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Proteins in pea seeds after 7 months of storage at 50 and 90% relative humidity

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Abstract

Two pea seed lots var. Flavanda were stored at 50 and 90% relative humidity (r.h.) at room temperature. After 7 months the germinability of seeds stored at 90% r.h. was reduced to 19%. Similarly, the vigor of these seeds was lower as compared with seeds stored at 50% r.h. Deterioration of seeds resulted in the reduction of the albumin content from about 14 to 8 mg per seed. The content of vicillin in non-viable seeds was twice as high than in fully vigorous ones. No changes in the level of legumin appeared. During germination of seeds with lowered viability and vigor, the protein content of their embryonic axes did not increase. Polyacrylamide gel electrophoresis of protein extracted from the cotyledons of aged seeds showed visible changes only in the electrophoretic pattern of the albumin fraction.

Key words: pea seed, protein, vigor, deterioration

INTRODUCTION

The ageing of seeds is accompanied by the loss of their vigor and germinability. The main causes of this phenomenon are: structural, compositional, and functional changes in membranes, bioenergetic disturbances, changes in enzymic activities and protein metabolism (Abdul-Baki 1980, Grzesiuk and Kulka 1981, Roberts and Ellis 1982, Cherry 1983).

Recently numerous papers have appeared on proteins during seed deterioration. Studies on populations of seeds exhibiting various degrees of viability have shown that the capacity for protein synthesis decreases with the reduction of germination and vigor (Abdul-Baki 1980, Bewley and Black 1982). Bray and Chow (1976) noticed lack of incorporation of

^{14}C-leucine into protein by non-viable embryonic axes of field pea and soybean. Harman et al. (1976) have also found a delay in protein synthesis in embryonic axes during imbibition of aged pea seeds. Similar results have been obtained from cereal grains (Roberts and Osborne 1973). Loss of the capacity to synthesize proteins in embryonic axes is accompanied by the inability to synthesize RNA or may be a consequence of lessions in transcription and translation (Sen and Osborne 1977, Osborne 1982).

There is a lack of information about changes in soluble and storage proteins during seed deterioration. Therefore it is the purpose of this study to determine changes of albumin, vicilin and legumin in pea seeds stored at high and low relative humidity.

MATERIAL AND METHODS

Pea (*Pisum sativum* L.) seed lots cv. Flavanda were stored in hygrostats at 90 and 50 per cent relative humidity (r.h.) at room temperature, for up to 7 months. Seeds were sampled periodically and viability and vigor were determined. Four replications of 50 seeds were germinated in rolled blotting paper at 20 °C. After 5 (speed of germination) and 8 days (germination capacity), the amount of viable seeds was counted according to ISTA rules (1976).

The vigor of seeds was estimated using the conductivity method, growth analysis of seedlings and by determination of overall dehydrogenase activity (Górecki 1982). Electrolyte leakage from seeds was determined by adding 250 cm^3 deionized water to 50 seeds. After 24 hr at 25 °C the electrical conductivity of the soaking water was measured using an OK-102 conductivity bridge (Radelkis). To analyse the growth of seedlings, 25 seeds from each sample were placed between blotting paper and incubated for 3 days at 25 °C. Seedlings were removed from these seeds and the dry weight of whole seedlings was determined. To determine total dehydrogenase activity, 20 embryonic axes were soaked in 1% (w/v) solution of 2,3,5-triphenyl tetrazolium chloride (ph 7.0) at 20 °C. After 24 hr the embryonic axes were homogenized in 15 cm^2 acetone using a mortar and pestle. The homogenate was centrifuged (3000 × g, 10 min) and extraction was repeated. The content of red formazane in the collected supernatants was estimated colorimetrically at E_{510} nm. Synthetic formazane (Sigma) was used to prepare the standard curve.

