I. BOUBRIAK [1, 2], V. POLISCHUK [3],
A. GRODZINSKY [4], DAPHNE J. OSBORNE [5]

[1] School of Biological and Molecular Sciences,
Oxford Brookes University Gipsy Lane, Headington, Oxford, OX3 0BP, UK
[2] Institute of Cell Biology and Genetic Engineering,
Ukrainian Academy of Sciences 148 Zabolotnogo street,
Kiev, 03143, Ukraine
[3] Department of Virology, Kiev National University,
64, Volodymyrska street, Kiev, 01033, Ukraine
[4] Institute of Botany, Ukrainian Academy of Sciences,
2, Tereshchenkivska st., Kiev, 01601, Ukraine
[5] Oxford Research Unit, The Open University, Foxcombe Hall,
Boars Hill, Oxford OX1 5HR, UK

TELOMERES AND SEED BANKS

We have found that a progressive loss of telomeric sequences occurs from high molecular weight DNA with an increasing appearance at low molecular weight as the periods of storage in the dry state were extended in time to provide seed germination loss from 98 to 0 %. Telomere distribution would appear to follow the general pattern of DNA random fragmentation that occurs in the embryos of seeds stored in the dry state, but there are also indications of an overall telomere loss from DNA as a consequence of storage. There is a need for a convenient «quality marker» for the seeds that can be monitored over time. Reviewing the implications of our results very carefully we believe that there is considerable potential in the use of telomere sequences to mark embryo ageing of seeds held in Seed Banks.

Introduction. The problem for Seed Banks is how long can a seed be stored and retain its useful viability? Not all species survive equally well and stored life expectancy of any seed much depends upon the environmental history it has experienced before it enters the Bank.

So there has to be a known *starting marker* for each lot to be stored and it has to be one that can be monitored over time. The marker must inform on the germination (vigour) of any new seeds collected for bank storage. It must be one that either increases or decreases as the seed ages and loses viability. It must be one on which reliance can be placed for determining the germination (vigour) of that seed when taken from store.

The easiest and most reliable method is without doubt the germination test, carried out under controlled conditions. When 50 % of a sample of the stored seed fails to germinate within a chosen period, the time has come to rear new seeds from those plants still germinating from the stored lot. Alternatively, a new lot must be obtained from the wild. This is a far too time consuming and labour intensive operation to be feasible for the thousands of species that will be stored in the world banks of plant genetic resources, so another, quicker, less labour intensive, preferably automated, but equally reliable method has to be found.

There are already a few long standing candidates used already to good effect in the seed testing world. Loss of mitochondrial dehydrogenase activity, increasing damage to membranes leading to enhanced solute leakage on imbibition, the lowering of protein synthesis in the early hours of water uptake. All can be used [1—3].

One feature is very clear from all the studies on seeds. The integrity of nuclear DNA is critical to fast and successful germination and where DNA has become damaged before or during storage then the ability of the embryo to repair that damage on imbibition is critical to survival. These tests too are time consuming, expensive and require considerable expertise for reliability.

Since the 1940's the ends of chromosomes have been shown to perform a special function in maintaining the linear structure of DNA [4] and it is clear that loss of the DNA sequences that make up these chromosome ends is, in many examples, associated with loss of DNA integrity and shortened life spans [5—8]. It is evident that maintaining genomic integrity is essential to the wellbeing of all cells and their performance, and so the role

of the telomere in cell survival has become of increasing interest.

Although for the embryos of seeds, DNA repair by an unscheduled DNA synthesis was established as early as 1981 [9], the question whether all the DNA then synthesized was by an excision and patch — repair was questioned [10—12]. Distinct from repair synthesis and the onset of replicative DNA synthesis in which nuclear DNA contents proceed from 2C to 4C in the first cell cycle, starting first in the cells of the root tip, there was evidence of a third DNA synthesis of unknown type, which appeared to be neither of repair nor replicative origin.

