

Viability and vigour of ageing winter wheat grains

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(Received: September 7, 1981)

Abstract

The viability and vigour of ageing winter wheat caryopses of the cvs. Grana and Jana were tested. Viability was determined on the basis of germination capacity and rate, and vigour on the basis of the over-all activity of hydrogenases in the sprouts, exudate conductometry, analysis of sprout growth, oxygen uptake and mitochondrial protein content in the sprouts. What is called energy (or rate) of germination and over-all dehydrogenase activity in embryos and sprouts and the electroconductivity of exudates were found to be very good measures of the vigour of ageing caryopses. The latter two indices of vigour should be determined at a strictly defined moment of swelling and germination. Good measures of caryopse vigour are also respiration during swelling and at the beginning of germination and mitochondrial protein content in the sprouts or seedlings. There is a high correlation between the vigour of ageing grain and its bioenergetic indices.

INTRODUCTION

One of the most important features of seed for sowing is its vigour that is ability to active or dormant life and production, under suitable environmental conditions, of normal sprouts. Viability of seeds is mainly determined as their germination capacity or additionally by the tetrazole topographic method (International rules for seed testing 1976, Metody badania nasion 1979 (Methods of seed testing. Polish Standard)).

Seed viability determined in the laboratory does not always correspond to the field germination of plants as found in seeds at various stages of development — immature, aged etc. (Heydecker 1972, Maguire 1977, Ellis and Roberts 1980, Knypl 1979b, Matthews et al. 1980, Grzesiuk and Górecki 1981). These findings encouraged further investigations and led to a new criterion for

qualification of seeds for sowing, namely the seed vigour (Woodstock 1969, Heydecker 1972, Perry 1973, 1978, 1980, Maguire 1977).

Seed vigour is their potential capability to form healthy and well developing seedlings and plants within a wide range of environmental factors. Vigour characterises the potential productiveness of seeds, that is their useful value. It is a physiological character determined by the genotype and modified by the environment. Vigour is manifested in the capacity of rapid seedling development, high tolerance to stress factors in the environment, good growth and development of the plants (Maguire 1977, Perry 1978, 1980, Grzesiuk and Górecki 1981). Seed vigour is, thus, the potential influencing the course of the entire plant ontogenesis.

The principles and methods of evaluation of seed viability have been established by the International Seed Testing Association (ISTA) (International... 1976) and by Polish standards (Metody... 1979) and arouse no doubts. There are, however, certain difficulties in the choice and usefulness of methods for seeds of various plant species (Knypl 1979a, Perry 1978, 1980).

From among the presently applied methods of vigour determination (Grzesiuk and Górecki 1981) noteworthy are: evaluation in the embryos or sprouts of the over-all dehydrogenase activity (Woodstock 1973) and conductometric determination in exudates of the cytomembrane permeability in embryos and sprouts (Perry 1970, 1978, Heydecker 1972, Wojke and Ostrzycka 1975, Knypl 1979a). These methods are simple and rapid, and vigour determined by them shows for many plant species good agreement with field germination and plant productivity (Grzesiuk and Górecki 1981).

The use of a single method of seed vigour evaluation is often unreliable or gives controversial and even erroneous results. The fullest data may be obtained by the simultaneous use of several methods (Perry 1978, 1980, Grzesiuk and Górecki 1980). This is obvious since vigour is a group of characters requiring the application of more than one method (Grzesiuk and Górecki 1981).

The caryopses of cereals age during storage. This is manifested in a decrease of their viability (germination capacity) and vigour (abnormal sprouts, slowed down growth of seedlings, reduced productivity of plants). The rate of ageing of seeds under artificial conditions has even been used as a measure of their vigour (Delouche and Baskin 1973, Ellis and Roberts 1980, Matthews et al. 1980).

The mechanism of seed ageing consists mainly in: 1) damage to the genome and disturbances in transcription and translation of genetic information; 2) destruction of the system of cell cytomembranes of the embryo; 3) bioenergetic disturbances in the embryo (Grzesiuk 1980, Tluczkiewicz 1980, Grzesiuk and Kulka 1981).

The aim of the present investigations was the determination by several methods of the viability and vigour of different-aged caryopses of two winter wheat varieties (harvested in the years 1974, 1975, 1976, 1977 and tested in 1978).

