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REVIEW

Carbohydrate metabolism and anoxia tolerance in cereal grains

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INTRODUCTION

Few plant species can survive when the availability of oxygen drops below the needs of aerobic respiration. Under these conditions, arising frequently from soil flooding, a limited number of plant species can avoid a rapid death, thanks to morphological and metabolic adaptations allowing them to avoid or tolerate the anaerobic stress. In the recent years, a number of reviews about plant responses to anaerobiosis have been published (Drew 1990; Kennedy *et al.* 1992; Perata & Alpi 1993; Armstrong *et al.* 1994; Crawford & Braendle 1996; Drew 1997). This review will therefore not include reference to many aspects on this subject but will rather focus on the response of a group of cultivated plants (cereals), with particular emphasis on their carbohydrate metabolism during grain germination. Indeed, in recent years, increasing attention has been paid to the possible role of carbohydrates availability and utilization in conferring anoxia

tolerance (Perata & Alpi 1993; Armstrong *et al.* 1994; Ricard *et al.* 1994; Hanhijärvi & Fagerstedt 1995).

One of the critical aspects when reviewing data about stress physiology arises from the non-homogeneous conditions used by researchers to impose the stress condition. Anoxic/hypoxic conditions can be obtained by incubating the plant material in anaerobic incubators, in flasks flushed with pure nitrogen gas, or by flooding under air-free water or buffer. All these methods are reliable ones, but the data obtained using different methods are not easy to compare (e.g. leakage of metabolites is different depending on the volume of liquid media in which the plant material is incubated, and obviously the volume is greater when using flooding as a method to get the anaerobic environment). An additional source of variability arises from the heterogeneous variety of plant material used: dry seeds/grains incubated under anoxia since the imbibition time, aerobically germinated seedlings transferred to anoxia, dissected tissues (coleoptiles, roots, root tips, etc.). This is fully justified by the different hypotheses to be tested, but comparison of data obtained from different plant species/cultivar/tissues is hardly possible. Most of the data discussed in this review were obtained in the author's laboratory using comparable anoxia-treatment conditions (pure nitrogen gas atmosphere) and thus allowing an unambiguous comparison of data.

ANOXIA-TOLERANT AND INTOLERANT CEREALS

A few plant species show tolerance to relatively prolonged anaerobic conditions (see Perata & Alpi 1993; Crawford & Braendle 1996 for recent reviews). Among cereals, only rice can germinate under anoxia, showing coleoptile elongation (Alpi & Beevers 1983). A similar behaviour is observed in some species of the rice field weed *Echinochloa* (Kennedy *et al.* 1992). The physiology of *Echinochloa* has been reviewed elsewhere, and we will therefore not include reference to this interesting plant species (Kennedy *et al.* 1992).

While all cereals but rice show reduced anoxia tolerance, maize, barley and wheat are those most frequently used as experimental material, and we will therefore make reference to these cereals as anoxia-intolerant when compared to the anoxia-tolerant rice.

ENERGY PRODUCTION UNDER ANOXIA

Reduced oxygen availability results in a variety of responses in the plant. Gene expression will be redirected to the synthesis of a specific set of polypeptides (Sachs *et al.* 1980; Mujer *et al.* 1993; Perata & Alpi 1993; Ricard *et al.* 1994), anatomical modification will occur and the whole metabolism will be seriously affected (Drew 1997 for a recent review). However, there is no doubt concerning the metabolic pathway that will be affected more profoundly: respiration. In the absence of oxygen aerobic respiration cannot proceed, and ATP production will drop from the 32 ATP moles produced for each mole of glucose (Taiz & Zeiger 1991) to only 2 ATP moles under anaerobiosis, about 6% of the aerobic value. This calculation is valid assuming the conversion of glucose into ethanol (see below) but it should be remembered that glucose represents an available substrate for the anaerobic metabolism only in few cases. In most cases, starch and sucrose are the real substrates for plant metabolism, either under aerobic and anaerobic conditions.

ENERGY PRODUCTION THROUGH FERMENTATION

When oxygen is not available for the reoxydation of NADH, not only ATP production through mitochondrial respiration is hampered, but also the glycolytic flux may slow down unless an alternative way for the reoxydation of NADH is operative. This is usually achieved by plants through the fermentative metabolism, leading to an initial production of lactic acid (lactate dehydrogenase-driven, allowing the reoxydation of NADH to NAD+) followed by the massive production of ethanol (driven by the action of pyruvate decarboxylase and alcohol dehydrogenase, the latter allowing the reoxydation of NADH to NAD+). Details about the regulation of the fermentative pathways, as well as about the possible self-poisoning effects resulting from the accumulation of the products of fermentation, have been reviewed in detail elsewhere (Perata & Alpi 1993, Drew 1997). Assuming that the production of ethanol is not poisonous to cereal grains/seedlings (Alpi *et al.* 1985), this pathway can provide energy to the anaerobic cell through the conversion glucose $\rightarrow$2 ethanol +2 CO_2+2ATP.

