



Mobilization of Endosperm Reserves in Cereal Seeds under Anoxia

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The great majority of seeds from higher plants fail to germinate under anoxic conditions. While the metabolic pathways operating during the aerobic process of seed germination are well described, there is only limited evidence concerning metabolic pathways operating under conditions of limited oxygen availability. This is despite the importance of carbohydrate availability for plant tolerance to anaerobic conditions.

We review and discuss carbohydrate metabolism in cereal seeds and seedlings subjected to anaerobic conditions. Analysis of recent results reveals that, among cereal seeds, only rice is able to degrade the starchy endosperm. This is achieved by the concerted action of the complete set of amylolytic enzymes. In anoxic wheat and barley seeds, the enzymes involved in starch degradation are either absent or present as inactive forms. Differences are also observed in the metabolism of soluble carbohydrates and in status of related enzymes, with rice showing anoxia-enhanced activities of sucrose synthase, fructokinase, glucose-6P dehydrogenase, and nucleoside diphosphate kinase. The possible occurrence of a sucrose synthase pathway for sucrose utilization under anoxia is discussed.

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Key words: *Hordeum vulgare* L., *Oryza sativa* L., *Triticum aestivum* L., anoxia, barley, carbohydrate metabolism, fermentative metabolism, rice, wheat.

INTRODUCTION

Cereals play a central role in human nutrition. Consequently cereal seed germination is one of the most widely studied physiological processes, and a large amount of information is available concerning the pathways of starch and sucrose metabolism (Beck and Ziegler, 1989; Fincher, 1989; ap Rees, 1992). The starchy endosperm contains most of the nutrients needed for the growth and development of germinating cereal seedlings. The majority of published papers deal with the aerobic process of seed germination and only a small number are devoted to pathways operating under conditions of limited oxygen availability. This is despite the importance of carbohydrate availability for plant tolerance to anaerobic conditions (Mohanty, Wilson and ap Rees, 1993; Perata and Alpi, 1993; Armstrong, Brändle and Jackson, 1994; Ricard *et al.*, 1994). The availability of readily fermentable carbohydrates is essential for the operation of the glycolytic pathway leading to ATP production in plants under hypoxic or anoxic conditions (Perata and Alpi 1993; Armstrong *et al.*, 1994; Hanhijarvi and Fagerstedt, 1995). Nevertheless, the amount of soluble sugars in dry cereal grains is usually very limited, starch being the main reserve carbohydrate. It is therefore inferred that the metabolism of starch is of primary importance for seed tolerance to anaerobic conditions. Moreover, the metabolism of sucrose, translocated to the developing parts of the seedling, is also required for the operation of the fermentative pathway in the growing embryo axis.

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CEREAL SEED GERMINATION UNDER ANOXIA

The great majority of seeds from higher plants fail to germinate under anoxic conditions (Kennedy, Rumpho and Fox, 1992; Perata and Alpi 1993). Some examples of plant species able to germinate without oxygen are the water plants *Trapa natans* L., *Nuphar luteum* L. and *Scirpus mucronatus* L. (Menegus *et al.*, 1992), *Erithrina caffra* (Small, Potgieter and Botha, 1989) and some *Echinochloa* species (Kennedy *et al.*, 1980, 1992). However, among cereals, only rice shows the peculiar ability to germinate and elongate the coleoptile when strictly anoxic (Kordan, 1972; Alpi and Beevers, 1983; Perata and Alpi, 1993).

Results of Raymond, Al Ani and Pradet (1985) indicate that starchy seeds are particularly tolerant to anaerobiosis. This is probably due to their ability to maintain a higher energy metabolism under conditions of anaerobiosis compared to fatty seeds. Therefore, the ability to maintain an active fermentative metabolism by fuelling the glycolytic pathway with readily fermentable carbohydrates (Atwell and Greenway, 1987; Perata *et al.*, 1992) is likely to be of importance in conferring tolerance to anaerobic conditions (Al-Ani *et al.*, 1985; Raymond *et al.*, 1985). However, among cereals, wheat, oat, rye and barley do not germinate under anoxia and only rice germinates, showing rapid coleoptile elongation but no root emergence (Taylor, 1946; Kordan, 1972; Alpi and Beevers, 1983; Perata *et al.*, 1992). This different behaviour suggests that the presence of starch is not itself enough to ensure tolerance to anaerobiosis presumably because it is not readily utilizable. Moreover,

the amount of readily fermentable carbohydrates in the dry cereal seeds is, in many cases, usually limited.

