THE INFLUENCE OF CO₂, TEMPERATURE AND α -TOCOPHEROL ON PHOSPHOLIPID CHANGES IN EMBRYONIC AXES OF FIELD BEAN SEEDS DURING STORAGE

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ABSTRACT

The paper presents the results of investigations of viability and phospholipids isolated from field bean seeds of different ages. Seeds were stored for seven years under controlled conditions in the Genes Bank in Radzików. No significant changes were detected in content or composition of phospholipids in seeds stored either at 4°C or in a CO₂ atmosphere, which seem to have maintained high viability. In embryonic axes of seeds stored at 18°C in air the levels of phosphatidylethanolamine and phosphatidylcholine declined, whereas those phosphatidic acid and unidentified fractions of the smallest polarity increased.

KEY WORDS: seeds, phospholipid, ageing, *Vicia faba* var. *minor*.

INTRODUCTION

Seed ageing and, consequently, seed storability depend on genetic and physiological properties of seeds (vigour and viability, chemical composition, condition of seed coat, structure of protein) as well as the effect produced by the environment in which seeds grow and are stored. Of the external factors affecting the longevity of seeds, conditions during seed maturation on parental plants seem most influential, as it is at the maturation phase that the potential vigour and viability of seeds are formed (Kulka 1994). It has been demonstrated on several occasions that seeds of the same variety (pea, yellow lupin) grown and harvested in various environments differ in their vigour and viability (Górecki 1986).

The following factors maintain relatively high seed vigour after harvest: air humidity and seed moisture, temperature, chemical composition of atmosphere, seed defects and other factors described earlier by many authors (Grzesiuk 1967; Berjak and Villiers 1972; Harrington 1973; Parrish and Leopold 1978; Lityński 1982; Grzesiuk and Kulka 1988; Kulka 1994; Zalewski, Lahuta 1998). Nowadays, it is widely acknowledged that loss of vigour and viability in ageing seeds stems mainly from destruction of cytoplasmic membranes (Wilson and McDonald 1986; Gidrol 1989; Leopold et al. 1990). Many scientists believe free radicals, formed also in dry seeds, are a fundamental, direct cause of unfavourable modifications occurring in cytoplasmic membranes. They are responsible for secondary changes, especially in phospholipids and glycolipids (Mazliak 1981; Bewley and Black 1982; Freeman and Crapo 1982; Wilson and McDonald 1986; Gidrol 1989; Hailstones and Smith 1989; Kulka 1994). Other researchers claim that seed longevity is connected with levels and composition of oligosaccharides, raffinose and sucrose in particular, present in seeds (Crowe et al. 1980; Koster and

Leopold 1988; Kermode 1990; Leopold 1990; Skriver and Mundy 1990; Bernal-Lugo et al. 1993; Blackman et al. 1993; Horbowicz and Obendorf 1994; Stil et al. 1994; Bernal-Lugo and Leopold 1995). The importance of these sugars in maintaining high seed viability during dessication has already been established. However, their protective function towards ageing seeds is still hypothetical.

MATERIAL AND METHODS

Field bean (*Vicia faba* var. *minor*) seeds of cv. Nadwiślański from 1987, 1994 and 1995 harvests were analysed. Following analyses of viability, samples of seeds harvested in 1987 were stored in the Genes Bank in Radzików (in tightly closed "twist" jars) from January 1988 to March 1995. The following storage conditions were maintained:

- air atmosphere, temperature 18°C,
- air atmosphere, temperature 4°C,
- CO₂ atmosphere, temperature 18°C,
- α -tocopherol, temperature 18°C.

Seeds collected in 1994 (to September 1995) were stored uncovered in a laboratory (temperature approx. 20-22°C, relative humidity 55-60%). Seeds were saturated in α -tocopherol by soaking in acetone solution containing 1% (w/v) α -tocopherol (Sigma). Surplus solution was poured out and residual acetone was evaporated. Seeds were then put into air-tight jars. Seed moisture was 9.2%.