It was Bucholc and Buchowicz [13] who first questioned and investigated whether this third type of DNA synthesis might be of the telomeric repeats that guard the terminal ends of chromosomal DNA. Following the methodologies of Richards and Ausubel [14] they probed the extracted nuclear DNA of wheat embryos from fresh and stored (12—14 months) seed with a synthetic oligonucleotide sequence common to eukaryotic (*Arabidopsis*) telomeric DNA. These samples, either before or after restriction enzyme cleavage with Bam H1, were used for Southern dot blot hybridizations with the ^{32}P labelled oligonucleotide sequence 5'-CCCTAAACCCTAAA-3'. Despite the fact that both lots of wheat embryos showed a 94 % germination, their results seem compelling — the ex-DNA fractions from the 12—24 month stored seeds were enriched with telomere hybridizing sequences, whilst those of the chromosomal DNA had become depleted.

The methods that Bucholc and Buchowicz used at this time required a considerable expenditure of time and careful manipulation to achieve their results and are not amenable to a rapid assessment of telomere distribution between high and low molecular weight DNA fractions. However, the fact that the differences between the fresh and stored seeds were so evident, despite their similarity in percentage germination indicates that telomere monitoring could provide the sought-for marker if the assay could be speeded.

To this end, we have explored a modified stratagem to follow what appears to be the accumulation of telomeric sequences in low molecular weight DNA during the process of ageing in storage in the embryos of rye, *Secale cereale*.

Materials and Methods. *Seed Material.* Fresh and stored seeds of different age harvests of rye, *Secale cereale*, held at the Oxford Research Unit were used for these experiments. For natural ageing the seeds were stored at room temperature at c. 40 % mean relative humidity. One lot (the newest, from the year 2000 harvest) gave 98 % germination in 48 h at 24 °C (designated 98 % G). The second lot, from the 1998 harvest was of lower viability giving 78 % germination after 1.5 year storage and 32 % germination after 2.5 year storage, both in 48 h at 24 °C and are designated 78 % G and 32 % G respectively. A third lot, from 1996, was completely non-viable and had been dead for at least a year. These seeds are described as NV. For accelerated aged seeds, those of the 2000 harvest were suspended in muslin bags over a saturated solution of NaCl in closed kilner jars maintained at 40 °C. Incubation in these conditions for 7 days (7AA) provided seeds that were mostly non-viable (8 % germination) and all at least 59 days dead by 72 days (samples designated 72AA).

Whole embryos were hand dissected from these seed samples before use.

DNA isolation from seed material. Three kits were selected for the isolation of DNA from intact embryos.

1. Bioline DNAce Clinipure Genomic Isolation Kit, based on the binding of DNA to a matrix binding milk and its subsequent elution. No enzymic treatments are involved in the kit. DNA precipitated with ethanol and washed with 70 % ethanol.

2. Promega Wizard™ Genomic DNA Purification Kit, based on the lysis of cell nuclei, removal of RNA and protein by RNase followed by high salt and precipitation of the DNA with isopropanol and the subsequent washing with 70 % ethanol.

3. Cruachem Annovis Phytosep DNA Magnetic Isolation Kit, based on the binding of DNA to paramagnetic matrix coated beads, the magnetic attachment of the beads to the tube wall, washing and release of the DNA from the beads by the elution buffer, the beads again immobilized to the tube wall and the released DNA in solution collected, RNased and the DNA precipitated with ethanol.

Each of these methods provided DNA suitable for subsequent Southern analysis.

DNA quantification for equal DNA gel loading. DNA was quantified by spectrophotometric analy-

sis at 260 nm followed by image analysis of the DNA run in ethidium bromide agarose gels and appropriate concentration adjustments made for equal DNA applications in subsequent gels. Alternatively fluorescent quantification of double stranded DNA in solution using Pico Green™ (Molecular Probes Europe) and subsequent concentration adjustment to permit equal DNA loading per gel lane was carried out.