STARCH DEGRADATION UNDER ANOXIA

While rice is able to degrade starch *in vivo* under anoxia, wheat and barley fail to do so (Atwell and Greenway, 1987; Perata *et al.*, 1992; our unpubl. res.). Evidence for starch breakdown and utilization in *Echinochloa crus-galli*, another plant species showing the ability to germinate under anoxia, was shown by Kennedy *et al.* (1980) from the analysis of the starch granules present in the endosperm. The inability of wheat seeds to break down starch under anoxia correlates with the inability to germinate under these conditions and it was also shown that wheat seeds can germinate under anoxia if exogenously fed with glucose or sucrose (Perata *et al.*, 1992).

The different behaviour of rice when compared with other cereal seeds in their ability to degrade starch under anoxia is primarily the consequence of the failure of wheat and barley seeds to respond to gibberellic acid under anoxic conditions, thus failing to produce starch-degrading α -amylase, while successful induction takes place in the anoxic

rice seeds (Perata *et al.*, 1993). However, in addition to α -amylase, the action of other enzymes, namely β -amylase, debranching enzyme and α -glucosidase are also needed to complete the process, leading to the production of glucose units. The complete set of enzymes needed to degrade starch completely was found to be present in anoxic rice seedlings (Guglielminetti *et al.*, 1995b; Table 1).

The two enzymes able to degrade native starch granules, α -amylase and α -glucosidase (Dunn, 1974; Sun and Henson, 1990), were absent in samples from anaerobic wheat and barley (Guglielminetti *et al.*, 1995b; Table 1) but present in rice. Debranching enzyme is also present in rice but absent from anoxic wheat and barley seeds. β -amylase is present in anaerobic wheat and barley, but the enzyme remains in the bound form (Sopanen and Lauriere, 1989). The starch granule-bound form of β -amylase has a very low ability to degrade soluble starch, and this can play no role in *in vivo* starch degradation (Sopanen and Lauriere, 1989; Guglielminetti *et al.*, 1995b; Table 1). As a consequence, only rice, showing an exceptional tolerance to anoxia, is able to carry out the entire process of starch degradation under anoxia. This is due to the successful production, or activation, of the full set of enzymes involved in the

TABLE 1. Comparison of the status of the complete set of amylolytic enzymes in cereal seeds under aerobic or anaerobic conditions. The comments in the table are based on data from Guglielminetti *et al.*, 1995b

Rice	Wheat	Barley
Total amylolytic activity <i>Dry seed</i> : very low activity. <i>Aerobic conditions</i> : activity increases as germination proceeds. <i>Anoxic conditions</i> : activity increases as germination proceeds.	<i>Dry seed</i> : very low activity. <i>Aerobic conditions</i> : activity increases as germination proceeds. <i>Anoxic conditions</i> : very low activity.	<i>Dry seed</i> : very low activity. <i>Aerobic conditions</i> : activity increases as germination proceeds. <i>Anoxic conditions</i> : very low activity.
α -Amylase <i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : activity increases as germination proceeds. <i>Anoxic conditions</i> : activity increases as germination proceeds, but at a slower rate when compared with the aerobic control.	<i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : activity increases as germination proceeds. <i>Anoxic conditions</i> : no activity detected.	<i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : activity increases as germination proceeds. <i>Anoxic conditions</i> : no activity detected.
β -Amylase <i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : activity increases as germination proceeds. <i>Anoxic conditions</i> : the enzyme is produced and activity increases as germination proceeds, but at a slower rate when compared with the aerobic control.	<i>Dry seed</i> : present in dry seed but in the bound form only. <i>Aerobic conditions</i> : activity increases as germination proceeds. The free form is released. <i>Anoxic conditions</i> : the free form is not released.	<i>Dry seed</i> : present in dry seed but in the bound form only (Sopanen and Lauriere, 1989). <i>Aerobic conditions</i> : activity increases as germination proceeds. The free form is released. <i>Anoxic conditions</i> : the free form is not released.
α -Glucosidase <i>Dry seed</i> : the enzyme is present as a latent form. <i>Aerobic conditions</i> : the enzyme is activated during germination. <i>Anoxic conditions</i> : the enzyme is activated during germination.	<i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : the enzyme is produced and activity increases as germination proceeds. <i>Anoxic conditions</i> : no activity detected.	<i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : the enzyme is produced and activity increases as germination proceeds. <i>Anoxic conditions</i> : no activity detected.
Debranching enzyme <i>Dry seed</i> : present as a latent, inactive form (Toguri 1991; Yamada 1981). <i>Aerobic conditions</i> : the enzyme is activated during germination. <i>Anoxic conditions</i> : the enzyme is activated during germination.	<i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : the enzyme is produced and activity increases as germination proceeds. <i>Anoxic conditions</i> : no activity detected.	<i>Dry seed</i> : no activity detected. <i>Aerobic conditions</i> : the enzyme is produced and activity increases as germination proceeds. <i>Anoxic conditions</i> : no activity detected.

