Desiccation and survival in the recalcitrant seeds of *Avicennia marina*: DNA replication, DNA repair and protein synthesis

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Abstract

An autoradiographic study was made of leucine and thymidine incorporation into the meristematic root primordia and hypocotyl tips of seeds of the recalcitrant mangrove species, *Avicennia marina*. The investigations show that although there is a temporary reduction of protein synthesis at shedding, root primordia and surrounding hypocotyl cells of the axis never wholly cease incorporation of [^{3}H]leucine and regain pre-shedding levels of activity within a day. Precursor studies using methyl-[^{3}H]thymidine show that, at shedding, there is a temporary cessation of incorporation into root meristem nuclei that lasts no longer than 48 h and, within a day, pre-shedding levels are regained in the meristem nuclei. Analysis of DNA fragmentation patterns in root tips at the time of shedding, and their ability to repair radiation-induced DNA damage, indicate that DNA repair processes are markedly compromised in these cells if water loss reaches 22%. Protein synthesis and DNA replication are reduced by more than half by a water loss of 18% and 16%, respectively. DNA replication does not fully recover on rehydration after only 8% water loss. DNA fragmentation to nucleosomes indicates a programme of cell death at a water loss of 10%. We suggest that the feature of continuous protein synthesis activity with only a temporary interruption in active cell cycling in *A. marina* root primordia helps to explain both the rapidity in seedling establishment and the extreme vulnerability to desiccation.

Keywords: Autoradiography, *Avicennia marina*, cell cycle, desiccation-sensitivity/tolerance, DNA repair, hypocotyl, root tips, mangrove, nucleosome, recalcitrant seed

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Introduction

During maturation drying of desiccation-tolerant (orthodox) seeds of most species, the embryos progressively lose some 80% of their water content whilst still on the mother plant, and are generally tolerant of a further water loss, often to 5% or less of the fully hydrated state, without impairment of viability (Roberts, 1972). In contrast, seeds of many tropical and sub-tropical species do not acquire desiccation tolerance at the time of shedding, and, like those of the mangrove, *Avicennia marina* (which are at 60–70% water content [wmb] at this stage), will die if, on dehydration, the level falls below about 50% (Berjak *et al*., 1984, 1989). The absence or inadequate expression of one or more of a relatively extensive suite of mechanisms present in orthodox seeds is held to underlie the desiccation sensitivity of recalcitrant types (Pammenter and Berjak, 1999). These authors also consider the continuous state of metabolic activity as a factor that characterises the desiccation sensitivity of most vegetative tissues and propagules.

In an early study of the biochemical changes associated with maturation drying in seeds of *Vicia faba*, Brunori (1967) showed that most cells are arrested in G_1, and DNA replication is one of the first events to be suppressed as the cells of the embryo lose water on the mother plant. This is followed by cessation of RNA transcription and finally of all protein synthesis.

On the subsequent addition of water, transcription and translation are restored within a matter of minutes, as shown in rye, *Secale cereale* (Sen *et al*., 1975). However, DNA replication is always a late event, not occurring until several hours after the start of imbibition, even in excised and isolated embryos. Until DNA synthesis (as part of the first round of S-

https://doi.org/10.1017/S0960258500000350 Published online by Cambridge University Press

phase replication) is resumed, embryos of orthodox seeds remain tolerant to desiccation and can be dried to less than 10% moisture and rehydrated more than once, without loss of viability. Indeed, certain desert seeds can hydrate and dehydrate as many as 90 times without detriment (Gutterman, 1993). However, the state of DNA replication is unknown. In the root meristems of rye, as soon as replication to 4C values occurs and cells enter G_2M, they again assume the desiccation sensitivity typical of vegetative tissue, and dehydration by as little as 20–30% then leads to cell death (Sen and Osborne, 1974).

On imbibition of orthodox seeds, cell division in the root meristem, followed by root extension, is an accepted marker of germination. We have questioned, therefore, whether desiccation sensitivity in the embryos of *Avicennia marina* can be linked to a continuous operation of the cell cycle, with replication of DNA that continues unabated throughout embryo maturation and germination, and have asked whether DNA damage occurs during dehydration and if it can afterwards be repaired.

