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Effect of Anoxia on Carbohydrate Metabolism in Rice Seedlings[1]

Lorenzo Guglielminetti, Pierdomenico Perata*, and Amedeo Alpi

Dipartimento di Biologia delle Piante Agrarie, Sezione di Fisiologia Vegetale, University of Pisa, Via Mariscoglio 34, I-56124 Pisa, Italy

The metabolism of carbohydrates was investigated in rice (*Oryza sativa* L.) seedlings grown under anoxia. Two phases can be recognized in the utilization of carbohydrates: during the first days of germination under anoxia, the metabolism of sugars is mainly degradative, whereas after the induction of α-amylase (EC 3.2.1.1) has taken place, the increased presence of glucose and sucrose indicates that both starch degradation and sucrose synthesis operate. The analysis of the enzymes involved in carbohydrate metabolism indicates that anoxic rice seedlings possess a set of enzymes that allow the efficient metabolism of starch and sucrose to fructose-6-phosphate. We propose that cytosolic sucrose metabolism in anoxic rice seedlings takes place mainly through a sucrose synthase (EC 2.4.1.13) pathway with nucleoside diphosphate kinase (EC 2.7.4.6), allowing the cycling of urydilates needed for the operation of this pathway.

Cereal seeds fail to germinate under anoxia, but rice (*Oryza sativa* L.) represents an exception to this rule (Alpi and Beevers, 1983; Perata and Alpi, 1993). The molecular and metabolic bases underlying the tolerance of rice to anoxia when compared with other cereal seeds are still unknown, but the ability to maintain an active fermentative metabolism, essential for the production of ATP under conditions of limited oxygen availability, is important for plant tolerance to anaerobiosis (Drew, 1990; Kennedy et al., 1992; Perata and Alpi, 1993).

Availability of readily fermentable carbohydrates is needed to sustain an active fermentative metabolism. Starchy seeds are generally more tolerant to anoxia than fatty seeds (Raymond et al., 1985), but among cereal seeds only rice appears to be able to respond to GA_3 under anoxia, thus producing α-amylase (Perata et al., 1993a). As a consequence, only rice is able to utilize the massive starchy reserves present in the endosperm, even if at a lower rate when compared to that taking place under aerobic conditions (Atwell and Greenway, 1987; Perata et al., 1992). Little is known about the metabolism of the soluble sugars, either present in the dry rice seed or resulting from starch degradation, during anaerobic germination of rice, apart from the reports that indicate that rice coleoptiles and roots are able to utilize Glc efficiently under anoxia (Alpi et al., 1985; Mayne and Kende, 1986; Atwell and Greenway, 1987).

SS is induced by an anaerobic treatment in rice (Ricard et al., 1991), suggesting that this enzyme may play a role in the metabolism of Suc under anoxia, but no data are available concerning the presence of other enzymes involved in the metabolism of Suc needed for the operation of the SS pathway (Huber and Akazawa, 1986).

In the present paper we report a detailed picture of the status of carbohydrates and related enzymes during the first phases of germination of the rice seed under anoxia.

MATERIALS AND METHODS

Plant Material

Rice (*Oryza sativa* L. cv Arborio) seeds were obtained from the university farm. Seed germination and anoxia treatments were carried out as previously described (Perata et al., 1992) with minor modifications: copper tubes were used to connect the flasks to avoid oxygen diffusion through plastic tubing. Root emergence was not observed under anoxia, but only the coleoptile elongated. Preliminary experiments determined whether microbial contamination was present in the flasks containing the seedlings: no differences in terms of carbohydrate levels or enzymatic activities were found when control flasks were compared with flasks with added rifampicin (7 μg/mL) and nystatin (2 μg/mL). Seedlings were dissected as previously described (Perata et al., 1992).

Chemicals

All the reagents used were purchased from Sigma.

Analysis of Carbohydrates

Samples (0.1–0.5 g fresh weight) were rapidly frozen in liquid nitrogen and ground to a powder. Samples were then extracted as described by Tobias et al. (1992).

Samples were assayed by coupled enzymatic assay methods measuring the increase in A_{340}. The efficiency of the methods was tested by using known amounts of carbohydrates. Incubations of the samples and standards were carried out at 37°C for 30 min. The reaction mixtures (1 mL)

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* Corresponding author; fax 39–50–570421.