MATERIAL AND METHODS

Winter wheat caryopses of the cultivars Grana and Jana were harvested at full ripeness from experimental plots of the Institute of Plant Biology of the Agricultural-Technical Academy at Olsztyn. They were further dried at room temperature and stored up to the year 1978 in linen bags at 16°-20°C and relative air humidity 40-70 per cent. The water content in the caryopses was 12-14 per cent of their mass.

For the investigations grains were selected of thickness exceeding 2.5 mm and 100-grain dry weight 3.7-4.1 g. Owing to fractionation, the material harvested in various years was uniform and comparable.

The caryopses were germinated on Petri dishes (8 dishes with 100 grains each) in a thermostat at 20°-21°C. The following determinations were carried out: germination energy (after 3 days), germination capacity (after 7 days), mean germination time (Grzesiuk 1967). Before germination the caryopses were soaked for 2 h in distilled water and washed with water repeatedly. This procedure ensured uniform swelling and germination of the seeds.

Coleoptile growth increment together with the plumule was measured from the apex to the germinal disk in 30 seeds after 4, 5, 7, 9 and 10 days. Dry and fresh weights (at 105°C) of the sprouts and seedlings were also determined.

Over-all dehydrogenase activity was evaluated in 50 freshly isolated embryos (without disks). Isolation was done after 12 h of soaking in water at 20°-21°C. The isolated embryos were flushed with 5 ml of 1 per cent 2,3,5-triphenyltetrazolin chloride solution in 0.1 M K-phosphate buffer (pH 7.2). The embryos were kept for 24 h at 20°-21°C. Then they were washed with water, dried and homogenised with absolute acetone (Piat and Springfield 1973). Extraction was repeated many times up to complete disappearance of phenylformazane in the homogenate. All the successive acetone eluates of phenylformazane were combined, mixed and left to stand for two hours in a refrigerator for clearing. Extinction of the solutions was measured in a spectrophotometre at wavelength 520 nm against absolute acetone (Grabe 1976). Phenylformazane content in the embryos was read from the standard curve prepared for phenylformazane within the concentration range 5-50 µg/ml. All operations connected with the use of triphenyltetrazolin chloride and isolation of phenylformazane were carried out in darkened rooms.

Electroconductivity of exudates was measured on samples consisting of 100 caryopses. Each caryopse was washed repeatedly with redistilled water, then flushed with 100 ml redistilled water and placed in a thermostat at 20°-21°C with stirring from time to time. After 2, 6, 10, 16, 24, 36 and 48 h conductivity of the exudate was measured with a Radelkis OK-102 conductometre. For comparison electroconductivity of water intended for flushing the caryopses was also measured and taken into account in calculation of the results.

Respiration intensity was determined by the manometric method in a Warburg apparatus. The number of caryopses in the sample after 1, 2, 3 and 4 days of soaking and germination was (for technical reasons) 25, 20, 15 and 10, respectively. Respiration measurements were conducted parallelly on four manometres and repeated four times on new material. Respiration intensity is expressed in $\mu\text{l O}_2$ taken up during one minute by one caryopse or sprout.

Mitochondria from the sprouts were isolated by differential centrifugation (Pomeroy 1974). The composition of the isolating mixture and the way of washing and suspension of the mitochondria followed the recommendations of Ikuma (1970). Protein content was determined by the biuret method with bovine blood albumin as standard (BSA).

The results were subjected to statistical analysis of variance with calculation of the smallest significant difference and of the correlations between viability and vigour of the caryopses and their bioenergetic properties.

RESULTS AND DISCUSSION

Winter wheat caryopses stored under laboratory conditions aged rather quickly. After four years their viability measured in terms of germination capacity decreased to several or a dozen or so per cent (Table 1). The process of ageing occurred faster in grain of the Grana than of the Jana cultivar. Ageing was most pronounced after three and four years of storage. This regularity was confirmed by vigour determination of the ageing caryopses (Table 2). The impairment of the cytomembranes probably increased in the aged caryopses, as indicated by the high exudation of electrolytes, and at the same time in the embryos of these caryopses the over-all activity of dehydrogenases decreased. As a consequence of destructive processes the ageing caryopses germinated slower and slower. The same was also manifested by slowing down of the rate of elongation growth of the sprouts and of the increase of their fresh and dry weight (Table 2).