To this end, we have followed by autoradiography the incorporation of methyl-[^{3}H]thymidine into DNA in the multiple root primordia and hypocotyl tip cells of *Avicennia marina* embryos at late maturation and at fruit shedding, and have assessed the response to dehydration of these cells by their ability to incorporate [^{3}H]leucine into protein following an imposed water loss. Additionally we have assessed the extent of DNA damage and the ability to repair DNA following different levels of dehydration.

Materials and methods

Plant material and preparation of embryos

Seeds (fruits) of *Avicennia marina* were picked directly from the trees at developmental stages previously classed from tagging experiments as days after fruit set (DAFS) (Farrant *et al*., 1992). Samples were harvested at 50, 65, 70 and 80 DAFS and, additionally, newly shed (within 12 h) 80 DAFS seeds were collected from the ground. All samples were immediately enclosed in polythene bags to prevent dehydration and brought to the laboratory. Sub-samples of the 80 DAFS shed seeds were placed on a plastic mesh above water in closed containers for 2 or 8 days.

Root primordia were excised as a group from the hypocotyl tips (Fig. 1) of the embryonic axes of seeds at each developmental stage. Each hypocotyl tip consisted of a cluster of nine to ten primordia, each of which is essentially a root meristem enclosed within enveloping parenchymatous tissue (Berjak *et al*., 1984). For the incorporation studies, each hypocotyl tip was cut into segments (approx. 2 mm^3), so as to include a single root meristem in each piece.

Incorporation procedures

Methyl-[^{3}H]thymidine

For the incorporation of methyl-[^{3}H]thymidine, 16–20 tissue pieces, each containing a single meristem, from four or five hypocotyls, were placed on a $\frac{1}{4}$ section of a GF/F 25 mm glass fibre filter disc with 750 µl of an aqueous solution containing 40 µCi of methyl-

Figure 1. *Avicennia marina*: (a) fruit; (b) embryonic axes (cotyledons removed); (c) hypocotyl protruding from between the cotyledon bases. The distal part of the hypocotyl shows the protrusions caused by the underlying multiple meristematic root primordia used for incorporation studies and DNA analyses. (a) and (b) Grid = 30 × 30 mm. (c) Scale bar = 4 mm.

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[^{3}H]thymidine (specific activity 2.0 TBq mmol^{-1}, 74 Ci mmol^{-1}, Amersham International, UK). Incubation was at room temperature (24°C) for 1.5 h. After incubation in the precursor solution, the samples were rapidly rinsed with 2 × 20 ml changes of distilled water and then in 20 ml of an 150-fold excess of non-radioactive thymidine. The samples were then transferred to Carnoy's fixative (ethanol: chloroform:glacial acetic acid, 6:3:1) overnight.

L-[4,5-^{3}H]leucine

For the incorporation of L-[4,5^{3}H]leucine, 16–20 tissue pieces, containing one root meristem each, were prepared as for the thymidine incorporation. The meristems (four from each hypocotyl, four or five hypocotyls) were placed on GF/F filter discs with 800 µl of an aqueous solution containing 40 µCi. L-[4,5-^{3}H]leucine (specific activity 2.33 TBq mmol^{-1}, 63 Ci mmol^{-1}, Amersham International, UK). Incubation was at 24°C for 60 min. After incubation the samples were rinsed rapidly with 2 × 20 ml changes of distilled water, followed by one rinse in 20 ml of an aqueous solution of non-radioactive L-leucine and then fixed in Carnoy's solution overnight.

Preparation of slides and autoradiographic analysis

After fixation, the tissue pieces were dehydrated through an alcohol series to 100% ethanol, embedded in wax and the tissue blocks sectioned serially at 2 or 6 µm. The ribbons were transferred to pre-coated slides (Blue Star, Chance Propper Ltd, Warley, UK), dewaxed in chloroform and chloroform/ethanol, finally rehydrated through a decreasing ethanol series to water and air dried before being coated directly with emulsion (Hypercoat nuclear emulsion, LM-1, Amersham International, UK). Coated slides were held over desiccant in the dark at 4°C for 2 weeks before developing.

