Plant Physiol. Biochem., 1988, **26** (3), 281-288.

Effect of accelerated aging on protein synthesis in two legume seeds

Xavier Gidrol, Abdelmajid Noubhani, Bernard Mocquot, Alain Fournier and Alain Pradet

Station de Physiologie Végétale, I.N.R.A., Pont de la Maye, France.

(Received for publication June 24, 1987; accepted December 1, 1987)

Gidrol, X., Noubhani, A., Mocquot, B., Fournier, A. and Pradet, A. 1988. Effect of accelerated aging on protein synthesis in two legume seeds. – Plant Physiol. Biochem. 26: 281-288.

Seed deterioration becomes very evident after 7 days under accelerated aging conditions (42°C and 100% relative humidity), as demonstrated by the loss of germinability. However soybean seeds (*Glycine max*) do not deteriorate to the same extent, nor at the same speed as pea seeds (*Pisum sativum*). In order to understand this different behaviour, the effect of accelerated aging on protein synthesis was investigated during early imbibition. The observed decrease of *in vivo* protein synthesis, after 12 h imbibition, could explain the reduction of the germinative response of seeds submitted to accelerated aging treatment, and the different behaviour of these two seed species. As demonstrated by *in vitro* protein synthesis, the deterioration of pre-existing "long-lived" mRNAs during the accelerated aging treatment seems relatively similar in both legume species. The difference between pea and soybean could originate from the reduction of the energy supply in soybean embryonic axes, as demonstrated by the low level of adenylate energy charge after 12 h imbibition, which results in the diminution of new mRNA synthesis and in the inefficiency of the *in vivo* translation. The possible role of deterioration of the mitochondrial membrane in the restriction of the energy supplied is evaluated.

Additional key words – Germination, membranes, mRNA, soybean, pea.

X. Gidrol (reprint requests), A. Noubhani, B. Mocquot and A. Pradet, Station de Physiologie Végétale, I.N.R.A., B.P. 131, 33140 *Pont de la Maye, France; A. Fournier, Laboratoire de Génétique, Université de Bordeaux-II, Avenue des Facultés,* 33405 *Talence Cedex, France.*

Résumé. *La dégradation des semences au cours du vieillissement accéléré (42°C et 100% d'humidité relative) devient considérable après 7 j de traitement, comme le montre la diminution de la faculté germinative. Cependant, les semences de Soja* (Glycine max) *se dégradent beaucoup plus vite et de façon plus importante que les semences de Pois* (Pisum sativum). *Afin de comprendre les origines de cette différence de comportement, l'effet du vieillissement accéléré sur la synthèse des protéines a été étudié. La diminution de la protéosynthèse* in vivo *pourrait expliquer la diminution de la germination des semences vieillies artificiellement et la différence de comportement des deux espèces étudiées. La synthèse protéique* in vitro *montre que la détérioration des ARN messagers « longue vie » préexistants est relativement similaire pour les deux espèces de légumineuses. La différence entre le Pois et le Soja pourrait provenir de la diminution de l'apport énergétique dans les axes des semences de Soja, comme le montre la faible charge énergétique mesurée après 12 h d'imbibition et qui entraînerait la diminution* in vivo *des nouvelles synthèse d'ARNm et de l'efficacité de la traduction. Le rôle que pourrait jouer la dégradation de la membrane mitochondriale dans la diminution de l'apport énergétique est discuté.* Mots clés additionnels : germination, membranes, ARNm, Soja, Pois.

Abbreviations. AA, accelerated aging; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

INTRODUCTION

Seeds deteriorate and lose their germinability during periods of prolonged storage. Many theories have attempted to explain seed aging: external factors such as irradiation, or fungal attack, and internal factors such as accumulation of toxic compounds, loss of vitamins or hormones, degradation of nucleic acids, proteins or membranes (Priestley, 1986). Several different processes have been implicated, but there is little definitive evidence for any primary process of aging damage.

It has been argued that membrane integrity plays a key role in seed viability (Simon, 1974; Harman and Mattick, 1976; Stewart and Bewley, 1980). Recently, a new hypothesis has considered lipid peroxidation as a model for the seed deterioration observed during aging (Wilson and McDonald, 1986). Authors working with the experimental accelerated aging model, in which seed storage is carried out under hot and wet conditions, *i.e.* 42°C and 100% relative humidity (Delouche and Baskin, 1973), do not always agree with the occurrence of a lipid peroxidation process. Harman and Mattick (1976) and Stewart and Bewley (1980) demonstrated the occurrence of lipid peroxidation in soybean seeds. In contrast, Priestley and Leopold (1983) did not find such a process in soybean. More recently, Buchvarov and Gantcheff (1984) made a compromise by demonstrating that in soybean embryonic axes lipid peroxidation occurs via the formation of free radicals, but not in soybean cotyledons.