TABLE 2. Comparison of the status of the enzymes involved in the pathway of alcoholic fermentation in cereal seeds under aerobic or anaerobic conditions

Rice	Wheat	Barley
<p>Alcohol dehydrogenase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity decreases as aerobic germination proceeds.</p> <p><i>Anoxic conditions:</i> a strong increase in the activity of the enzyme is observed in the coleoptile, and in the embryo, as anoxic germination proceeds.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity decreases as aerobic germination proceeds.</p> <p><i>Anoxic conditions:</i> an increase in the activity of the enzyme is observed in the embryo during the anoxic treatment.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity decreases as aerobic germination proceeds.</p> <p><i>Anoxic conditions:</i> an increase in the activity of the enzyme is observed in the embryo during the anoxic treatment.</p>
<p>Pyruvate decarboxylase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity does not increase as aerobic germination proceeds.</p> <p><i>Anoxic conditions:</i> an increase in the activity of the enzyme is observed in the coleoptile and in the embryo as anoxic germination proceeds.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity does not increase as aerobic germination proceeds.</p> <p><i>Anoxic conditions:</i> an increase in the activity of the enzyme is observed in the embryo during the anoxic treatment.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity does not increase as aerobic germination proceeds.</p> <p><i>Anoxic conditions:</i> an increase in the activity of the enzyme is observed in the embryo during the anoxic treatment.</p>

metabolism of starch. β -amylase plays no role in native starch granule degradation (Dunn, 1974) and, in wheat and barley is present only in the bound, high molecular weight form under anoxia.

Wheat and barley are, however, metabolically active under anoxic conditions. An active lactic and alcoholic fermentation was observed in anoxic barley half-seeds (Hanson and Jacobsen, 1984), possibly resulting from the utilization of the soluble sugars present in the dry seeds. Our preliminary results indicate that soluble sugars are rapidly used and converted to ethanol in anoxic wheat and barley seeds, suggesting that the synthesis and activation of starch degrading enzymes are somehow specifically inhibited under anoxia in wheat and barley, whereas this does not happen in rice. However, induction of fermentative enzymes takes place normally in barley and wheat seeds under anoxia (Table 2), suggesting that protein synthesis could be active under anaerobic conditions.

A physiological explanation for the different behaviour observed in rice when compared with anoxia-intolerant cereals has been proposed (Hanson and Jacobsen, 1984; Perata *et al.*, 1992): only rice has the ability to elongate the coleoptile under flooding conditions, thus allowing oxygen to be transported to the flooded seedling tissues. This process requires energy, obtained through the fermentative pathway (Perata and Alpi, 1993), but the small amount of soluble sugars present in the dry seed would allow this ATP-producing pathway to operate for only a few days. Thereafter, access to the starchy reserves present in the endosperm is needed to provide glucose for more prolonged fermentative metabolism that would support coleoptile growth (Perata *et al.*, 1992; Guglielminetti, Perata and Alpi, 1995a). Both wheat and barley seed may be able to elongate the coleoptile under flooded conditions if starch degradation were possible under anoxia. It may also be true that even with available sugar, coleoptile extension by wheat and barley may be inhibited by anoxia for other reasons. It is worth noting that ungerminated seeds can withstand longer periods of anoxia when compared to the germinated seeds

(unpubl. obs.) and anoxia-intolerant species could therefore take advantage of a delayed germination when the amount of oxygen present in the environment would not allow normal seedling growth.