Slides were viewed and grains counted using a Zeiss Ultraphot III RS photomicroscope. For viewing with the oil immersion objective, sections were mounted in Euparal (Raymond A. Lamb, Wembley, UK) and covered with thin coverglasses. For the thymidine incorporations, grains over nuclei in 10–18 median sections from 12–15 root meristems from each of four or five hypocotyl tips were counted for each treatment. Not less than 200 cells of each root tip meristematic region were counted as were a further 200 or more enveloping cells of the adjacent hypocotyl parenchyma.

For the time course study of DNA replication prior to, and immediately after, fruit shedding, nuclear grain counting was determined on sections cut at 2 µm and viewed on a video screen linked to a Nikon Eclipse E400 microscope. The results are presented as (a) the percentage of meristem cells viewed that contained nucleus-located grains above background; (b) the mean number of grains per labelled nucleus per labelled meristem. Meristems with labelled nuclei were considered to be undergoing an active DNA replicative synthesis.

Similar numbers of cells were assessed for the leucine incorporation experiments. Only in those desiccation treatments that caused less than 35% water loss were grains above background consistently recorded in the cells. Lack of incorporation was taken to indicate desiccation-induced cell death.

Desiccation and rehydration of embryonic axes

Axes were removed from 80 DAFS fruits and 20 hypocotyl tips (approximately 4 mm long) were excised, weighed and then dried at room temperature in a continuous flow of air that was first passed over activated silica gel. Each hypocotyl tip was re-weighed to determine the proportion of water lost. Tip segments with root primordia were then immediately excised and incubated in [^{3}H]thymidine or [^{3}H]leucine on GF/F filter discs as described for the fresh samples.

For these experiments the excised and dehydrated hypocotyl tips were held on fine wire mesh over (but not in) H_2O in a closed container for 24 h at 24°C. Segments containing root primordia were then excised and incubated with precursor as before.

DNA extraction and electrophoresis

For DNA isolation a commercial genomic DNA kit (InViSorb™, Bioline, UK) was used. The DNA content for each extracted sample was quantified using PicoGreen™ (Molecular Probes Europe, the Netherlands) and then adjusted so that an equal double-stranded DNA concentration was applied to each lane of the agarose gel for electrophoretic fractionation. Each DNA sample was run on neutral 0.8% agarose gel at constant current (50 mA) for estimation of the overall double-stranded DNA fragmentation pattern. DNA profiles are shown as scans (Ultrascan XL Laser Densitometer, LKB, Sweden) of the photographic negatives (Polaroid 665 film) from the original ethidium bromide stained gels. Each DNA sample represents an extraction of an original 65 mg of hypocotyl tips before dehydration.

Irradiation treatments

In order to introduce a standard damage to the DNA after different levels of dehydration, excised hypocotyl tips were γ-irradiated with 750 Gy (dose rate 0.14 Gy s^{-1}) from a ^{137}Cs-source of a Gravitron RX 30/55 Irradiator (Gravatom, UK). Samples were then either

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extracted directly for determination of the extent of DNA strand breaks or imbibed immediately in water for 2 h to permit DNA repair before extraction.

Nucleosome assays

During the programmed death of cells in mammalian tissues (Greenberg, 1996) and in the embryos of rye (Boubriak and Osborne, 2000), fragmentation of DNA to nucleosomes and nucleosome multimers is indicative of the end point of an organised and non-reversible apoptotic process. Nucleosome contents were measured using a Nucleosome ELISA kit (Oncogene Research Products, Calbiochem, USA). For this, equal fresh weights (65 mg) of hypocotyl tips containing eight to ten root meristems were ground in dry ice and lysed in 750 µl of the buffer supplied. Samples were diluted 1:8 for immunological analysis. Data are shown as units of nucleosomes per 1 ml of original lysis solution, calculated from kit standards that represent nucleosomes per initial fresh weight of tissue (before dehydration). [One nucleosome unit per ml = amount of nucleosomes produced from 444 UV-treated Daudi cells per ml; assay detection limit is equivalent to 67 irradiated Daudi cells (Nucleosome ELISA kit information leaflet)]. Assays are the mean of four replicate samples.