THE SUBSTRATES FOR FERMENTATION

Glucose is efficiently metabolized in rice seedlings kept under anoxia (Mayne & Kende 1986; Atwell & Greenway 1987). Indeed, the classical 'textbook' substrate for fermentation is obviously glucose, but this sugar is usually not a stored carbohydrate but results from starch degradation (the starchy endosperm, when considering cereal grains). The site of starch degradation does not necessarily coincide with the site of carbohydrate utilization (fermentation, in the embryo when considering cereal grains). Therefore, only a minor part of glucose resulting from starch breakdown will be fermented in cells near the site of starch storage, while the major part of glucose units must be transported to the other plant organs where starch is not stored but where energy production through fermentation is needed for survival. Sugars are usually transported as sucrose in plants. Sucrose synthesis, transport and degradation is thus needed to provide hexose units for fermentation. This pathway can be summarized as follows:

Starch degradation $\rightarrow$ glucose $\rightarrow$ sucrose synthesis and transport $\rightarrow$ sucrose degradation $\rightarrow$ fermentation of the hexoses.

The amount of energy produced by this pathway can deviate considerably from the theoretical 2 ATP/glucose: energy is consumed to synthesize sucrose, and the amount of energy stored in sucrose may be partly wasted depending on the pathway of sucrose degradation. Differences in the glycolytic pathway may also affect the amount of ATP produced (see below).

Fructans were found to accumulate in plants subjected to flooding (Albrecht *et al.* 1997). This is possibly an adaptative response for plants germinated under aerobic conditions and experiencing a transient period of low oxygen availability due to soil flooding (see Albrecht *et al.* 1997). We will not discuss this subject since fructans, when compared to starch, represent a minor source of carbohydrates in cereal grains.

THE FIRST STEP: STARCH DEGRADATION UNDER ANOXIA

Cereal grains store starch as the main reserve compound. This polysaccharide is stored in the starchy endosperm, and is hydrolysed during germination to provide soluble

carbohydrates (mainly glucose) to the germinating embryo. The process of starch degradation is regulated by a complex mechanism involving both hormonal and metabolic regulation (Perata *et al.* 1997b and references therein). A set of enzymes is produced to carry on starch breakdown: α-amylase, β-amylase, debranching enzyme and α-glucosidase (Dunn 1974; Sun & Henson 1991). Most of the information we have concerning the importance of a metabolic pathway in cereal grains under anoxia arises from a comparison of the enzymatic set present in the anoxia-tolerant rice with that of anoxia-intolerant cereals (wheat, barley) kept under either aerobic or anaerobic conditions. While this is merely a correlative approach, the conclusions obtained as far as starch degradation is concerned are quite convincing. Rice grains can induce α-amylase even under anoxia, while the anoxia-intolerant cereals fail to produce this enzyme, which plays a crucial role in the initiation of starch degradation (Perata *et al.* 1992; Guglielminetti *et al.* 1995b). Moreover, also the other enzymes needed to complete starch degradation are present in the anoxic rice grain, while they are either absent or inactive in the other cereals (Guglielminetti *et al.* 1995b). The final result is an efficient starch degradation in rice under anoxia, while the anoxia-intolerant cereals fail to degrade starch, and therefore cannot utilize the starchy reserves. This results in sugar starvation, eventually leading to death (Perata *et al.* 1996). In the forthcoming paragraphs the relative importance of the various starch degrading enzymes will be analysed further.

STARCH DEGRADATION UNDER ANOXIA: α-AMYLASE

Although both α-glucosidase and α-amylase are able to degrade native starch granules, the latter enzyme is considered to play a major role in this process, and is therefore the key enzyme for starch degradation (Dunn 1974; Sun & Henson 1991). A detailed review on the effects of anoxia on the induction of α-amylase has recently been published (Perata *et al.* 1997a), and we will therefore only summarize a few considerations about this subject:

- α-Amylase is produced in rice seeds under anoxia (Perata *et al.* 1992), while it is not induced in the anoxia-intolerant cereals (wheat, barley) (Guglieminetti *et al.* 1995b)
- The successful induction of α-amylase in rice is probably responsible for the subsequent successful degradation of starch taking place in the endosperm, as the other starch degrading enzymes are unlikely to be able to initiate the process of starch degradation.
- In the absence of α-amylase induction (anoxia-intolerant cereals: wheat, barley) starch is not degraded, and the grains suffer soon from sugar starvation (Perata *et al.* 1996).
- Anoxic rice embryoless half-grains respond to exogenous gibberellic acid (GA), while wheat and barley are insensitive to the hormone under anoxia (Perata *et al.* 1993).
- The induction of α-amylase in rice seeds under anoxia is GA-dependent (Perata *et al.* 1993), and this implies that GAs are either produced or already present in the grains. This subject has received little attention and would be worth a careful investigation.

