The metabolism of aged seeds. The involvement of the pentose phosphate pathway in respiration in germinating rye grains of various ages

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Abstract

Winter rye grains from different harvest years (having distinctly different viabilities) were studied in terms of germination, total dehydrogenase activity and growth analysis. Reduction of seed vigor and viability was accompanied by a decrease in the intensity of embryo respiration during germination. The use of ($1\text{-}^{14}\text{C}$) glucose and ($6\text{-}^{14}\text{C}$) glucose showed that germinating rye embryos catabolize glucose through glycolysis, Krebs cycle and the pentose phosphate pathway. It was also shown that the C_6/C_1 ratio in respiring embryos initially increased during the first 48 hours of germination, then dropped, which suggests a mounting contribution of the PP pathway to the overall catabolism. The oxidation of glucose in embryos from the most deteriorated grains proceeded through glycolysis and Krebs cycle only.

Key words: seed, ageing, rye, respiratory system

INTRODUCTION

The pentose phosphate pathway (PPP) is an alternative to catabolizing glucose through glycolysis and Krebs cycle. It was initially thought that the basic role of the PPP is the interruption of both plant and seed dormancy (Simonds and Simpson 1972, Roberts 1973, Hendricks and Taylorson 1975, Roberts and Smith 1977). It was also proven that in unripe seeds, the pentose phosphate pathway participates to a greater extent in the oxidation of glucose than in morphologically mature seeds. According to Grzesiuk and Kulka (1988), the oxidation of glucose in young tissues through the PPP is

minimal, but increases with age. It is known, however, that glycolysis with the accompanying Krebs cycle is the basic pathway of glucose oxidation. In most of the plant organs studied to date, both the PP and glycolytic pathways have been found to be active (Salisbury and Ross 1969).

Respiration must be acknowledged as one of the most conclusive indicators of vigor and intensity of life-processes in ageing seeds. The ageing of grain seeds, for example, is accompanied by an especially large fall in the activity of mitochondrial respiratory enzymes in the embryo. This is caused by the destruction of mitochondrial membranes (Roberts and Ellis 1982). Mainly it is the endpoint dehydrogenases and oxidases which undergo inactivation, resulting in the uncoupling of oxidative phosphorylation and decreases the adenylate energy charge (Grzesiuk et al. 1983, Grzesiuk and Tłuczkiwicz 1983). Ageing seeds are often characterized by a high respiratory index which reaches values of 1.5 or more (Anderson 1970, Koźmina 1976). In studies carried out to now, the participation of the particular pathways of glucose metabolism have not been determined for the overall balance of glucose oxidation, which was the reason for undertaking the presented study.

MATERIAL AND METHODS

The study was carried out on winter rye grains cv. Dańkowskie Żłote. The grains were harvested at full ripeness, dried and stored in linen bags in a laboratory, at a temperature of 18–20°C and average humidity of 50–55%. Grain harvested in 1980, 1982, 1984 and 1986 was analyzed. Grain harvested in 1989 of the same variety served as the control, and was analyzed immediately after post-harvest dormancy ended.

The viability of the grain was determined using the germination method according to the international ISTA rules (Anonymous 1976).

Imbibition and germination. Before germination the grain was sterilized in a 2% solution of sodium hypochlorite for 3 minutes, after which it was washed carefully in sterile water, its surface dried and placed in sterile Petri dishes containing a triple layer of Whatman No. 1 filter paper saturated with water. The seeds were germinated for 6–48 hours at 21°C in darkness. After the planned duration of germination, the embryos with their disk were isolated by hand from the grains and transferred to Warburg manometric vessels.

Determination of the C_6/C_1 ratio in embryos. The C_6/C_1 ratio was determined by measuring the amount of $^{14}\text{CO}_2$ emitted during the catabolism of glucose labelled at the sixth or first carbon, according to the method of Agrawal and Canvin (1971). The C_6/C_1 ratio was determined in a Warburg apparatus using 100 (when germination was conducted for 6–24 hrs) or 50 embryos (when germination lasted 48 hours). The embryos were incubated in manometric vessels at a temperature of 25°C for 3 hrs in the presence of solutions of 1- ^{14}C or 6- ^{14}C -glucose (74 KBq/cm³, 81.6 MBq/mmol) in 0.05M Tris-HCl buffer, pH 7.2.

