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Amylolytic Activities in Cereal Seeds under Aerobic and Anaerobic Conditions[1]

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An adequate carbohydrate supply contributes to the survival of seeds under conditions of limited oxygen availability. The amount of soluble, readily fermentable carbohydrates in dry cereal seeds is usually very limited, with starch representing the main storage compound. Starch breakdown during the germination of cereal seeds is the result of the action of hydrolytic enzymes and only through the concerted action of α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2), debranching enzyme (EC 3.2.1.41), and α-glucosidase (EC 3.2.1.20) can starch be hydrolyzed completely. We present here data concerning the complete set of starch-degrading enzymes in three cereals, rice (*Oryza sativa* L.), which is tolerant to anaerobiosis, and wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), which are unable to germinate under anoxia. Among the cereal seeds tested under anoxia, only rice is able to degrade nonboiled, soluble starch, reflecting the ability to degrade the starch granules in vivo. This is explained by the presence of the complete set of enzymes needed to degrade starch completely either as the result of de novo synthesis (α-amylase, β-amylase) or activation of preexisting, inactive forms of the enzyme (debranching enzyme, α-glucosidase). These enzymes are either absent or inactive in wheat and barley seeds kept under anaerobic conditions.

Cereal seeds other than rice (*Oryza sativa* L.) fail to germinate in anoxic environments (Alpi and Beevers, 1983; Perata and Alpi, 1993). The metabolic basis explaining the different degree of tolerance among plant species is still unknown (Drew, 1990; Kennedy et al., 1992; Perata and Alpi, 1993). Starchy seeds are usually more tolerant to anoxia than fatty seeds, which is a possible consequence of the higher fermentative metabolism observed in these seeds (Al-Ani et al., 1985; Raymond et al., 1985) leading to an adequate ATP production. It is therefore inferred that an adequate supply of carbohydrates contributes to the survival of seeds under conditions of limited oxygen availability. The amount of soluble, readily fermentable carbohydrates in dry cereal seeds is usually very limited, because starch is the main storage compound in these seeds.

Starch breakdown during the germination of cereal seeds is the result of the action of hydrolytic enzymes, and it is generally accepted that phosphorylases are not involved in this process (Beck and Ziegler, 1989; Fincher, 1989). α-Amylase plays a major role during the degradation of native starch granules (Dunn, 1974; Sun and Henson, 1991), but only through the concerted action of α-amylase, β-amylase, debranching enzyme, and α-glucosidase can starch be hydrolyzed completely (Sun and Henson, 1991). The possible role of α-glucosidase in the process of degradation of native starch granules in aerobic barley (*Hordeum vulgare* L.) seedlings has been reported (Sun and Henson, 1990).

Although several papers have been published about the process of starch breakdown under aerobic conditions (Akazawa et al., 1988; Beck and Ziegler, 1989; Fincher, 1989), the effects of anoxia on starch-degrading enzymes have been poorly studied. Few reports are available, all dealing with the effects of anoxia on α-amylase. The induction of α-amylase in submerged deep-water rice has been described by Kende and associates (Raskin and Kende, 1984; Smith et al., 1987), and a similar phenomena has been reported to occur in flooded tobacco plants (Hurng and Kao, 1993). Hanson and Jacobsen (1984) showed that barley aleurone layers do not respond to GA_3 under anoxia and thus fail to produce α-amylase. Under anoxia, only rice seeds appear to be able to induce α-amylase, whereas barley, wheat (*Triticum aestivum* L.), oat, and rye do not produce the enzyme in the absence of oxygen (Perata et al., 1992, 1993). As a consequence, among the cereal seeds studied to date, only rice is able to degrade starch under anoxia (Perata et al., 1992).

Nevertheless, a description of the status of the complete set of starch-degrading enzymes in seeds kept under anaerobic conditions is missing, despite the importance of this process to plant tolerance to anaerobiosis (Perata and Alpi, 1993; Ricard et al., 1994). No data are available concerning α-glucosidase, debranching enzyme, and β-amylase.

We present here data regarding the complete set of starch-degrading enzymes in three cereals: rice, tolerant to anaerobiosis, and wheat and barley, intolerant to anaerobiosis, with the aim of describing in detail the effects of anoxia on these enzyme activities.

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Abbreviation: DNS, 3,5-dinitrosalycilic acid.