Table 1

Germination of different-aged wheat grains (two varieties)

Age of caryopses (harvested in:)	Germination rate (energy), %		Germination capacity, %		Mean germination time, days	
	cv. Grana	cv. Jana	cv. Grana	cv. Jana	cv. Grana	cv. Jana
1 year (1977)	96.6	98.0	99.3	99.4	2.06	2.03
2 years (1976)	88.0	76.0	93.1	87.3	2.23	2.78
3 years (1975)	46.8	65.5	58.1	85.1	3.06	3.09
4 years (1974)	5.0	1.8	12.5	18.5	4.14	5.01
LSD P=1% for years	3.93	4.62	4.35	3.09	0.15	0.26

Slower germination of aged wheat caryopses was best demonstrated by what is called their germination energy (Table 1) which, according to the ISTA, is actually the germination rate (Grzesiuk and Górecki 1981) and the measure of seed vigour (Heydecker 1972). Analysis of the dynamics of germination of the Grana wheat determined within a 10-day period (Fig. 1) indicated the same. It is seen in this figure that the youngest caryopses reached full germination capacity as early as after three days, the two-year-old ones after five days, the three-year-old ones after 7-8 days, whereas the four-year-old ones did not reach this stage up to the 9th day. Figure 1 leads also to the conclusion that in aged caryopses germination energy diminishes very rapidly. Thus, in ageing caryopses germination energy is a very good measure of vigour.

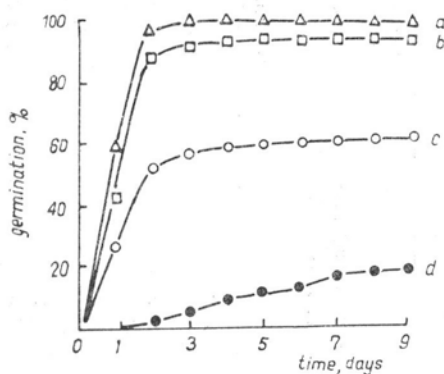


Fig. 1. Germination of wheat grains cv. Grana harvested in: 1977 (a), 1976 (b), 1975 (c), 1974 (d). Experiments of 1978

Table 2
Vigour of ageing winter wheat grains

Age of caryopses (harvested in:)	Electroconductivity of exudates after 24 h of seed soaking, $\mu\text{S}/\text{cm}$		Over-all dehydrogenase activity after 24 h of soaking, mg phenylfor- mazane/100 embryos		Sprout length after 7 days of germination, mm		Weight of wheat sprout, after 5 days of germina- tion, mg	
	cv. Grana	cv. Jana	cv. Grana	cv. Jana	cv. Grana	cv. Jana	cv. Grana	cv. Jana
1 year (1977)	72.33	52.03	1.950	2.626	83.7	67.5	77.0	9.42
2 years (1976)	112.93	76.39	1.690	2.288	75.1	57.0	65.8	8.52
3 years (1975)	133.33	105.75	1.170	1.560	53.4	45.0	49.0	6.57
4 years (1974)	173.83	169.77	0.442	0.624	11.5	10.5	16.8	2.99
LSD $P=1\%$ for years	8.12	10.15	0.104	0.130	6.3	4.5	5.6	0.45

The commonest methods of seed vigour evaluation are conductometric testing of exudates in the period of seed swelling and determination of dehydrogenase activity in the sprouts (Perry 1978, 1980). Seeds with low vigour excrete much exudate in which electrolytes prevail. The lower the electric conductivity of the exudates the higher is the seed vigour. The accuracy of this method depends, however, on the moment of its application during seed swelling. For ageing caryopses of the wheat Grana (Fig. 2) the optimal period for measurement was between the 6th and 24th hour of swelling. Earlier measurements (before 4 h of swelling, Fig. 2) showed but small differences, whereas at later periods, owing to germination, the proportions between caryopses of various vigour and variously aged were obliterated. Thus, by the use of accurate conductometers the vigour of caryopses can be evaluated after several hours of swelling. The most suitable moment for dehydrogenase activity determination was after 24 h of germination.