Abbreviations: FK, fructokinase; Fru6P, Fru-6-P; GK, glucokinase; Glc1P, Glc-1-P; Glc6P, Glc-6-P; HK, hexokinase; NDPK, nucleoside diphosphate kinase; PFP, PPi:Fru-6-P phosphotransferase; PGI, Glc-6-P isomerase; PGM, phosphoglucomutase; SS, Suc synthase; UDPGlc, UDP-Glc; UDPGlc-PPase, UDP-Glc pyrophosphorylase.

were as follows. Glc: 100 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 2 mM ATP, 0.6 mM NADP, 1 unit HK, 1 unit Glc6P dehydrogenase; Fru was assayed as described for Glc plus the addition of 2 units of PGI; the increase in A_{340} was recorded. Suc was first broken down using 85 units of invertase (in 15 mM sodium acetate, pH 4.6) and the resulting Glc and Fru were assayed as described above. Hexose monophosphates were assayed as described by Tobias et al. (1992) and Mohanty et al. (1993) with minor modifications: 100 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 0.6 mM NADP, 1 unit of Glc6P dehydrogenase for Glc6P; 2 units of PGI were added to the previous reaction mixture when Fru6P was measured; 4 units of PGM and 20 μM Glc-1,6-bisphosphate were added for the determination of Glc1P.

Recovery experiments evaluated losses taking place during the extraction procedures. Two tests were done for each metabolite by adding known amounts of authentic standards to the samples prior to the extraction. The concentrations of the standards added were similar to those estimated to be present in the tissues in preliminary experiments. The percentage recovery ranged between 98 and 133% for Suc, Glc, and Fru, whereas lower recoveries were obtained for hexose monophosphates (57–83%). Data were corrected on the basis of the recovery percentages obtained for each sample.

Analysis of Enzymes of Carbohydrate Metabolism

Samples (0.2–0.5 g fresh weight) were extracted in 100 mM Hepes-KOH, pH 7.5, containing 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, 10 mM $NaHSO_3$. Inclusion of insoluble polyvinylpolypyrrolidone did not affect the activity of any of the enzymes under investigation and was therefore not included in the extraction buffer. 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 invertase, SS, HK, and NDPK were dialyzed against the extraction buffer for 12 h to remove soluble sugars present in the extracts that may interfere with the assays. Assays were optimized for extracts from both aerobic and anaerobic plant material.

Because an enzyme inactivator has been reported to be present in aerobically germinated rice seedlings (Shimomura and Beevers, 1983), we routinely tested whether mixing an extract from aerobic seedlings with that of anoxic seedlings could result in losses of enzymatic activities. No inactivation of the activities under investigation was found.

Samples were assayed for the enzymatic activities at 25°C in 0.5-mL reaction mixtures using the following methods. α-Amylase (Doehlert et al., 1982): samples pretreated at 70°C in the presence of 3 mM $CaCl_2$ to eliminate interferences from β-amylase were incubated with 2.5% (w/v) soluble starch; activity of enzyme (1 unit) is defined as the amount of enzyme required to produce 1 μmol Glc min^{-1}. Invertase and SS (Huber and Akazawa, 1986): UDPGlc-PPase (Sowokinos et al., 1993); NDPK (method 1, described by Perata et al., 1993b); GK and FK (50 mM Hepes-KOH, pH 7.5, 2 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, 2 mM ATP or UTP, 0.75 mM NADP, 4 mM Glc or Fru, 1 unit of Glc6P dehydrogenase, 1 unit of PGI); PGI (50 mM Hepes-KOH, pH 7.5, 2 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, 0.75 mM NADP, 4 mM Fru6P, 1 unit of Glc6P dehydrogenase); PGM (50 mM Hepes-KOH, pH 7.5, 2 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, 0.75 mM NADP, 4 mM Glc1P, 20 μM Glc-1,6-bisphosphate, 1 unit of Glc6P dehydrogenase).

Cycling of urydilates was assayed as described by Xu et al. (1989) with modifications: the occurrence of urydilates cycling through a UTP-dependent FK was verified using 50 mM Hepes-KOH, pH 7.5, 2 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, 0.75 mM NADP, 80 mM Suc, 8 μM UDP, 4 mM PPi, 30 μM Glc-1,6-bisphosphate, 1 unit of Glc6P dehydrogenase. The role of NDPK was tested by adding 80 μM ADP to the reaction mixture reported above.

