 6.8) together with 100 mg polyvinyl polypyrrolidone. Homogenates were kept at 4 °C and centrifuged for 10 min at 4 °C and 10,000 g. The supernatants were snap-frozen in liquid N₂ in 100 μl aliquots until required. Lactate dehydrogenase (LDH; EC 1.1.1.27) was determined as described earlier (Bergmeyer, 1983). Alcohol dehydrogenase (ADH; EC 1.1.1.1) was assayed as given previously (Waters et al., 1991). Activity of pyruvate decarboxylase (PDC; EC 4.1.1.17) was measured spectrophotometrically at 25 °C in a final volume of 1 ml. The reaction mixture contained 100 μl enzyme extract, 10 U ADH, 50 mM MES (pH 6.8), 25 mM NaCl, 1 mM MgCl₂, 2 mM DTT, 0.5 mM TPP, 0.2 mM NADH, and 50 mM sodium oxamate (inhibitor of LDH). After preincubation for 30 min, the reaction was started by adding pyruvate (final concentration 25 mM). Activity measurements were corrected by subtracting values obtained in the absence of substrate. All enzymatic assays were checked to be dependent on each substrate and linearity with respect to time and amount of extract.

Extraction and determination of metabolic intermediates
Frozen material was extracted with trichloroacetic acid (TCA) (Herbers et al., 1997). Lactate was determined spectrophotometrically by coupling the oxidative reaction of LDH with alanine:2-oxoglutarate aminotransferase (ALT; to remove pyruvate from the equilibrium) at 25 °C in a final volume of 0.8 ml. The reaction mixture contained 100 μl TCA extract, 150 mM glutamic acid, 1 mM NAD, and 3 U ALT (pH 8.9). Samples were preincubated for 10 min and the reaction was started by adding 10 U LDH. Nucleotides and their sugars were determined by HPLC (DX-500, Dionex, USA). Separation was carried out on a Vydac 302 IC column (4.6 x 250 mm) at 25 °C. Column effluents were monitored at 260 nm. The column was equilibrated with buffer A at a flow rate of 2 ml min⁻¹. The gradient was accomplished with buffer A (NaH₂PO₄-Na₂HPO₄, 1:1 molar ratio, 25 mM, pH 2.8) and buffer B (NaH₂PO₄-Na₂HPO₄, 1:1 molar ratio, 125 mM, pH 2.9). The gradient was produced by the following concentration changes: 2 min 0% B, 9 min 11% B, 18 min 100% B, hold 100% B for 2 min, return to 0% B in 1 min. Recovery rates were estimated to be 139% (AMP), 104% (ADP), 111% (ATP), 108% (ADP-glucose), 92% (UDP-glucose), and 82% (lactate).

**Results**

**Oxygen concentrations in seeds of Vicia faba**
Fine glass microsensors (tip diameter ~ 30 μm) were used to measure O₂ concentration along a transect through a developing V. faba seed of ~ 900 mg fresh weight in darkness. The microsensors penetrate the seed along the longitudinal axis and straight across one of the cotyledons (Fig. 1A). Before the measurement the pod was removed from the plant and a window was cut into the pod wall to get access for the electrode. The O₂ level immediately declined to 70% when the microsensor penetrated the outer layer of the seed coat (atmospheric O₂ level of approximately 21 kPa is set to 100%). Within the seed coat the O₂ concentration sharply decreased to ~ 3% towards the inner border. After 950 μm the microsensor tip entered the embryo. The endospermal vacuole is already lacking at this stage. Mean and minimum O₂ levels within the embryo was 3.3 ± 0.6 and 2.4%, respectively. Within the embryonic tissue there was no O₂ gradient present. When the tip of the microsensor entered the seed coat again (right side in Fig. 1A), O₂ levels increased nearly symmetrically compared to the left side. The insertion channel of the tip is shown in the enlargement of Fig. 1B.