Proteins were extracted and fractioned on the base of differential solubility according to Thomson et al. (1978) with slight modifications. One g seed

powder, pairs of 5 cotyledons or 50 embryonic axes were homogenized in a mortar in 10 cm^3 of 0.1 M Na-phosphate buffer, containing 1 M NaCl and 0.02 NaN_3. Homogenates were contrifuged (20000 × g, 20 min), and the extraction of proteins in the pellet was repeated twice. Collected supernatants were dialyzed for 24 hr at 1-2°C against McIlwain's buffer (0.1 M citric acid, 0.2 M Na_2HPO_4) pH 4.7, containing 0.2 M NaCl and 0.02% NaN_3. After centrifugation (20000 × g, 30 min) the pellet of legumin was dissolved in 10 cm^3 of extract buffer. The supernatant was dialyzed again, centrifuged as above and the residue legumin was collected. The albumin and vicilin fractions in the supernatant were dialyzed against deionized water at 1-2°C for 48 hr and centrifuged (28000 × g, 30 min). The vicilin pellet was dissolved in 10 cm^3 phosphate buffer (0.15 M KH_2PO_4, 0.2 M Na_2HPO_4) pH 7.5 containing 1 M NaCl and 0.02% NaN_3. The remaining supernatant contained the albumin fraction. Except for dialysis, all extraction and fractionation steps were conducted at room temperature.

The protein content in each fraction was determined colorimetrically by the Lowry et al. (1951) method with bovine serum albumin as the standard. All protein extractions and determinations were made in four replicates.

Albumin, vicilin and legumin extracted from cotyledons were also used for electrophoretic analysis. Polyacrylamide disc electrophoresis (anodic system) of freshly extracted albumin was carried out on 7.5% gels according to the procedure described by Keleti and Lederer (1975).

Urea-PAGE of vicilin and legumin. Protein preparations, dissolved in 0.2 M NaCl, pH 7.5, were reduced by addition of 2-mercaptoethanol, 8 M urea, and subjected to electrophoresis in a catodic system according to Orrick et al. (1973) and Przybylska et al. (1979). Electrophoresis was performed on 10% slab gels containing 4.5 M urea. Before applying protein samples (ca 20 μg), pre-electrophoresis with 0.9 M acetic acid (with addition of 4.5 M urea), as the electrolyte, was conducted. The electrophoresis was performed at a constant voltage (ca 20 V/cm) for 3 hrs. All gels were stained with 0.02%. Coomassie brilliant blue in 12% trichloroacetic acid for 1 hr (Diezel et al. 1972) and destained in 7% acetic acid.

All protein analyses were made on seeds after 7 months of storage.

RESULTS AND DISCUSSION

Pea seed lots used in this study had good intial quality indicators (Table 1). After 7 months of storage at 90% r.h., the seeds had deteriorated to a high extent; their germination decreased to below 20%. Lowered

Table 1

Viability and vigor of pea before and after storage during 7 months at low and high relative air humidity (r.h.) at 18–21°C

Indicators of seed quality	Before storage	After storage at	
		50% r.h.*	90% r.h.
Germination capacity, %	98	96	19
Speed of germination, %	95	92	17
Sprout dry weight, mg	409	389	111
Conductivity of leachates, $\mu S \cdot cm^{-1}$ per 100 seeds	380	440	1100
Total dehydrogenases activity, μg phenylo-formazane per 100 embryonic axes	245	240	50

* Seeds later called seeds with high viability and vigor

viability of seeds correlated with high leaching of solutes from their tissues (increment of conductivity from 380 to 1100 μS). The total dehydrogenase activity in embryonic axes was reduced by about five times. These seeds also produced smaller seedlings. The viability and vigor of seeds stored at 50% r.h. were practically identical as compared with those of control seeds.

Deterioration of pea seeds resulted in marked changes in their proteins. In the powdered tissues from whole seeds with decreased vigor and viability, the level of the albumin fraction was lowered by about 40% (Fig. 1).