STARCH DEGRADATION UNDER ANOXIA: β-AMYLASE

β-Amylase is synthesized de novo in the anoxic rice grains (Guglielminetti *et al.* 1995b). In the anoxia-intolerant cereals such as wheat and barley the enzyme is present in the dry seed, stored as a starch-bound form (Hara-Nishimura *et al.* 1986; Lauriere *et al.* 1986; Sopanen & Lauriere 1989). Release of the bound form to the free, active enzyme is triggered by an endoproteolytic activity that cleaves a 5 kD peptide allowing the release of the free β-amylase (Sopanen & Lauriere 1989; Guerin *et al.* 1992; Grime & Briggs 1996; Loreti *et al.* 1998). However, under anoxia β-amylase remains in the bound form, and cannot play any role in starch degradation (Guglielminetti *et al.* 1995b). The failure to release β-amylase is probably related to the inability of these cereals to respond to GA, since at least one of the endoproteases involved in the process of β-amylase release (the endoprotease EP-A), is under hormonal control with GA playing an inductive role (Koehler & Ho 1990a,b; Loreti *et al.* 1998) and is not produced in barley grains kept under anoxia (Loreti *et al.* 1998). However, even assuming that β-amylase would be released under anoxia, this would have a minor impact on starch degradation:

- β-Amylase cannot degrade native starch granules (Sun & Henson 1991).
- Cereal mutants devoid of β-amylase show a normal germination under aerobic conditions (Daussant *et al.* 1981; Kreis *et al.* 1987), indicating that this enzyme plays a minor role even in the aerobic starch degradation.

STARCH DEGRADATION UNDER ANOXIA: DEBRANCHING ENZYME AND α-GLUCOSIDASE

In rice both debranching enzyme and α-glucosidase are present in the dry seed as latent, inactive forms, which are activated during germination under either aerobic or anaerobic conditions (Guglielminetti *et al.* 1995b). On the other hand, in the anoxia-intolerant cereals (wheat and barley) these enzymes are produced de novo during the aerobic germination, but are absent in the anoxic grains (Guglielminetti *et al.* 1995b). Overall, the presence of debranching enzyme and α-glucosidase appears to be somehow co-ordinated with that of α-amylase, since both enzymes degrade the products of α-amylase action. Since α-amylase is absent under anoxia in the anoxia-intolerant cereals, these enzymes are not produced. Little is known about the regulatory processes controlling the induction of these enzymes, and it is therefore not possible to discuss further the reasons behind their absence under anoxia in the anoxia-intolerant cereals.

STARCH DEGRADATION AND THE FERMENTATIVE METABOLISM

As reported above, only rice can degrade starch under anoxia, while the failure of wheat and barley to produce starch degrading enzymes hampers the utilization of the starchy reserves present in these grains. However, a limited amount of soluble carbohydrates is present in the dry cereal grains (Perata *et al.* 1996). Therefore, those cereals which are unable to carry on starch degradation under anoxia also show ethanol production when incubated under anaerobic conditions (our unpublished data). However, while in rice the rate of ethanol production is stable for several days, in barley and wheat it declines after a few days: the limited pool of soluble carbohydrate

reserves rapidly declines as it fuels the fermentative metabolism (our unpublished data). This behaviour is reflected in the ATP production rate. Raymond *et al.* (1985) calculated that in rice grains the ATP production is 12% of the aerobic rate during the first 4 h under anoxia, a value very close to that of wheat grains (10% of the aerobic rate). However, during the 24–48 h interval, in rice the anaerobic ATP production rises to 21% of the aerobic value while it drops to 5% in wheat. These differences can be explained by the drop of 'fuel' for the fermentative metabolism in the anoxia-intolerant cereals being unable to utilize the starchy reserves (Perata *et al.* 1996). The inability to induce α-amylase, a process usually taking place during the first 24–48 h of imbibition, may therefore arise from differences in the energy status of the cereal grains.

It is worth remembering that starch degradation by starch phosphorylase would allow an higher ATP production (3 moles/mole of glucose fermented), since no ATP is needed for the phosphorylation of the glucose units resulting from starch degradation (see Fig. 1). While the activity of starch phosphorylase is not detectable in cereal seeds (including anoxic rice), this starch degrading enzyme shows a 10-fold greater activity when compared to amylases in the rhizomes of the flood-tolerant bulrush (*Schoenoplectus lacustris* L.) (Steinmann & Braendle 1984), indicating that plants tolerant to anaerobiosis other than rice may save more ATP by using starch phosphorylases instead of amylases for anaerobic starch degradation (Fig. 1).