Results

Incorporation of methyl-[^{3}H]thymidine by root meristems

Only those cells in the immediate meristematic region were assessed; occasionally, cells proximal to the meristematic region showed grains above background, but these never reached levels observed in the meristems. Grain counts of nuclei of root meristems of the 50 DAFS embryos showed that almost all were incorporating [^{3}H]thymidine into DNA at a relatively high rate, and were, therefore, considered, to be actively replicating (Table 1). Similar results were apparent for the 65–70 DAFS embryos, except that the number of nuclei with grains above background in 70% DAFS material had fallen to 13%. Root meristems are not all at the same stage of development, with one or two always appearing in advance of the other nine to ten primordia in a single hypocotyl tip. However, it was clear that by 80 DAFS, in those fruits that were naturally shed, DNA replicative synthesis as measured over a 1.5 h incorporation period had essentially ceased in almost all the root meristems analysed. At this stage DNA labelling was also almost zero in the surrounding hypocotyl tissue (Fig. 2).

The period of arrested synthesis in the root meristems was temporary only. Within 48 h of collection of shed 80 DAFS fruits, all had resumed nuclear DNA synthesis, again showing incorporation of [^{3}H]thymidine at a high rate and indicating resumption of S-phase replicative activity. The number of replicating nuclei then rose the longer the mature fruits were maintained in hydrated storage, with many grains per nucleus occurring for most of the nuclei labelled by 8 days (Table 1).

The effect of different levels of dehydration on incorporation was studied in 80 DAFS fruits picked from the trees since these were still replicating at a low level. Grain counting of the thicker (6 µm) sections showed that even at 19% water loss the level of incorporation was reduced in all root meristem cells (Table 2). To determine if the meristems of hypocotyl tips recovered from the water stress, hypocotyl tips were dehydrated and then rehydrated again by holding them over water for 24 h in a humid atmosphere at 24°C before transferring them to an incorporation medium and processing for autoradiography. Grain counting of these samples showed that no recovery of replication occurred in the rehydrated root meristem cells (Table 3) and as little as 10% dehydration led to a sustained impairment of replicative activity.

Table 1. Replicative activity in cells of excised root meristems from hypocotyl tips of embryos from fruits of increasing developmental stage. Incorporation of [^{3}H]thymidine into nuclear DNA.

Developmental stage (days after fruit set)	Meristem cells with grained nuclei* (%)	Number of grains per labelled nucleus
50 DAF	95	7.8 ± 0.6
65 DAF	32	5.3 ± 0.8
70 DAF	13	4.7 ± 0.4
80 DAF	4	3.5 ± 0.7
80 DAF, shed	0	1.7 ± 0.2
80 DAF, shed + 2 days over H_2O	10	7.7 ± 1.0
80 DAF, shed + 8 days over H_2O	20	6.5 ± 0.7

*Mean values for 35–40 root meristems. Section thickness 2 µm.

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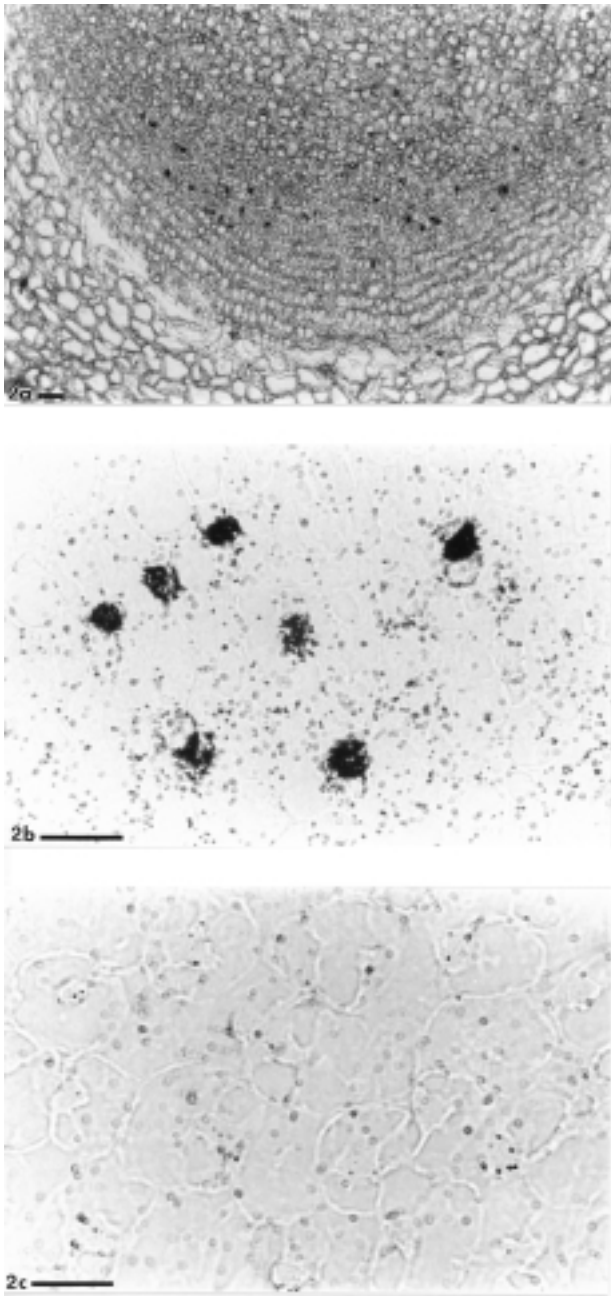

