The metabolism of aged seeds. The formation of polyribosomes in germinating field bean (*Vicia faba* sp. *minor*) seeds of different ages

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(Received: December 8, 1989. Revision accepted: April 19, 1991)

Abstract

Total dehydrogenase activity and formation of polyribosomes in embryonic axes and cotyledons of field bean seeds from different harvest years were studied. ^3H -uridine incorporation experiments showed that seed ageing was accompanied by decreased capability for RNA synthesis and polyribosome formation. The embryonic axes of seeds with reduced vigor contained lower levels of polyribosomes.

Key words: RNA, ribosomes, ageing, field beans, seeds

INTRODUCTION

Stored seeds age at a rate dependent on individual traits, conditions of maturation on the parent plant and on the influence of external factors during storage. The last group of factors in which the humidity of the environment and water content of the seeds play an important role along with temperature and the gaseous composition of the atmosphere, exerts a decisive influence on the life-span of seeds (Barton 1961, Grzesiuk 1967, Kulka 1971, Lityński 1977, Priestley 1986). In spite of numerous studies, the mechanism of seeds ageing is still not fully elucidated. The main causes of seed ageing are believed to be deterioration of cell membranes, bioenergetic changes, disorders of the genetic apparatus and protein biosynthesis system, accumulation of toxic substances and free radicals, loss of vitamins and hormones (Simon 1974, Harman and Mattick 1976, Anderson 1977, Wilson and McDonald 1986).

The integrity of nucleic acids and the intensity with which the processes of transcription and translation are carried out have been studied relatively well in ageing pea and soybean seeds and in wheat grains (Bray 1979, Anderson

1977, Perrish and Leopold 1978, Osborne 1982, 1983, Gidrol et al. 1988). The seeds of the remaining species, including the field bean, have been studied in this respect only very slightly, which was the reason this study was undertaken.

MATERIAL AND METHODS

The study was carried out on field bean seeds cv. Grot. The seeds in all the experimental variants were harvested at full ripeness, dried and stored in linen bags at 18-20°C and mean air humidity of 50-55%. Seeds harvested in 1984 are denoted as D, 1985 — C, 1986 — B. The control seeds were seeds from the same cultivar harvested in 1987 — A, characterized by a high viability index.

The viability of the seeds was determined by the germination method according to the International ISTA regulations (Anonymous 1976).

Before imbibition and germination the seeds were sterilized in a 2% solution of sodium hypochlorite for 3 minutes, after which they were washed carefully with sterilized water, wiped with gauze and placed in sterilized Petri dishes. Imbibition was carried out for 10 hours at 0°C, germination at 22°C for 24-72 hours. The seeds germinated in the dark and with constant (only for germination conducted for 1-2 days) supply of ^3H -uridine ($0.40 \text{ MBq} \cdot \text{cm}^{-3}$). After the set duration of germination, the remaining unused precursor was washed off of the seeds and the embryonic axes were removed by hand from the cotyledons, which were stored in closed containers at -25°C.

Isolation and fractionation of ribosomes from field bean seeds was carried out according to the method of Davies et al. (1972). The ribosome concentration in solution was calculated according to the method of Gualerzi and Cammarano (1969).

The total dehydrogenase activity was determined after 24 hours of imbibition in redistilled water at 20°C. The embryonic axes were isolated by hand and incubated in 0.7% tetrazolium chloride for 24 hours (29°C), after which they were homogenized in acetone in a porcelain mortar. After decantation, the amount of formazane produced was determined.

The study was carried out in four (viability and dehydrogenase activity) or three replicates (remaining experiments).

RESULTS

The varied duration of storage of field bean seeds resulted in unequal falls of viability and vigor. This was manifested by, among others, the reduced rate and capability of germination, as well as by the decrease in the epicotyl and embryonic root lengths after 10 days of growth (Table 1). The greatest differences between the control seeds (1987 harvest — A) and seeds stored for 2-4

Table 1

Viability and vigour of field bean seeds of different age

Harvest year	Rate of germination (%)	Germination capacity (%)	Epicotile length (cm) after 10 days of germination	Radicle length after 10 days of germination	Total dehydrogenases activity in embryonic axes (mg of formazan · 100 axes ⁻¹)
1987 — A	98.4±1.81	98.6±1.34	12.17±0.65	15.10±0.82	19.90±0.99
1986 — B	95.4±1.23	96.1±0.98	11.72±0.71	13.87±0.94	16.50±1.25
1985 — C	81.0±1.45	82.4±1.32	10.80±0.63	10.67±0.66	13.40±1.07
1984 — D	90.4±1.47	93.0±1.41	10.40±0.64	12.87±0.58	13.95±0.93

years was observed in their dehydrogenase activities (Table 1). These differences ranged from 17 to 33%.