STARCH DEGRADATION AND SUCROSE SYNTHESIS

An increasing sucrose content is detected when analysing the carbohydrate content of rice grains germinating under anoxia (Guglielminetti *et al.* 1995a). This implies that sucrose is synthesized under anoxia in rice. On the other hand, sucrose is not synthesized in barley grains kept under anoxia (our unpublished data). Both these results are not surprising: in barley anoxia depresses the activity of sucrose phosphate synthase, the key enzyme for sucrose synthesis (our unpublished data), but even in the presence of an adequate enzymatic machinery, the absence of starch degradation (glucose production) is not compatible with the synthesis of sucrose, glucose and fructose being almost absent in the barley grain incubated under anoxia for a few days (Perata *et al.* 1996). In anoxic rice grains starch is degraded, glucose accumulates and sucrose can be synthesized (Guglielminetti *et al.* 1995a). Indeed, the level of sucrose phosphate synthase is not affected by anoxia in rice (our unpublished data). A few additional comments are needed, however: sucrose synthesis is an energy-consuming process (2 moles ATP needed for the synthesis of 1 mole of sucrose), and this is suggestive of the importance of sucrose translocation to the growing rice coleoptile (where it will be degraded and utilized through the fermentative pathway). Sucrose transport in the phloem does not appear to be negatively affected by anoxia, but sucrose unloading from the phloem is inhibited in maize roots (Saglio 1985); sugar transport under anoxia is poorly studied, but we may speculate that in rice coleoptiles sucrose transport/unloading is not negatively affected by anoxia, since feeding sugars to coleoptiles (or maintaining the coleoptiles attached to their natural source of sugars, see below) has positive effects on the survival of this tissue.

Glucose resulting from starch degradation is taken up by the epithelium cells of the scutellum, where sucrose synthesis takes place. Glucose fed embryos utilize only a minor part of the glucose supply for sucrose synthesis, while the great majority of glucose is metabolized through glycolysis and alcoholic fermentation (our unpublished

data). For each mole of sucrose synthesized (2 ATP moles consumed), one mole of glucose must be channelled through glycolysis/fermentation (2 ATP moles produced), but it is obviously expected that the rate of glucose utilization through glycolysis/fermentation will largely exceed that of sucrose synthesis, to provide enough energy for the survival of the scutellum cells.

THE PATHWAYS OF SUCROSE DEGRADATION

Sucrose can be degraded in the cytosol through the activity of two distinct pathways, one involving invertase and another with sucrose synthase as key enzyme (ap Rees 1992; see Fig. 2). Interestingly, one of the most heavily labelled proteins detected after two-dimensional electrophoresis of *in vivo* ^{35}S-methionine labelled protein extracts from anoxic maize seedlings is a 87 kD polypeptide, later identified as the product of the *Shrunken* gene (*Sh1*), encoding the SS1 subunit of the tetrameric enzyme sucrose synthase (Springer *et al.* 1986). This indicates clearly that sucrose synthase is synthesized under anoxia in maize (Springer *et al.* 1986), but a report by McElfresh & Chourey (1988) shows that sucrose synthase is induced only at the transcriptional level, without significant increase in the sucrose synthase protein amount. Therefore, mRNA encoding sucrose synthase is translated under anaerobic conditions (Springer *et al.* 1986), but not with a rate resulting in an increased sucrose synthase activity under anoxia (McElfresh & Chourey 1988). In *Sorghum* a significant elevation in the level of sucrose synthase mRNAs was also observed, but again without a corresponding increase at the protein level (Chourey *et al.* 1991). In *Sorghum*, both genes coding for sucrose synthase subunits were induced by the anaerobic treatment, while only the *Sh1* gene is induced by anoxia in corn, and the other maize sucrose synthase gene (*Sus*) is unaffected by the anaerobic treatment (McElfresh & Chourey 1988).

In rice seedlings incubated under anoxia sucrose synthase is induced at both the transcriptional and the translational level (Ricard *et al.* 1991), suggesting that differences between species showing a different tolerance to anaerobiosis can be observed in the efficiency of translation of mRNA coding for anaerobic polypeptides.

A recent re-evaluation of the induction of sucrose synthase in both maize and rice led to the following conclusions (Guglielminetti *et al.* 1997):

- Aerobically germinated maize and rice seedlings subjected to air→anoxia transition induce sucrose synthase at the transcriptional level, and a correspondingly increased level of sucrose synthase activity is observed in both rice and maize seedlings kept under anoxia in long-term experiments.
- Seedlings subjected to the air→anoxia transition can also cleave sucrose through the activity of alkaline invertase.
- Alkaline invertase activity is virtually absent in cereal seeds directly sown under anoxia
- The sucrose synthase pathway plays a major role in the cereal seeds kept under anoxia since the imbibition time.