DNA electrophoresis and membrane transfer. The equal loading DNA samples were fractionated on neutral 0.8 % agarose (Type 1, Sigma-Aldrich) mini-gels containing 0.0005 % ethidium bromide at 45 mA for 1.5 h. Then washed in IO N HCl for 15 min to partially depurinise the DNA followed by ca. 30 min in high pH denaturing buffer before neutralizing to pH 8.5. DNA was capillary transferred to Hybond N (Amersham) membrane overnight and under pressure. The blot was then air-dried (1 h) and the DNA bound to the membrane by 1 min. exposure to a UVC light source. Membranes were then stored for use.

Telomere Probe Labelling. The telomer PTELC3 5'-$(CCCTAAA)_3$-3' probe was obtained from Sigma-Genosys and used for all the hybridisations [15]. Probe Labelling was with the Gene Images 3'-oligolabelling module kit (Amersham-Pharmacia) using fluoresceine-11-dUTP and 3'OH-terminal transferase for 1.5 h.

Southern Hybridization. Membranes were prehybridized in a rotating hybridizer at 52 °C in 50 ml 5×SSC buffer containing 0.1 % SDS and 0.5 % dextransulphate (0.25 ml/cm²) for 1 h. The fluoresceine labelled probe was then added (10 ng/ml) and rotation continued overnight at 52 °C. Membranes were then washed free of probe with 5×SSC buffer + 0.1 % SDS then 2×SSC + 0.1 % SDS and finally 1×SSC + 0.1 % SDS at 55 °C.

Detection of fluorescein probe binding. For this, the Gene Images CDP-Star Detection Module (Amersham Pharmacia) was used. Membranes were first incubated in blocking agent in100 mM pH 9.5 TrisHCl containing 300 mM (0.75 ml/cm²) NaCl at room temperature for 1h. Solution was then replaced by similar buffer containing the anti-fluorescein alkaline phosphatase conjugate in 0.5 % BSA (0.3 ml/cm²) membrane for 1 h at room temperature. Membranes were washed (3 × 10 min) in TrisHCl containing NaCl and 0.3 % Tween 20 (2 ml/cm²) to remove excess conjugate and drained.

Fig. 1. Presence of telomere sequences by Southern hybridization in low molecular weight DNA of embryos of dry stored seeds and the redistribution of sequence recognition as an early event of germination. Bioline DNA extraction; A_{260} quantification; 5 µg DNA per lane. Lane 1 — stored seed (1.5 years) 78 % G. Lane 2 — same imbibed for 3 h

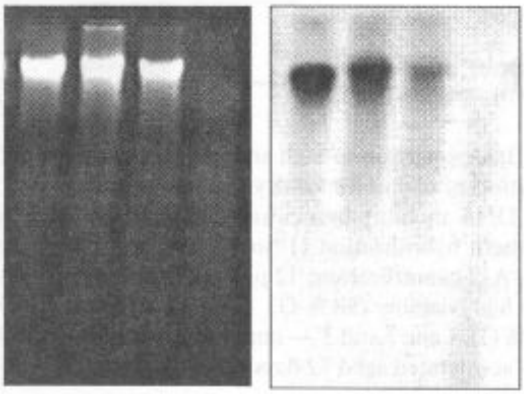

Fig. 2. DNA mobilities by neutral agarose gel fractionation (1 to 3) and telomere distribution by Southern hybridization (1' to 3') for embryos of dry stored seeds. Bioline DNA extraction; image analysis quantification 'X' µg DNA per lane. Lanes 1 and 1' — high viability (98 % G); 2 and 2' — low viability (32 % G); 3 and 3' — non-viable (0 % G)

Damp membranes were enclosed in Saran wrap and the fluorescein detection reagent (40 µl/cm²) was applied to the membrane surface. After 5 min. during which the probe-bound phosphatase conjugate catalyses light production from the stabilized dioxetane substrate, the membrane was drained only, and exposed at once to Hyperfilm-MP film in the kit exposure bag for different periods of time for optimal quantification (2 h → 16 h).

