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

KAZIMIERZ ZALEWSKI¹, DOROTA WIDEJKO¹, RYSZARD J. GÓRECKI²

¹Department of Biochemistry, ²Department of Plant Physiology and Biotechnology
Warmia and Masuria University, Olsztyn 10-957, Plac Łódzki 3/312, Poland

(Received: October 14, 1999. Accepted: March 31, 2000)

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 phosphatidyethanolamine 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. Nadwisłański 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. Nadwisłanski before (C - 1987) and after 7 years storage in Gene Bank under different conditions.

<table>
<thead>
<tr>
<th>Sample of seeds</th>
<th>Germination rate (%)</th>
<th>Germination capacity (%)</th>
<th>Conductivity of lichenates (μS · cm⁻² · g⁻¹)</th>
<th>Seedling length (mm)</th>
<th>Fresh mass of seedling (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>86.0</td>
<td>94.5</td>
<td>23.80</td>
<td>73.20</td>
<td>0.366</td>
</tr>
<tr>
<td>1994</td>
<td>90.0</td>
<td>95.0</td>
<td>21.20</td>
<td>79.40</td>
<td>0.379</td>
</tr>
<tr>
<td>C - 1987</td>
<td>99.0</td>
<td>99.0</td>
<td>19.23</td>
<td>84.06</td>
<td>0.396</td>
</tr>
<tr>
<td>1987 - CO₂, 18°C</td>
<td>95.5</td>
<td>95.0</td>
<td>44.24</td>
<td>71.00</td>
<td>0.332</td>
</tr>
<tr>
<td>1987 - 4°C</td>
<td>98.7</td>
<td>99.0</td>
<td>38.48</td>
<td>78.43</td>
<td>0.352</td>
</tr>
<tr>
<td>1987 - α-toc. 18°C</td>
<td>90.6</td>
<td>91.0</td>
<td>46.74</td>
<td>69.30</td>
<td>0.316</td>
</tr>
<tr>
<td>1987 - 18°C</td>
<td>58.5</td>
<td>64.4</td>
<td>67.51</td>
<td>20.77</td>
<td>0.102</td>
</tr>
<tr>
<td>LSD p=0.01</td>
<td>3.65</td>
<td>4.1</td>
<td>4.86</td>
<td>7.80</td>
<td>0.048</td>
</tr>
</tbody>
</table>


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 Redelikis OK 102/1 apparatus (20°C, Prusinski 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 (Gorecki 1986).

Isolation of phospholipids

From embryonic axes was carried out according to the method of Allen et al. (1966), as described by Pukačka (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 Gene 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), phosphatic acid - (PA) and digalactosylglycerol - (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 phosphatic 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.

<table>
<thead>
<tr>
<th>Sample of seeds</th>
<th>Percentage participation P of individual fractions in total quantity P of phospholipids in embryonic axes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PX</td>
<td>PI</td>
</tr>
<tr>
<td>1995</td>
<td>tr</td>
</tr>
<tr>
<td>1994</td>
<td>tr</td>
</tr>
<tr>
<td>1987 - CO₂, 18°C</td>
<td>9.68</td>
</tr>
<tr>
<td>1987 - 4°C</td>
<td>2.62</td>
</tr>
<tr>
<td>1987 - α-toc. 18°C</td>
<td>1.77</td>
</tr>
<tr>
<td>1987 - 18°C</td>
<td>10.98</td>
</tr>
<tr>
<td>LSD p=0.01</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Symbols: PI - phosphatidylinositol; PC - phosphatidylcholine; PG - phosphatidylglycerol; PE - phosphatidylethanolamine; PA - phosphatic acid; PX and PY - unidentified fractions.
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 Kontraktowicz 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).

ACKNOWLEDGMENTS

We wish to thank Professor A. Rejowski for the seeds probes.

LITERATURE CITED


Wpływ CO₂, Temperatury i α-Tokoferolu na Zmiany Fosfolipidów w Osiąach Zarodkowych Nasion Bobiku w Czasie Ich Przechowywania

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 fosfatydylkolinolaminy i fosfatydylcholiny zmniejszyła się, a udział procentowy kwasu fosfattydylowego i fosfolipidów niezidentyfikowanych o najmniejszej polarności zwiększył się.

SŁOWA KLUCZOWE: nasiona, fosfolipidy, starzenie, bobik.