The wells of the manometric vessels contained 0.25 cm³ 40% KOH in order to absorb the released ¹⁴CO₂. After incubation, 50 mm³ samples of the KOH were transferred to scintillation vials, 10 cm³ Tritosol (Fricke 1973) added and counted.

In order to measure the amount of radioactive glucose taken up, the embryos or sprouts were washed for 2–3 minutes under running distilled water, transferred to porcelain mortars and homogenized in 10 cm³ distilled water. The homogenate was centrifuged at 12 000 x g for 10 minutes. The supernatant was collected and the pellet homogenized again as before two more times and centrifuged. The supernatants were pooled and 1 cm³ was taken for determination of radioactivity.

In order to check if the experiment had in fact been carried out under sterile conditions, samples were taken from the glucose incubation mediums (after completion of the experiments) and cultured on full bacterial mediums. The plates containing the mediums and samples were incubated at 37°C for 48 hours. The lack of bacterial and fungus colonies showed that the influence of microflora on the results of the experiment had been eliminated.

The radioactivity of the samples was measured using a Beckman LS-1801 scintillation counter.

The total activity of dehydrogenases was determined after 24 hrs of imbibition of the grains in redistilled water at 20°C. The embryos (200) were isolated by hand and incubated in a 0.7% solution of tetrazolium chloride for 24 hrs (20°C), then homogenized in a porcelain mortar with 25 cm³ acetone. After decantation, the amount of formazan produced was determined.

The experiments were carried out in four replicates.

RESULTS

The analyzed rye grains had significantly different germination capacities in their harvest years (Table 1). In each case, however, it exceeded 90%. Storage caused a decrease in viability and vigor. This was manifested by a decrease in germination capacity, decrease in dehydrogenase activity in the embryos and lower sprout mass and length increases after 2–3 days of germination (Table 1).

After 6, 12, 24 or 48 hrs of germination in water at 21°C, embryos along with disks were isolated from the grain and incubated in solutions of radioactive glucose (25°C). After incubation, the remaining amount of radioactive glucose was determined in the embryos (Table 2). The presented results show that the control grain (from the 1989 harvest) had the highest take-up ability for both of the forms of glucose used. This trait was not always clearly visible after all of the incubation times (eg. after 12 hrs). The highest significant differences occurred between samples after 48, while the least after 12 hours of germination of grain, regardless of the type of glucose used. It was also after 12 hours of germination

that the lowest content of both types of glucose was observed in the embryos of all of the analysed samples with the exception of the most aged grain. In the embryos of this grain (1980), the quantitative changes in ^{14}C -labelled glucose were the most irregular, in spite of the significant influence of factor III (duration of germination, Table 2) on this process.

T a b l e 1
The viability and vigor of rye grains of different ages

Years of harvest	Germination capacity %		Total dehydrogenase activity (mg phenylformazan per 100 embryos)	Dry mass of 100 embryos (mg)		Coleoptile length after 72 hours germination (21°C) (mm)
	In year of harvest (control)	After period of ageing		Imbibition (8 h, 2°C)	Germination (48 h, 21°C)	
1989	93.7	93.7	3246	0.118	0.291	8.5
1986	92.8	52.0	1998	0.108	0.149	4.6
1984	90.3	31.8	1242	0.112	0.142	3.4
1982	94.2	12.3	770	0.106	0.108	3.5
1980	95.2	4.5	271	0.123	0.120	2.3
LSD, P=0.001	3.12	4.05	104.8	0.008	0.023	0.24

The intensity of respiration of rye grains of various ages was measured only in embryos germinated for 6 to 48 hrs. A measure of the intensity of respiration of embryos (sprouts) was the amount of $^{14}\text{CO}_2$ emitted, which increased along with the duration of germination (Fig. 1). The amount of $^{14}\text{CO}_2$ coming from the oxidation of glucose was also distinctly dependent on the degree of ageing (factor I) and decreased as the viability of the grain decreased. This regularity was not observed after 6 hrs of germination. Significant differences in the emission of $^{14}\text{CO}_2$ were also observed depending on the type of labelled glucose used (factor II, Fig. 1).