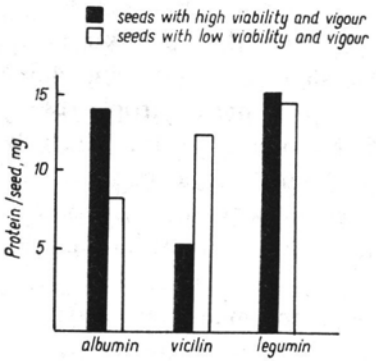

Fig. 1. The content of different protein fractions in the flour from pea seeds with high and low viability and vigor

In contrast, the content of vicilin was by about twice as high in the deteriorated seeds as compared with that of the non-deteriorated ones. No changes in the level of legumin were noted.

Other authors (Abdul-Baki and Anderson 1972, Grzesiuk and Kulka 1981) report that during seed deterioration a reduction of soluble protein (especially albumin) occurs. The increased level of vicilin observed in our study might result from changes of the solubility or isoelectric point of other proteins. Herman et al. (1976) and Cherry (1983) stated that often hydrolysis of storage proteins under high moisture is a consequence of fungal attack.

Embryonic axes isolated from seeds with high viability and vigor showed a gradual increase of total protein content during germination (Fig. 2).

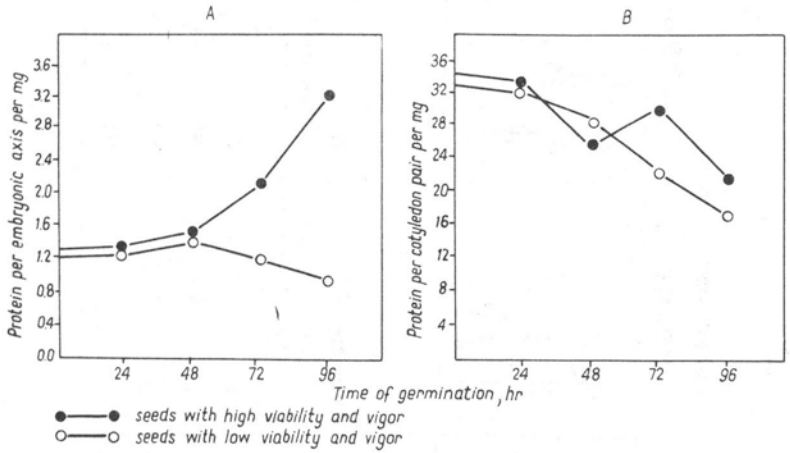

Fig. 2. The changes of the total soluble protein contents in embryonic axes (A) and cotyledons (B) of germinating pea seeds with high and low viability and vigor

Aged seeds exhibited lack of accumulation of proteins in embryonic axes up to 48 hr of germination. After 48 hr of incubation evident reduction of these compounds was noted. Lack of protein synthesis in embryonic axes and embryos of seeds with lowered viability have been found in many species (Harman et al. 1976, Abdul-Baki 1980, Grzesiuk and Kulka 1981, Bewley and Black 1982). Bray and Dasgupta (1976) pointed out that in axes of non-viable pea, the inability to synthesize proteins is accompanied by loss of the capacity to synthesis RNA. In wheat embryos, a gradual loss of viability during seed aging is associated with a decline in incorporation of ^{3}H-uridine into all major classes of RNA (Zalewski and Weidner 1981, Weidner and Zalewski 1982). It has been proven that loss of viability and capacity for protein synthesis is a consequence of an impared transcription and translation mechanism as a result of damage to nuclear DNA (Abdul-Baki 1980, Bewley and Black 1982). Small increases in the protein level in embryonic axes of pea with lowered

quality during the first 48 hr of germination rather fell into the range of experimental error, but a more detailed study showed that in pea seeds there is pre-exiting mRNA, which provides protein synthesis during early hours of incubation (Sutcliffe and Bryant 1977).