RESULTS

Carbohydrates in Aerobic and Anaerobic Rice Seedlings

Suc, Glc, and Fru were present in the dry seed and their amount increased during aerobic germination (Table I). The increase was particularly prominent after day 2, when the induction of α-amylase, allowing starch degradation, took place (Perata et al., 1992).

Under anoxia, a different behavior was observed. During the first 2 to 3 d the metabolism of carbohydrates appeared to be mainly degradative, with a decrease in the amount of Suc, Glc, and Fru (Table I). After 3 d, when α-amylase induction occurred (Perata et al., 1992; Table II), an increase in the amount of Glc resulting from starch breakdown was observed.

Several papers have been published concerning the time sequence of carbohydrate breakdown and synthesis during aerobic germination of rice (Murata et al., 1968; Nomura et al., 1969; Palmiano and Juliano, 1972); therefore, we focused our subsequent experiments on the anaerobic germination of rice by analyzing carbohydrates in the seed tissues dissected from anaerobic seedlings. Glc was mainly

Table I. *Carbohydrate contents of rice seedlings grown in air and anoxia*

Data are from two separate experiments.

Treatment and Days	Suc	Glc	Fru
	nmol per seedling		
Air			
0	547	492	313
	603	560	343
1	612	125	97
	714	147	111
2	1172	249	258
	1368	291	322
3	2328	709	487
	2572	831	573
Anoxia			
0	547	492	313
	603	560	343
1	332	343	187
	414	417	211
2	154	224	60
	186	274	84
3	212	427	93
	256	535	155

Table II. *Effects of anoxia on enzymes of carbohydrate metabolism of rice seedlings*

Data (milliunits mg^{-1} protein) are means ± SE of three replicates. nd, Not detectable.

Enzyme and Tissue	Air			Anoxia		
	0 d	4 d	8 d	0 d	4 d	8 d
α-Amylase						
Endosperm	nd	3580 ± 130	8600 ± 340	nd	670 ± 70	2460 ± 300
Embryo	nd	2300 ± 270	1910 ± 480	nd	1680 ± 150	4110 ± 190
Shoot	–	nd	nd	–	nd	nd
Root	–	nd	nd	–	–	–
GK						
Endosperm	30 ± 5	72 ± 2	54 ± 2	30 ± 5	32 ± 4	75 ± 9
Embryo	24 ± 2	63 ± 3	63 ± 22	24 ± 2	120 ± 15	228 ± 59
Shoot	–	43 ± 4	38 ± 17	–	44 ± 5	111 ± 8
Root	–	44 ± 8	48 ± 9	–	–	–
FK						
Endosperm	59 ± 9	105 ± 12	88 ± 2	59 ± 9	67 ± 19	150 ± 19
Embryo	44 ± 3	95 ± 11	62 ± 4	44 ± 3	284 ± 15	301 ± 42
Shoot	–	57 ± 4	55 ± 6	–	312 ± 1	428 ± 16
Root	–	70 ± 9	55 ± 16	–	–	–
Invertase						
Endosperm	nd	19 ± 4	28 ± 6	nd	nd	nd
Embryo	4 ± 1	44 ± 7	27 ± 2	4 ± 1	9 ± 2	6 ± 1
Shoot	–	52 ± 8	42 ± 5	–	19 ± 1	18 ± 2
Root	–	89 ± 9	84 ± 16	–	–	–
SS						
Endosperm	88 ± 6	15 ± 7	nd	88 ± 6	58 ± 16	54 ± 2
Embryo	43 ± 1	41 ± 8	49 ± 9	43 ± 1	168 ± 1	102 ± 15
Shoot	–	117 ± 26	212 ± 12	–	362 ± 12	540 ± 13
Root	–	120 ± 2	92 ± 27	–	–	–
PGI						
Endosperm	659 ± 115	924 ± 63	862 ± 54	659 ± 115	576 ± 24	763 ± 17
Embryo	603 ± 8	1198 ± 29	1114 ± 26	603 ± 8	1259 ± 99	1437 ± 68
Shoot	–	570 ± 74	567 ± 101	–	703 ± 98	993 ± 114
Root	–	483 ± 38	609 ± 23	–	–	–
PGM						
Endosperm	1600 ± 320	1260 ± 210	1180 ± 310	1600 ± 320	1110 ± 280	700 ± 10
Embryo	1150 ± 340	2580 ± 60	2600 ± 140	1150 ± 340	1620 ± 150	1750 ± 120
Shoot	–	1410 ± 190	910 ± 20	–	880 ± 70	870 ± 10
Root	–	1100 ± 60	660 ± 30	–	–	–
UDPGlc-PPase						
Endosperm	3430 ± 870	2360 ± 350	4190 ± 960	3430 ± 870	1870 ± 30	2050 ± 250
Embryo	2000 ± 260	6420 ± 1260	5140 ± 440	2000 ± 260	4450 ± 730	5220 ± 70
Shoot	–	2530 ± 110	2660 ± 760	–	2510 ± 110	1940 ± 30
Root	–	1880 ± 50	1630 ± 110	–	–	–
NDPK						
Endosperm	2360 ± 120	1740 ± 110	1010 ± 20	2360 ± 120	2720 ± 690	1460 ± 20
Embryo	820 ± 50	1470 ± 180	1630 ± 30	820 ± 50	1590 ± 190	2210 ± 320
Shoot	–	1020 ± 90	910 ± 20	–	960 ± 150	1200 ± 30
Root	–	680 ± 90	810 ± 20	–	–	–