O₂ profiles were then measured within illuminated Vicia seeds at four developmental stages of 30, 150, 300, and 900 mg of seed fresh weight (Fig. 2). In principle, similar gradients were found for all stages: O₂ concentrations declined strongly across the seed coat tissue whereas no gradient was seen within the embryo. The slope of the concentration gradient within the seed coat was similar at all stages. Lowest O₂ levels (down to 1.3%) were always

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**Fig. 1.** Oxygen concentration measured in darkness along a longitudinal transect of a Vicia faba seed of 900 mg fresh weight. (A) Profile of oxygen concentration. The boundary of the embryo to the seed coat is indicated by arrow heads. (B) Magnification of insert b in (A) shows the insertion channel of the microsensor (arrow heads); sc, seed coat; co, cotyledon; bar = 1 mm.**
detected within the endospermal vacuole between the seed coat and embryo. Within the embryo tissue $O_2$ levels rose to higher values. Young embryos in 30 mg seeds contained the lowest $O_2$ concentrations. Levels were higher in embryos of 150 mg seed fresh weight and reached the highest levels at a seed weight of 300 mg. Thereafter, in embryos of 900 mg seeds, $O_2$ concentration decreased again. The results show that $O_2$ concentration in embryonic tissue changed during development. The relationship between mean $O_2$ concentration measured in embryos and seed fresh weight is given in Fig. 3. A steady increase in $O_2$ level was found.

**Oxygen concentrations in seeds of Pisum sativum**

The $O_2$ concentration was measured in developing pea seeds of 30 and 300 mg seed fresh weight. Similar patterns were detected compared to those described above for *V. faba*. A strong decline of $O_2$ across the seed coat towards the interior was observed. Inside the endospermal cavity $O_2$ levels were lowest (<1%) but increased again within the embryo. Measured in darkness the mean $O_2$ levels were $\sim 7\%$ in an embryo of a 30 mg seed and $\sim 3\%$ in the embryo of a 300 mg seed (Fig. 4A, B). The effect of light was then investigated. When seeds were illuminated, $O_2$ levels increased only slightly within the

![Fig. 3. Relationship between mean cotyledonary oxygen levels and seed fresh weight measured in illuminated seeds of *V. faba*.](image)

![Fig. 4. Oxygen profiles within developing *Pisum sativum* seeds measured in darkness (○) and light (●). Two developmental stages were analysed: 30 mg (A) and 300 mg (B) of seed fresh weight. For further information see text. The relative positions of the seed coat, endospermal vacuole and embryo are indicated by the dark grey colour, little lines and the light grey colour, respectively.](image)
embryos of a 30 mg seed (Fig. 4A), but increased from ~3% to 50% within the 300 mg seed (Fig. 4B). The discontinuity in O2 concentration in the illuminated 300 mg seed (Fig. 4B) corresponded to the vacuolar space separating the two cotyledons at that stage. These results indicate that O2 levels within pea cotyledons increase considerably upon illumination. However, this effect was mainly found in older embryos.

Oxygen increase upon illumination was probably caused by photosynthetic activity within the cotyledons. Therefore photosynthetic rates were estimated within illuminated pea embryos. A steady-state O2 profile was measured at a depth of 1 mm inside illuminated pea cotyledons. Then two dark impulses of 30 s each were applied. The decrease in O2 concentration during the first s of darkness was measured (Fig. 5). Constant respiration and diffusive import of O2 before and immediately after darkening was assumed. By fitting a linear regression line to the data points obtained within the first seconds after light extinction the photosynthetic rates from the slope of regression were estimated. The calculated value was $68.6 \pm 3.1 \text{nM O}_2 \text{ min}^{-1}$. Assuming an average water concentration of 75% in the cotyledons studied here, a gross photosynthetic rate of $51.4 \pm 2.3 \text{ nmol O}_2 \text{ g}^{-1} \text{ fresh weight min}^{-1}$ was calculated. Regression coefficients ($r^2$) were higher than 0.97, clearly indicating both a constant rate of respiration and constant diffusive fluxes (i.e. virtually unchanged oxygen profiles) within the first seconds of the dark period.

**Adenine nucleotides and sugar nucleotides**

Hypoxic zones within tissues could influence the energy status, which is a good indicator for the overall metabolic state. Adenine nucleotide levels and their ratio are regarded as indicators for the energy status of tissues.

Therefore adenine nucleotides, energy charge as well as nucleotide sugars within developing *V. faba* cotyledons harvested during the light phase were measured. ATP content was ~70 nmol g$^{-1}$ in early embryos (fresh weight < 30 mg), followed by a sharp increase to values of ~250 nmol g$^{-1}$ fresh weight in 100 mg embryos (Fig. 6A). In embryos from this stage onwards, ATP content declined slightly until the embryo reached 450 mg of...
fresh weight. At later stages the ATP content increased again to ~300 nmol g\(^{-1}\) fresh weight. ADP levels were ~40–60 nmol g\(^{-1}\) throughout development without significant changes. AMP decreased initially concomitant with the increase of ATP and remained at ~80–100 nmol g\(^{-1}\) fresh weight throughout development (Fig. 6A). The time-course of the adenylate energy charge calculated by (ATP + 0.5 ADP)/(ATP + ADP + AMP) followed closely that of ATP. Low values (<0.4) at very early stages increased to a maximum of 0.76 in 100 mg embryos followed by a slight decrease to ~0.65 (Fig. 6B).

