EFFECT OF AGEING ON THE MAJOR RESERVE MOLECULES AND THEIR RELATED ENZYME IN NATURAL AGED SEEDS OF FLAX

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SUMMARY: The natural aged seeds of flax (Linum usitatissimum) displayed a marked decline in germinability and the quantity f disarccharides. This decrease was not paralleled to the quantity of ∝-amylase which remains constant even in the non-viable seeds. The colorimetric determination of the total free amino acids, water soluble proteins, and buffer extracted proteins indicated that quantities of water soluble proteins and buffer extracted proteins were decreased on ageing, while that of the total free amino acids showed no variation. It was also found that there was no close correlation between the protein level and carboxypeptidase, amino-peptidase and proteinase enzymes. Electrophoretic separation of the total seed proteins on SDS-PAGE exhibited that the highest molecular weight subunits (legumin-like protein) were disintegrated into low molecular weight subunits. These data indicate that a complex family of substances are involved in ageing processes.

Key Words: Linum usitatissimum, ∝-amylase, amino peptidase, carboxy peptidase.

INTRODUCTION

The physiology and biochemistry of seed ageing represent a controversial field of research. A considerable amount of work has been done on the following aspects: membranes (2,23,24,32), lipid related changes (3,10,12, 13,25-27), biochemical pathways (1,31), changes in nucleic acids (5,28,29), and changes in activity of proteolytic enzymes (6,11,20) of deteriorating seeds. However, although Floris (9) reported that ageing process seems to involve both endosperm and embryo and that the full activities of these seeds organs are probably required for the correct expression and regulation process of the seed reserves, none of these studies investigated the effect of ageing on the major reserve molecules (Carbohydrates and proteins).

In this report we describe the effect of ageing on the major reserve molecules and their related enzymes in natural aged seeds of flax.

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MATERIALS AND METHODS

Flax (*Linum usitatissimum*) seeds were obtained from Botanischer Garten und Botanisches Museum, Berlin Dahlem, D 1000 Berlin 33, West Germany; and Royal Botanic Garden, Edinburgh, EH3 5LR, Scotland. Two year harvested high virous seeds of 99 per cent viability were obtained from Agriculture Research Center El-Giza, Egypt.

Germination tests were performed using 10 samples of 10 seeds spread on 10 cm Petri dishes with two layers of Whatman filter paper wetted with 10 ml of the test solution at 20°C. Seeds were considered germinated when radicals and coleoptiles were evident.

Carbohydrate Determination

Monosaccharides and polysaccharides were determined according to the method of Naguib (16, 18).

∝-Amylase Activity

This was carried out as described by Okamoto and Akazawa (1979).

Determination of Free Amino Acids

Free amino acids were extracted from the seed meals with modifications of methods used by Russell (30) and Naguib (17).

Protein Determination

The total proteins of the meal extracts were determined using the method of Lowery *et al.* (15).

Extraction and Analyses for Proteolytic Enzyme Activities

The seed meals were extracted as described by Feller and Erismann (7) and desalted by dialysis against distilled water.

The hydrolysis rate of L-leucine-p-nitroanilide (aminopeptidase), N-CB2-L-phenyl-alanine-L-alanine (carboxypeptidase), and azocasein (proteases) were determined as described by Feller (8).

Protein Electrophoresis

Polyacrylamide-gel electrophoresis (PAGE) was carried out in 17% acrylamide gel slabs according to Laemmli (14). Protein extracts were prepared by extracting seed meals with sample buffer (60 mg/ml buffer) overnight at 4°C).

The gel was scanned in LKB Recording Laser Densitometer equipped with LKB 2220 Recording Integrator.

RESULTS

Germination capacity was used as main criterion to estimate the physiological status of the seeds of flax (Table 1). The 1986 seeds (2 years old) had a high germination capacity, whereas in those of 1981 (7 years old) germinability was reduced, while 1974 (14 years old) had lost germinability.

When monosaccharide, disaccharides, polysaccha-

Table 1: Germination percentage; amylase activity; and monosaccharide, disaccharide, and polysaccharide quantities in aged seeds of flax.

Year of harvest	Germination (%)	Amylase activity (Unites)	Monosaccharides (mg/g)	Disaccharides (mg/g)	Polysaccharides (mg/g)	Total Carbohydrates (mg/g)
1986	99	62.4	07.88	300	113.3	421.18
1981	80.5	63.5	12.16	280	113.3	405.46
1978	68	62.1	16.16	250	110.2	376.18
1974	00.0	64.3	16.39	237	115.6	168.45
1969	00.0	63.6	16.23	215	93.4	324.27
1956	00.0	63.2	20.20	190	91.2	301.22
1943	00.0	62.5	23.47	170	85.3	278.50

Table 2: Free amino acids, buffer extracted protein; and water extracted protein quantities in aged seeds of flax.