The results presented in Fig. 2 point to the dependence between the size of the C_6/C_1 ratio on the viability and vigor of the grain. After 6 hours of germination, the C_6/C_1 value equalled 0.45 for embryos from the control grain, and 0.8 for the grain harvested in 1982 and analyzed after 7 years of storage in a laboratory. Germination of the grain for a further 6 hours led to a high rise in the C_6/C_1 ratio, after which an equally large fall in this value was observed (after 24 hrs germination) in all of the samples, with the exception of the grain harvested in 1980. The opposite direction of the changes in the C_6/C_1 ratio in this sample also applies to the results obtained after 48 hrs of germination (Fig. 2).

Table 2

The ($1\text{-}^{14}\text{C}$) glucose and ($6\text{-}^{14}\text{C}$) glucose content in the isolated embryos after 3h incubation at 25°C preceded by 6-48 h germination at 21°C of rye grains of different ages

Years of harvest	Quantity of ¹⁴ C-glucose incorporated by 100 embryos (% of initial ¹⁴ C-glucose concentration in incubation mixture)							
	(1- ¹⁴ C) glucose				(6- ¹⁴ C) glucose			
	time of germination (h)							
	6	12	24	48	6	12	24	48
1989	9.75±0.38	4.55±0.31	24.58±1.48	28.88±0.80	10.31±0.78	5.41±0.41	23.24±1.17	29.59±1.26
1986	6.72±0.20	4.40±0.37	12.18±0.96	13.47±0.43	7.31±0.39	5.19±0.39	11.10±0.77	13.16±0.85
1984	7.92±0.43	3.93±0.19	12.88±0.57	13.05±0.40	8.12±0.37	3.94±0.41	12.02±1.22	11.99±0.88
1982	5.68±0.33	4.01±0.41	5.37±0.19	6.35±0.63	6.04±0.54	4.31±0.54	8.44±0.47	9.43±0.58
1980	5.94±0.47	5.52±0.79	6.87±0.80	5.61±0.85	6.57±0.57	8.02±0.53	8.93±0.80	7.03±0.72

Data in the table are supplemented by standard deviation. LSD, $P = 0.001$ for: factor I (years of harvest) — 0.28; factor II (1 or $6\text{-}^{14}\text{C}$ glucose) — 0.18; factor III (duration of germination) — 0.26; interactions for factors: $I \times II$ — 0.40; $I \times III$ — 0.57; $I \times II \times III$ — 0.81; $II \times III$ — 0.03.

Fig. 1. The intensity of embryo respiration (measured by the amount of liberated ¹⁴CO₂ during incubation (3 h, 25°C) with (6-¹⁴C) or (1-¹⁴C) glucose) isolated from germinating rye grains (6-48 h, 21°C) of various ages

Fig. 2. Changes in the C₆/C₁ ratio in germinating embryos isolated from rye grains of various ages

DISCUSSION

The ageing of the analyzed rye grains from several harvest years manifested itself in a decrease in their vigor and viability (Table 1). The vigor of the grain aged to various extents was determined by growth analysis of the sprouts (Górecki and Grzesiuk 1982) and by a method assessing the total dehydrogenase activity in the embryos (Woodstock 1973). The obtained results indicate a high correlation between vigor and germination capacity of grain (Table 1).

In order to determine in what way does ageing affect the uptake of various forms of glucose and if the possible differences in the uptake of (1- ^{14}C) and (6- ^{14}C) glucose by embryos affects the C_6/C_1 ratio, the content of the labelled precursors in the embryos was determined (Table 2). A high glucose content after 3 hours of incubation following 6 hours germination of whole grains in water indicates that this time period (6h) was too short for the full hydration of embryos. After a further period of germination (12 hrs), a significant fall, then a significant rise (24 h) in the content of both forms of glucose was observed in the control grains and those aged for 3 to 7 years. This increase was probably caused by increased takeup of water along with the precursors dissolved in it due to the passing of the embryos from the phase of germination to intense increase of mass and sprout length. The relatively high labelled glucose content in the embryos of the most severely aged grain (1980) could have been caused by the good development of the embryos (the highest dry matter content per 100 embryos among all of the analyzed samples), and, on the other hand, by the lowest intensity of respiration (catabolism of glucose) due to loss of vigor and viability (table 1). The correlation of seed viability and vigor with the amount of labelled glucose in the embryo, which decreased with the age of the grain, was especially visible after two days of germination (Table 2). In addition, it seems that the amount of labelled glucose in the embryos was high enough and did not have a decisive influence on the intensity of its catabolism or the value of the C_6/C_1 ratio (Fig. 2, Table 2).