Starch is a main storage product in developing embryos of *V. faba* and pea. The activated sugars UDP-glucose and ADP-glucose act as direct precursors. Therefore, their levels during development were measured. The time-course of ADP-glucose and UDP-glucose is shown in Fig. 6C. The content of ADP-glucose was low throughout development (~20–50 nmol g\(^{-1}\)). Only in later stages were contents higher than 100 nmol g\(^{-1}\) fresh weight detected. UDP-glucose content was considerably higher (~300 nmol g\(^{-1}\)) and increased slightly from the early to the late stages. The results show that, only in very young embryos, were ATP levels and energy charge low but otherwise were maintained at a relatively high and constant value. ADP-glucose and UDP-glucose did not show large fluctuations.

**Fermentation enzymes and their products**

Low oxygen levels within plant tissues might cause hypoxia or even anoxia followed by the induction of fermentative pathways. Therefore, the activity of fermentation enzymes as well as lactate within *V. faba* embryos were analysed. Alcohol dehydrogenase (ADH, Fig. 7A) and pyruvate decarboxylase (PDC, Fig. 7B) increased from the early to the late developmental stages from 100–300 nmol g\(^{-1}\) fresh weight min\(^{-1}\). Ethanol as the end-product of fermentation could not be detected throughout development (measured by gas chromatography). Lactate dehydrogenase (LDH, Fig. 7C) was present at a nearly constant level with activities of 300–400 nmol g\(^{-1}\) fresh weight min\(^{-1}\) and lactate accumulated slightly at mid-term development to levels of 0.5–0.6 mmol g\(^{-1}\) (Fig. 7D). Levels did not correlate to the activity profile of LDH. These results indicate that throughout embryo development fermentative activity does not play a major role.

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**Fig. 7.** Enzyme activities of the fermentative pathway and lactate levels in developing *V. faba* embryos. (A) Alcohol dehydrogenase (ADH), (B) pyruvate decarboxylase (PDC), (C) lactate dehydrogenase (LDH), and (D) lactate.
**Discussion**

**Low seed coat permeability provides an hypoxic environment for legume embryo development**

Growing seeds of *V. faba* and pea are bulky tissues with high metabolic activity. However, as compared to leaves, photosynthetic activity is low possibly affecting oxygen supply. This study therefore aims to analyse O₂ distribution within developing seeds of *V. faba* and pea and its implications for energy state and levels of key metabolites. Using optical microsensors it is demonstrated that O₂ concentration sharply decrease to 3% from the outer to the inner border of the seed coat in both *Vicia* and pea seeds. This suggests that oxygen entry from the surrounding gas space into the seed is strongly restricted by the seed coat. It was reported that O₂ intake into pea seeds occurs entirely through the micropylar region whereas the seed coat is nearly impermeable (Wager, 1974a). Therefore, the low O₂ concentration within the embryo is partly due to the low gas permeability of the seed coat. The lowest O₂ levels were always detected within the endospermal vacuole between the seed coat and embryo, indicating some respiratory activity within the endospermal cytoplasmic strands. Endosperm tissue is metabolically active and accumulates storage products (Borisjuk et al., 1995).

Minimum O₂ levels measured in darkness were <1% in *Vicia* and pea embryos. Pronounced gradients as measured across the seed coat were not detected within embryos. A possible interpretation could be that the embryonic tissue offers relatively little diffusional impedance. This would allow efficient gas exchange and would compensate locally-distinct O₂ consumption rates (due to varying metabolic activity, cf. Borisjuk et al., 1995). O₂ concentrations increased significantly upon illumination of seeds. Light supply and the corresponding ability for photosynthetic O₂ production modulate oxygen levels within the embryo. The O₂ increase in light versus darkness is relatively low at earlier but higher in later developmental stages (Fig. 3). Lower O₂ levels in young embryos can principally be explained by lower photosynthetic activity. Because embryos become fully green only during the later stage it is concluded that photosynthetic activity is still low in early embryos and increases during development. The gross photosynthetic rates are about one-third of the corresponding respiration rate. In addition to photosynthesis, changes in respiration activity and seed coat permeability (diffusive O₂ influx) could affect internal O₂ levels. Respiration had been measured in pea embryos of >200 mg (Kolöffel and Matthews, 1983). These authors found that O₂ uptake of cotyledons increased, reaching the highest levels during the storage phase and then declined when desiccation starts (Kolöffel and Matthews, 1983). However, whether respiration in the very early stages is higher than in later ones remains unclear. In addition, respiration is regulated by mechanisms other than seed age, for example, by temperature and light (Ribas-Carbo et al., 2000) as well as CO₂-level (Wager, 1974b).