Year of harvest	Free amino acids (mg/g)	Water soluble protein (mg/g)	Buffer soluble protein (mg/g)
1986	15.00	70.30	275
1981	13.00	68.30	270
1978	14.60	68.40	270
1974	13.80	66.66	266
1969	15.10	56.60	229
1956	14.90	53.30	218
1943	14.75	46.60	208

Table 3: Proteolytic activity in aged seeds of flax at 0 and 4-d of imhibition.

Year of harvest	Proteinase activty in		Carboxypedtidase activity in		Amino peptidase activity in	
	dry seeds	4-d imhibition	dry seeds	4-d imhibition	dry seeds	4-d imhibition
1986	28	54	280	246	114	128
1981	17	32	295	253	107	122
1978	19	37	278	243	100.2	087
1974	22	-	298	-	95.0	-
1969	10	-	301	-	93.0	-
1956	03	-	299	-	65.0	-
1943	02	-	303	-	64.0	-

Result in units per 50 seeds.

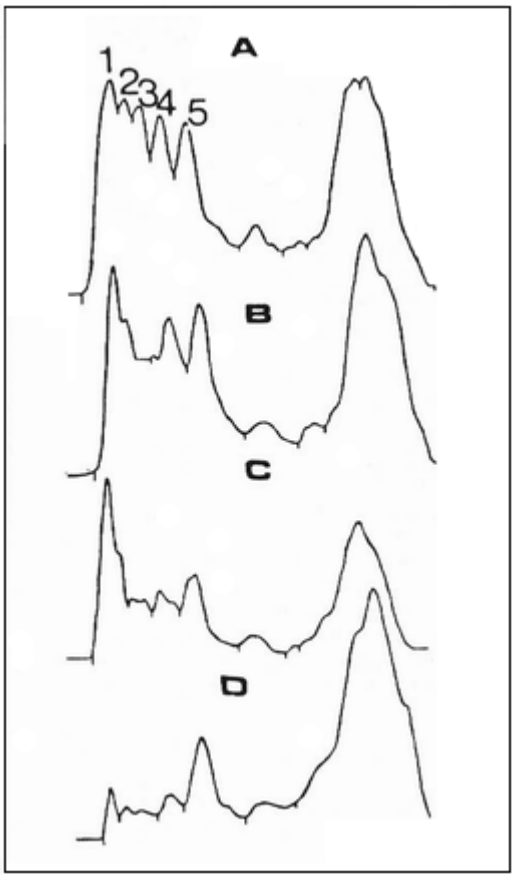

Figure 1: SDS-PAGE of the total proteins of the different ageing flax seeds, extracted with Tris/HCI buffer pH 6.8. 1, the 1986 seeds; 2, the 1981 seeds; 3, the 1978 seeds; 4, the 1974 seeds; 5, the 1969 seeds; 6, the 1956 seeds; 7, the 1943 seeds.

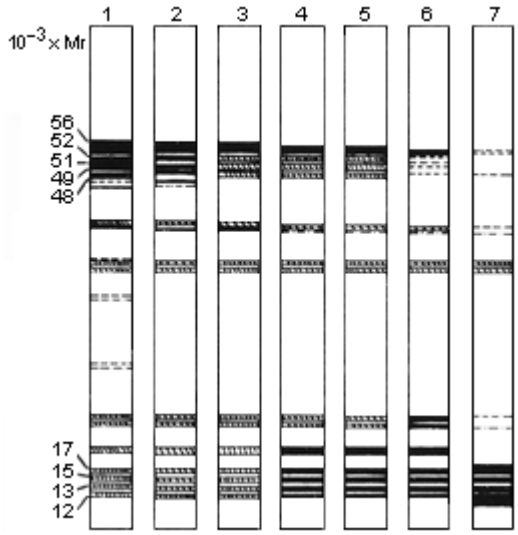

Figure 2: Scans of gel patterns of the different ageing flax seeds. A, the 1986 seeds; B, the 1978 seeds; C, the 1969 seeds; D, the 1974 seeds; E, the 1943 seeds; The five peaks observed in the 1986 seeds are those of the leguminlike protein of flax seed proteins.

rides were estimated in the dry seeds (Table 1), it was found that quantities of monosaccharides decreased. The quantities of polysaccharides exhibited slight variations during seed ageing.

Amylase activity was assayed for each harvest (Table 1). The data displayed that amylase activity remained constant during seed ageing, even when the seeds had lost their germinability.