localized in the endosperm (Fig. 1), as expected from the degradation of the starchy endosperm, but a considerable amount of Glc was also detected in the incubation medium (up to 9 μmol/seedling after 10 d under anoxia), as the result of leakage from the germinating seeds.

The amount of Suc present in the germinating seedling increased (4–10 d of germination), indicating that Suc synthesis had taken place in the anoxic rice seedling. The site of Suc synthesis is likely the scutellum, whereas the presence of Suc in the endosperm (Fig. 1) is explained by a possible "backflow" from the embryo to the endosperm as shown previously for aerobic rice seedlings (Nomura et al., 1969).

Sugar monophosphates were not detectable in aerobic seedlings. Glc6P was found to be present in the dry seed and Fru6P was detected in the dry-seed embryo (Table III), but their concentrations decreased (below the detection limit of the method used) during aerobic germination in all of the organs examined (data not shown).

A higher content of sugar monophosphates was observed in anaerobic seedlings (Table III): although Glc1P was not detectable in the tissues examined, the Glc6P content in the anoxic embryo decreased during the first 6 d of anaerobic germination but subsequently increased. A stable Glc6P concentration was found to be present in the

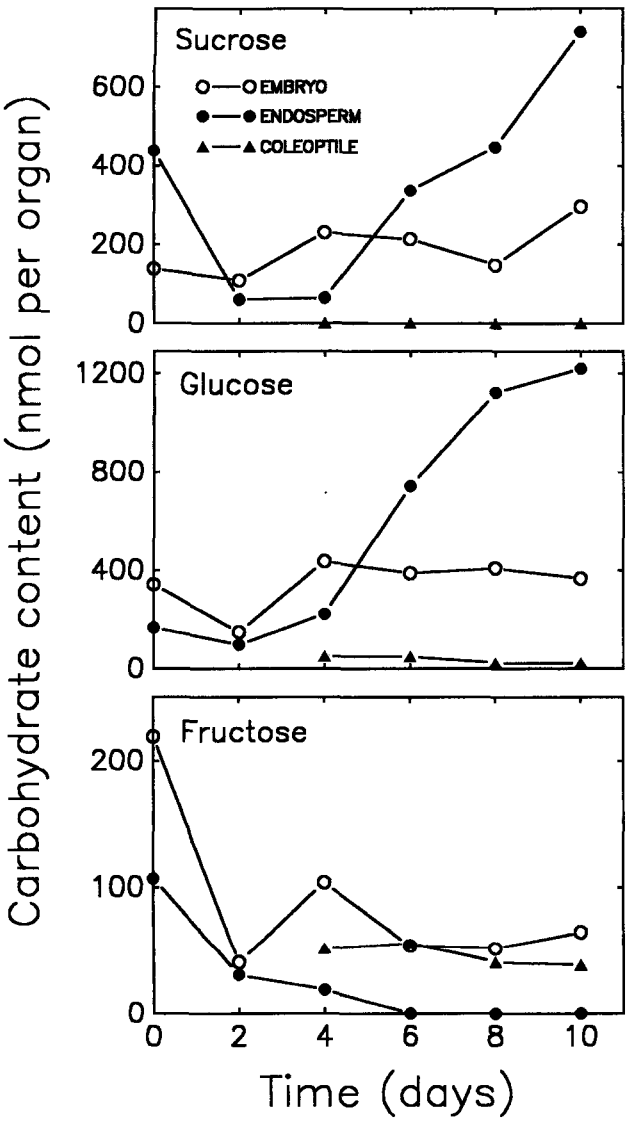

Figure 1. Content of Suc (top), Glc (middle), and Fru (bottom) in rice seeds germinating under anoxic conditions. Data are means of two replicates. Variation width did not exceed 15% of the given values.

anoxic coleoptile. The amount of Fru6P present in the dry-seed embryo decreased after 2 d of germination and this concentration remained relatively stable during the following days. These results are in agreement with those reported by Mohanty et al. (1993), indicating that anoxia increased the concentration of hexose monophosphate in rice suspension cultures.

Enzymes of Carbohydrate Metabolism in Aerobic and Anoxic Rice Seedlings

As previously reported, the induction of α-amylase also took place under anoxic conditions in rice (Perata et al., 1992). These results have been confirmed for the rice variety under investigation (Table II). Under anoxia α-amylase activity was lower than under aerobic conditions in the endosperm, whereas a higher activity was found to be present in the embryo at 8 d (Table II).