A distinct difference in the ribosome content of embryonic axes was found in all of the analyzed groups of seeds. A correlation was noted between the number of ribosomes and the viability of the seeds (Table 2).

Table 2

Changes in the ribosome contents in embryonic axes of field beans seeds of different age, during germination

Sample of seeds	Ribosome contents (mg · 100 axes ⁻¹)				
	Time of germination				
	10 h, 0°C	24 h, 22°C	48 h, 22°C	72 h, 22°C	96 h, 22°C
A	0.530	0.722	0.795	1.288	1.592
B	0.428	0.455	0.603	1.248	1.375
C	0.320	0.325	0.409	0.508	0.964
D	0.439	0.442	0.482	0.561	1.190
LSD P=5%	0.0265	0.0383	0.0527	0.0888	0.1081

The process of germination caused an increase in the number of ribosomes in embryonic axes and epicotyls. The highest increases were characteristic for the germinating seeds with the greatest vigor and viability (harvested in 1987), and equalled 45.6% after 24 hours and 200.3% after 96 hours of germination. In the seeds stored for 2-4 years (the analysis was carried out in the summer of 1988), these increases were smaller and the rate of biogenesis of ribosomes did not rise noticeably until after 3 days of germination (Table 2).

The seeds harvested in 1984 (D) and stored the longest, were characterized by higher viability and vigor indexes as well as increases in the number of ribosomes in embryonic axes during germination than the seeds collected in 1985 (C).

Polysomes and monosomes were isolated from the post-mitochondrial

fraction of the supernatant using a high ionic strength and high pH buffer for homogenization. This facilitated effective isolation of ribosomes and prevented the degradation of polysomes. The total fraction of ribosomes was fractionated on a step sucrose gradient, which made the distinct separation of 80 S monosomes from the polysome fraction possible.

Polysomes comprised 40% and monosomes 60% of the total ribosome fraction from embryonic axes in the control sample (A) after 24 hours of germination (Fig. 1). Extending germination for a further 3 days increased the proportion of polysomes in this sample to 78%. In the remaining combinations, the percentage of polysomes was somewhat lower, while that of monosomes greater than in the control sample, both after 24 and 96 hours of germination (Fig. 1).

Fig. 1. Percentage participation of polyribosomes fractions isolated from embryonic axes of germinating field beans seeds of different age. A, B, C, D — as in Table 1

The cotyledons of field beans of various ages were found to contain from 10 (D) to 16% (B) polysomes after 16 hours of germination and from 34 (D) to 45.5% (B) after a further 24 hours (Fig. 2). No correlation was found between the polysome to monosome ratio and the vigor and viability of the seeds.

The varied time of storage of the seeds (under equal conditions) affected the degree of incorporation of ^3H -uridine into the polysome RNAs (mRNA + rRNA) in the cotyledons and embryonic axes during 24 and 48 hours of germination of the seeds (Table 3). The highest radioactivity of polysomal RNA

Fig. 2. Ribosome fractions isolated from cotyledons of germinating field beans seeds of different age. A, B, C, D — as in Table 1

isolated from the cotyledons and embryonic axes was observed in seeds from the control group both after 1 and 2 days of germination. In the remaining samples, the intensity of RNA synthesis (in the same time period) measured by incorporation of the labelled precursor was lower and did not correlated distinctly with the viability of the seeds. It was also noticed that in all of the samples, the embryonic axes exhibited a higher specific radioactivity as compared with cotyledons. However, the ratio of the specific activity of polysomes to monosomes increased with the age of the seeds (Table 3).