MATERIALS AND METHODS

Plant Materials

Rice (*Oryza sativa* L. cv Arborio) and barley (*Hordeum vulgare* L. cv Himalaya) seeds were obtained from the University of Pisa farm and wheat (*Triticum aestivum* L. cv Hatsuho) seeds were obtained from Nagoya University. Seed germination and anoxia treatments were carried out as previously described (Perata et al., 1992; Guglielminetti et al., 1995): a moistened gas stream of nitrogen was passed through in a 500-mL flask containing the seeds at 30 mL/min throughout the experimental period. All of the germination experiments were performed in the dark. Rice root emergence was not observed under anoxia, but only the coleoptile elongates. Seedlings were collected when they reached the age shown in the figures and then were frozen in liquid nitrogen and stored at −80°C.

Chemicals

All the reagents used were purchased from Sigma.

Extraction of Plant Material

Preliminary experiments were performed to verify the presence of the enzymes under investigation in the different seedling components (endosperm, scutellum, shoot, root). The results showed that all of the starch-degrading enzymes were mainly localized in the endosperm (more than 80%, enzyme activity expressed on organ basis). However, dissection of the endosperm from aerobically germinated seedlings of wheat and barley resulted in considerable losses (up to 60% of the activity detected using whole seedlings) of starch-degrading enzymatic activities, a consequence of the liquefactent consistence of the partially degraded starchy endosperms. We therefore extracted whole seedlings to avoid losses that would be highly variable and difficult to be evaluated. Samples (0.5 g fresh weight) were extracted in 1.5 mL of 100 mM Hepes-KOH, pH 7.5, containing 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, and 10 mM $NaHSO_3$. The presence of reducing agents in the extraction buffer may affect some enzyme activities, including debranching enzyme (Yamada, 1981a, 1981b, 1981c; Toguri, 1991); we therefore extracted an additional set of samples using a basal buffer (100 mM Hepes-KOH, pH 7.5) to test the importance of reducing agents and other additives on the enzyme activities under investigation. Enzyme activities were lower when extracts were prepared using the basal buffer, but the pattern of activities with respect to time course of germination was similar for all of the enzymes under investigation except for the pattern of activities of debranching enzyme and α-glucosidase from rice. Unless specified otherwise in the figure legends, the results reported are those for samples extracted in the presence of reducing agents. Extracts were centrifuged (13,000*g*, 15 min), the resulting pellets were washed with the extraction buffer and centrifuged again, and the resulting supernatants were combined and used for the enzymatic assays. Extracts to be assayed for debranching enzyme and α-glucosidase were dialyzed against the extraction buffer for 12 h to remove soluble sugars that were present in the extracts and that may interfere with the assays. All of the enzymes were stable to dialysis and to freeze-thaw treatments.

Analysis of Starch-Degrading Enzymes

All assays were performed in duplicate and checked for linearity with respect to time and sample volume for each individual sample. Blanks, conducted in duplicate, were obtained by treating the sample with DNS solution (see below) prior to the addition of the substrate, with the exception of samples used for assaying α-glucosidase, for which boiled samples were used. The amount of reducing sugars present in the substrates was routinely evaluated. The variability of duplicate assays never exceeded 3% of the mean value.

All amylolytic activities, except α-glucosidase, were assayed by testing for the release of reducing sugars from soluble starch or pullulan. An aliquot (200 μL) was taken from the assay mixture and treated with 150 μL of DNS solution (40 mM DNS, 400 mM NaOH, 1 M K-Na tartrate, heated at 50°C, and filtered through filter paper) for 5 min at 105°C. After dilution with distilled water (up to 5 mL) the A_{530} was taken and reducing power evaluated using a standard curve obtained with Glc (0–9 μmol).

Total amylolytic activity was assayed using crude extracts. The assay buffer consisted of 50 mM Na-acetate, pH 5.2, and 10 mM $CaCl_2$. The substrate was 2.5% boiled soluble potato starch; incubation time was up to 15 min. As reported by Sun and Henson (1991), nonboiled, soluble starch can be used to predict in vivo starch granule hydrolysis. Total amylolytic activity was therefore also assayed as described by Sun and Henson (1991).

α-Amylase (EC 3.2.1.1) was assayed using heat-treated crude extracts (70°C for 15 min in the presence of 3 mM $CaCl_2$) to inactivate β-amylase, debranching enzyme, and α-glucosidase (Sun and Henson, 1991). The assay buffer consisted of 50 mM Na-acetate, pH 5.2, and 10 mM $CaCl_2$. The substrate was 2.5% boiled, soluble starch; incubation lasted for up to 15 min.