This is supported by the following experimental evidence (Guglielminetti *et al.* 1995a; 1997):

- A decreased activity of alkaline invertase under anoxia.
- A strong increase of sucrose synthase activity under the same conditions that results

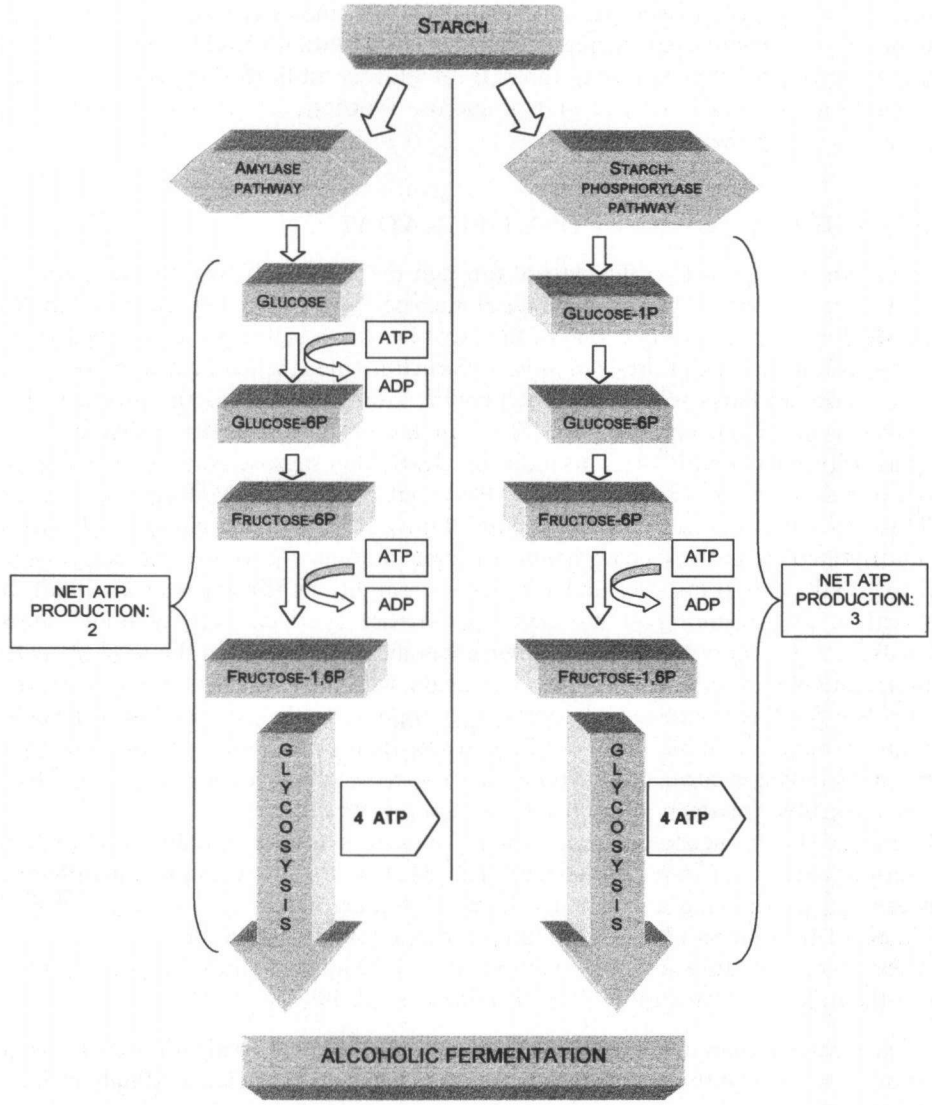

Fig. 1. Pathways for starch degradation under anoxia. The amylase pathway (left) is operative in rice seeds (Perata *et al.* 1997a) , while the starch-phosphorylase pathway (right) is operative in Schoenoplectus lacustris (Steinmann & Braendle 1984). The net ATP production is calculated as [(ATP moles produced) – (ATP moles consumed)] in the conversion of 1 mole of glucose into ethanol. The conversion glucose→glucose-6P (catalysed by hexokinase) and fructose-6P→ffructose-1,6P (catalysed by phosphofructokinase ATP-dependent, as shown in the figure; this step can also be catalysed by the activity of the PPi-dependent enzyme, pyrophosphate: fructose 6-phosphate 1-phosphotransferase) are ATP consuming steps, while ATP is produced in the fructose-1,6P→→→ethanol conversion (alcoholic fermentation).

in a 1/30 ratio of activities between invertase and sucrose synthase in 8-day-old rice coleoptiles.

- The presence of the enzymatic set needed for the operation of the sucrose synthase