There are few reports on the status of nucleic acids during the accelerated aging treatment (Dourado and Roberts, 1984*a* and *b*), whereas, in the case of cellular oxidations, nucleic acids constitute a privileged target as do biomembranes (Slater, 1984). Furthermore, the integrity of nucleic acids and the efficiency of the repair processes are very important for seed viability and vigour under natural aging conditions (Bray, 1979; Osborne, 1982, 1983; Ghosh and Chaudhuri, 1984). However, no data is available on the intensity of protein synthesis in seeds artificially aged.

The aim of the present study is, on the one hand, to determine the effect of accelerated aging on the efficiency of protein synthesis during early germination and, on the other hand, to attempt to point out the essential events of the aging process by comparing the deteriorations in two legume seeds having different behaviours under hot and wet storage conditions.

MATERIALS AND METHODS

Plant material and accelerated aging treatment. Soybean (*Glycine max* L. var. Weber) and pea (*Pisum sativum* L. var. Kalife) seeds were provided by the Rustica and Clause companies, respectively. Control seeds were kept dry by storing them at 4°C with desiccant silica gel. Seeds were artificially aged by exposing them to 42°C and 100% relative humidity in a covered water bath placed in a regulated incubator. Seeds were never in direct contact with water. The water uptake was followed during the accelerated aging and, at the end of the treatment, seeds were returned to their initial moisture content by drying them at room temperature in a vacuum chamber with desiccant silica gel. These dry seeds were used for extraction of total RNA. In all other experiments, control and artificially aged seeds were imbibed during 12 h: in this stage seeds were in germination phase II, *i.e.* when respiration of seed stabilizes, before the rootlet protrusion which completes the germination process. Therefore all the seeds used in the following experiments were ungerminated.

Germination test. Approximately 100 seeds were used for the test. Seeds subjected to various aging conditions were dispensed into 70 mm Petri dishes lined with filter paper on a layer of glass beads (6 mm diameter) and filled with 10 ml of distilled water. Germination was carried out at 25°C in a dark room. Seeds were considered as having sprouted when a 2 mm long rootlet had emerged from the seed.

***In vivo* protein synthesis.** Seeds were previously surface-sterilized according to the technique described by Mocquot *et al.* (1977). The absence of contamination was verified at the end of the experiment by spreading samples of the seed imbibition medium on an appropriate bacteria culture medium in Petri dishes. Duplicate samples of 5 seeds were decontaminated and imbibed for 12 h. In this stage, seed germination did not occur. The embryonic axes were then excised and used for a 2 h pulse-labeling of proteins. The axes were labeled for 2 h at 25°C with [^{35}S]methionine (2.1 MBq for 5 axes, New England Nuclear, 42 TBq mmol^{-1}) in 1 ml H_2O under continuous stirring. After the labeling, the samples were washed 3 times with 250 ml of 1 mM methionine and fixed in liquid nitrogen. The axes were ground in a glass homogenizer and the radioactivity incorporated into the trichloroacetic acid precipitable fractions was counted according to Mocquot *et al.* (1981).

RNA isolation. Total RNA was extracted from 50 dry seeds, or from 200 axes excised from ungerminated seeds imbibed for 12 h, as described by Laroche-Raynal *et al.* (1984). Typically, 4 mg (pea) and 1.5 mg (soybean) of total RNA was obtained from 50 dry seeds and 4 mg

(pea) and 3 mg (soybean) from 200 axes excised from imbibed seeds. Poly-A^+ RNA was fractionated from the total RNA of the excised axes, using messenger affinity paper according to Werner *et al.* (1984). The quantity of poly-A^+ RNA recovered from axes was about 40 µg for pea and 18 µg for soybean, thus representing about 0.6-1% of total RNA.

***In vitro* translation and gel electrophoresis.** Total RNA (5 µg) and poly-A^+ RNA (2 µg) were translated *in vitro* using a rabbit reticulocyte lysate system (New England Nuclear kit) and 740 kBq of [^{35}S]methionine per sample (New England Nuclear). The [^{35}S]methionine-labeled products were separated by one-dimensional 15% acrylamide SDS-PAGE (Mocquot *et al.*, 1981). The gels were impregnated with dimethyl sulfoxide and 2,5-diphenyloxazole, dried and fluorographed as described by Bonner and Laskey (1974).

