Effect of winter wheat (cv. Jana) seed ageing on its viability and vigour

EUGENIUSZ SÓJKA, RIAD ZAIDAN

Institute of Plant Biology, Agricultural-Technical Academy,
10-957 Olsztyn-Kortowo, Poland

(Received: December 1, 1981)

Abstract

Viability and vigour of freshly collected 1, 2 and 3 years old seeds of the winter wheat cv. Jana were determined, on the base of the rate of germination, germinative capacity and the mean germination time, as well as the length of the shoot after 4, 7 and 10 days of the germination test. The intensity of respiration of whole caryopses, of isolated embryos and of the mitochondria was also measured. It was shown that the seed vigour and viability decreases with ageing. In seeds stored over longer periods the respiratory activity decreases as consequence of disturbance of the biogenesis of mitochondria, decline of oxidative phosphorylation and a decrease of the ability to oxidize the respiratory substrate.

INTRODUCTION

The viability of grain is conditioned by genetic maternal and environmental factors prevailing during ripening and storage (Abdul-Baki and Anderson 1971, Ching and Schoolcraft 1968, MacKay 1972). The grain quality greatly deteriorates owing to mechanical injury (Abdul-Baki and Anderson 1971, Ching and Schoolcraft 1968) as well as with prolonged time of storage. A basic symptom of depressed viability of ageing seeds is a decrease of the germination capacity and the appearance of abnormal sprouts in large quantities (Berjak and Villiers 1972, Kulka 1971).

Maintenance of a high viability in stored grain and retardation of the ageing process could increase the reserves of seed grain. In agricultural practice, however, ageing grain is seldom used for sowing.

MATERIAL AND METHODS

The wheat grain of the variety Jana originated from the harvests of 1974, 1975, 1976, and 1977. For analyses grain of 2.5-2.8 mm size was used. Caryopses from the years 1974-1976 were stored in bags at room temperature and air moisture 60-70 per cent. The analyses were performed in 1977 after 24, 48, 72 and 96 h of soaking and germination on whole grain and isolated embryos. Germination tests were run on Petri dishes on moist filter paper at 20-21°C.

The germination rate and capacity were determined according to the modified I S T A method (1976). The germination rate was calculated after three and germination capacity after seven days of germination. Mean germination time was determined after Grzesiuk (1967). The length of the hypocotyl was measured from the tip to the scutellum on 50 specimens after 4, 7 and 10 days of germination. Grain and embryo respiration were measured in a Warburg apparatus at 25°C. Mitochondria were isolated from the homogenate by the method of centrifugal fractionation (Pomeroy 1974). The homogenate was prepared by the method of Ikuma (1970).

The respiratory activity of isolated mitochondria was measured by the polarographic method with the use of an oxygen electrode of Clark type. The electrode was placed in a reaction vessel thermostated at 25°C.

RESULTS AND DISCUSSION

CHARACTERISTIC OF VIABILITY AND VIGOUR ON THE BASIS OF GERMINATION AND SEEDLING GROWTH

The stored grain in a state of the normal dormancy underwent ageing and gradually lost its viability. The ageing wheat grain from earlier harvests as compared with fresh grain (1977 harvest) showed a lower germination rate and capacity and a longer mean time of germination (Table 1). Wheat grain from 1976 as compared with that from 1977 exhibited a rate and capacity depressed by 18 and 12 per cent, respectively. The decrease in germination rate (29%) was still more pronounced with a similar germination capacity (13%) in two-year-old grain from the 1975 harvest. The viability decrease was greatest in the grain from 1974 which germinated in only a low per cent (Table 1).

These relations found their reflection in the mean germination time (Table 1). In grain from the 1976 and 1975 harvests, with germination rate and capacity lower than that of grain from 1977, the germination time of one caryopse was prolonged by 16-25 h, thus the germination time of the whole seeds was extend by 30 and 45 per cent, respectively.