One of the reasons for the ageing of seeds is the disruption of bioenergetic processes in the embryo or embryonic axes, which becomes apparent during germination (Ching 1973, 1982, Anderson 1977, Leopold and Musgrave 1980, Grzesiuk nad Tłuczkiewicz 1983). Studies on the intensity of glucose oxidation by embryos of rye grains of various degrees of deterioration fully support the above hypothesis (Figs. 1-2). Regardless of the type of glucose used and the duration of germination preceding incubation, the amount of $^{14}\text{CO}_2$ emitted by the embryos was the highest in the control grains and decreased along with loss of viability due to long storage of grain. This was first correlated with the low ATP level in embryo cells of ageing seeds (Ching 1973, Van Onckelen et al. 1974). Further studies showed that the low ATP level in the embryos is caused by numerous unfavorable changes in mitochond-

rial membranes (Roberts and Ellis 1982), inactivation of respiratory enzymes and uncoupling of oxidative phosphorylation (Tłuczkiewicz 1980, Grzesiuk et al. 1983). As the result of these changes, the ageing embryonic axes or embryos have a lowered adenylate energy charge (Anderson 1977, Osborne 1982, Grzesiuk et al. 1983).

The participation of the PP pathway is high and increases as germination proceeds in imbibing and germinating grain, eg., in barley (Roberts and Smith 1977). In the control sample of germinating rye grains, the C_6/C_1 ratio initially rose (1–12h), then fell, which indicates the increasing role played by the PP pathway in respiration (Fig. 2). Changes in the value of the C_6/C_1 ratio in rye grains from the 1989–1982 harvests were very similar. It can therefore be said that storage of rye grains for a period of 2–7 years causes a drop in its viability and vigor and is associated with a proportional reduction of respiration processes in the germinating embryos. This drop does not, however, change the proportion between Krebs cycle and the pentose phosphate pathway (Table 1, Fig. 2). However, the embryos of the most severely aged grain (the 1980 harvest) showed a different pattern of changes in the C_6/C_1 ratio (Fig. 2), which after 48 hrs of germination equalled 1. This indicates that after this time, oxidation of glucose occurred exclusively through glycolysis and Krebs cycle. The total inactivation of the PP pathway in ageing rye grains therefore occurs earlier than inactivation of glycolysis and Krebs cycle.

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Metabolizm starych nasion. Udział cyklu pentozofosforanowego w oddychaniu kielkujących ziarniaków żyta różnego wieku

Streszczenie

Zbadano kielkowanie, ogólną aktywność dehydrogenaz oraz wykonano analizę wzrostową siewek wyrosłych z ziarniaków żyta ozimego pochodzących z różnych lat zbioru, o wyraźnie zróżnicowanej żywotności. Obniżeniu żywotności i wigoru ziarna żyta towarzyszyło zmniejszenie natężenia oddychania zarodków podczas kielkowania. Stosując ($1\text{-}^{14}\text{C}$) glukozę i ($6\text{-}^{14}\text{C}$) glukozę stwierdzono, że kielkujące zarodki żyta katabolizują glukozę na drodze glikolizy i cyklu Krebsa oraz jednocześnie szlakiem pentozofosforanowym. Ponadto wykazano, że stosunek C_6/C_1 w oddychających zarodkach żyta różnego wieku podczas pierwszych 48 godzin kielkowania początkowo rośnie (0-12h) później zaś obniża się, co świadczy o zwiększeniu udziału cyklu PP w oddychaniu. W zarodkach ziarna najbardziej zestarzałego utlenianie glukozy zachodziło wyłącznie drogą glikolizy i cyklu Krebsa.