Adenine nucleotide assays. Adenine nucleotides were assayed during the imbibition. Imbibition was performed by placing 5 seeds in 2 ml of distilled water in 20 ml vials under continuous shaking. Each experimental point was the mean of three samples assayed in triplicate. Imbibition was stopped by freezing the vials in cold diethyl ether (−100°C). The samples were then freeze-dried at −20°C, in order to eliminate the incubation medium. The axes were then excised from the seeds in liquid nitrogen. Adenine nucleotides were extracted from the axes and assayed as previously described (Mocquot *et al.*, 1981).

Electrolyte leakage. Imbibitional leakage was determined by placing 50 seeds in 50 ml of distilled water, and measuring the conductivity of the external medium with a Tacusel CD6N conductimeter equipped with a CM01/G electrode.

RESULTS

Germination response

As shown in figure 1, a large decrease of germinability was observed for both types of the legume seeds studied. However, pea and soybean did not deteriorate to the same extent, nor at the same speed. After 7 days, under accelerated aging conditions, pea seeds had a maximum germination of about 85%, and this percentage reached 75% after 8 days. Soybean seeds deteriorated faster, having a maximum germination of 6% after 7 days and, after 8 days, no seeds were able to germinate. Besides the modification of the maximum cumulative percentage of germination, the germination rate was also modified, the T_{50} (time required to obtain 50% germination) being significantly increased when the time spent under accelerated aging conditions was prolonged.

Therefore, accelerated aging treatment leads to a dramatic decrease of germinability in both types of seeds, the decrease being greater in the case of soybean seeds.

Seed deterioration at the nucleic acid level

A decrease in the rate of *in vivo* protein synthesis (as expressed by the ratio of the radioactivity incorporated/radioactivity uptake) was observed with the time spent under the accelerated aging conditions, in both seed species (*tab.* 1). Nevertheless, the incorporation of [^{35}S]methionine in excised soybean axes was much lower than that observed

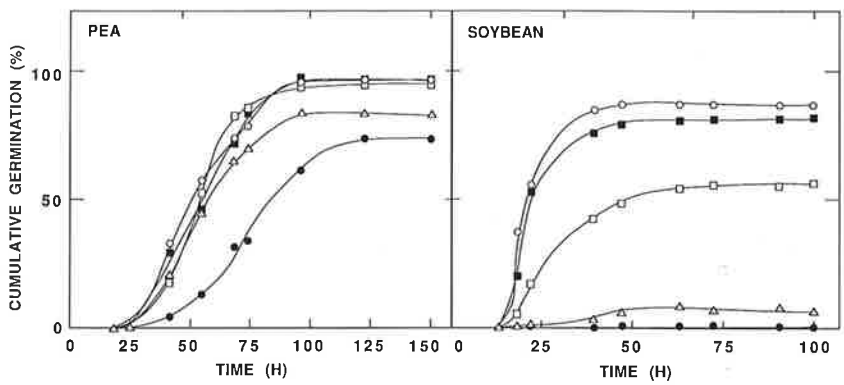

Figure 1. *Effect of accelerated aging on germination.* Pea and soybean seeds were placed at 25°C in a dark room as described in Materials and Methods. Cumulative percentages of germination are calculated as a function of time of imbibition. (○), control; (■), 3 days AA; (□), 5 days AA; (△), 7 days AA; (●), 8 days AA.

Table 1. *[^{35}S]methionine uptake and incorporation into the acid-insoluble fraction of pea and soybean axes for 2 h and rate of protein synthesis.* Seeds previously submitted or not to accelerated aging treatment were decontaminated and imbibed 12 h in sterile water. Embryonic axes were then excised and labeled during 2 h. Results are the mean of two experiments.

Treatment before the labeling	(cpm axis^{-1}) × 10^{-6}		Incorporation/ Uptake
	Uptake	Incorporation	
PEA			
Control	7.36	2.67	0.36
3 days AA	7.12	2.51	0.35
5 days AA	6.04	2.32	0.38
7 days AA	3.84	0.95	0.25
SOYBEAN			
Control	5.96	2.10	0.35
3 days AA	5.92	2.25	0.38
5 days AA	1.36	0.13	0.10
7 days AA	1.20	0.02	0.02

in the excised pea axes. After 7 days, practically no more proteins were synthesized in the soybean axes, while some proteins were still synthesized in the pea axes. In the case of the excised soybean axes submitted to 5 days or 7 days of the accelerated aging treatment, no more labeled protein bands could be observed on the fluorograms (*fig.* 2), whereas in the case of the pea axes, even after 7 days in the hot and wet atmosphere, some proteins were still synthesized *in vivo*. The observed depletion in [^{35}S]methionine incorporation affected all of the proteins synthesized and no qualitative variation could be seen on the fluorograms (*fig.* 2).