Figure 3 shows that the increase of protein content in embryonic

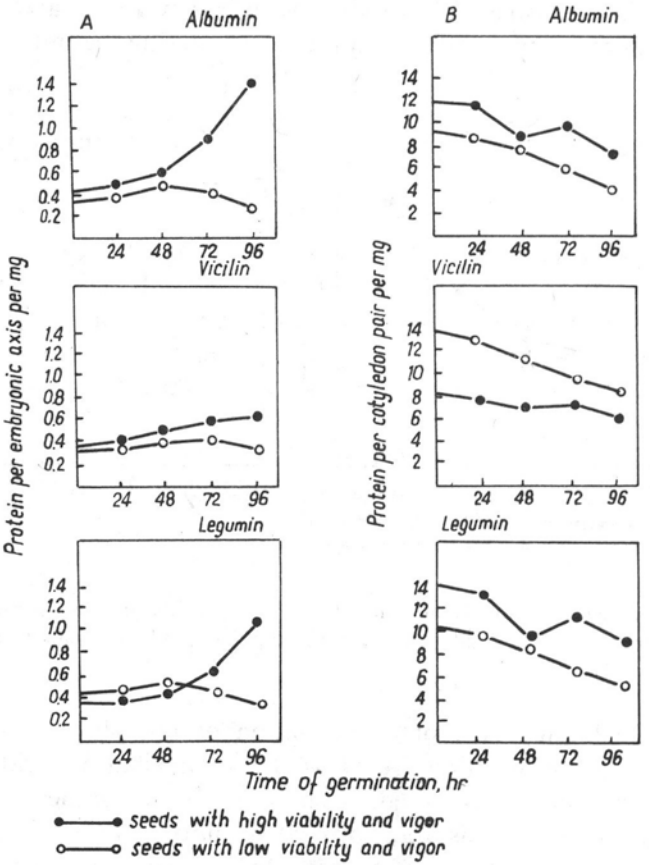

Fig. 3. The dynamics of the protein fractions content in embryonic axes (A) and cotyledons (B) of germinating pea seeds with high and low viability and vigor

axes of fully vigorous seeds was due to the accumulation of different protein fractions, however, albumin and legumin appeared to be accumulated more rapidly than vicilin during the first four days of germination.

In the cotyledons of viable seeds the typical pattern of protein changes was observed. From 0 to 48 hr of germination, a decrease of all protein fraction occured (Figs. 2 and 3), which might have been a result of translocation of these compounds into developing embryonic axes. Between 48 and 72 hr

an increase (ca 20%) of protein (mainly albumin and legumin) was noted. One can presume that this phenomenom was due to protein synthesis *de novo*. Gumilevskaya (1975) and Grzesiuk and Kulka (1981) reported that in the cotyledons of germinating seeds, the synthesis of enzymic

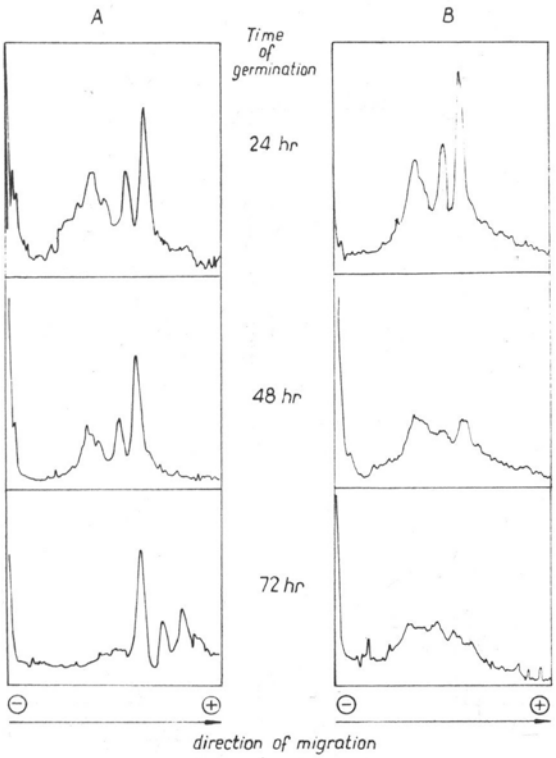

Fig. 4. Absorbance scans of the stained albumin from pea cotyledons of viable (A) and non-viable (B) seeds

and storage proteins occurs, besides their degradation. In the last studied period (72-96 hr) a further decrease of protein content occurred. The presented data are similar to those of Basha and Beevers (1975).