GK activity was needed for the phosphorylation of the Glc units resulting from the amylase-catalyzed starch breakdown. Table II shows the effect of anoxia on GK activity (ATP as the phosphate donor). Results indicate that the activity of GK was almost unaffected by anoxia in the endosperm, whereas a strong increase in GK activity was observed in both the embryo and the shoot. Under both aerobic and anaerobic conditions GK activity was mainly localized in the embryo tissue, including the scutellum, where Glc resulting from starch degradation was taken up and phosphorylated for further metabolism.

The conversion of Glc6P to Fru6P, a step needed for entering Glc6P in the glycolytic flux, is catalyzed by the enzyme PGI, whose activity, which was high under both aerobic and anaerobic conditions (Table II), increased under anoxia in both the embryo and shoot tissue.

As reported above, Suc was synthesized in anaerobic rice seedlings. Therefore, the presence of Suc-metabolizing enzymes is expected if Suc utilization is important for anaerobic metabolism. Two enzymes are involved in the cleavage of Suc: alkaline invertase and SS (ap Rees, 1992). Table II shows the activity of alkaline invertase in aerobic and anaerobic rice seedlings: although in the presence of oxygen invertase activity increased during the germination of the seeds, the activity was strongly reduced under anoxia.

Table II also shows the effects of anoxia on SS activity. Under aerobic conditions SS activity increased during the germination and was mainly localized in the shoot where Suc is likely broken down. A higher activity of SS was found under anoxia, with a more than doubled activity in the anoxic shoot when compared with the aerobic shoot.

Table III. *Hexose monophosphate content of rice seedlings grown under anoxia*

Glc1P was not detectable in any of the tissues. nd, Not detected (detection limit: 100 nmol g^{-1} fresh weight). Data are from two separate experiments.

Tissue and Days under Anoxia	Glc6P	Fru6P
	nmol g^{-1} fresh wt	
Endosperm		
0	288–348	nd
2	nd	nd
4	nd	nd
6	nd	nd
8	nd	nd
10	nd	nd
Embryo		
0	4895–5359	1216–1580
2	876–1132	231–317
4	nd	200–302
6	nd	213–289
8	208–272	214–264
10	833–1063	333–455
Coleoptile		
4	1063–1241	nd
6	1144–1430	nd
8	996–1138	nd
10	979–1201	nd

The marked enhancement of SS activity under anoxia, in contrast to the reduced invertase activity, resulted in a ratio between SS and invertase of 30/1 and 5/1 in 8-d-old anoxic and aerobic coleoptiles, respectively. This may indicate that under aerobic conditions Suc can be broken down by both invertase and SS, whereas under anoxia the SS pathway plays a major role. Granted that such a mechanism really operates, we might expect to find related enzyme activities, such as FK, UDPGlc-PPase, and PGM (Huber and Akazawa, 1986; Black et al., 1987). Germination under anoxia resulted in a clear increase in FK activity (Table II): the activity found in the coleoptiles was about 8 times higher under anoxia than in air.