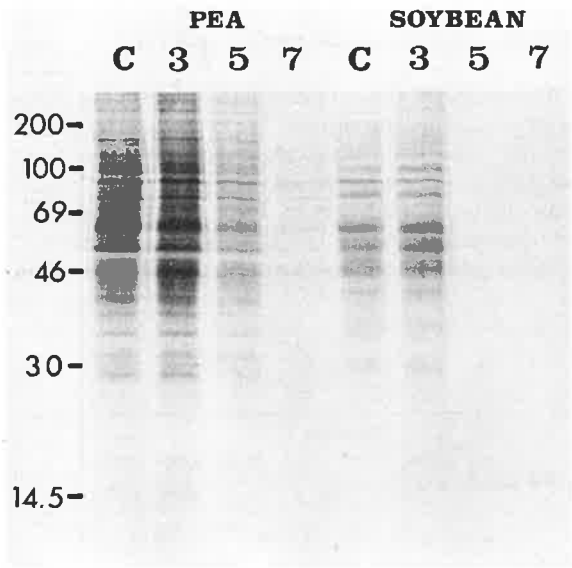

Figure 2. *Autoradiogram of* in vivo *protein synthesis.* Embryonic axes excised from pea and soybean seeds, after 12 h imbibition, were incubated with [^{35}S]methionine as described in Materials and Methods. Labeled proteins were then extracted and separated by monodimensional 15% acrylamide SDS-PAGE. Neosynthesized polypeptides were revealed by direct exposition of the gel to a hypersensitive film (β-max Amersham). The molecular weights of the marker proteins are listed on the left. C, control; 3, 3 days AA; 5, 5 days AA; 7, 7 days AA.

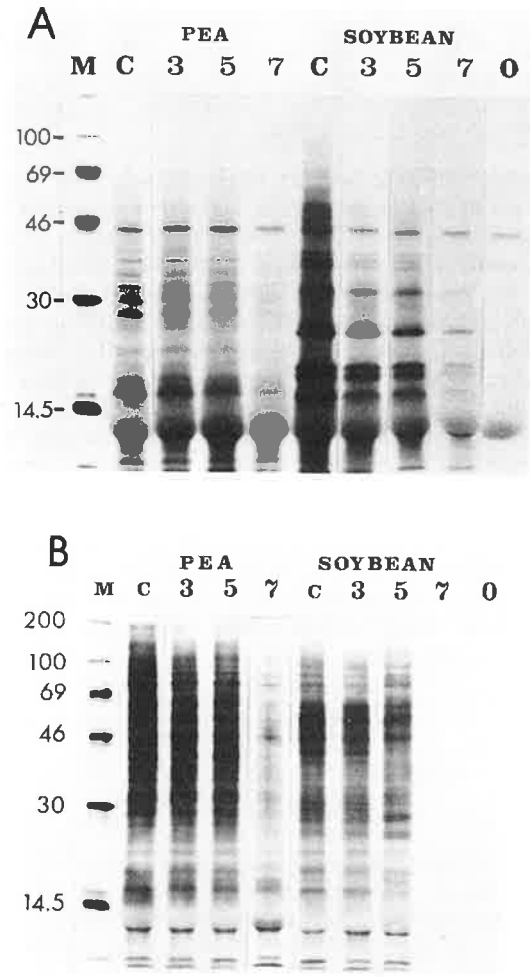

Figure 3. *Fluorogram of* in vitro *protein synthesis primed by RNA from dry seeds* (**A**) *and imbibed axes* (**B**). Total RNA of pea and soybean seeds were extracted and translated in a cell-free system with [^{35}S]methionine. Synthesized polypeptides were separated and analyzed by SDS-PAGE. Labeled polypeptides were revealed by fluorography. The molecular weights of the marker proteins are listed on the left (M). The right lane is the control without added RNA (0). The other lanes are the same as in figure 2.