Vigour and viability determination

The viability of the seeds was determined by the germination method according to the ISTA rules (Anonymous 1976). Before imbibition and germination the seeds were sterilized in a 2% solution of sodium hypochlorite for 3 minutes, after

TABLE 1. The indicators of vigour and viability of field bean (*Vicia faba* var. *minor*) seeds cv. Nadwislanski before (C – 1987) and after 7 years storage in Gene Bank under different conditions.

Sample of seeds	Germination rate (%)	Germination capacity (%)	Conductivity of leachates ($\mu\text{S} \cdot \text{cm}^{-2} \cdot \text{g}$)	Seedling length (mm)	Fresh mass of seedling (g)
1995	86.0	94.5	23.80	73.20	0.366
1994	90.0	95.0	21.20	79.40	0.379
C – 1987	99.0	99.0	19.23	84.06	0.396
1987 – CO ₂ , 18°C	95.5	95.0	44.24	71.00	0.332
1987 – 4°C	98.7	99.0	38.48	78.43	0.352
1987 – α -toc. 18°C	90.6	91.0	46.74	69.30	0.316
1987 – 18°C	58.5	64.4	67.51	20.77	0.102
LSD p=0.01	3.65	4.1	4.86	7.80	0.048

Symbols: 1987 – α -toc. – seeds treatment acetone with α -tocopherol before storage in Gene Bank; C – 1987 – control (analyzed in 1987).

which it was washed carefully in sterile water, wiped dry with sterile gauze and placed in Petri dishes. Germination was conducted for 7 (germination rate) or 14 days^{*} in redistilled water at 20°C in the dark. After 5 days seed germination at 20°C, the growth of seedlings and their fresh mass was determined. Conductivity of exudates of seeds was measured with a Redelkis OK 102/1 apparatus (20°C, Prusiński 1992). The total dehydrogenase activity was determined after 24 hours of imbibition in redistilled water at 20°C. The embryonic axes were excised by hand and incubated in 0.7% tetrazolium chloride for 24 hours (25°C), after which they were homogenized in acetone. After decantation, the amount of formazane produced was determined by measuring the A₅₂₀ with an Beckman recorder (Górecki 1986).

Isolation of phospholipids

From embryonic axes was carried out according to the method of Allen et al. (1966), as described by Pukacka (1983). Using silica gel 60G plates (Merck), lipids extracts were separated by thin-layer chromatography in chloroform: methanol:acetic acid:water (85:15:10:3.5, v/v, Nichols et al. 1965) using phospholipid standards from Sigma. Spots with phospholipids were scraped off for phosphorus analysis (Ames 1966).

RESULTS

The results of analyses of vigour and viability of field bean seeds harvested in the three years, made before and after storage in different conditions, are presented in Table 1. The highest pre-storage vigour and viability were found for seeds

collected in 1987, which had the highest vigour indices, i.e. the lowest electro conductivity and best formed seedlings after 5 days germination (Table 1), contrary to seeds harvested in 1955.

Investigations on seeds stored in the Genes Bank for six years showed that seeds kept at 4°C had higher vigour and viability than seeds stored at 18°C, CO₂ atmosphere or treated with α -tocopherol. The significantly lower results obtained for seeds stored at 18°C indicate a lowering of biological value (Table 1). Seeds pre-treated with α -tocopherol produced intermediate results.

Lipids extracted from embryonic axes of field bean seeds immediately after harvest, after one years storage (1994 harvest) or after seven years storage (1987 harvest) with chloroform: methanol mixture, were separated by the TLC method into a series of fractions, among which phosphatidylinositol – (PI), phosphatidylcholine – (PC), phosphatidylglycerol – (PG), phosphatidylethanolamine – (PE), phosphatidic acid – (PA) and digalactosilglycerol – (DG) were identified. Irrespective of storage conditions and time or age of seeds, phosphatidylcholine was a dominant fraction in embryonic axes of faba bean seeds. Phosphatidylcholine phosphorus made up 50% of the total phosphorus of all phospholipids. Percentage of phosphatidylethanolamine and phosphatidylinositol was between 10 and 20%, percentage of phosphatidic acid and phosphatidylglycerol – below 10%.