The activity of β-amylase (EC 3.2.1.2) was measured in crude extracts from which α-amylase was precipitated with specific antibodies raised against either rice α-amylase (rice samples) or barley α-amylase (barley and wheat samples). The assay buffer consisted of 50 mM Na-acetate, pH 5.2, containing 10 mM $CaCl_2$. The substrate was 2.5% boiled, soluble starch; incubation time lasted for up to 15 min.

Debranching enzyme (EC 3.2.1.41) was assayed using dialyzed crude extracts. The assay buffer consisted of 50 mM Na-acetate, pH 5.2, and 10 mM $CaCl_2$. The substrate was 2.5% pullulan containing 10 mM DTT; the incubation time lasted for up to 60 min.

α-Glucosidase (EC 3.2.1.20) was assayed using dialyzed crude extracts. The assay buffer consisted of 50 mM Na-acetate, pH 5.2, containing 10 mM $CaCl_2$. The substrate was 10 mM maltose. The samples were incubated for up to 60 min. The release of Glc was followed by measuring the changes in NADPH at 340 nm in a coupled enzymic reaction of hexokinase (EC 2.7.1.1) and Glc-6-P dehydrogenase (EC 1.1.1.49). The reaction mixture contained 150 mM Tris-

HCl, pH 7.6, 4 mM $MgCl_2$, 3 mM ATP, 0.8 mM NADP, and 1 unit of hexokinase and Glc-6-P dehydrogenase. Quantitative results were obtained by comparing A_{340} obtained for the samples with those of a standard curve (0–896 nmol Glc). One unit is defined as the amount of enzyme releasing 1 μmol of Glc in 1 min.

Specificity and Optimization of the Enzyme Assays

In recent years, many assay methods have been proposed to assay specifically amylolytic activities in plant tissues (Doehlert and Duke, 1983; Ziegler, 1990; Sun and Henson, 1991). Some methods, based on the use of synthetic substrates (Doehlert and Duke, 1983; Ziegler, 1990), give very reliable results but the use of larger, branched substrates, more typical of native starch (Ziegler, 1990), is, in our opinion, preferred.

The main interference in the assay for α-amylase using soluble starch may derive from the high β-amylase activities present in the samples. Treatment of the sample at 70°C destroys completely β-amylase activity; samples from ungerminated, dry barley and wheat seeds (that are devoid of α-amylase activity) lose the ability to degrade boiled, soluble starch if heat treated. No activity of debranching enzyme and α-glucosidase was found in heat-treated samples. On the other hand, α-amylase activity is completely unaffected by the heat treatment. The effect of the heat treatment on α-amylase was evaluated by using the starch azure assay (Doehlert and Duke, 1983), a method highly specific for α-amylase. No differences in α-amylase activity were found in heat-treated samples when compared with nontreated samples, indicating that α-amylase activity is not affected by the heat treatment. The starch azure assay (a dye-releasing assay) was not routinely used to assay α-amylase to allow a comparison between the activities of the different starch-degrading enzymes, assayed in this work using reducing sugar-releasing substrates (soluble starch and pullulan).

β-Amylase was assayed using boiled, soluble starch as substrate after removal of α-amylase from the samples by immunoprecipitation. α-Amylase was completely absent in the immunoprecipitated samples, as demonstrated by the inability of these samples to degrade β-limit dextrins (Panabieres et al., 1989). Debranching enzyme and α-glucosidase activities in wheat and barley samples were too low when compared with β-amylase to interfere significantly with the assay. The positive correlation between the immunoblot and enzyme activity analysis of β-amylase in rice was used to validate results for rice samples.

Debranching enzyme and α-glucosidase assays were based on the use of specific substrates and no interferences have been reported from other amylolytic activities on these substrates (Sun and Henson, 1991).

Immunoblotting

Equal amounts of proteins were subjected to SDS-PAGE on 12.5% polyacrylamide gels. Electrofocusing of proteins was performed as previously reported (Perata et al., 1992). After electrophoresis, proteins were transferred to nitrocellulose paper as previously described (Perata et al., 1992). Analysis was performed using the following antibodies: anti-rice α-amylase (Daussant et al., 1983; for rice α-amylase immunoblotting), anti-barley α-amylase (for barley and wheat α-amylase immunoblotting), anti-barley β-amylase (for rice, wheat, and barley β-amylase immunoblotting; the antibody was raised against barley β-amylase obtained from Sigma and fractionated by SDS-PAGE), anti-rice debranching enzyme (for rice debranching enzyme immunoblotting; the antibody was raised against rice debranching enzyme purified from 8-d-germinated rice seedlings according to the method of Iwaki and Fuwa [1981]. The specific antibody was raised against the purified protein sample fractionated by SDS-PAGE, and this antibody was specific to rice debranching enzyme and could therefore not be used to detect wheat and barley debranching enzyme). Antibodies against a protein from rice or barley were used to detect protein(s) from a different species only when preliminary experiments demonstrated a good degree of cross-reactivity (data not shown). An alkaline phosphatase-labeled second antibody was used to detect immunoreactive bands.