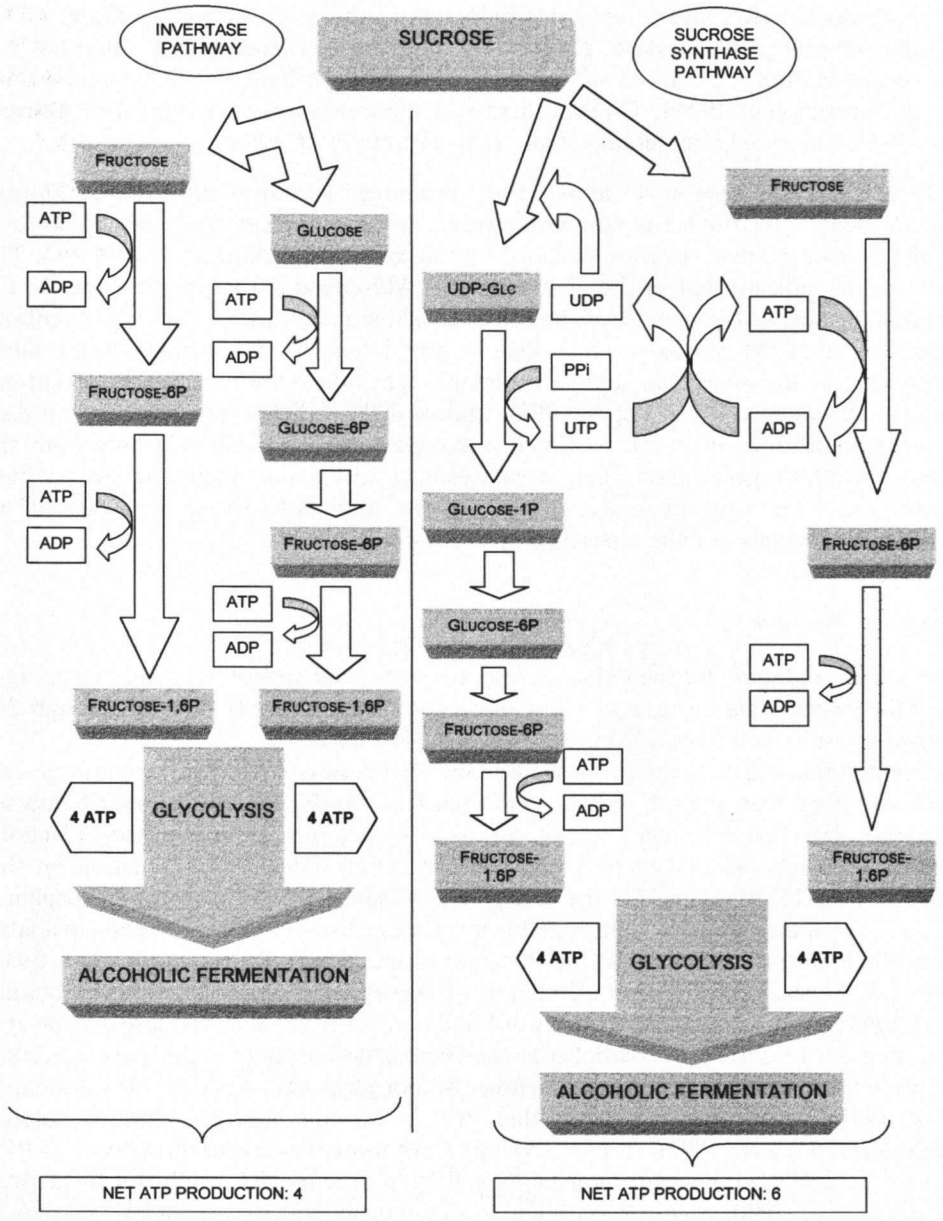

Fig. 2. Pathways for sucrose degradation under anoxia.Sucrose can be degraded either through the invertase (left) or sucrose synthase pathway (right) (see text). The net ATP production is calculated as [(ATP moles produced) – (ATP moles consumed)] in the conversion of 1 mole of sucrose into ethanol. The conversion glucose→glucose-6P (catalysed by hexokinase), fructose→fructose-6P (catalysed by fructokinase) and Fructose-6P→Fructose -1,6P (catalysed by phosphofructokinase ATP-dependent, as shown inthe figure; this step can also be catalysed by the activity of the PPi-dependent enzyme, pyrophosphate:fructose 6-phosphate 1-phosphotransferase) are ATP consuming steps, while ATP is produced in the fructose-1,6P→→→ethanol conversion (alcoholic fermentation). The conversion UDP-Glc (UDP-glucose) →glucose-1P is catalysed by UDP-glucose pyrophosphorylase. Cycling of urydilates UDP↔UTP is accomplished through the nucleoside diphosphate kinase activity (UDP+ATP ↔ UDP+ATP).

pathway. In rice, anoxia does not reduce the activity of sucrose synthase, UDP-glucose-pyrophosphorylase, phosphoglucomutase and fructokinase activities but, on the contrary, enhanced the activity of sucrose synthase and fructokinase (Guglielminetti *et al.* 1995a). On the other hand, these enzymatic activities are negatively affected in anoxia-intolerant cereal grains (Perata *et al.* 1996).

In maize grains kept under anoxia for a prolonged period of time, anoxia induces the *Sh1* gene at the transcriptional level, with a corresponding enhancement in the level of the sucrose synthase isoform encoded by this gene (Guglielminetti *et al.* 1996). The results also indicate that in the absence of the *Sh1*-encoded sucrose synthase (in the *sh1* mutant) the *Sus1*-encoded isoform can easily allow a readjustment of the metabolism, suggesting that the *Sh1*-isoenzyme offers no physiological advantages over the *Sus1*-isoenzyme in the anaerobic sucrose→ethanol transition. However, a very recent experimental evidence (Ricard *et al.* 1998) confirmed the critical role of sucrose synthase in anoxia tolerance in maize seedlings: a double mutant of maize lacking both the sucrose synthase genes shows decreased tolerance to anoxia, suggesting that sucrose synthase is needed for tolerance to anaerobiosis, and that sucrose is a substrate of primary importance for the anaerobic metabolism.