The storage of seeds in different thermal conditions and modified atmosphere, as well as the treatment of seeds with α -tocopherol changed the quantitative relations among phospholipids (Fig. 1, Table 2). Loss of viability was linked with significantly decreased amounts of PE, PC and PG in embryonic axes and relatively increasing amounts of phos-

TABLE 2. Composition of phospholipids fraction isolated from embryonic axes of field bean seeds stored at various conditions.

Sample of seeds	Percentage participation P of individual fractions in total quantity P of phospholipids in embryonic axes						
	PX	PI	PC	PG	PE	PA	PY
1995	tr	13.13	54.51	6.52	18.01	3.81	2.16
1994	tr	14.65	54.82	3.88	14.57	2.26	6.97
1987 – CO ₂ , 18°C	tr	9.68	54.36	8.96	18.59	2.26	4.75
1987 – 4°C	2.62	11.88	53.21	6.07	15.39	1.89	5.10
1987 – α -toc. 18°C	1.77	9.77	52.55	5.84	14.95	2.25	7.01
1987 – 18°C	10.98	10.90	49.51	3.89	7.25	7.09	10.38
LSD p=0.01	1.38	0.96	1.65	1.46	1.48	1.21	1.03

Symbols: PI – phosphatidylinositol; PC – phosphatidylcholine; PG – phosphatidylglycerol; PE – phosphatidylethanolamine; PA – phosphatidic acid; PX and PY – unidentified fractions.

Fig. 1. The basic phospholipids content in embryonic axes of *Vicia faba* var. *minor* seeds stored at various conditions.

phatidic acid and unidentified phospholipids. The unidentified phospholipids (PX and PY) appeared before PI and behind PA on a chromatographic plate.

DISCUSSION

Vigour and viability are the primary indicators of seed ageing. Loss of vigour and viability during seed storage is determined not only by genetic properties of seeds but also by external conditions, of which temperature, atmospheric humidity and composition, light supply, ionizing radiation and biotic factors (Anderson 1977; Litynski 1982; Roberts and Ellis 1982; Blackman et al. 1993; Kulka 1994; Zalewski and Konradowicz 1997).

The results of the studies have shown that among the examined factors relatively high temperature had the greatest adverse effect on vigour and viability and resulted in the most significant changes of phospholipids in embryonic axes. A negative influence of high storage temperature on seed vigour and viability was reported earlier (Roberts 1972; Roberts and Roberts 1972; Litynski 1982). It was also established that alterations in humidity and temperature, however small, may induce a significant, negative influence on seed longevity. The effect of high temperature on "dry seed storage" involves stimulated development of seed mass microorganisms and accelerated catabolic processes, especially respiration. Based on the data shown in Table 2 it may also be concluded that a temperature 18°C combined with an oxygen atmosphere stimulates phospholipid autooxidation and decomposition (PC, PE, PG).

The data presented in Fig. 1 seem indicative of differences in the rate at which phospholipids are decomposed relative to the seeds storage conditions. High temperature and presence of oxygen in the atmospheric air cause decomposition of all primary phospholipids, with phosphatidylethanolamine being decomposed most rapidly.

Although application of a natural antioxidant (α -tocopherol) greatly inhibited the process, it did not stop it, which is in accordance with the earlier findings by Górecki (1986). Replacing atmospheric air by carbon dioxide had a more positive effect on the maintenance of vigour, viability and integrity of phospholipids than treating seeds with α -tocopherol. Biological value and phospholipid content of seeds changed least in the case of seeds stored at 4°C. The experimental data seem to support the view that while storing seeds of moisture content over 10%, loss of vigour and viability is due primarily to chain reactions of free radicals (Wilson and McDonald 1986). Autooxidation could be slowed by lowering temperature, eliminating oxygen from atmosphere and applying antioxidants. However, they cannot be completely eliminated owing to extremely low activation energy (Wilson and McDonald 1986; Gidrol 1989; Hailstones and Smith 1989).