The activity of UDPGlc-PPase, catalyzing the conversion of UDPGlc to Glc1P and UTP, was very high under both aerobic and anaerobic conditions (Table II), even if under anoxia a slightly lower activity was observed in the endosperm and shoot tissues.

Relatively high activities of PGM were detectable in both aerobic and anaerobic conditions (Table II), even if under anoxia lower activities were observed in all the tissues examined.

Cycling of Urydilates

Suc degradation through SS and UDPGlc-PPase needs a mechanism of recycling UTP to UDP. We investigated whether the UTP formed by UDPGlc-PPase could be used by HK, leading to cycling of urydilates needed for the operation of the SS pathway (Huber and Akazawa, 1986; Xu et al., 1989). Results indicate that neither GK nor FK can use UTP as an efficient phosphate donor under either aerobic or anaerobic conditions (data not shown). A significant increase in the UTP-dependent activity of GK and FK was observed when aerobic seedlings were exposed to anoxia, but these activities were only about one-sixth of those observed with ATP as phosphate donor (data not shown).

Since the UTP-dependent activities of GK and FK are too low to allow the recycling of uridylates at a high rate as proposed by Huber and Akazawa (1986), we tested whether, as suggested by Renz and Stitt (1993), NDPK activity could allow the cycling of urydilates through the ADP-mediated conversion of UTP to UDP with ATP production.

Our results show that a high NDPK activity was present under both aerobic and anaerobic conditions, with a slightly higher activity under anoxia (Table II). We further investigated cycling of urydilates by testing whether crude extracts of rice seedlings could cycle the urydilates during the breakdown of Suc through the coupled activity of SS, UDPGlc-PPase, and PGM present in the dialyzed preparations. First we tested the stoichiometry of the conversion of Suc to Glc1P using a limited amount of UDP: if cycling of uridylates takes place through the action of FK, the ratio between the NADPH produced (through the oxidation of Glc6P catalyzed by the addition of Glc6P dehydrogenase from *Leuconostoc mesenteroides*) and the UDP added for the activity of UDPGlc-PPase should be higher than 1, as shown by Xu et al. (1989) using potato extracts. Our results, presented in Figure 2, indicate that in rice extracts no cycling of uridylates took place through the action of FK, but addition of ADP to the reaction mixture resulted in a clear cycling of urydilates. The ratio NADPH/UDP became rapidly higher than 1 in the presence of ADP, indicating that the NDPK-mediated conversion of UTP to UDP occurred (Fig. 2). Moreover, cycling of the adenylates resulting from the NDPK activity, likely through the activity of FK, was also observed using a limited amount of both UDP and ADP. The reaction, monitored through the recording of NADPH production, lasted longer than 10 h despite the presence of UDP and ADP in amounts theoretically allowing the operation of the coupled enzymatic reaction for only 20 min (data not shown). This was likely the result of the cycling of both urydilates and adenylates through the combined action of NDPK and FK, as represented in Figure 3. An increase in the rate of NADPH production was also observed in the presence of both UDP and ADP, as was expected from the production of ATP and its utilization by FK leading to a ratio of 2 mol of NADPH produced per mol of Suc broken down (when compared with the initial equimolar ratio occurring when no phosphorylation of Fru takes place).

DISCUSSION

The ability of rice to induce α-amylase under anoxia and to degrade the starchy reserves present in the endosperm was reported previously (Perata et al., 1992, 1993a) but no information has been available concerning the anaerobic fate of the soluble carbohydrates present in the dry seed or resulting from starch degradation.

Our results indicate that two phases can be recognized in the metabolism of carbohydrates in rice seeds germinating