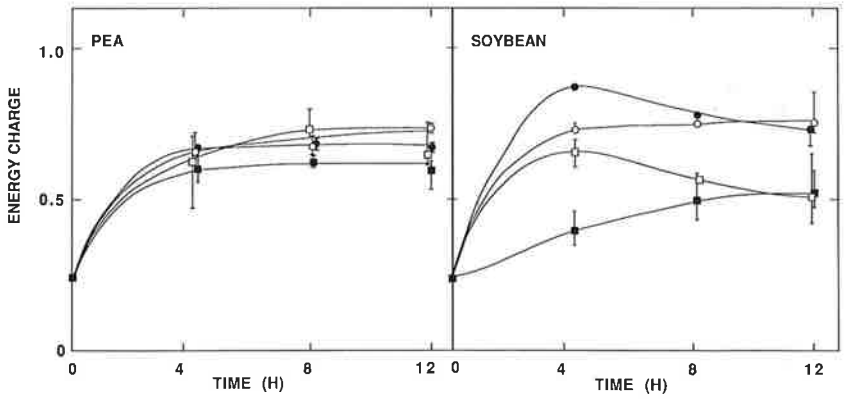

Figure 4. *Effect of accelerated aging on the energy charge time-course during seed imbibition under normoxia.* Adenine nucleotides were extracted from isolated axes and assayed as described in Materials and Methods. Energy charge values are obtained by calculation with the adenine nucleotide concentrations, each value being the mean of 9 determinations. Vertical bars represent the maximal amplitude of variation. (○), control; (●), 3 days AA; (□), 5 days AA; (■), 7 days AA.

In order to determine the extent of nucleic acid damage that could occur during the storage under hot and wet conditions *per se*, cell-free translations of total RNA extracted from dry seeds, previously submitted to accelerated aging treatment, were performed. Cell-free translation products from total RNA extracted from the dry seeds were analysed by SDS-PAGE. The fluorography of the [^{35}S]methionine-labeled proteins synthesized *in vitro* showed that, for both kinds of seeds, there was a progressive decrease of the proteins synthesized as a function of the time spent under the accelerated aging conditions, suggesting the degradation of pre-existing "long-lived" mRNAs during the treatment (*fig.* 3 A). The diminution of the methionine incorporated into proteins *in vitro* concerned all the proteins synthesized and no qualitative differences in the intensity of the label could be observed, with the technique used (*fig.* 3 A).

In order to determine whether repair processes could occur during imbibition of previously artificially aged seeds, cell-free translation of total RNAs extracted from imbibed axes (12 h imbibition), was performed. The analysis of the translation products by SDS-PAGE and fluorography, showed that the accelerated aging induced a large decrease of the proteins synthesized *in vitro*, especially after 7 days of treatment (*fig.* 3 B). In this case, a difference between pea and soybean was observed. After 7 days under hot and wet conditions and 12 h imbibition, soybean axes did not contain any translatable mRNA, whereas no such advanced degradation was observed in the pea axes after 7 days and numerous proteins were still synthesized *in vitro*. The decrease of the *in vitro* protein synthesis during the accelerated aging treatment concerned all the translation products and no qualitative variations were observed by monodimensional SDS-PAGE (*fig.* 3 B). The cell-free translation of poly-A$^+$ RNA (presumed messenger RNA) gave the same type of results (data not shown).

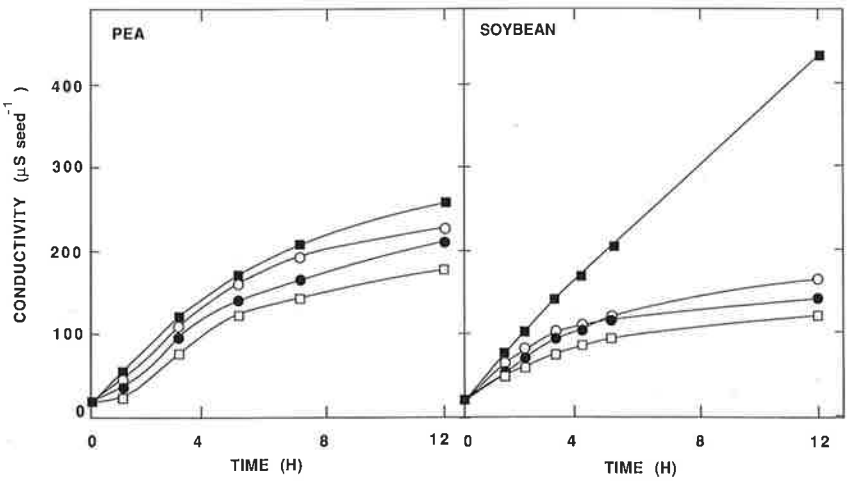

Figure 5. *Effect of accelerated aging on seed electrolyte leakage.* The time-course of the conductivity of the imbibition medium was followed. (○), control; (●), 2 days AA; (□), 4 days AA; (■), 7 days AA.