Film development and telomere probe quantification. Exposed films were developed using a GBX Developer and Fixer Pack (Sigma-Aldrich) and

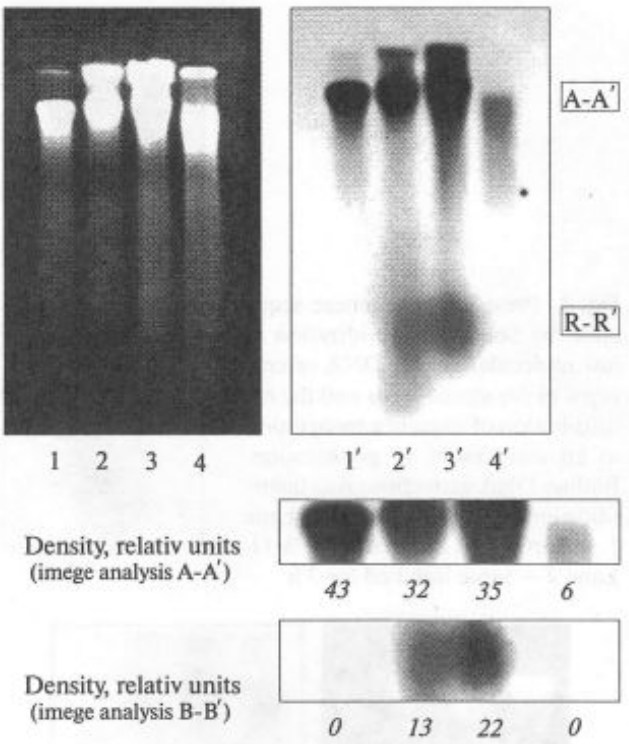

Fig. 3. Comparison of high and low molecular weight distribution of telomeres for dry stored and accelerated ageing. DNA mobility by neutral gel fractionation (1 to 4); Southern hybridization (1' to 4'). Promega DNA extraction; A_{260} quantification; 12 μg DNA per lane. Lane 1 and 1' — high viability (98 % G). Lane 2 and 2'— low viability (32 % G). Lane 3 and 3' — non-viable (0 % G). Lane 4 and 4' — accelerated aged 72 days (0 % G)

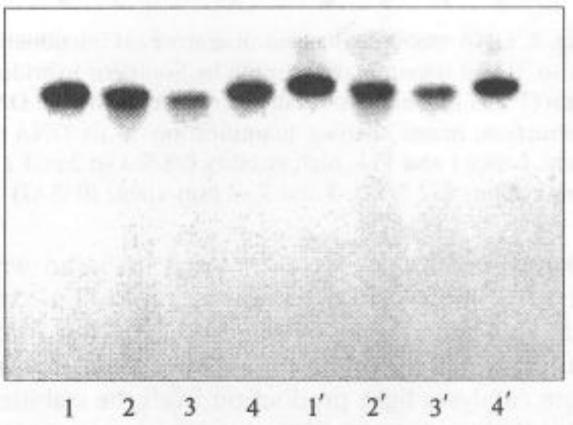

Fig. 4. Comparison of different DNA loadings for Southern hybridizations of electrophoretically fractionated DNA. Magnetic DNA extraction; Pico Green quantification, lanes 1—4 (8 μg DNA); lanes 1' to 4' (6 μg DNA). Lane 1 and 1' — high viability (98 % G). Lane 2 and 2' — low viability (32 % G). Lane 3 and 3' — non-viable (0 % G). Lane 4 and 4' — accelerated aged 7 days (8 % G)

the intensity of probe-labelling assessed with the UVP (UK) Image Analyser and Gelworks™ Advanced Gel Analysis Software.