When the free amino acids of the aged seeds were determined (Table 2), it was found that their quantities decreased during seed ageing. However, the seeds had germinability had nearly the same quantities of the free amino acids.

The seed meals of the dry seeds were extracted with borate buffer, pH 8, and distiller water. Total proteins were estimated in both buffer and water extracts (Table 2). The data exhibited a decrease in the quantities of both the buffer and water extracted proteins during the seed ageing, even when the seeds had lost their germinability. However, the degree of the loss in water extracted proteins was higher than that of the buffer extracted proteins.

When proteinase, amino-peptidase, and carboxypeptidase activities were assayed in the meals of the dry seeds and 4-day germinating seeds (Table 3), it was found that the highest level of proteases was found to present in the youngest seeds having the highest viability, whereas the lowest level was found in the oldest seeds. However, this decrease was not proportional to the reduction in the viability of the seeds.

Carboxypeptidase activity remained nearly constant during seed ageing, even when the seed lost germinability, while it decreased on germination.

Amino peptidase activity already present in the dry seeds, increased during germination of 1986 and 1981 year seeds, while it decreased in 1978 seeds.

When the total proteins of the aged seeds were electrophorectically analyzed on 17% SDS-PAGE (Figure 1), it was found that the highest molecular weight subunits were disintegrated into low molecular weight polypeptides. However, it is very interesting to notice that disintegration

was mainly in the disulphide bonded subunits. As it is seen in Figure 1, the intensity of the high molecular weight subunits decreased with the loss of viability of the seeds. The rate of disintigration is shown in Figure 2. However, the highest molecular weight subunit (56 kilo Dalton) resisted more than the others.

DISCUSSION

It was found that flax seeds retain their viability for 12 years. However, there was a progressive loss of viability after 7 years. The loss of viability may take place after decades or within a few days of seeds being shed (9, 22). Bhattacharyya and Sen-Mandi (4) attributed the loss of viability to the lack of supply of monosaccharides and disaccharides and suggested that glucose in aged seeds may converted to sucrose. In view of the data presented here, the previous suggestion can not be upheld because the quantity of monosaccharides in flax seeds increased an ageing, even in non-viable seeds, while the quantity of disaccharides decreased. The data also displayed that flax seeds retain their amylolytic activity on ageing, even in non viable seeds. On the contrary, the seeds of wheat was found to loss their amylolytic activity on ageing (4). This indicated that firstly seed retain of amylolytic activity on ageing varied from one species to other, secondly there was no close correlation between the decrease in the quantity of disaccharides on ageing and amylolytic activity.

By determining the quantity of the free amino acids, the results showed that their quantities remained nearly constant on ageing. This confirmed the suggestion that a reduced supply of amino acids to the embryonic axis was not responsible for the decreased germination percentage (11). In the light of the data of carbohydrate and free amino acids analysis, it can be concluded that the lack of the utilizable substrates may not be the reason of the loss of viability. This data also confirmed the conclusion drawn by Roberts *et al.* (29) that the main reasons for the loss of viability was the loss of ribosomal integrity, impairment of t-RNA, amino acyl-t-RNA, synthetase of transfer enzymes, transfer enzyme activity of the non-viable embryo supernatant.

It is well established that proteolytic enzymes had an important role in seed protein degradation during germination (19). However, on ageing it has been found that activity of carboxypeptidase, amino peptidase, and proteinase enzymes was not closely correlated with germination and protein level. The same finding was reported by Galleschi *et al.* (11). In addition, it was found that activity of the proteolytic enzymes was not correlated with the rate of degradation of the total proteins (analyzed on SDS-PAGE) of the aged seeds. Although the electrophoretic profiles of the aged seeds were similar to those of the germinating seeds (R. Sammour unpublished data), especially in the upper part of the electrophoregram, the way of degradation in both cases is different.

This work indicated that the attention should be concentrated on both embryo and endosperm as Floris (9) reported because the full activities of these seed organs are probably required for the correct expression and regulation process of the seed reserves.

REFERENCES

1. Abdul-Baki A-Anderson J: Physiological and biochemical deterioration of seeds. In: T. KOZLOWSKI ed., Seed Biology, Vol. II. Academic Press, New York, 1972.

2. Berjak P, Villiers TA: Age induced damage and its repair during germination. New Phytol, 71:1069-1074, 1972.

3. Berjak P: Viability extension and improvement of stored seeds. S Afr J Sci, 74:365-368, 1978.

4. Bhattacharyya (Gang Uli) S, Sen-Mandi S: Studies into causes of non-germination of aged wheat seeds. Annals of Botany, 56:475-479, 1985.