Seed deterioration at the energy supply level

In order to determine whether the energy supply could explain the differences observed between the pea and soybean seeds after 12 h imbibition, the adenine nucleotide ratios was estimated within the first hours of seed imbibition, as a function of the time spent under the accelerated aging conditions. The determination of the adenine nucleotide contents is an indirect method allowing the estimation of the efficiency of oxidative phosphorylation.

Figure 4 demonstrates that in embryonic axes, the accelerated aging induced a decrease of the adenylate energy charge values, reached after 12 h of imbibition. For pea axes, only small variations occurred, whereas for soybean axes, the accelerated aging conditions induced a large decrease of the maximum adenylate energy charge to 0.5, reached after 5 or 7 days spent under a hot and wet atmosphere. The results show that the energy supply is restricted in the case of soybean axes and suggest that the oxidative phosphorylation becomes less efficient according to the time spent under hot and wet conditions.

Seed deterioration at the membrane level

To confirm or invalidate the proposition concerning the occurrence of membrane degradation dependent on the time spent under accelerated aging conditions, the integrity of the plasma membrane was investigated by an indirect method, the extent of electrolyte leakage. The conductivity of the imbibition medium increased dramatically with aging: in soybean seeds submitted to 8 days of accelerated aging conditions, leakage was 420% as compared to untreated seeds, whereas there was only a slight (34%) increase in pea seeds (*fig.* 5).

DISCUSSION

The *in vivo* incorporation of [^{35}S]methionine into the newly synthesized proteins shows that the accelerated aging greatly decreases the protein synthesis during the first hours of germination in both kinds of seeds. Furthermore, large differences are observed between pea and soybean seeds (*fig.* 2). These differences in the intensity of protein synthesis could explain both, the diminution in the germinative capacities induced by the accelerated aging treatment and the different behaviour observed between pea and soybean during storage under hot and wet conditions. These results must be related to previous studies which showed that the rate of protein synthesis during the first hours of germination is closely correlated with embryo performances and can be recognized as an index of embryo vigour (Blowers *et al.*, 1980) or viability (Sen and Osborne, 1977) under natural aging conditions.

In vivo protein synthesis does not indicate whether the translatable pool of RNA or the translation machinery, have been modified during the accelerated aging treatment. Therefore, we performed the cell-free translation of RNA extracted from dry seeds that had previously been artificially aged. The results obtained suggest that there is a degradation of translatable pre-existing "long-lived" mRNAs during the treatment. Nevertheless, even after 7 days spent under hot and wet conditions, numerous RNAs are still translatable *in vitro* in both seed species. The degradation of "long-lived" mRNAs during the accelerated aging treatment cannot explain the difference between pea and soybean seeds and the very low germination response (6%) of soybean seeds after 7 days under accelerated aging treatment.

In order to determine if repair processes and new mRNA synthesis occur during imbibition, cell-free translations of RNA extracted from axes excised after 12 h imbibition was performed. The decrease in the intensity of label due to the accelerated aging treatment, was observed in embryonic axes after 12 h imbibition, much as described in dry seeds.

The comparison of *in vivo* and *in vitro* protein synthesis in excised axes after 12 h imbibition shows that, for soybean seeds which have undergone 5 days of treatment, numerous mRNAs are still present, but there is no protein synthesis *in vivo*. This result suggests that the translation system becomes ineffective during the accelerated aging. In contrast, in the case of the pea, the *in vitro* and *in vivo* patterns are relatively similar, suggesting a good efficiency of the translation system in embryonic axes *in vivo* and a regulation of the protein synthesis by the mRNA content. Therefore, the differences observed between the two seed species could principally result from the impossibility for artificially aged soybean seeds to synthesize new mRNA species and to translate messengers during the first hours of imbibition. This result corroborates those of Sen and Osborne (1977), who pointed out the fundamental role of early-synthesized RNA in the vigour of rye seeds under natural aging conditions.