Results. The first objective of this investigation was to determine if telomeres in the embryos of ageing rye seed behaved in a similar way to those of the ageing wheat seed as described by Bucholc and Buchowicz [13]. It was questioned if telomere sequences present in both high and low molecular weight DNA in a reduced viability stored rye sample (78 % G) would be found only in the high molecular weight DNA fraction after the start of germination. This would indicate that part of the very early DNA repair synthesis could include the restoration of telomere sequences to high molecular weight chromosomal DNA.

Using the Bioline extraction kit and DNA quantification at A260 it was demonstrated that for the embryos of the stored seed (78 % G) a distribution of telomeres to both high and low molecular weight DNA was present in the dry state with an accumulation in the smallest DNA fragments, but that after 3 h germination the hybridization profile was restricted to telomeres only of the high molecular weight DNA (Fig. 1). As Bucholc and Buchowicz had shown for wheat embryos, DNA-containing telomere sequences were cleaved during storage, but were restored in rye embryos as part of the rehabilitation of genomic integrity during DNA repair in the first few hours of germination.

The question was then addressed as to how closely telomere sequences follow the concentrations of fragmented DNA and how far overall telomere binding changes with seed storage. In a comparison of equal loadings of a Bioline extracted DNA and electrophoresis on neutral agarose gels Fig. 2 (lanes 1—3) the Southern hybridizations probes for telomeres on blots of these gels (lanes 1'—3') show that the distribution of telomere sequences essentially follows the distribution of DNA. Most importantly, these gels show that at high molecular weight, the 98 % G sample shows highest probe binding, declining in the 32 % G to a low level of binding in the 0 % G samples during seed storage.

In a Promega extraction experiment with a higher DNA loading per lane than in Fig. 2 it can be seen again from the quantification of the Southern hybridizations (Fig. 3) that the greatest proportion of telomeres remains with the DNA at high molecular weight in the 98 % G embryos (lane 1') but

with less than the 98 % G at this mobility after storage to 32 % G or 0 % G (lanes 2' and 3'). In addition, these higher DNA loadings allow the visualization of an increasing level of telomere sequences present in the small DNA fragments particularly of the 0 % G (lane 3'). The hybridizations of DNA from seeds embryos of accelerated aged for 7 days (after which some 8 % only can still germinate) shows a remarkably high proportion of telomeres linked to the DNA at high molecular weight (Fig. 3, lane 4'), but very little hybridization to the DNA fragments — indicating the already known difference in the DNA degradation pattern between the normal and accelerated progress of ageing [16].

Using a Magnetic DNA separation kit and quantification by Pico Green, a comparison was made of two different DNA loadings and the subsequent Southern telomeric hybridizations (Fig. 4). Lanes 1 to 4 (8 μg DNA per lane) and 1' to 4' (6 μg DNA per lane) show the progressive decrease in high molecular weight DNA and telomere frequency as the age of the dry stored embryos increases and the percentage germination declines.

Also of interest here is the maintenance of high molecular weight DNA (and telomere sequences) in the 8 % germinating 7 day accelerated aged embryos compared with the very low level of telomeres present in the 72 day accelerated aged material (Fig. 3, lanes 4 and 4'). Whereas the low molecular weight telomere distribution in the 32 % G and 0 % G dry stored material is just visible (lanes 2 and 3, 2' and 3') no fragment hybridization is detectable in these 7 day accelerated aged samples (lanes 4 and 4').

Length of exposure of the hybridized membrane to the Hyperfilm is important for maximizing information as seen from the comparisons of 4 h and 12 h in Fig. 5. With the Magnetic kit, and quantification by A_{260}, equal DNA loadings were run of the 98 % G, 32 % G, 0 % G and 7 day (8 % G) accelerated aged seed embryos. In order to optimally visualize the low level of telomere hybridization, a 12 h exposure time is required (lanes 1' 2' 3' and 4') but this then overexposes the high molecular weight DNA. Quantification of the latter becomes possible only when the exposure time is reduced to 4 h.