RESULTS

The ability of extracts from aerobic and anaerobic samples to degrade nonboiled, soluble starch was tested to predict in vivo starch granule hydrolysis (Sun and Henson, 1991). The results, shown in Figure 1, indicate that, whereas

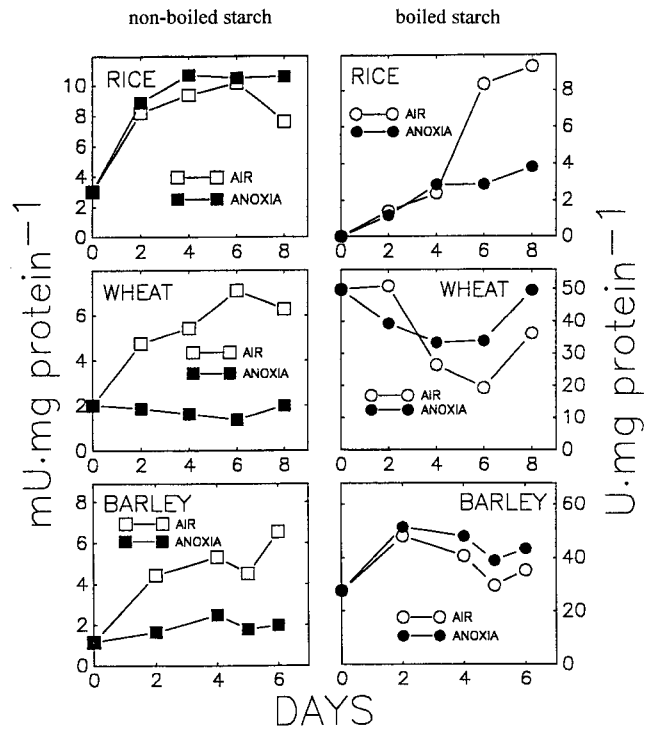

Figure 1. Time course of total amylolytic activity in cereal seeds under aerobic and anaerobic conditions. The substrate was nonboiled, soluble starch (□, ■) or boiled, soluble starch (○, ●). Data are means from three separate experiments (±SE did not exceed 15% of the given values). mU, Milliunits; U, units.

rice appears to be unaffected by anoxia, starch hydrolysis is greatly hampered in wheat and barley seeds kept under anaerobic conditions.

If boiled, soluble starch was used as substrate to test the total amylolytic activity present in the samples under investigation, different results were obtained (Fig. 1). Wheat and barley showed high amylolytic activity under both aerobic and anaerobic conditions, whereas in rice starch degradation seemed to take place at a slower rate under anoxia. We are aware that hydrolysis of boiled, soluble starch does not correlate significantly with starch granule hydrolysis (Sun and Henson, 1991), but the clear indication of the presence of activities able to degrade starch even under anoxia in wheat and barley is intriguing. We therefore analyzed the samples for the presence of the single amylolytic activities, with the aim of assessing their importance in the process of starch degradation under aerobic and anaerobic conditions.

Activity of α-amylase was absent in dry seeds of rice, wheat, and barley (Fig. 2), but its activity rapidly appeared and increased in all three seed types, as the process of germination occurred. Only rice was able to induce α-amylase under anoxia, confirming the data reported previously (Perata et al., 1992, 1993). These results were further confirmed by immunoblot analysis of protein extracts obtained from aerobic and anoxic seeds and seedlings (Fig. 3). A single immunoreactive band of α-amylase was detected in rice and wheat, whereas two bands were found in samples from aerobic barley seedlings (Sticher and Jones, 1991).

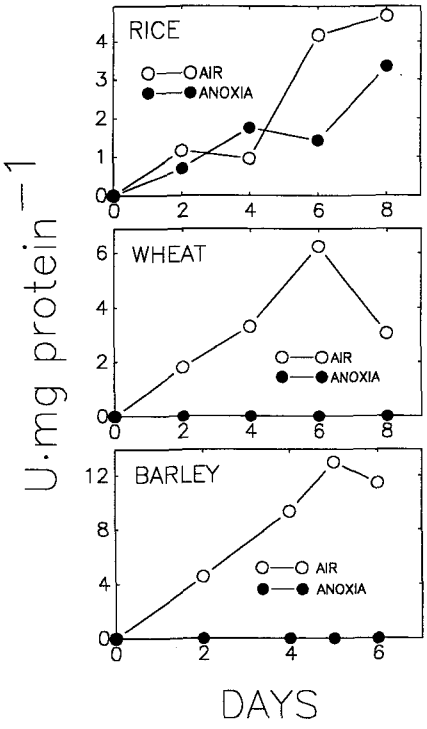

Figure 2. Time course of α-amylase activity in cereal seeds under aerobic and anaerobic conditions. Data are means from three separate experiments (±SE did not exceed 10% of the given values). U, Units.