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LITERATURE CITED

- ALLEN C.F., GOOD P., DAVIS H.F., CHISUM P., FOWLER S.D. 1966. Methodology for the separation of plant lipids and application to spinach leaf and chloroplast lamellae. *J. Am. Oil Chem. Soc.* 43: 223-230.
- AMES B.N. 1966. Assay of inorganic phosphate and phosphatases. *Methods in Enzymology* 8: 115-118.
- ANDERSON J.D. 1977. Adenylate metabolism of embryonic axes from deteriorated soybean seeds. *Plant Physiol.* 59: 610-614.
- ANONYMOUS, 1976. International rules for seeds testing. *Seed Sci. Technol.* 4: 3-18.
- BERJAK P., VILLIERS T.A. 1972. Age-induced damage and its repair during early germinations. *New Phytol.* 71: 1069-1074.

- BERNAL-LUGO I., DIAZ de LEON F., CASTILLO A., LEOPOLD A.C. 1993. Embryo sugar composition and seed storage performance. In: Proceedings of the Fourth International Workshop on Seeds: Basic and Applied Aspects of Seed Biology. Come D., Corbineau F. (eds). Universite Pierre et Marie Curie, Paris, pp. 789-792.
- BERNAL-LUGO I., LEOPOLD A.C. 1995. Seed stability during storage: Raffinose content and seed glassy state. *Seed Science Research* 5: 75-80.
- BEWLEY J.D., BLACK M. 1982. Physiology and biochemistry of seeds in relation to germination. Vol. 2, Springer-Verlag, Berlin, Haidelberg, N.Y.
- BLACKMAN S., LEOPOLD A.C., COME D. 1993. Chemical and physical factors in seed deterioration. In: Proceedings of the Fourth International Workshop on Seeds: Basic and Applied Aspects of Seed Biology. Come D., Corbineau F. (eds). Universite Pierre et Marie Curie, Paris, pp. 731-738.
- CROWE L.M., MOURADIAN R.R., CROWE J.H., JACKSON S.A., WOMERSLEY C. 1980. Effects of carbohydrates on membrane stability at low water activity. *Biochim. Biophys. Acta* 769: 141-150.
- FREEMAN B.A., CRAPO J.D. 1982. Free radicals and tissue injury. *Lab. Invest.* 47: 412-426.
- GIDROL X. 1989. Accumulation de peroxyde d'hydrogene et vieillissement accelere des semences de soja. *Comptes Rendus de l'Academie des Sciences* 308: 223-228.
- GÓRECKI R.J. 1986. The study on vigour of some legume seeds. *Acta Acad. Techn. Olszt., Agricult.* 42: 1-45, in Polish.
- GRZESIUK S. 1967. Physiology of seeds. PWRiL, Warszawa, pp. 3-108, in Polish.
- GRZESIUK S., KULKA K. 1988. Biology of grains. PWN, Warszawa, pp. 318-401, in Polish.
- HAILSTONES M., SMITH D. 1989. Increased lipid peroxidation during imbibition of deteriorated seeds and the invigorating effect of ferrous ions. *S. Afr. Sci.* 85: 670-671.
- HARRINGTON J.F. 1973. Biochemical basis of seed longevity. *Seed Sci. Technol.* 1: 453-463.
- HORBOWICZ M., OBENDORF R.L. 1994. Seed desiccation tolerance and storability: Dependence on flatulence-producing oligosaccharides and cyclitols-review and survey. *Seed Science Research* 4: 385-404.
- KOSTER K.L., LEOPOLD A.C. 1988. Sugars and desiccation tolerance in seeds. *Plant Physiol.* 88: 829-832.
- KERMODE A.R. 1990. Regulatory mechanism involved in the transition from seed development to germination. *Critical Rev. Plant Sci.* 3: 155-195.
- KULKA K. 1994. The causes of seeds ageing. In: The crops physiology. Grzesiuk S., Górecki R.J. (eds). ART Olsztyn, p. 332-357.
- LEOPOLD A.C. 1990. Coping with desiccation. In: Stress responses in plants: adaptation and acclimatization mechanisms. Alscher R.G., Cumming J.R. (eds). Wiley-Liss, New York, pp. 22-56.