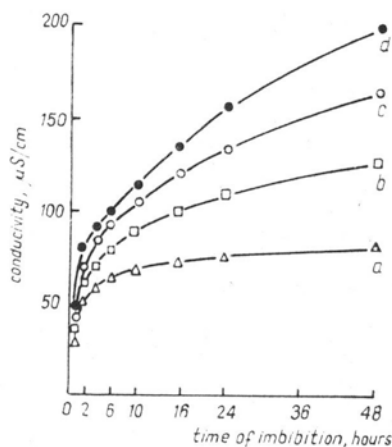


Fig. 2. Electroconductivity of seed exudates from wheat grains cv. Grana harvested in 1977 (a), 1976 (b), 1975 (c), 1974 (d). Experiments of 1978

Both seed viability and vigour are associated with their bioenergetics (Woodstock 1973, Perry 1978, 1980), the basic index of which is respiration. In ageing wheat caryopses this process measured in terms of O_2 uptake occurred slower and slower with ageing (Table 3). One- and two-year-old caryopses, that is those of high vigour (particularly their embryos and sprouts) took up oxygen intensively, whereas in the 4-year ones the process was hardly noticeable. The differences in respiration intensity of different-aged seeds appeared as early as the first day of germination, but were most pronounced after four days. In this period the respiration rate of 4-year-old caryopses was thirteen times lower

Table 3

Respiration (uptake of O_2 in μl) in the sprouts and mitochondrial protein content in ageing grains of winter wheat Grana

Age of caryopses (harvested in:)	Amount of O_2 (μl) taken up per 1 min. by:								Mitochondrial protein content after germination, mg/100 sprouts			
	caryopse after germination				sprout after germination							
	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days
1 year (1977)	0.20	0.28	0.78	1.30	0.12	0.20	0.66	1.15	1.00	1.55	2.52	3.15
2 years (1976)	0.16	0.20	0.71	1.12	0.08	0.15	0.55	0.97	—	—	—	—
3 years (1975)	0.10	0.15	0.38	0.80	0.05	0.09	0.09	0.58	0.75	1.10	1.70	2.51
4 years (1974)	0.03	0.05	0.06	0.10	0.02	0.03	0.03	0.04	0.55	0.60	0.70	0.75
LSD $P=1\%$ for years	0.016	0.032	0.048	0.054	0.020	0.026	0.052	0.065	0.15	0.20	0.10	0.25

that of one-year-old ones, and the same values for the sprouts were as much as 28 times lower.

Protein content in the mitochondria of germinating caryopses may serve as measure of the amount and biogenesis of these organelles. In aged caryopses mitochondrial protein content was very low, and in young seeds it was high. The content of this protein increased in the sprouts of young caryopses more than threefold, while in aged ones