Low gas exchange between maternal and filial parts of seeds is a common feature found in several species. Possibly the low gas exchange is helpful for refixation of respired CO₂ which would otherwise escape from the seed. Accordingly, it has been shown that CO₂ levels are elevated within seeds up to 11% v/v (Wager, 1974a). High CO₂ promotes phosphoenolpyruvate carboxylase which is high in both pod walls and seeds of legumes and catalyses CO₂ refixation (Harvey et al., 1976; Wager, 1974b; Flinn, 1985; Golombek et al., 1999). Minimizing CO₂ loss is therefore important for the carbon economy of the seed (Flinn, 1985).

**Low oxygen effects on seed metabolism**

It is demonstrated here that legume embryos develop in a hypoxic environment. This could affect the overall metabolic state with respect to energy status, adenylate nucleotides and the levels of key metabolic precursors. Adenylate energy charge as well as ATP levels are lowest in early embryos concomitantly with lowest O₂ levels. A similar nucleotide pattern has been reported for soybean seeds (Quebedeaux, 1981). This suggests that early embryo growth may be energy-limited and that respiration does not cover energy demand. However, there is no evidence for a significant induction of the fermentative pathway at this time of development (and later on). Energy status is much higher during the storage phase. Energy charge only shows a slight depression between 150–600 mg of embryo weight, but never falls below 0.6. It is concluded that except for the youngest stages, energy supply does not become limiting due to insufficient oxygen supply. In accordance, the levels of adenylate and uridinylate sugars are stable without large fluctuations.

The lowest oxygen levels measured here within embryos are still sufficient for cytochrome oxidase due to its very high affinity for oxygen (0.013%, Drew, 1997). However it has been shown that already at 1–5% of oxygen concentration within tissues metabolic adaptations occur (Geigenberger et al., 2000). Thereby, the low O₂ content found here could represent a signal which induces adaptive energy-saving metabolic responses, including reduced respiratory and glycolytic activity. The change from an invertase to a sucrose synthase pathway of sucrose degradation occurs during the switch from the pre-storage to the storage phase (Weber et al., 1995, 1996). Sucrose synthase saves one ATP as compared to invertase and, therefore, represents an energy-saving mechanism of sucrose breakdown. In maize roots, low oxygen leads to a rapid repression of the invertase to
Embryo tissue does not become anoxic during development

Although O₂ falls to very low levels in darkness, anoxic zones could not be detected within Vicia or pea seeds. Anoxia only occurs under experimental conditions when seeds are aerated with 100% nitrogen. Accordingly, no significant induction of fermentative pathways was detected throughout embryo development. Anoxia has to be avoided by the plant because of its detrimental effects on cell metabolism (Andrews et al., 1994; Drew, 1997; Zeng et al., 1999; Geigenberger et al., 2000). Among others, anoxia interrupts nutrient supply and biosynthetic activity (Thorne, 1982). Oxygen content within Vicia embryos in darkness is strikingly stable at a basal level of ~2–3% (Figs 1A, 4B). To maintain such a minimum O₂ level within embryonic tissue either O₂ influx is sufficient or O₂ consumption is adapted to supply rates. Seeds may possess a mechanism to buffer O₂ at a minimum level, mediated by non-symbiotic haemoglobins (Hill, 1998). These proteins are induced by low O₂. Under higher O₂ pressure they are oxygenated but release O₂ at lower partial pressure. Thereby haemoglobins could also act as a sensor connecting the fall of O₂ to metabolic adaptations. The mechanism could help to avoid anoxia when O₂ becomes limited (Sowa et al., 1998). Experiments with transformed maize cells indicate that haemoglobins act to improve the energy status of cells under low oxygen stress (Hill, 1998).

In summary, oxygen falls to very low levels inside developing embryos. They maintain a minimum level which has been shown to affect the overall rate of metabolism. That would mean oxygen plays a limiting, i.e. regulatory, role in seed development. Mechanisms of both sensing the oxygen and adaptive responses preventing anoxia have to be further investigated.

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References