- LEOPOLD A.C., VERTUCCI C.W., STANWOOD P.C., McDONALD M.B. 1990. Seed moisture. CSSA Special Publication, Cornell University 14: 51-67.
- LITYŃSKI M. 1982. The biological basis of seeds production. PWN, Warszawa, p. 340-356.
- MAZLIAK P. 1981. Plant membrane lipids: changes and alternations during ageing and senescence. In: Postharvest physiology and crop preservation. Liberman M. (ed.). Plenum Press, N.Y., London, pp. 144.
- NICHOLS B.M., HARRIS R.V., JAMES A.T. 1965. The lipid metabolism of blue-green algae. *Biochemical at Biophysical Research Communication* 20: 256-262.
- PARRISH D.J., LEOPOLD A.C. 1978. On the mechanism of ageing in soybean seeds. *Plant Physiol.* 61: 365-368.
- PRUSIŃSKI J. 1992. Zastosowanie testu elektroprowadnictwa wód nastoinowych w ocenie wigoru i w przewidywaniu wschodów polowych roślin strączkowych. *Hod. Rośl. Aklim. Nasien.* 3: 13-17.
- PUKACKA S. 1983. Phospholipids changes and loss of viability in Norway maple (*Acer plantanoides* L.) seeds. *Z. Pflanzenphysiol* 112: 199-205.
- ROBERTS E.H. 1972. Viability of seeds. Chapman and Hall, London, p. 321-359.
- ROBERTS E.H., ROBERTS D.L. 1972. Viability momographs. In: Viability of seeds. Chapman and Hall, London, p. 417-423.
- ROBERTS E.H., ELLIS R.H. 1982. Physiological, ultrastructural and metabolic aspects of seed viability. In: The physiology and biochemistry of seed development, dormancy and germination. Khan A.A. (ed.). Elsevier Biomedical Press, p. 465-485.
- SKRIVER K., MUNDY J. 1990. Gene expression in response to abscisic acid and osmotic stress. *Plant Cell* 2: 120-131.
- STILL D.W., KOVACH D.A., BRADFORD K.J. 1994. Development of desiccation tolerance during embryogenesis in rice (*Oryza sativa*) and wild rice (*Zizania palustris*). *Plant Physiol.* 104: 431-438.
- WILSON D.O., McDONALD M.B. 1986. The lipid peroxidation model of seed ageing. *Seed Sci. Technol.* 14: 269-300.
- ZAŁEWSKI K., KONTRAKTOWICZ M. 1997. Metabolism of ageing seeds. Phosphatidylinositol biosynthesis in germinating lupin seeds of different ages. In: Lupin in modern agriculture "Lupin – Protein – Ecology", Proceedings, Olsztyn-Kortowo, Poland, pp. 131-141, in Polish.
- ZAŁEWSKI K., LAHUTA L.B. 1998. The metabolism of ageing seeds. Changes in the raffinose family oligosaccharides during storage of field bean (*Vicia faba* var. *minor* Harz) seeds. *Acta Soc. Bot. Pol.* 67: 193-196.

WPŁYW CO₂, TEMPERATURY I α -TOKOFEROLU NA ZMIANY FOSFOLIPIDÓW W OSIACH ZARODKOWYCH NASION BOBIKU W CZASIE ICH PRZECZOWYWANIA

STRESZCZENIE

Praca zawiera wyniki badań nad żywotnością i składem fosfolipidów izolowanych z nasion bobiku różnego wieku. Nasiona przechowywano siedem lat w kontrolowanych warunkach w Banku Genów w Radzikowie. W nasionach przechowywanych w temperaturze 4°C i w atmosferze CO₂ nie zauważono zmian w składzie fosfolipidów ani zmian w ich ilości. Nasiona te cechowały się też wysoką żywotnością. W osiach zarodkowych nasion bobiku przechowywanych w temperaturze 18°C w atmosferze powietrza ilość fosfatydyloetanolaminy i fosfatydylocholino zmniejszyła się, a udział procentowy kwasu fosfatydowego i fosfolipidów niezidentyfikowanych o najmniejszej polarności zwiększył się.

SŁOWA KLUCZOWE: nasiona, fosfolipidy, starzenie, bobik.