Many factors can be responsible for the inability to synthesize new mRNA *in vivo:* the degradation of DNA and the deficiency of the DNA repair

system (Sen and Osborne, 1977), the absence of sufficient energy supply (Anderson, 1977). From our results, it can be seen that the restriction of the energy supply as a function of the time spent under accelerated aging conditions could explain, at least partially, the difference observed in the *in vivo* protein synthesis between both type of seeds. Indeed, Hourmant and Pradet (1981) have demonstrated that oxidative phosphorylation is effective within the first minutes of seed imbibition; furthermore the adenylate energy charge is a good marker of the energy supply under restricted-energy conditions (Pradet and Raymond, 1983). In air, 95% of the energy supply of soybean seeds is ensured by oxidative phosphorylation (Raymond *et al.*, 1985). So it appears that in soybean axes the oxidative phosphorylation has become ineffective following accelerated aging. However, from our results one cannot definitively determine whether the high adenylate energy charge values observed in pea axes result from an efficient oxidative phosphorylation, via well-functioning mitochondrial membranes, or from fermentation. Indeed, under aerobic germination conditions, 40% of the energy supply in pea seeds is provided by fermentation (Raymond *et al.*, 1985).

Woodstock *et al.* (1984) demonstrated that compared to mitochondria isolated from high vigour soybean axes, those from low vigour soybean axes were characterized by a lower O_2 uptake, and by decreased ADP/O and respiratory control ratios. Similarly, Anderson (1977) indicated that the lowered rates of RNA and protein syntheses of deteriorated soybean axes were associated with a reduced ATP content of the tissue. The demonstration of the importance of the energy supply in the early biosynthetic events occurring during seed imbibition does not eliminate the fundamental role of DNA repair in the efficiency of germination as pointed out by Osborne (1982).

The inefficiency of oxidative phosphorylation could be due to membrane deterioration as already suggested by different authors (Anderson, 1977; Parrish and Leopold, 1978). But direct evidence for deterioration of the mitochondrial membrane is difficult to get, considering the complexity to isolate intact mitochondria from partially imbibed seed. The existence of plasma membrane degradation in soybean seeds during the accelerated aging treatment is confirmed by the fact that a large increase in the conductivity of the external medium was observed, suggesting that the plasma membrane of soybean seeds submitted to 7 days of accelerated aging is significantly deteriorated, whereas in pea seeds no such increase in the conductivity of the external medium could be observed. As pointed out by Perl *et al.* (1978), the increased conductivity of the imbibition medium is a marker of an established degradation. Many authors have described a similar leakage resulting from membrane degradation in seeds of various plants submitted to natural aging (Ching and Schoolcraft, 1968), or accelerated aging (Harman and Mattick, 1976; Stewart and Bewley, 1980; Buchvarov and Gantcheff, 1984).

In conclusion, our results show similar deterioration at nucleic acid level in both species during the storage under hot and wet conditions. In contrast, cellular deterioration at membrane level seems more important in soybean seeds. The ability to synthesize new mRNAs and proteins during early imbibition could explain the difference observed between pea and soybean after the accelerated aging. Cellular deterioration at membrane and nucleic acid levels observed during the storage under hot and wet conditions could be the reflect of cellular oxidations as already suggested by different authors in the case of seed aging (Stewart and Bewley, 1980; Wilson and Mc-Donald, 1986) or more generally in the case of plant senescence and wounding (Thompson *et al.*, 1987). The comprehension of the mechanisms whereby the degradation of nucleic acids and biomembranes occurs under accelerated aging conditions will be the aim of further studies.

Acknowledgements. We thank Mrs. C. Prat for excellent technical assistance.

REFERENCES

Anderson J. D., 1977. — Adenylate metabolism of embryonic axes form deteriorated soybean seeds. *Plant Physiol.*, **59**, 610-614.

Blowers L. E., Stormonth D. A. and Bray C. M., 1980. — Nucleic acid and protein synthesis and loss of vigour in germinating wheat embryos. *Planta*, **150**, 19-25.

Bonner W. M. and Laskey R. A., 1974. — A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.*, **46**, 83-88.

Bray C. M., 1979. — Nucleic acid and protein synthesis in the embryo of germinating cereals. In *Recent advances in the biochemistry of cereals*, Laidman D. L. and Wyn-Jones R. G., ed., Academic Press, New York, 147-173.

Buchvarov P. and Gantcheff T., 1984. — Influence of accelerated and natural aging on free radical levels in soybean seeds. *Physiol. Plantarum*, **60**, 53-56.