Table 3

Incorporation of ^3H -uridine (0.4 MBq cm^{-3}) into ribosomes isolated from embryonic axes and cotyledons of germinating field bean seeds of different age

Seed sample	Specific radioactivity (c.p.m. $\times$ mg of ribosomes $^{-1}$)								Total radioactivity			
	axes				cotyledons				c.p.m. $\times$ axes $^{-1}$		c.p.m. $\times$ cotyledons $^{-1}$	
	24 h		48 h		24 h		48 h		24 h	48 h	24 h	48 h
	R	P/M	R	P/M	R	P/M	R	P/M				
A	20 163	0.82	54 896	1.37	14 436	1.01	31 215	1.75	155	436	4 114	9 832
B	14 121	0.77	33 676	1.37	13 105	0.80	28 611	1.52	64	203	4 285	9 498
C	12 862	0.75	27 788	1.63	7 259	0.79	15 956	1.31	41	113	2 192	9 498
D	13 008	0.83	30 182	2.03	8 301	0.95	17 024	2.06	57	145	2 365	4 647
LSD P=5%	1 432	0.03	3 827	0.14	826	0.07	2 139	0.14	9	27	395	709

P — polyribosomes; M — monoribosomes; R — polyribosomes + monoribosomes.

DISCUSSION

The presented data dealing with the vigor and viability of ageing field bean seeds are generally in agreement with the results of studies published earlier (Anderson 1977, Perrish and Leopold 1978, Blowers et al. 1980, Priestley 1986, Zalewski 1988).

The question of polyribosome formation in germinating seeds of various viabilities and the mRNA and rRNA synthesis related to this are to this day still controversial. It has not been determined yet for the basic harvest crops exactly which form of mRNA takes part in initiating protein synthesis during seed germination (Brooker et al. 1978, Chavkin 1982, Müntz 1982). On the other hand, the changes which long-lived and storage seed mRNAs undergo during the process of loss of viability, have also not been determined yet.

It seems that the overall drop in the number of ribosomes in embryonic axes demonstrated in this study (Table 2) was associated with the complete disintegration of 80 S monosomes or ribosomal subunits. Numerous studies have shown that intense rRNA degradation occurs in older seeds (Bray and Chow 1976, Bray and Dasgupta 1976, Weidner et al. 1980). In addition, a decrease in the mass of ribosomes isolated from old seeds has also been reported (Roberts and Osborne 1973) as have modifications in their protein content (Zalewski and Weidner 1982, Weidner et al. 1984).

According to Blowers et al. (1980) and Nowak et al. (1984), the proportion of polysomes in the total ribosome fraction is considered to be an unerring indicator of protein biosynthesis under various experimental conditions.

A lag in polyribosome formation in embryonic axes of seeds harvested in 1984 and 1985 was observed during the first three days of germination (Table 2) and probably reflects the energetic status of these seeds (Ching 1973, Anderson 1977, Grzesiuk and Tłuczkiewicz 1982) and the condition of the storage RNAs (Gidrol et al. 1988, Zalewski 1989).

In comparison with other species, field bean seeds age rather slowly. This is supported by the data in Table 1. In spite of the small differences in germination rate and capacity of seeds from the various harvest years (reaching 16%), very large differences occurred in them in the intensity of their RNA synthesis as measured by incorporation of ^3H -uridine. For example, after 24 hours of germination, the differences in the radioactivity levels between the control (A) and sample (C) was 278%, and still increased over the next 24 hours. It therefore seems that the level of RNA synthesis better characterizes the condition of the embryonic axis cells of aged field bean seeds than viability indexes.

Smaller differences were found in *in vitro* RNA synthesis in cotyledons among the various samples of seeds, but the role of these organs in the process of germination is decidedly smaller than that of embryonic axes.

Similarly as in rye and wheat (Zalewski 1982 and 1985), in the embryonic

axes of germinating field bean seeds of decreased vigor (C, D) the ribosomes synthesized "de novo" occurred mainly in the form of polysomes. This is indicated by the high specific radioactivities of these samples (Table 3). The small specific radioactivity of the monosome fraction indicates that their biogenesis occurred during the formation and maturation of the seeds.

Acknowledgment

The study was supported by IHAR within the framework of project PBP 05.04.01.41.

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Metabolizm starych nasion. Formowanie się poliribosomów w kielkujących nasionach bobiku różnego wieku

Streszczenie

Zbadano kielkowanie, ogólną aktywność dehydrogenaz, proces formowania się poliribosomów w osiach zarodkowych i liścieniach nasion bobiku o zróżnicowanej żywotności i wigorze, pochodzących z różnych lat zbioru. Stosując ³H-urydynę i metodę ultrawierowania stwierdzono, że starzenie się nasion bobiku związane jest ze zmniejszeniem się ich zdolności do syntezy RNA i formowania poliribosomów. Wykazano również, że starzenie nasion bobiku związane jest ze zmniejszeniem się ich wigoru oraz obniżeniem zawartości rybosomów w osiach zarodkowych.