An example of how maximum information can be achieved from telomere probe recognition is provided in Fig. 6. Here, different loadings of DNA were used per lane (1 to 4 9 μg; 1' to 4' 6 μg). By

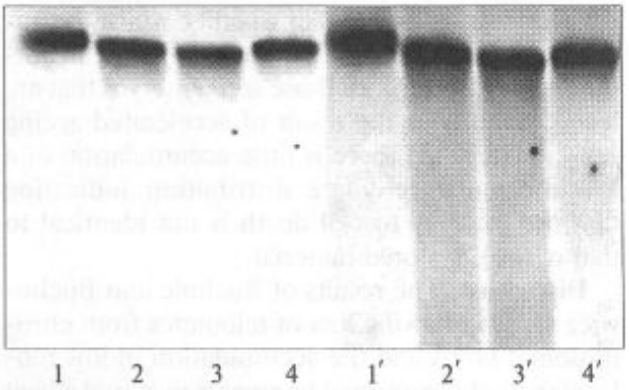

Fig. 5. Comparison of membrane exposure times to Hyperfilm. Lanes 1 to 4 (4 h); lanes 1' to 4' (12 h). Magnetic DNA extraction; A_{260} and Pico Green quantification; 8 μg DNA per lane. Lane 1 and 1' — high viability (98 % G). Lane 2 and 2' — low viability (32 % G). Lane 3 and 3' — non-viable (0 % G). Lane 4 and 4' — accelerated aged 7 days (8 % G)

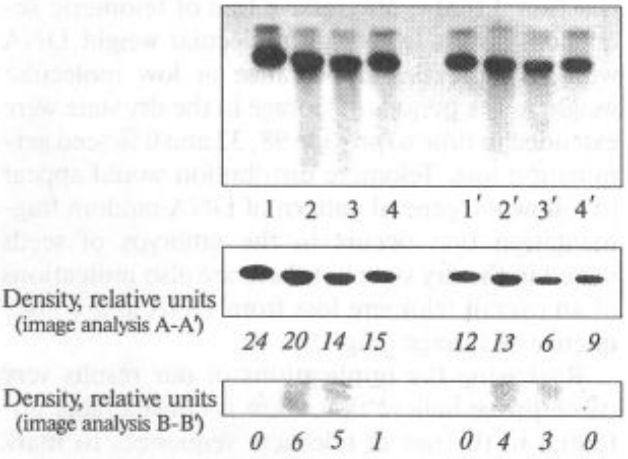

Fig. 6. Optimization by image analysis of high (A—A') and low molecular weight (B—B') quantification of telomere binding in Southern hybridizations of electrophoretically fractionated DNA. Magnetic DNA extraction; A_{260} and Pico Green quantification; lanes 1 to 4 9 μg DNA; lanes 1' to 4' 6 μg DNA. Lane 1 and 1' — high viability (98 % G). Lane 2 and 2' — low viability (32 % G). Lane 3 and 3' — non-viable (0 % G). Lane 4 and 4' — accelerated aged 7 days (8 % G)

adjusting the light exposure to the developed film in the image analyser, both high and low molecular weight probe binding can be measured independently and compared for the two levels of DNA loading.

Very clearly it can be seen that the extent of high molecular weight binding decreases with time of

dry seed storage and loss of viability, whilst the appearance of low molecular weight fragment binding rises from zero. In those seed embryos that are losing viability as the result of accelerated ageing however (8 % G) there is little accumulation of a low molecular telomere distribution indicating that the pathway to cell death is not identical to that of the dry stored material.

Discussion. The results of Bucholc and Buchowicz [13] in showing loss of telomeres from chromosomal DNA and the accumulation in low molecular ex-chromosomal fragments in stored wheat was confirmed for the ageing embryos of stored rye [17].

So also is the disappearance of the small telomeric DNA early in germination and restoration of the chromosomal telomeric DNA at high molecular weight.