α-Amylase was undetectable in samples from seeds of barley and wheat kept under anoxic conditions.

Activity of β-amylase was almost absent in dry seeds of rice, whereas a strong activity was present in barley and wheat seeds (Fig. 4). During the aerobic germination of rice, β-amylase was synthesized de novo and a latent, starch granule-bound form was released (Okamoto and Akazawa, 1980). Our results indicate that anoxia reduces the process of synthesis/release of β-amylase in rice seeds. The results were confirmed by the immunoblot analysis (Fig. 5), which showed the presence of a faint β-amylase signal in the dry seed that clearly increased as germination proceeded.

In barley, β-amylase is present as both a free and starch granule-bound form. Both forms are present in the dry seed and have a molecular mass of 64 kD (Sopanen and Lauriere, 1989). During seedling growth both forms are converted to a low-molecular-weight form (Sopanen and Lauriere, 1989). The bound, high-molecular-weight form is easily extracted using a DTT-containing buffer and is enzymatically active even if the free, low-molecular-weight form is slightly more active (Sopanen and Lauriere, 1989). The immunoblot analysis (Fig. 5) shows that the high-molecular-mass form was gradually transformed to the low-molecular-mass form under aerobic conditions. Under anoxia this process did not take place. The amount of β-amylase remained high under anoxia, whereas under aerobic conditions both the activity and the signal in the immunoblot decreased in wheat.

We also examined the processing of β-amylase in barley by using IEF on the protein extracts, followed by immunoblot analysis. The results (Fig. 6) show that during the aerobic germination β-amylase is gradually converted to forms having a higher pI; under anoxia this conversion did not take place.

In wheat a similar situation was observed: β-amylase activity was higher under anoxia (Fig. 4), but the conversion to the free form(s) did not occur under anoxia (Fig. 5). A multiple β-amylase SDS-PAGE pattern was observed in wheat, in agreement with the results reported by Bureau et al. (1989).

Debranching enzyme was assayed during the aerobic and anaerobic treatments of the cereal seeds under investigation. Since the occurrence of a latent, inactive form of the enzyme has been reported (Yamada, 1981a, 1981b, 1981c, 1981d; Toguri, 1991), we assayed two different sets of extracts prepared in the presence or absence of reducing agents, with the former being known to convert the latent form into the active form. The conversion takes place in vivo during aerobic rice seed germination (Toguri, 1991). Fewer data are available about the presence of a latent form in wheat and barley seeds, but reductant activation of debranching enzyme has been reported for several cereal seeds, including those described in this paper (Yamada, 1981b).

The results we obtained by analyzing rice extracts confirm the results of Toguri (1991). A latent form was easily extracted using a buffer containing reducing agents as shown by enzyme assay (Fig. 7) and immunoblot analysis

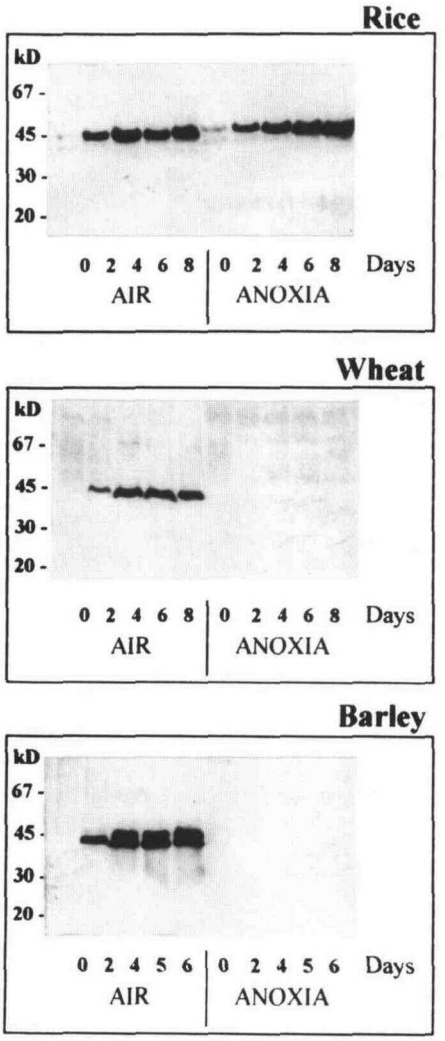

Figure 3. Immunoblot of α-amylase enzyme in cereal seeds under aerobic and anaerobic conditions. Protein extracts were subjected to SDS-PAGE before electrophoretic transfer and immunoblotting with α-amylase-specific antibodies. Data are from a representative experiment.