THE SUCROSE–ETHANOL TRANSITION

Sucrose degradation through the sucrose synthase pathway offers some advantages over the invertase pathway, as the flow of carbon from sucrose to ethanol through this pathway results in a considerable ATP saving (see Fig. 2).

Granted that the pathway proposed by Guglielminetti *et al.* (1995a) operates *in vivo*, and assuming that pyrophosphate (PPi) needed for the activity of UDP-glucose-pyrophosphorylase is present in the cell as a by-product of biosynthetic reactions (Smyth & Black 1984; Mertens *et al.* 1990; see Drew 1997 for a discussion on this subject) no ATP is needed for the conversion of sucrose to hexose monophosphates while 2 ATP moles are needed through the invertase pathway (see Fig. 2). Data available from the literature indicate that the PPi pool in the plant cells (Smyth & Black 1984; Huber & Akazawa 1986) is not affected by anoxia (Dancer & ap Rees 1989; Mohanty *et al.* 1993). PPi needed for the activity of UDPGlucose-pyrophosphorylase may be also provided by the activity of pyrophosphate:fructose 6-phosphate 1-phosphotransferase (PFP) in the gluconeogenic direction (Huber & Akazawa 1986; Xu *et al.* 1989; Mohanty *et al.* 1993), even if it is not likely that PFP is the only source of PPi for sucrose metabolism (ap Rees 1992). Anoxia not only leads to an increase in the activity of PFP but to an increased concentration of its activator, fructose 2,6 bisphosphate as well (Mertens *et al.* 1990). A marked decline in the phosphofructokinase (PFK) activity is also observed in rice seedlings under anoxia (Mertens *et al.* 1990), when an increased glycolytic flux would be expected to counteract the decreased ATP production in the absence of oxygen; PFP may therefore act in the glycolytic direction under anaerobic conditions, allowing an increase in the ATP yield of glycolysis. Hajirezaei *et al.* (1994) suggested that PFP catalyses a net glycolytic reaction in potato tubers. The source of PPi needed for the activity of UDP-glucose pyrophosphorylase is therefore still unclear at the present stage and requires further study.

The cycling of the uridylates (UTP/UDP), necessary for the sucrose synthase pathway, could be accomplished by the UTP-dependent phosphorylation of the fructose units

resulting from the cleavage of sucrose (Huber & Akazawa 1986; Xu *et al.* 1989), but our results suggest that the cycling of the UTP/UDP pool may be accomplished by the action of nucleoside diphosphate kinase (Renz & Stitt 1993; Guglielminetti *et al.* 1995a; see Fig. 2). The conversion UTP→ATP not only allows the re-cycling of UDP needed for the activity of sucrose synthase, but also provides ATP used by fructokinase for the phosphorylation of the fructose units resulting from sucrose cleavage.

ATP production in the sucrose→ethanol transition can range from 4 moles for each mole of sucrose (invertase pathway; see Fig. 2) to 6 moles/mole of sucrose (sucrose synthase pathway; see Fig. 2) up to 8 moles/mole of sucrose (sucrose synthase pathway together with PFP activity instead of PFK activity). If the whole cereal seedling is considered, when the invertase pathway is operative only 2 ATP moles are produced for each sucrose mole degraded, since the synthesis of 1 mole of sucrose (from glucose resulting from starch degradation) has a metabolic cost of 2 ATP moles.

INFLUENCE OF SUGAR AVAILABILITY ON ANOXIA TOLERANCE IN CEREAL GRAINS

The soluble sugar content of wheat grains incubated under anoxia decreases to an almost undetectable level within a few days of incubation under anoxia (Perata *et al.* 1996). Interestingly, but not surprisingly, sugar depletion correlates with loss of viability of the grains (e.g. they fail to resume germination if transferred back to aerobic conditions). It is a logical hypothesis to assume that survival is correlated to an adequate supply of fermentable carbohydrates, since energy production under anoxia is restricted to glycolysis/fermentation: indeed, feeding glucose to wheat grains enhances significantly their ability to withstand prolonged anaerobiosis, and even promotes root elongation (Perata *et al.* 1992). Vartapetian *et al.* (1976) proposed that rice coleoptiles are tolerant to anoxia as a consequence of their ability to transport organic compounds from the seed to the anaerobic coleoptile. The availability of readily fermentable sugars could therefore play a role in anoxia tolerance. Some authors reported data indicating that carbohydrates supplied exogenously enhance anoxia tolerance of plant tissues (Andreev *et al.* 1991; Webb & Armstrong 1983; Waters *et al.* 1991; Perata *et al.* 1992) while others proposed an opposite view (Malki *et al.* 1989; Roberts *et al.* 1985). Evidence suggesting an important role for sugar supply in conferring anoxia tolerance has been reviewed recently (Vartapetian & Jackson 1997, and references therein). This evidence and others can be summarized as follows:

- Dependence on exogenous sugar supply for rice coleoptiles survival.
- Mitochondrial damage in coleoptiles separated from sugar supply (endosperm).
- Enhanced tolerance to anoxia in roots fed with exogenous sugars (Vartapetian *et al.* 1977; Webb & Armstrong 1983).
- Increased adenylate energy charge in maize root tips fed with glucose (Saglio & Pradet 1980; Saglio *et al.* 1980).
- Increased anoxia tolerance in wheat grains fed with glucose or sucrose (Perata *et al.* 1992).

Further evidence is available regarding other plant species. A recent report by Germain *et al.* (1997) demonstrated that sucrose but not glucose or fructose allows anoxic tolerance in tomato roots, a consequence of a marked inhibition of hexokinases in the anoxic tomato roots resulting in the inability of this tissue to utilize hexoses. Sucrose

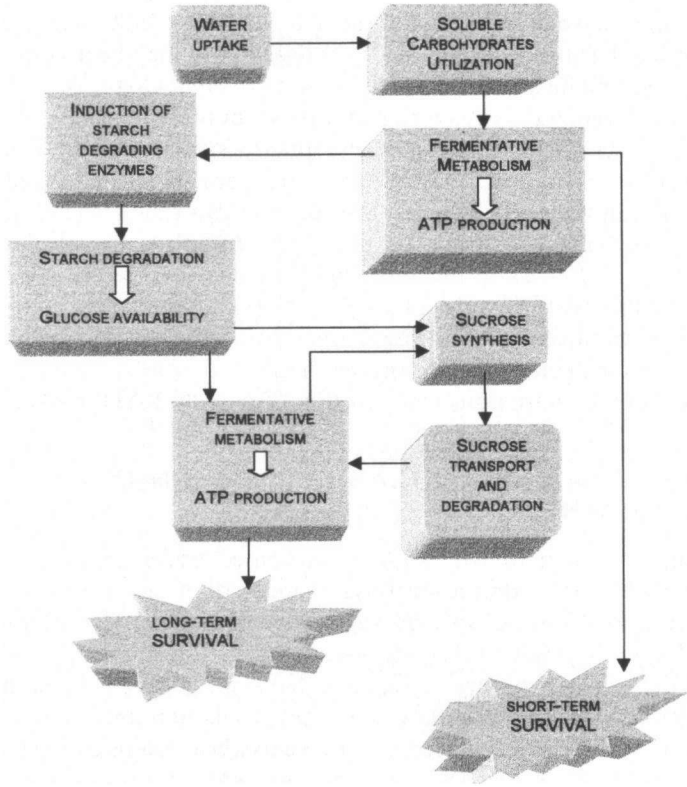

Fig. 3. Overall schematic hypothesis for carbohydrates utilization linked to anoxia tolerance in germinating rice grains. Soluble carbohydrate utilization through the fermentative pathway allows energy production and allows a short-term survival of the embryo. Only if the induction of starch degradation is successful can the embryo avoid sugar starvation and death thanks to long-term energy production through fermentation (long-term survival), not only in tissues adjacent to the starchy endosperm but, thanks to sucrose synthesis→transport→degradation, also in distal tissues such as the elongating coleoptile.

was utilized instead, thanks to a sucrose synthase pathway allowing the hexokinase-dependent hexose-phosphorylating step to be by-passed (Germain *et al.* 1997).

CONCLUDING REMARKS

Plant tolerance to anaerobiosis is probably the result of the interaction of a series of adaptive traits (see Drew 1997, for a recent review), and cannot be simplified by assuming that a single metabolic pathway is responsible for tolerance/susceptibility to anoxia. In rice, many factors contribute to the adaptation of this species to anaerobic environments (see Setter *et al.* 1997, for a review on this subject). However, there is little doubt that energy production through glycolysis/fermentation is the basis for all the other adaptive responses. Unless glycolysis is continuously fuelled by a non-limiting amount of readily fermentable carbohydrates, ATP produced via this pathway would soon be exausted: this is the case in cereal grains such as barley and wheat while, in rice, starch degradation under anoxia results in the availability of a large amount of glucose which is used for ATP production, sucrose synthesis and transport to the

growing coleoptile, sucrose degradation linked to additional glycolysis/fermentation (long-term survival). The proposed pathway for carbohydrate metabolism in the anoxic rice grain is shown in Fig. 3.

It is worth emphasizing that the hypothesis and proposed pathways described in this paper are based on experimental evidence obtained in our laboratory by comparing rice, wheat, barley and maize grains. However, it might be possible that the same pathways are operative in plant groups different from cereals, or during developmental stages of cereals different from the germinating phase.

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