Using three convenient DNA extraction kits it was found that a progressive loss of telomeric sequences occurs from high molecular weight DNA with an increasing appearance at low molecular weight as the periods of storage in the dry state were extended in time to provide 98, 32 and 0 % seed germination loss. Telomere distribution would appear to follow the general pattern of DNA random fragmentation that occurs in the embryos of seeds stored in the dry state but there are also indications of an overall telomere loss from DNA as a consequence of storage (Fig. 2).

Reviewing the implications of our results very carefully we believe that there is considerable potential in the use of telomere sequences to mark embryo ageing of seeds held in Seed Banks.

Firstly, the telomere sequence itself is common to *almost* all seeds so could be a standard probe in all but a few species [18—20].

Secondly, it would appear that even if the telomere sequences do not show a high preferential loss in stored seeds of every species, loss will follow the pattern of the overall random DNA fragmentation that is common to all seeds held dry that have so far been examined [13, 17].

Thirdly, given good seed and good extraction kit procedures, no hybridization at low molecular weight should be found in freshly banked seed - in fact, that seed should probably not be accepted at all for banking.

Fourthly, the probe will recognize not only terminal telomeres but also interstitial sequences, so affording the maximal recognition for any sized fragments of genomic DNA.

Fifthly, if a density centrifugation step were included to follow upon DNA isolation (instead of electrophoretic fractionation in gels) high and low molecular weight fractions would become available in solution, then a dot-blot hybridization of these fractions could give an alternative means of obtaining the ratio of distribution of telomeres to each. If time had permitted with the experiments with ageing rye, we would have been able to fit such telomere ratios to the percentage germination values of each stored seed lot as we have done for gel fractionations and to quantify with accuracy the extent of telomere sequence loss per unit DNA with storage ageing.

We are quite certain, that given further improvements, telomere values would provide satisfactory markers for alerting when the need had come to restock any banked seed lot that was reaching an unacceptable loss of viability.

РЕЗЮМЕ. Нами показано, что при продолжительном хранении семян, во время которого жизнеспособность семян снижается от 98 до 0 %, происходит прогрессивная потеря теломерных последовательностей из высокомолекулярной ДНК и их накопление в низкомолекулярной фракции. Распределение теломер по ДНК в целом соответствует стандартным профилям деградации ДНК у семян, хранящихся в сухом виде; при этом также наблюдается общая потеря теломер из ДНК как следствие такого хранения. Существует необходимость в подборе подходящего маркера «качества семян», который можно легко отслеживать в течение хранения. Анализируя возможность применения наших результатов, мы убеждены, что есть значительный потенциал для использования теломерных последовательностей как маркера старения семян, хранящихся в Банках семян.

РЕЗЮМЕ. Нами встановлено, що при тривалому зберіганні насіння, під час якого життєздатність його зменшується з 98 до 0 %, відбувається прогресивна втрата теломерних послідовностей із високомолекулярної ДНК та їх накопичення в низькомолекулярній фракції. Розподіл теломер по ДНК в цілому збігається зі стандартними профілями деградації ДНК у насіння, що зберігається в сухому вигляді; при цьому як наслідок такого зберігання спостерігається також загальна втрата теломер із ДНК. Існує необхідність в добиранні зручного маркера «якості насіння», який можна легко контролювати під час зберігання. Аналізуючи можливість використання наших резуль-

татів, ми впевнились, що існує значний потенціал для використання теломерних послідовностей як маркера старіння насіння, що зберігається в Банках насіння.