5. Cheah KSE, Osborne DJ: DNA lesions occur with loss of viability in embryos of ageing rye seed. Nature (Lond), 272:593-599, 1978.

6. Cheng SH, Kao CH: The role of proteolytic enzymes in protein degradation during senescence of rice leaves. Physiol Plant, 62:231-237, 1984.

7. Feller U, Erismann KH: Veranderungen des.Gaswechsels und der Aktivitaten proteolytischer Enzyme wahrend der Seneszenz von Weizenblatten (Triticum aestivum L.). Z Pflanzenphsiol, 90:235-244, 1978.

8. Feller U: Nitrogen mobilization and proteolytic activities in germinating and maturing bush beans (Phaseolus vulgaris L.). Z Pflanzenphysiol, 95:413-422, 1979.

9. Floris C: Ageing in Triticum during seeds: behaviour of embryos and endosperms from aged seeds as revealed by the embryo-transplantation technique. J Exp Botany, 21:462-468, 1970.

10. Franscis A, Coolbear P: A comparison of changes in the germination responses and phospholipid composition of naturally

and artificially aged tomato seeds. Annals of Botany, 59:167-172, 1981.

11. Galleschi L, Spano C, Bedini G, Floris C, Saviozzi F, Capocchi A: Proteolytic activities in X Haynaldoticum sardoum seeds of different ages. Annals of Botany, 61:93-97, 1988.

12. Harman GE, Mattick LR: Association of lipid oxidation with seed ageing and death. Ibid, 260:323-324, 1976.

13. Koostra PT, Harrington JF: Biochemical effects of age on membranal lipids of Cucumis sativus L. seeds. Proceedings of the International Seed Testing Association, 34:329-340, 1968.

14. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227:680-685, 1970.

15. Lowery OH, Rosebrough NJ, Farr AL, Randall RJ : Protein measurement with the folin-phenol reagent. J Biol Chem, 193:265-275, 1957.

16. Naguib MI : Colorimetric estimation of plant polysaccharides. Zucker, 16:15-18, 1963.

17. Naguib MI : Effect of benzoic acid and its derivatives on plant metabolism. Can J Bot, 42:197-202, 1964.

18. Naguib MI : Effect of sevin on the carbohydrate and nitrogen metabolism during germination of cotton seeds. Ind J Expt Biol, 2:149-152, 1964.

19. Nielsen SS, Liener IE : Degradation of the major storage protein of Phaseolus vulgaris during germintaion: Role of endogenous proteases and protease inhibitors. Plant Physiol, 74:494-498, 1984.

20. Nowak J, Mierzwinska J : Activity of proteolytic enzymes in rye seeds of different ages. Z Pflanzenphysiol, 86:15-22, 1978.

21. Okamoto K, Akazawa T : Enzymic mechanism of starch breakdown in germinating rice sees: 7, Amylase formation in the epithelium. Plant Physiology, 63:336-340, 1979.

22. Pandita PN : Effect of storage on seed germination in pyrethrum (Chrysanthemum cinerariaefolium) visl. Pyrethrum Post, 16:91-92, 1986.

23. Parrish DJ, Leopold AC : On the mechanism of ageing in soybean seeds. Plant Physiology, 61:365-368, 1978.

24. Petruzzelli L, Carella G : The effect of ageing conditions on loss of viability in wheat (T. durum). J Exp Botany, 34:21-225, 1983.

25. Petruzzelli L, Taranto G : Phospholipid changes in wheat embryos aged under different storage conditions. J Exp Botany, 35:517-520, 1984.

26. Pearce RS, Abdel Samad IM : Change in fatty acid content of polar lipids during ageing of seeds of peanut (Arachis hypogea L.). J Exp Botany, 31:1283-1290, 1980.

27. Priestley DA, Leopold AC : Absence of lipid oxidation during accelerated ageing of soybean seeds. Plant Physiology, 63:726-729, 1979.

28. Roberts BE, Osborn DJ : Protein synthesis and viability in rye grains. In seed ecology Ed. W. Heydecker. Butterworths, London. pp 99-114, 1973.

29. Roberts BE, Payne PI, Osborne DJ : Loss of activity of protein synthesizing systems in vitro associated with a loss of viability. Biochem J, 131:275-285, 1973.

30. Russell JA : Note on the colorimetric determination of amino nitrogen, J Biol Chem, 156:467-469, 1944.

31. Sen S, Osborne DJ : Decline in ribonucleic acid and protein synthesis with loss of viability during early hours of imhibition of rye (Secale cereale L.) embryos. Biochem J, 166:33-38, 1977.

32. Simon EW : Phospholipids and plant membrane permeability. New Phytologist, 73:377-420, 1974.

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