Table 1

Characteristic of viability of wheat grain cv Jana stored for various periods on the basis of germination tests

Grain age — year of harvest	Germination rate, %	Germination capacity, %	Mean germination time, days/1 grain
3 years — 1974	6	18	5.10
2 years — 1975	70	86	3.08
1 year — 1976	81	87	2.78
Fresh grain — 1977	99	99	2.13

The oldest grain (1974 harvest) as compared with fresh grain (from 1977) exhibited a germination time protracted by 72 h.

Measurements of seedling growth from wheat grains of different ages and viability showed that the old grains had the shortest shoots (Fig. 1). Between days 4 and 10 of germination the mean length of the shoots in grain from the 1976 and 1975 harvest (as compared with those of 1977) was by 16-20 per cent and 26-30 per cent shorter, respec-

Fig. 1. Growth of shoots from wheat cv. Jana grain harvested in the years: 1974 (a), 1975 (b), 1976 (c) and 1977 (d)

tively. In three-year-old grain (1974) the shoots growth was hardly noticeable, indicating a very low viability. It was, thus, found that with prolongation of the storage period the grain regularly lost its viability and this was accompanied by inhibition of the initial shoots growth. Storage for three years (under the above described conditions) caused an almost complete loss of grain viability.

Germination is a generally used routine test for evaluation of grain viability (Dorywalski et al. 1964, Grzesiuk 1967, MacKay 1972, McDonald 1975, Lityński 1977). It is considered that a high vital activity of seeds is better characterised by the rate of germination and the mean time of germination than by the germination capacity (MacKay 1972, Woodstock 1973). Germination of ageing seeds proceeds slower. Fresh seeds germinate rapidly (Abdul-Baki 1969, Ching 1972, Ovčarov and Košev 1974). With prolonged germination time (in the present experiment to more than 3 days), the amount of germinating seeds increased more in old than fresh grain (Kulka 1971, Woodstock 1973). This suggests that during germination of ageing seeds (with depressed viability) a repair mechanism is in operation in the embryo cells, although the growth of the seedlings is weaker.

INTENSITY OF RESPIRATION OF GERMINATING GRAIN AND EMBRYOS
AND RESPIRATORY ACTIVITY OF MITOCHONDRIA ISOLATED
FROM EMBRYONAL CELLS

The differences in the viability and growth of different-aged wheat seedlings served as basis for determination of the course of respiration of swelling and germinating grain and isolated embryos. The respiratory activity of isolated embryos and whole wheat grains of different ages increased in general with prolongation of germination time (Figs. 2, 3). Older grains and embryos took up much more oxygen than did fresh ones. The depressed oxygen uptake by germinating grain and embryos progressed with the age and decreasing viability. The differences in oxygen uptake by embryos and grain were noticeable in all the successive stages of germination, the disproportion in respiratory intensity being more pronounced in embryos than in whole grain. Respiration of embryos and fresh grain as compared with ageing ones with decreased viability was characterised by a higher dynamics of oxygen utilisation in later germination stages (Figs. 2, 3). Oxygen uptake in embryos and grain from the 1977 harvest between the first and fourth days of germination increased 10-11-fold. During the same time the increase in oxygen consumption by embryos and grain from the 1976 and 1975 harvest was correspondingly higher, 8-10 and 7-10 times, respectively, whereas respiration of embryos and grain from the

Fig. 2. Respiration intensity of germinating wheat cv. Jana grains harvested in the years: 1974 (a), 1975 (b), 1976 (c) and 1977 (d)

Fig. 3. Respiration intensity of germinating embryos from wheat grain cv. Jana harvested in the years 1974 (a), 1975 (b), 1976 (c) and 1977 (d)

year 1974 underwent hardly doubled. Numerous authors (Abdul-Baki 1969, Ching 1972, Ching and Schoolcraft 1968, Kittock and Law 1968, Kulka 1971, McDaniel 1969, McDonald 1975, Throneberry and Smith 1955, Tłuczkiewicz 1980) point to an exact relation between seed viability and their respiration. These associations occur as early as the first stages of swelling and germination (Woodstock 1973, Woodstock and Grabe 1967). They have been also confirmed in the present investigations.