(Fig. 8), whereas the process of in vivo activation was observed using a buffer devoid of reducing agents (Fig. 7). The process of in vivo activation took place normally also under anoxic conditions.

We were unable to extract a latent form from wheat and barley. Samples from dry seeds never showed activity of the debranching enzyme, even if extracted in the presence of reducing agents (Fig. 7, wheat and barley). The enzyme was produced during the aerobic germination, and higher activity was extracted using a buffer containing reducing agents when compared with that obtained from samples extracted using a basic buffer. Whether this was the result of enhanced extraction efficiency or enzyme stabilization by reducing agents is presently unknown and requires further studies. Regardless of these considerations, the enzyme was clearly absent in extracts from anoxic seeds (Fig. 7).

The status of α-glucosidase resembles that of debranching enzyme: in rice the enzyme was present in the dry seed but was extracted using only a buffer containing reducing agents (Fig. 9). If samples are extracted using a buffer devoid of reducing agents, α-glucosidase activity increases during seed germination under both aerobic and anaerobic germination. A different situation was observed in wheat and barley. Regardless of the extraction buffer used, the enzyme was not found in the dry seeds, but its activity rapidly increased during aerobic germination. No activity was detected in any of the anaerobic samples.

DISCUSSION

Whereas rice was able to degrade starch in vivo under anoxia, wheat and barley failed to do so (Perata et al., 1992; L. Guglielminetti, P. Perata, A. Alpi, unpublished results). This clearly distinct behavior is primarily the consequence of the failure of wheat and barley seeds to respond to GA_3 under anoxic conditions, thus failing to produce α-amylase, while successful induction takes place in the anoxic rice seeds (Perata et al., 1993). Nevertheless, α-amylase alone is unable to carry on the complete process of starch breakdown. The action of other enzymes, namely β-amylase, debranching enzyme, and α-glucosidase, is needed to complete the process, leading to the production of Glc units.

Among the cereal seeds tested under anoxia, only rice appears to be able to degrade nonboiled, soluble starch,

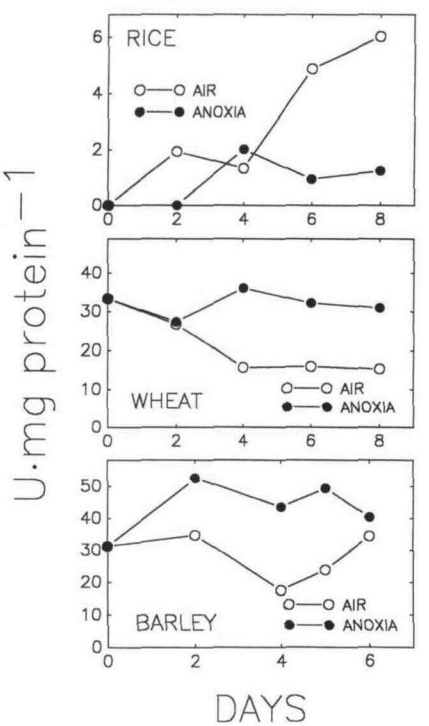

Figure 4. Time course of β-amylase activity in cereal seeds under aerobic and anaerobic conditions. Data are means from three separate experiments (±SE did not exceed 20% of the given values). U,