EFFECTS OF ANOXIA ON THE INDUCTION OF α -AMYLASE

The induction of amylolytic activity in stems of partially submerged rice (deep-water rice) has been described by Kende and associates (Raskin and Kende, 1984; Smith, Jacobsen and Kende, 1987). These rice varieties show a stem-growth rate of 20–25 cm d⁻¹ responding to the rising of the water level (Raskin and Kende, 1983). The induction of amylolytic activity allows the degradation of the starchy reserves present in the internodes into readily fermentable carbohydrates providing energy to support the enhanced elongation of deep-water rice when submerged (Raskin and Kende, 1984; Smith *et al.*, 1987). The induction of amylolytic activity has also been reported in flooded tobacco plants (Hung and Kao, 1993).

During germination, α -amylase synthesis in anoxia (see Fig. 1) allows rice to degrade the starchy reserves present in the endosperm (Atwell and Greenway, 1987; Perata *et al.*, 1992), thus obtaining readily-fermentable carbohydrates providing ATP for the germinating embryo, and this contributes to rice tolerance to anaerobic conditions (Perata *et al.*, 1992). In cereal seeds, the synthesis of α -amylase is controlled, at the transcriptional level, by the plant hormone gibberellic acid (Akazawa, Mitsui and Hayashi, 1988). Rice embryoless half-seeds are able to respond to GA₃, consequently producing and translating α -amylase mRNA under anoxic conditions while both wheat and barley are unable to respond in these ways to the hormone when anaerobic (Perata *et al.*, 1993). Lactic and ethanolic fermentation have been observed in aleurone layers of barley (cv. Himalaya) incubated under anoxic conditions and both alcohol dehydrogenase and lactate dehydrogenase activities are induced during anaerobiosis in barley aleurone layers

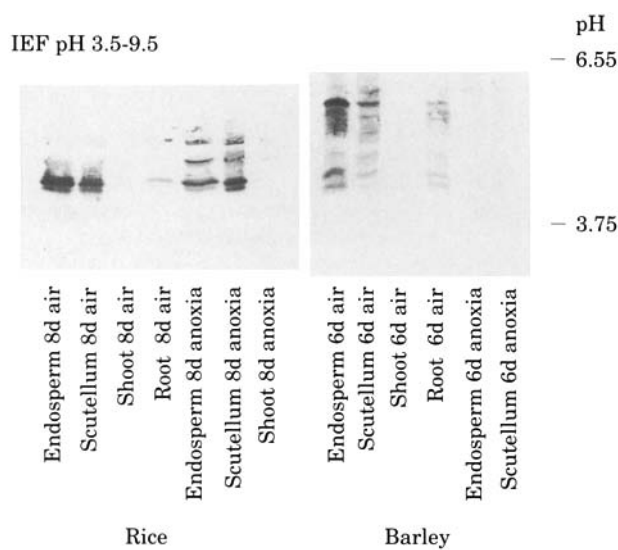


FIG. 1. Pattern of α -amylase isozymes from seeds of rice and barley kept under aerobic (air) or anaerobic (anoxia) conditions for the time indicated. Seeds and seedlings were dissected, and extracts subjected to isoelectrofocusing (pH range 3.5–9.5) prior to immunoblotting using α -amylase specific antibodies (Perata *et al.*, 1992). α -amylase isozymes are detectable in both aerobic and anoxic samples of rice, with a preferential localization of the enzyme in the endosperm and scutellar tissues. The significance of the slightly different isoenzymatic pattern observed under anoxia is presently unknown. In barley, α -amylase isozymes were detectable in the aerobic samples only, confirming the inability of barley to produce α -amylase under anoxia (Perata *et al.*, 1993).