The respiratory activity of isolated mitochondria from embryos taken from grain of the 1977 and 1975 harvests was determined after 48 h of germination. The respiratory activity of the mitochondria at various physiological stages (determined by the polarographic technique) concerns mainly oxygen utilisation at definite ADP concentrations (Chance and Williams 1956). The active state (state 3) occurs in the presence of ADP as energy acceptor when phosphorylation of ADP to ATP takes place. Then oxygen uptake by the mitochondria is rapid (Ikuma 1970, Pomeroy 1974). On the other hand, in the controlled state (state 4) oxygen consumption by mitochondria in the absence of ADP is very low (Chance and Williams 1956, Estabrook 1967, Ikuma 1970, Pomeroy 1974).

The rate of oxygen uptake in state 3 by mitochondria isolated from embryos of ageing grain was lower in the oxidation of all the substrates used than in the case of fresh grain (Table 2). Oxygen uptake in state 3 (in the presence of ADP) by mitochondria from embryos of aged grains (1975) was lower as compared with that in fresh grain for α -ketoglutarate by 25 per cent, L-malate with glutamate by 32 per cent and for succinate with glutamate by 11 per cent. The rate of oxidation of the substrates in the mitochondria in state 4 of the tested grain was similar in the case of α -ketoglutarate and L-malate, whereas for oxidation of succinate oxygen utilisation in state 4 was higher in the mitochondria of embryos from grain of the 1975 harvest by 16 per cent as compared to that in the grain of the 1977 harvest (Table 2).

The following conclusions may be advanced on the basis of the above described investigations:

1. Fresh grain as compared with ageing grain (3-year-old) is characterised by a higher germination rate and capacity and a lower mean germination time. With prolongation of the storage time of the grain and its loss of viability these indexes decrease.
2. Respiration intensity (measured by the amount of oxygen taken up) in the germinating wheat grain, particularly in the embryos, depends on the age and viability of the grain and shows with ageing a decreasing tendency.
3. The mitochondria from embryos of grain with lowered viability and vigour exhibit a much lower ability of oxidation (stimulated by

Table 2

Respiratory activity of isolated mitochondria from embryos of germinating wheat cv Jana grains stored for various periods

Substrates	Year of harvest	Oxygen uptake, μmol/min/mg protein		Respiratory control (RCR)	ADP: O
		state 3	state 4		
α-ketoglutarate	1975	64±19.0	22± 5.8	2.90±0.57	2.22±0.10
	1977	88± 8.5	24± 5.5	3.54±0.50	2.57±0.11
α-malate with glutamate	1975	116±24.0	35± 7.8	3.31±0.09	2.04±0.0
	1977	169±43.8	36±11.1	4.73±0.50	2.35±0.0
Succinate with glutamate	1975	151±20.1	75±13.9	2.00±0.0	1.48±0.14
	1977	168±40.2	63±13.1	2.66±0.27	1.74±0.15

Results (means) and standard deviations (±) calculated from four lots of separately isolated mitochondria and three oxidation cycles (states 3 and 4) for each substrate

- ADP) of various substrates, and in the case of some substrates a higher oxygen consumption in energetically ineffective respiration.
4. In mitochondria from grain of low viability (as compared with the rapidly germinating one) there is a distinctly defective coupling of the oxidation and phosphorylation reactions (depression of RCR value) and there occurs a marked dissociation of oxidative phosphorylation (decreased ADP:O ratio).
 5. A reliable and objective evaluation of viability at various grain ages may be achieved by complex application of physiological and biochemical methods.