Ching T. M. and Schoolcraft I., 1968. — Physiological and chemical differences in aged seeds. *Crop Sci.*, **8**, 407-409.

Delouche J. C. and Baskin C. C., 1973. — Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Sci. Technol.*, **1**, 427-452.

Dourado A. M. and Roberts E. H., 1984*a*. — Chromosome aberrations induced during storage in barley and pea seeds. *Ann. Bot.*, **54**, 767-779.

Dourado A. M. and Roberts E. H., 1984*b*. — Phenotypic mutations induced during storage in barley and pea seeds. *Ann. Bot.*, **54**, 781-790.

Ghosh B. and Chaudhuri M. M., 1984. — Ribonucleic acid breakdown and loss of protein synthetic capacity with loss of viability of rice embryos (*Oryza sativa*). *Seed Sci. Technol.*, **12**, 669-677.

Harman G. E. and Mattick R. L., 1976. — Association of lipid oxidation with seed aging and death. *Nature*, **260**, 323-324.

Hourmant A. and Pradet A., 1981. — Oxidative phosphorylation in germinating lettuce seeds (*Lactuca sativa*) during the first hours of imbibition. *Plant Physiol.*, **68**, 631-635.

Laroche-Raynal M., Aspart L., Delseny M. and Penon P., 1984. — Characterization of radish mRNA at three developmental stages. *Plant Sci. Lett.*, **35**, 139-146.

Mocquot B., Pradet A. and Litvak S., 1977. — DNA synthesis and anoxia in rice coleoptiles. *Plant Sci. Lett.*, **9**, 365-371.

Mocquot B., Prat C., Mouches C. and Pradet A., 1981. — Effect of anoxia on energy charge and protein synthesis in rice embryo. *Plant Physiol.*, **68**, 636-640.

Osborne D. J., 1982. — Deoxyribonucleic acid integrity and repair in seed germination: the importance in viability and survival. In *The Physiology and biochemistry of seed development, dormancy and germination*, Khan A. A., ed., Elsevier Biomedical Press, Amsterdam, 435-463.

Osborne D. J., 1983. — Biochemical control systems operating in the early hours of germination. *Can. J. Bot.*, **61**, 3568-3577.

Parrish D. J. and Leopold A. C., 1978. — On the mechanism of aging in soybean seeds. *Plant Physiol.*, **61**, 365-368.

Perl M., Luria I. and Glemond H., 1978. — Biochemical changes in sorghum seeds affected by accelerated aging. *J. Exp. Bot.*, **29**, 497-509.

Pradet A. and Raymond P., 1983. — Adenine nucleotide ratios and adenylate energy charge in energy metabolism. *Annu. Rev. Plant Physiol.*, **34**, 199-224.

Priestley D. A. and Leopold A. C., 1983. — Lipid changes during natural aging of soybean seeds. *Physiol. Plantarum*, **59**, 467-470.

Priestley D. A., 1986. — *Seed aging. Implications of seed storage and persistance in the soil.* Cornell University Press, Ithaca.

Raymond P., Al-Ani A. and Pradet A., 1985. — ATP production by respiration and fermentation, and energy charge during aerobiosis and anaerobiosis in twelve fatty and starchy germinating seeds. *Plant Physiol.*, **79**, 879-884.

Sen S. and Osborne D. J., 1977. — Decline in ribonucleic acid and protein synthesis with loss of viability during the early hours of imbibition of rye (*Secale cereale* L.) embryos. *Biochem. J.*, **166**, 33-38.

Simon E. W., 1974. — Phospholipids and plant membrane permeability. *New Phytol.*, **73**, 377-420.

Slater T. F., 1984. — Free-radical mechanisms in tissue injury. *Biochem. J.*, **222**, 1-15.

Stewart R. R. C. and Bewley J. D., 1980. — Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.*, **65**, 245-248.

Thompson J. E., Legge R. L. and Barber R. F., 1987. — The role of free radicals in senescence and wounding. *New Phytol.*, **105**, 317-344.

Werner D., Chemla Y. and Herzberg M., 1984. — Isolation of poly(A)$^+$ RNA by paper affinity chromatography. *Anal. Biochem.*, **141**, 329-336.

Wilson D. O. and Mc-Donald M. B., 1986. — The lipid peroxidation model of seed ageing. *Seed Sci. Technol.*, **14**, 269-300.

Woodstock L. W., Furman K. and Solomos T., 1984. — Changes in respiratory metabolism during aging in seeds and isolated axes of soybean. *Plant Cell Physiol.*, **25**, 15-26.