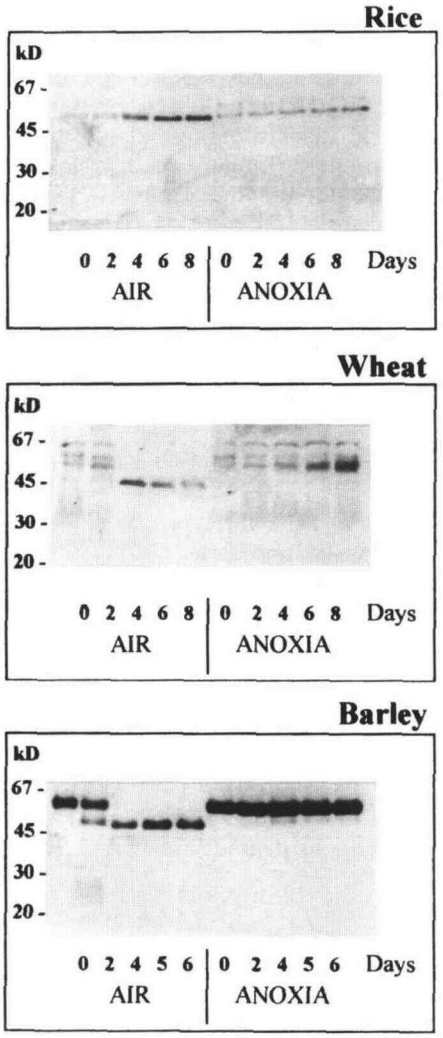

Figure 5. Immunoblot of β-amylase enzyme in cereal seeds under aerobic and anaerobic conditions. Protein extracts were subjected to SDS-PAGE before electrophoretic transfer and immunoblotting with β-amylase-specific antibodies. Data are from a representative experiment.

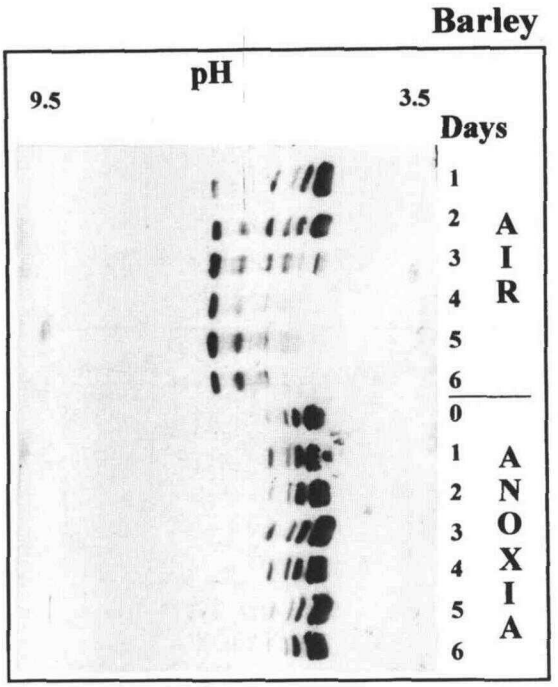

Figure 6. Immunoblot of β-amylase enzyme in cereal seeds under aerobic and anaerobic conditions. Protein extracts were subjected to IEF before electrophoretic transfer and immunoblotting with β-amylase-specific antibody. Data are from a representative experiment.

reflecting the ability to degrade the starch granules in vivo (Sun and Henson, 1991). This is explained by the presence of the complete set of enzymes needed to degrade starch completely, as the result either of de novo synthesis (α-amylase, β-amylase) or of the activation of preexisting, inactive forms of the enzyme (debranching enzyme, α-glucosidase).

Samples from anaerobic wheat and barley are unable to degrade nonboiled, soluble starch because of the absence of both of the enzymes able to degrade native starch granules, α-amylase and α-glucosidase (Dunn, 1974; Sun and Henson, 1990). Debranching enzyme is also absent in anoxic wheat and barley seeds, whereas β-amylase is present, but the enzyme remains in the bound form. Since the bound form released using reducing agents is active in vitro (Sopanen and Lauriere, 1989), the presence of a strong β-amylase activity under anoxia accounts for the high total amylolytic activity detected using boiled, soluble starche

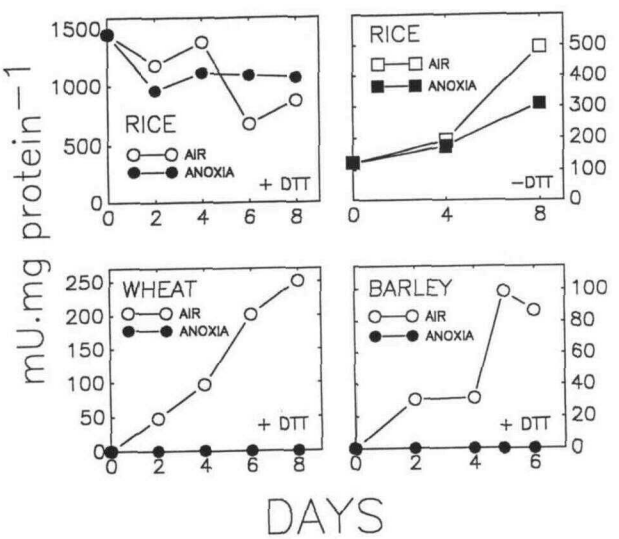

Figure 7. Time course of debranching enzyme activity in cereal seeds under aerobic and anaerobic conditions. Samples were extracted in the presence (○, ●) or absence (□, ■) of reducing agents. Data are means from three separate experiments (±SE did not exceed 15% of the given value). mU, Milliunits.