REFERENCES

- Abdul-Baki A. A., 1969. Relationship between seed viability and glucose metabolism in germinating barley and wheat seeds. *Crop Sci.* 9: 732-737.
- Abdul-Baki A. A., Anderson J. D., 1971. Glucose metabolism of embryos and endosperms from deteriorating barley and wheat seeds. *Plant Physiol.* 48: 270-272.
- Berjak P., Villiers T. A., 1972. Ageing in plant embryos. II. Age induced damage and its repair during early germination. *New Phytol.* 71: 135-144.
- Chance B., Williams G. R., 1956. The respiratory chain and oxidative phosphorylation. *Adv. Enzymol.* 17: 65-134.
- Ching T. M., 1972. Metabolism of germinating seeds. In: *Seed biology*, Kozlowski T. T. (ed.), vol. 2. Acad. Press, New York-London, pp. 103-219.
- Ching T. M., Schoolcraft I., 1968. Physiological and chemical differences in aged seeds. *Crop Sci.* 8: 407-409.
- Dorywalski J., Wojciechowicz M., Bartz J., 1964. *Metodyka oceny nasion*. PWRiL, Warszawa.

- Estabrook R. W., 1967. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. In: Methods in Enzymology, Estabrook R. W., Pulman M. (eds.), vol. 10, Academic Press, New York, pp. 41-47.
- Grzesiuk S., 1967. Fizjologia nasion. PWRiL, Warszawa.
- Ikuma H., 1970. Necessary conditions for isolations of tightly coupled higher plant mitochondria. Plant Physiol. 45: 775-781.
- International Seed Testing Association, 1976. International rules for seed testing. Annexes 1976. Seed Sci. Technol. 4: 357-411.
- Kittock D. L., Law A. G., 1968. Relationship of seedling vigor to respiration and tetrazolium chloride reduction by germinating wheat seeds. Agron. J. 60: 286-288.
- Kulka K., 1971. Biochemiczne aspekty starzenia się ziarna owsa i jęczmienia. Zesz. Nauk. WSR, Olsztyn 6: 3-79.
- Lityński M., 1977. Biologiczne podstawy nasiennictwa. PWN, Warszawa.
- MacKay D. B., 1972. The measurement of viability. In: Viability of seeds, Roberts E. H. (ed.), Chapman and Hall, London, pp. 172-208.
- McDaniel R. G., 1969. Relationship of seed weight, seedling vigor and mitochondrial metabolism in barley. Crop Sci. 9: 823-827.
- McDonald M. B., 1975. A review and evaluation of seed vigor tests. Proc. Assoc. Seed Anal. 65: 109-139.
- Ovčarov E. K., Košelev Y. P., 1974. Soderzhanye sakharov v siemenakh kukuruzhy raznoj zhiznesposobnosti. Fizjol. Rast. 21: 969-974.
- Pomeroy M. K., 1974. Studies on the respiratory properties of mitochondria isolated from developing winter wheat seedlings. Plant Physiol. 52: 653-657.
- Throneberry G. O., Smith F. G., 1955. Relation of respiratory and enzymatic activity to corn seed viability. Plant Physiol. 30: 337-342.
- Tłuczkiewicz J., 1980. Ageing-induced changes of respiratory activity in tissues and mitochondria from germinating rye grains. Acta Physiol. Plant. 2: 9-17.
- Woodstock L. W., 1973. Physiological and biochemical test for seed vigor. Seed Sci. Technol. 1: 127-157.
- Woodstock L. W., Grabe D. E., 1967. Relationship between seed respiration during inhibition and subsequent seedling growth in *Zea mays* L. Plant Physiol. 42: 1071-1076.

Wpływ wieku na żywotność i wigor ziarna pszenicy ozimej odmiany Jana

Streszczenie

Badano żywotność i wigor świeżego i zestarzałego ziarna pszenicy ozimej odmiany Jana, określając szybkość, zdolność i średni czas kiełkowania oraz długość łodyżek siewek po 4, 7 i 10 dniach kiełkowania. Zmierzono także intensywność oddychania całego ziarna, zarodków i wyizolowanych z nich mitochondriów. Wykazano, że wigor i żywotność ziarna zmniejszają się w miarę jego starzenia. W ziarnie przechowywanym przez dłuższy czas, zachodzą wyraźne, niekorzystne zmiany, polegające m.in. na obniżeniu aktywności oddechowej. Wiązało się to z zaburzeniami w biogenezie mitochondriów, obniżeniem fosforylacji oksydacyjnej oraz osłabieniem zdolności utleniania substratów oddechowych.