(Hanson and Jacobsen, 1984). A 100-fold increase in the level of alcohol dehydrogenase mRNA was observed after about 1 d under anoxic conditions (Hanson, Jacobsen and Zwar, 1984), suggesting active transcription. Therefore, the

inhibitory effect of anoxia on the transcription of the α -amylase gene is not a general effect of anaerobiosis on gene transcription. The failure of α -amylase mRNA to accumulate under anoxia in barley may result from either an inhibition of the transcription of α -amylase genes or from a rapid degradation of the newly synthesized α -amylase mRNA after GA₃ application under anoxia (Perata *et al.*, 1993). The half-life of α -amylase mRNA was estimated to be about 100 h in barley aleurone layers (Belanger, Brodl and Ho, 1986) and, results from Perata *et al.* (1993) do not indicate an enhanced degradation of α -amylase mRNA under anoxia as reported for heat-shock stress (Belanger *et al.*, 1986; Brodl and Ho, 1991). Thus inhibition of transcription seems to explain the failure of α -amylase mRNA accumulation in anoxic barley seeds. It is not known how anoxia inhibits this α -amylase mRNA accumulation nor why rice seeds can circumvent this inhibitory effect. Among numerous possibilities Perata *et al.* (1993) proposed a series of hypotheses. A more efficient anaerobic metabolism may operate in the rice aleurone, or a differential abscisic acid production and/or sensitivity to ethanol may exist between rice and barley seeds under anoxic conditions. Indeed, the data summarized and discussed in this paper suggest that several enzymes involved in the metabolism of carbohydrates have anoxia-enhanced activities only in rice. Thus, it is tempting to speculate that this may well result in a more efficient utilization of carbohydrates under anoxia. Moreover, α -amylase production by barley aleurone layers is depressed in the presence of relatively low ethanol concentrations (Perata, Alpi and LoSchiavo, 1986), and this metabolite is detectable in considerable amounts in anoxic barley seeds. Additional research is therefore needed to elucidate further this aspect.

TABLE 3. Comparison of the status of the principal enzymes involved in sucrose degradation in cereal seeds under aerobic or anaerobic conditions. For rice, the comments reported in the table are based on data from Guglielminetti *et al.*, (1995a)

Rice	Wheat	Barley
Invertase <i>Dry seed:</i> very low activity detected. <i>Aerobic conditions:</i> activity increases as germination proceeds. <i>Anoxic conditions:</i> a limited increase in the activity of the enzyme is observed (coleoptile) as germination proceeds.	<i>Dry seed:</i> very low activity detected. <i>Aerobic conditions:</i> activity increases as germination proceeds. <i>Anoxic conditions:</i> very low activity detected.	<i>Dry seed:</i> very low activity detected. <i>Aerobic conditions:</i> activity increases as germination proceeds. <i>Anoxic conditions:</i> very low activity detected.
Sucrose synthase <i>Dry seed:</i> activity detected. <i>Aerobic conditions:</i> activity increases as germination proceeds. <i>Anoxic conditions:</i> activity increases as germination proceeds. Activity in the anoxic embryo and coleoptile is higher than in the same tissues under aerobic conditions.	<i>Dry seed:</i> activity detected. <i>Aerobic conditions:</i> activity increases in growing tissues as germination proceeds. <i>Anoxic conditions:</i> activity increases in the anoxic embryo only.	<i>Dry seed:</i> activity detected. <i>Aerobic conditions:</i> activity increases in growing tissues as germination proceeds. <i>Anoxic conditions:</i> activity increases in the anoxic embryo only.
Invertase vs. sucrose synthase The activity of sucrose synthase is five times higher than invertase activity in the aerobic coleoptile. This ratio increases to 30:1 (sucrose synthase:invertase) in the anoxic coleoptile.	The activity of sucrose synthase is slightly lower than invertase activity in the aerobic coleoptile and a comparable activity was found in the aerobic embryo. This ratio changes to 4:1 (sucrose synthase:invertase) in the anoxic embryo.	The activity of sucrose synthase is slightly lower than invertase activity in the aerobic coleoptile and a comparable activity was found in the aerobic embryo. This ratio changes to 4:1 (sucrose synthase:invertase) in the anoxic embryo.