In cotyledons of aged seeds the amount of each protein fraction decreased during the entire studied time of incubation (Fig. 3B).

In the next step of the experiments, albumin, vicilin and legumin from cotyledons were fractioned by means of PAGE. Polyacrylamide disc gel electrophoresis of albumin from viable seeds indicated the presence of three major and two minor components (Fig. 4). This high heterogenity of pea albumin proteins was also found by Przybylska et al. (1977),

Konopska (1983) and Croy et al. (1984). As one can see, loss of seed vigor was accompanied by evident changes in albumin composition; in aged seeds only three subfractions were detected. During the first three days of germination some components of albumin disappeared. The protein content in each band also changed. Konopska (1983) found significant degradation of albumin during pea seed germination.

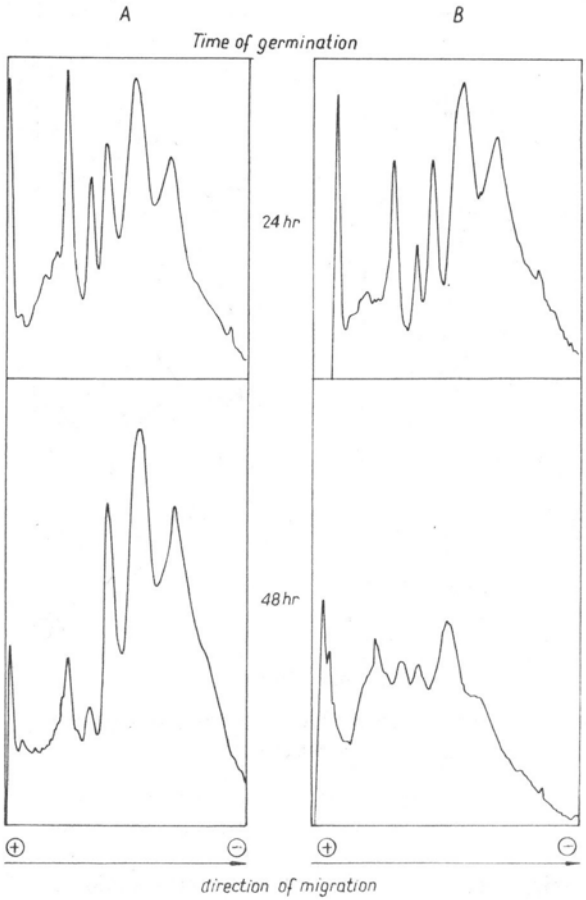

Fig. 5. Absorbance scans of the stained vicilin from pea cotyledons of viable (A) and non-viable (B) seeds

The scanning profiles of albumin components of cotyledons of non-viable seeds showed much more distinct changes during the whole time of incubation than in viable ones.

Slab gel electrophoresis of vicilin showed its high heterogenity which was also reported by others (Gatehouse et al. 1981, Hurkman and

Beevers 1982, Miege 1982, Müntz 1982). In the presence of urea and mercaptoethanol, we were able to detect five major basic subunits (cathodic electrophoresis) of vicilin from viable as well as non-viable seeds (Fig. 5). The number of vicilin fractions did not change during germination, however the amount of protein in each subfraction changed significantly (Fig. 5). The legumin electrophoretic pattern showed high also diversity. During