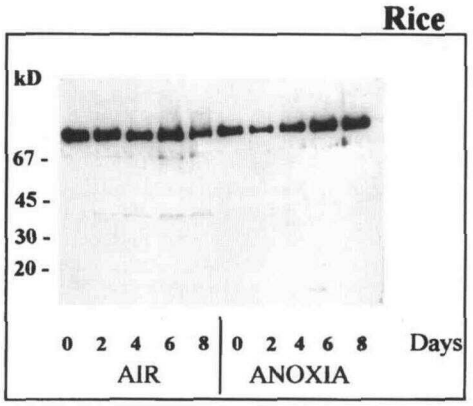

Figure 8. Immunoblot of debranching enzyme in rice seeds under aerobic and anaerobic conditions. Protein extracts were subjected to SDS-PAGE before electrophoretic transfer and immunoblotting with debranching enzyme-specific antibody. Data are from a representative experiment.

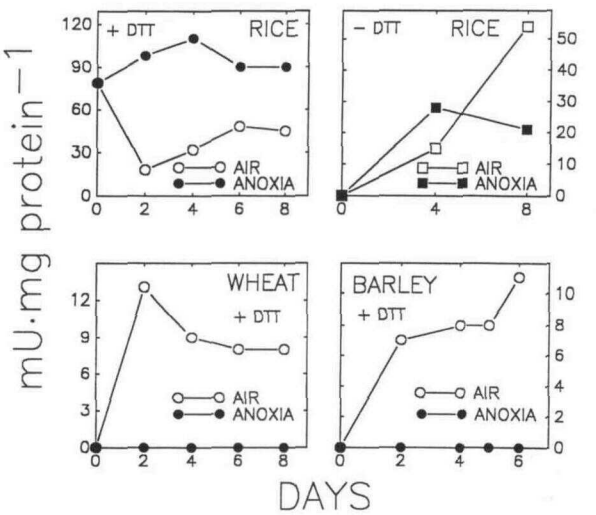

Figure 9. Time course of α-glucosidase activity in cereal seeds under aerobic and anaerobic conditions. Samples were extracted in the presence (○, ●) or absence (□, ■) of reducing agents. Data are means from three separate experiments (±SE did not exceed 20% of the given values). mU, Milliunits.

Therefore, whereas rice is able to degrade starch even under anoxia because of the presence of the enzymes involved in starch degradation, wheat and barley seeds cannot take advantage of the starchy reserves, since they do not have the majority of the enzymes needed to degrade starch. β-Amylase plays no role in native starch granule degradation (Dunn, 1974) and is present only in the high-molecular-weight form under anoxia. Since the process of release of the free, low-molecular-weight β-amylase form is likely due to the action of a sulfhydryl proteinase (Sopanen and Lauriere, 1989), it is inferred that anoxia prevents the action of this proteinase or, alternatively, that the proteinase is not synthesized under anoxia.

This may suggest that anoxic conditions prevent the synthesis of several enzymes and that under these conditions wheat and barley are metabolically inactive. Experimental evidence suggests that this does not hold true. Anoxic barley half-seeds are able to carry on an active lactic and alcoholic fermentation, likely taking advantage of the soluble sugars present in the dry seeds (Hanson and Jacobsen, 1984). Indeed, our preliminary experimental results indicate that soluble sugars are rapidly used and converted to ethanol in anoxic wheat and barley seeds, even if only in rice can a continuous ethanol production be observed under long-term anoxic treatments (Raymond et al., 1985; L. Guglielminetti, P. Perata, A. Alpi, unpublished results).

Under anaerobic conditions energy is produced 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. Therefore, access to the starchy reserves present in the endosperm is needed to provide Glc for the fermentative metabolism (Perata et al., 1992; Guglielminetti et al., 1995). On the other hand, both wheat and barley seeds are unable to elongate the coleoptile under flooding conditions; starch degradation under conditions of limited oxygen availability would therefore be detrimental to these species. It is worth noting that ungerminated seeds can withstand longer periods of anoxia when compared to the germinated seeds (L. Guglielminetti, P. Perata, A. Alpi, unpublished observation) 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.

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