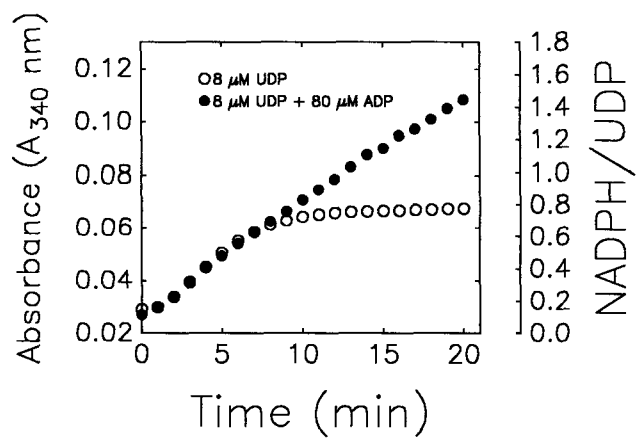

Figure 2. Urydilates cycling via NDPK in dialyzed extract from 9-d-old anoxic rice seedlings. Suc conversion to Glc6P via the SS pathway (involving the activity of SS, UDPGlc-PPase, and PGM) was measured by recording NADPH formation by the *Leuconostoc* Glc6P dehydrogenase. NADPH production was dependent on the presence of both PPi and UDP (not shown). In the absence of ADP the reaction stopped when UDP was consumed (open symbols) leading to the NADPH/UDP ratio shown in the figure. Cycling of urydilates was observed if ADP was added to the reaction mixture (filled symbols), since the reaction continued even when virtually all of the UDP added was consumed.

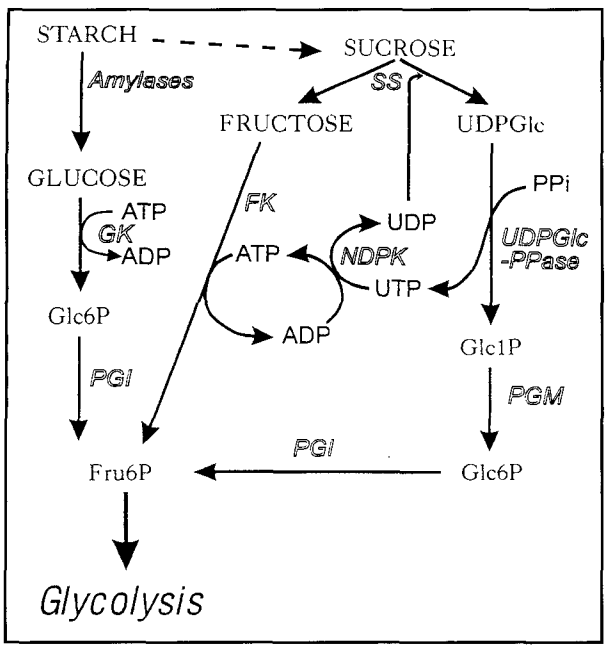

Figure 3. Proposed pathway of Suc degradation in anoxic rice seedlings. The products of starch degradation are mainly converted to Suc in the scutellum (dashed arrow; Nomura et al., 1969) and partially channeled to the glycolytic pathway. Suc is metabolized through the SS pathway with NDPK, allowing the cycling of urydilates. See "Discussion" for details.

under anoxia. The first phase is characterized by the catabolism of the sugars present in the dry seed, and in the second phase, starting only after the induction of α-amylase, the increased concentration of Glc and Suc indicates that starch breakdown and Suc synthesis are occurring in the anaerobic seedling.

Among the enzymes involved in the metabolism of carbohydrates that we examined, anoxia depressed considerably only the activity of invertase. On the contrary, the activities of GK, SS, FK, and, to a certain extent, PGI and NDPK were higher under anoxia compared to aerobic conditions. Our results are in agreement with those reported by Ricard et al. (1991) and Bertani et al. (1981), showing the induction of SS and PGI under anoxia, but we failed to confirm the enhancement of PGM activity under anoxia as reported by Rivoal et al. (1989). A report indicating a progressive reduction of both acid and alkaline invertase in rice roots under anaerobic conditions has been published (Bertani et al., 1981).

The rate of starch degradation in rice under anoxia is slower than in air (Perata et al., 1992). Therefore, it is likely that the enhanced activity of GK found in the anaerobic seedlings can ensure the phosphorylation of Glc resulting from starch breakdown. Nevertheless, it should be remembered here that HKs may be regulated by the ATP/ADP ratio, as demonstrated in potato tubers by Renz and Stitt (1993). More studies are required to establish whether the ATP/ADP ratio present in rice seedlings under anoxia may affect the activity of HK, even if results from Mohanty et al. (1993) indicate that long-term anoxia does not alter the ATP/ADP ratio in suspension-cultured rice cells.

Concerning the ability of rice to metabolize Glc under anoxia, Mayne and Kende (1986) found that rice seedlings aerobically germinated and subsequently transferred to anaerobic conditions are able to metabolize Glc at a rate similar to that of the tissue incubated under aerobic conditions, indicating that anoxia does not interfere with Glc metabolism in rice.