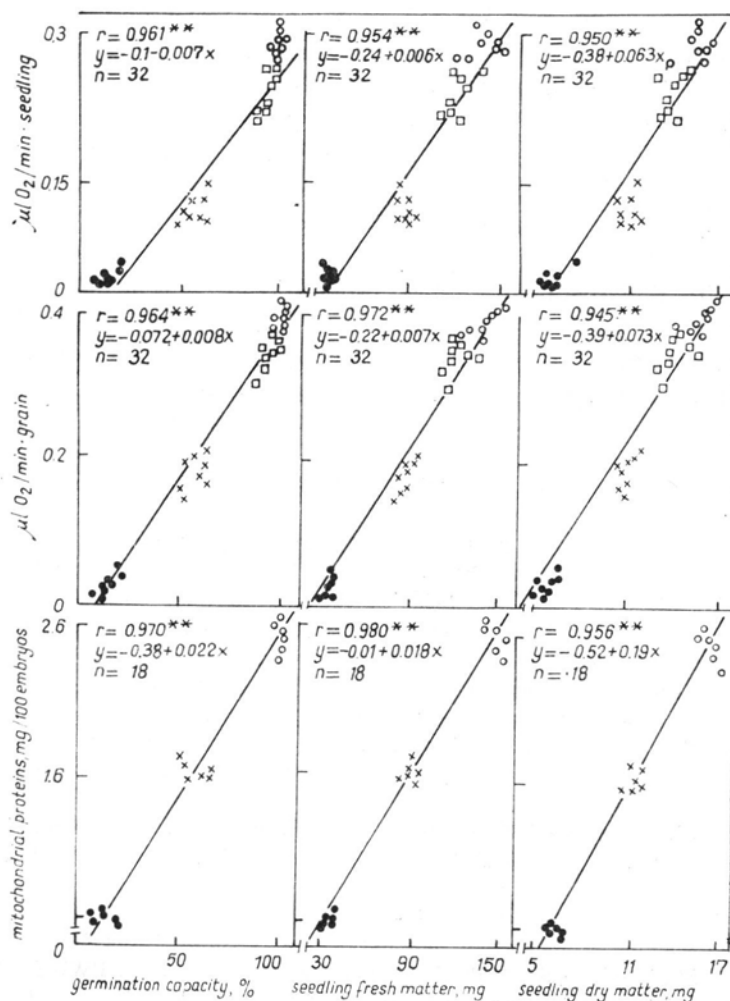


Fig. 3. Relationships between respiration intensity of 2-day sprouts, respiratory activity of grains soaked for 2 days, mitochondrial protein content in 3-day sprouts and germination capacity of grains, and fresh and dry weight of 9-day seedlings. Explanations: sprouts and seedlings obtained from wheat grains cv. Grana harvested in 1977 (o), 1976 (\square), 1975 (x), 1974 (\bullet)

r — correlation coefficient, y — regression lines, n — number of observations, ** — significant correlations at 1% level

barely by 50 per cent. This slow biogenesis of mitochondria in aged caryopses was one of the causes of the low respiration intensity of these grains. It results from the above quoted data that respiration intensity of wheat sprouts or of whole germinating caryopses may be a good index of the viability and vigour from the first days of germination, whereas the mitochondrial protein content can play this role not earlier than after five days of germination.

Calculation of some correlations between respiration and biogenesis of mitochondria, and germination as well as fresh and dry weight of the seedlings from ageing grain indicated (Fig. 3) close relations between some bioenergetic indices and both viability (germination capacity) and vigour (measured as fresh and dry weight of seedlings). Adoption of other vigour parameters than fresh and dry weight of seedlings for correlation calculations would probably reveal a still higher dependence of vigour on bioenergetics (Kittock and Law 1968, McDonald 1975, Woodstock 1973, Woodstock and Grabe 1967, Grzesiuk 1980). The results obtained indicate (Fig. 3) that the bioenergetic properties of caryopses may serve for determination of both their viability and vigour and that both these characters are interconnected. The notion of seed vigour, namely, implies its viability.

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Żywotność i wigor starzejących się ziarniaków pszenicy ozimej

Streszczenie

Zbadano żywotność i wigor starzejących się ziarniaków pszenicy ozimej odm. Grana i Jana zebranych w latach: 1974, 1975, 1976, 1977. Doświadczenia przeprowadzono w 1978 roku. Żywotność określano na podstawie zdolności i szybkości kiełkowania, zaś wigor na podstawie ogólnej aktywności dehydrogenaz, konduktometrii

eksudatów, analizy wzrostu kielków oraz zużycia tlenu i zawartości białka mitochondrialnego w kielkach czy siewkach. Stwierdzono, że w warunkach laboratoryjnych ziarniaki pszenicy dość szybko się starzeją tracąc po 3-4 latach zarówno żywotność jak i wigor. Bardzo dobrymi miernikami wigoru zestarzałych ziarniaków jest tzw. energia (czyli szybkość) kiełkowania oraz ogólna aktywność dehydrogenaz w zarodkach lub kielkach i elektroprzewodnictwo eksudatów. Dwa ostatnie wskaźniki wigoru należy oznaczać w ściśle określonym momencie pęcznienia i kiełkowania. Dobrym miernikiem wigoru ziarna jest także jego oddychanie podczas pęcznienia i na początku kiełkowania oraz biogeneza mitochondriów w kielkach i siewkach. Pomiedzy wigorem starzejącego się ziarna a jego wskaźnikami bioenergetycznymi istnieje duża korelacja.