REFERENCES

1. *Osborne D., Boubriak I., Leprince O.* Chapter 12: Rehydration of dried systems: membranes and the nuclear genome // Desiccation and Plant Survival / Eds M. Black and H. Prichard. — Cambridge : CABI Publ., 2002. — P. 343—364.
2. *Berjak P.* Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms // Seed Sci. Res. — 2006. — **16**, № 1. — P. 1—15.
3. *Stupnikova I., Benamar A., Tolleter D., Grelet J., Borovskii G., Dorne A.J., Macherel D.* Pea seed mitochondria are endowed with a remarkable tolerance to extreme physiological temperatures // Plant Physiol. — 2006. — **14**, № 1. — P. 326—335.
4. *McClintock B.* The stability of broken ends of chromosomes in Zea mays // Genetics. — 1941. — **26**, № 3. — P. 234—282.
5. *Kilian A., Stiff C., Kleinhofs A.* Barley telomeres shorten during differentiation but grow in tissue culture // Proc. Nat. Acad. Sci. USA. — 1995. — **92**. — P. 9555—9559
6. *Sharikov E.V., Surovtseva Y.V., Osbun N., Shippen D.E.* The Arabidopsis Pot1 and Pot2 proteins function in telomere length homeostasis and chromosome end protection // Mol. and Cell. Biol. — 2005. — **25**, № 17. — P. 7725—7733.
7. *Fajkus J., Sykorova E., Leitch A.R.* Telomeres in evolution and evolution of telomeres // Chromosome Res. — 2005. — **13**, № 5. — P. 469—479.
8. *Zhang L.L., Tamura K., Shin-ya K., Takahashi H.* The telomerase inhibitor telomestatin induces telomere shortening and cell death in Arabidopsis // Biochim. Biophys. Acta — Mol. Cell Res. — 2006. — **1763**, № 1. — P. 39—44.
9. *Osborne D.J. Sharon R., Benishai R.* Studies of DNA integrity and DNA repair in germinating embryos of rye (Secale cereale) // Israel J. Bot. — 1981. — **29**, № 1/4. — P. 259—272.
10. *Vazquez-Ramos J.M., Osborne D.J.* Analysis of DNA synthesized during early germination of rye embryosusing BND-cellulose chromatography // Mutat. Res. — 1986. — **166**, № 1. — P. 39—47.
11. *Marciniak B., Bucholc M., Buchowitz J.* Early DNA synthesis during the germination of wheat embryos // Phytochemistry. — 1987. — **26**, № 2. — P. 331—334.
12. *Bucholc M., Buchowitz J.* Replicative, repair, and a 3rd type of nuclear DNA synthesis in germinating wheat embryos // Acta Physiol. Plant. — 1989. — **11**, № 1. — P. 13—18.
13. *Bucholc M., Buchowicz J.* Synthesis of extrachromosomal DNA and telomere-related sequences in germinating wheat embryos // Seed Sci. Res. — 1992. — **2**, № 1. — P. 141—144.
14. *Richards E.J., Ausubel F.M.* Isolation of a higher eukaryotic telomere from Arabidosis thaliana // Cell. — 1988. — **53**, № 1. — P. 127—136.
15. *Fajkus J., Sykorova E., Leitch A.R.* Techniques in plant telomere biology // Biotechniques — 2005. — **38**, № 2. — P. 233—243.
16. *Boubriak I., Dini M., Berjak P., Osborne D.J.* Desiccation and survival in the recalcitrant seeds of Avicennia marina: DNA replication, DNA repair and protein synthesis // Seed Sci. Res. — 2000. — **10**, № 3. — P. 307—315.
17. *Бубряк І.І., Осборн Д., Гродзінський Д.М.* Деградація теломер при зберіганні насіння // Доп. НАН України. — 2002. — **8**. — С. 158—162.
18. *Osborne D.J., Boubriak I.* Telomeres and their relevance to the life and death of seeds // Crit. Rev. Plant Sci. —2002. — **21**, № 2. — P. 127—141.
19. *McKnight T.D., Riha K., Shippen D.E.* Telomeres, telomerase and stability of the plant genome // Plant Mol. Biol. — 2002. — **48**, № 4. — P. 331—337.
20. *McKnight T.D., Shippen D.E.* Plant telomere biology // Plant Cell. — 2004. — **16**, № 11. — P. 2910—2922.

Received 25.05.06