Under aerobic conditions both invertase and SS activities are present; it is therefore not easy to assess the contribution of SS in the metabolism of Suc under aerobic conditions, but our results suggest that under anoxia the SS pathway plays a major role in the process of Suc degradation in the cytosolic compartment. This is supported by (a) the decreased activity of alkaline invertase activity under anoxia, (b) the strong increase of SS activity under the same conditions that result in a 1/30 ratio of activities between invertase and SS in 8-d-old coleoptiles, and (c) the presence of the enzymatic set needed for the operation of the SS pathway. Activities of SS, UDPGlc-PPase, PGM, PGI, and FK are high under aerobic conditions, and anoxia did not reduce the activity of these enzymes. On the contrary, anoxia enhanced the activity of SS, GK, PGI, and FK. Moreover, the flow of carbon from Suc to hexose monophosphates through the SS pathway results in a considerable ATP saving when compared with the invertase pathway. Granted that the pathway proposed in Figure 3 operates in vivo, and assuming that PPi is present in the cell as a by-product of biosynthetic reactions (see below), no ATP is needed for the conversion of Suc to hexose monophosphates, whereas two ATPs are needed through the invertase pathway.

The SS gene is induced in several plant species under anoxia (McElfresh and Chourey, 1988; Chourey et al., 1991; Ricard et al., 1991). Nevertheless, until now only rice appeared to be able to induce SS at both the transcriptional and translational level (Ricard et al., 1991), whereas in corn and *Sorghum*, two plant species showing a low tolerance to anoxia, the induction has been observed only at the transcriptional level (McElfresh and Chourey, 1988; Taliercio and Chourey, 1989; Chourey et al., 1991). These results, together with the data reported in this paper, may indicate that the SS pathway could play a role in the tolerance to anaerobiosis.

It has been proposed that the cycling of the uridylates (UTP/UDP), necessary for the SS pathway, can be accomplished by the UTP-dependent phosphorylation of the Fru units resulting from the cleavage of Suc (Huber and Akazawa, 1986; Xu et al., 1989). Our results suggest that the cycling of the UTP/UDP pool may be accomplished by the action of NDPK (Renz and Stitt, 1993).

PPi, needed for the operation of UDPGlc-PPase, is a by-product of several biosynthetic reactions (Smyth and Black, 1984; Mertens et al., 1990); data available from the literature indicate that its pool in plant cells (Smyth and Black, 1984; Huber and Akazawa, 1986; Dancer and ap Rees, 1989a) is not affected by anoxia (Dancer and ap Rees, 1989b; Mohanty et al., 1993). It has been suggested (Huber and Akazawa, 1986; Xu et al., 1989; Mohanty et al., 1993) that the bulk of PPi needed for the activity of UDPGlc-PPase may be provided by the activity of PFP in the glu-

coneogenic direction, even if it is unlikely that PFP is the only source of PPi for Suc metabolism (ap Rees, 1992). Activity of PFP is indeed present in anaerobic rice seedlings (Mertens et al., 1990) and anoxia leads to an increased activity of this enzyme as well as to an increased concentration of its activator, Fru-2,6-bisphosphate. Nevertheless, data from Mertens et al. (1990) also indicate that a marked decline in the phosphofructokinase activity is observed in rice seedlings under anoxia. Since an increased glycolytic flux is expected under anoxia to counteract the decreased ATP production in the absence of oxygen, it is quite likely that PFP acts in the glycolytic direction under anaerobic conditions. Moreover, as stressed by Mertens et al. (1990), PFP activity in the glycolytic direction would allow a 50% increase in the ATP yield of glycolysis. A recent report by Hajirezaei et al. (1994) indicates that PFP catalyzes a net glycolytic reaction in potato tubers.

In conclusion (Fig. 3), under anoxia rice is able to utilize its starchy reserves through the action of α-amylase, and the set of enzymatic activities needed for the metabolism of the soluble sugars, either present in dry seed or resulting from starch breakdown, is available in the anoxic rice seedling. Suc is synthesized under anoxia and its metabolism likely occurs through the SS pathway, including NDPK for the cycling of uridylates. The source of PPi needed for the activity of UDPGlc-PPase is unknown at present and requires further studies.

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