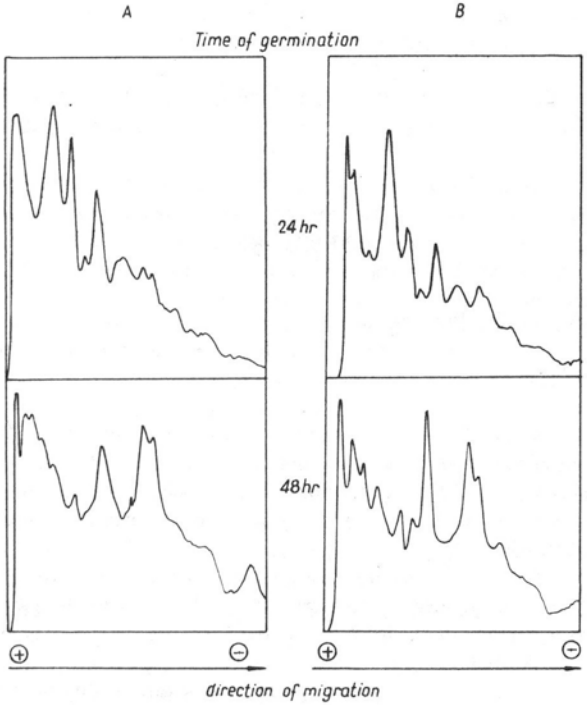

Fig. 6. Absorbance scans of the stained legumin from pea cotyledons of viable (A) and non-viable (B) seeds

cathodic electrophoresis, legumin was separated into six main components (Fig. 6). Seed germination resulted only in quantitative changes of proteins in all bands. It is worth however noticing that these changes were more evident for legumin isolated from seeds with low viability and vigor.

We found no significant changes in the electrophoretic patterns of both globulin fractions (vicilin and legumin) using an anodic electrophoretic system (unpublished data).

Taking into consideration all of the results from the presented study, one can state that loss of seed vigor and viability is accompanied by evident quantitative changes in three main protein fractions. Much smaller alterations occur in the subunit composition of these proteins, especially globulins.

Our findings fit well to Abdul-Baki's report (1980). In his opinion seed vigor is more closely related to the integrity of the protein synthesis system than to the amount of protein *per se*, and protein instability. The concept of protein instability fits rather well with the concept of impairment of the protein synthesis system when vigor is reduced or lost. Seed vigor determination on the base of protein studies does not seem to be a promising tool.

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Białka grochu po 7 miesiącach przechowywania nasion w warunkach 50 i 90% wilgotności względnej powietrza

Streszczenie

Próbki nasion grochu odm. Flawanda przechowywano w higrostatach o 50 i 90% wilgotności względnej powietrza w temperaturze pokojowej. Po upływie 7 miesięcy, nasiona przechowywane w warunkach podwyższonej wilgotności, w przeciwieństwie do nasion przetrzymywanych w 50% wilgotności względnej powietrza utraciły w znacznym stopniu żywotność i wigor (badane kilkoma metodami). Badania białek w zmielonej tkance z całych nasion wykazały mniejszą zawartość albuminy zaś zwiększoną ilość wiciliny w nasionach zestarzałych niż w nie zestarzałych. Nie stwierdzono natomiast istotnych zmian w poziomie leguminy. Podczas pęcznienia i kiełkowania obserwowano spadek ogólnej zawartości białka w liścieniach i wzrost jego poziomu w osiach zarodkowych i kiełkach nasion w pełni żywotnych. Nasiona zestarzałe nie były zdolne do gromadzenia białka w osiach zarodkowych. Za pomocą elektroforezy w żelu polyakryloamidowym wykazano istotne zmiany w obrazie elektroforetycznym albumin wyizolowanych z liścieni nasion o dużym i małym wigorze. Stosując elektroforezę w żelu z dodatkiem 4,5 M mocznika nie stwierdzono różnic w składzie podjednostkowym wiciliny i leguminy.