TABLE 4. Comparison of the status of the additional enzymes involved in sucrose degradation in cereal seeds under aerobic or anaerobic conditions. For rice the comments reported in the table are based on data from Guglielminetti et al., (1995a)

Rice	Wheat	Barley
<p>Glucokinase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity increases as germination proceeds. Activity in the anoxic embryo and coleoptile is higher than in the same tissues under aerobic conditions.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase during the anaerobic treatment.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase during the anaerobic treatment.</p>
<p>Fructokinase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity increases as germination proceeds. Activity in the anoxic embryo and coleoptile is higher than in the same tissues under aerobic conditions.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase during the anaerobic treatment.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase during the anaerobic treatment.</p>
<p>Glc-6-P Isomerase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity increases as germination proceeds. Activity in the anoxic seedling is slightly higher than under aerobic conditions.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases slightly as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases slightly as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase.</p>
<p>Phosphoglucomutase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity increases as germination proceeds.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase.</p>
<p>UDP-Glc Pyrophosphorylase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity increases as germination proceeds.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase.</p>
<p>Nucleoside diphosphate kinase</p> <p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity increases as germination proceeds.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> activity does not increase.</p>	<p><i>Dry seed:</i> activity detected.</p> <p><i>Aerobic conditions:</i> activity increases as germination proceeds.</p> <p><i>Anoxic conditions:</i> Activity does not increase.</p>

THE METABOLISM OF SOLUBLE CARBOHYDRATES IN CEREAL SEEDS UNDER ANOXIA

Little information is available concerning the anaerobic fate of the soluble carbohydrates either originally present in the dry seed or resulting from starch degradation during imbibition and germination. Mayne and Kende (1986) found that rice seedlings aerobically germinated and subsequently transferred to anaerobic conditions are able to metabolize glucose at a rate similar to that of the tissue incubated under aerobic conditions, indicating that anoxia does not interfere with the potential for glucose metabolism in rice. Our recent results (Guglielminetti *et al.*, 1995a) indicate that two phases can be recognized in the metabolism of carbohydrates in rice seeds germinating under anoxia. The metabolism of the sugars present in the dry seed is

mainly degradative during the first days after imbibition, while, after the induction of α -amylase has taken place, an increased concentration of glucose and sucrose indicates that starch breakdown and sucrose synthesis are occurring in the anaerobic seedling. In wheat and barley seeds kept under anoxic conditions, only the first phase can be observed, characterized by the progressive degradation of all the small amount of soluble sugar reserves present in the dry seeds (our preliminary observation).

Vartapetian, Andreeva and Kozlova (1976) reported that mitochondria from excised rice coleoptiles are degraded. This can be avoided by supplying glucose to the excised coleoptiles, suggesting the importance of the fuelling of anaerobic metabolism with readily fermentable carbohydrates. Vartapetian *et al.* (1976) therefore proposed that rice coleoptiles are tolerant to anoxia as a consequence of receiving organic compounds transported from the seed.

unable to utilize the massive starchy reserves, due to the inability to respond to gibberellic acid under anoxia, resulting in the failure to induce α -amylase. Furthermore, the other enzymes involved in starch degradation are either absent, or present in an inactive form in the two cereals unable to germinate without oxygen. The set of enzymatic activities needed for the metabolism of the soluble sugars, either present in the dry seed or resulting from starch breakdown, is available in the anoxic rice seedling. Sucrose is synthesized under anoxia in rice and its metabolism probably occurs through the sucrose synthase pathway (Fig. 2). It is not currently known whether the inability of wheat and barley to increase the activity of several enzymes involved in the metabolism of soluble carbohydrates would affect the efficiency of the anaerobic metabolism in these seeds. However, an inability of cereal seeds such as wheat and barley to degrade starch under anoxia coupled with the rapid consumption through fermentation of the soluble sugars present in the dry seed, results in a rapid sugar starvation that must compromise seed germination and viability.

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