Figure 2. Autoradiographs showing incorporation of methyl-[^{3}H]thymidine into DNA in root meristems of hypocotyl tips from embryos of 80 DAFS fruit. (a) and (b) Fruit hand picked. (c) Fruit shed. Scale bars = 10 µm.

Incorporation of [^{3}H]leucine by hypocotyl tips

Incorporation was evident in all root meristems and hypocotyl cells throughout development, including shedding (Fig. 3) and post-shedding. Incubation of excised root meristems in [^{3}H]leucine immediately following the dehydration treatments of 80 DAFS naturally-shed seeds showed that incorporation was more than halved by a water loss of 18% and was essentially zero after 35% water loss (Table 4). By 41% fresh weight loss, no graining above background occurred in any cell. This complete failure of incorporation was taken to indicate cell death.

Table 2. Effect of dehydration on subsequent incorporation of [^{3}H]thymidine for 1.5 h by excised root meristem cells of hypocotyl tips from embryos of freshly picked 80 DAFS fruits.

	Water loss (%)			
	0	11	19	25
Labelled meristems (%)	100	100	84	25
Number of nuclei labelled per meristem*	32 ± 5	37 ± 7	9 ± 4	12 ± 3

*Mean value for all labelled meristems (n=400 cells). Section thickness 6 µm.

Table 3. Dehydration of hypocotyl tips from embryos of freshly picked 80 DAFS fruits followed by their rehydration over H_2O for 24 h and subsequent incorporation of [^{3}H]thymidine by excised root meristems for 1.5 h.

	Water loss (%)			
	0	10	16	22
Labelled meristems (%)	100	86	77	20
Number of nuclei labelled per meristem*	22 ± 6	10 ± 4	6 ± 3	7 ± 1

*Mean value for all labelled meristems (n=400 cells). Section thickness 6 µm.

Table 4. Dehydration and subsequent incorporation of [^{3}H]leucine for 1.5 h by (a) excised root meristem cells and (b) surrounding parenchyma cells of hypocotyl tips from embryos of freshly shed 80 DAFS fruits.

	Water loss (%)			
	0	18	35	41
(a) Root meristem				
% Cells labelled	97[a]	39[b]	2[c]	0[d]
Grains per cell*	22.9 ± 1.9	4.3 ± 0.3	1.3 ± 0.2	0
(b) Hypocotyl parenchyma				
% Cells labelled	100[e]	37[f]	15[g]	0[h]
Grains per cell*	5.6 ± 1.0	3.1 ± 0.3	°3.4 ± 0.3	0

*Mean value for all labelled cells.
[a]n>400 cells; [b,c,d]n>600 cells; [e,f,g,h]n>800 cells.

DNA fragmentation and DNA repair in hypocotyl tips

Following dehydration of hypocotyl tips to 22 and 42% water loss, isolation of the DNA followed by electrophoretic fractionation showed considerable

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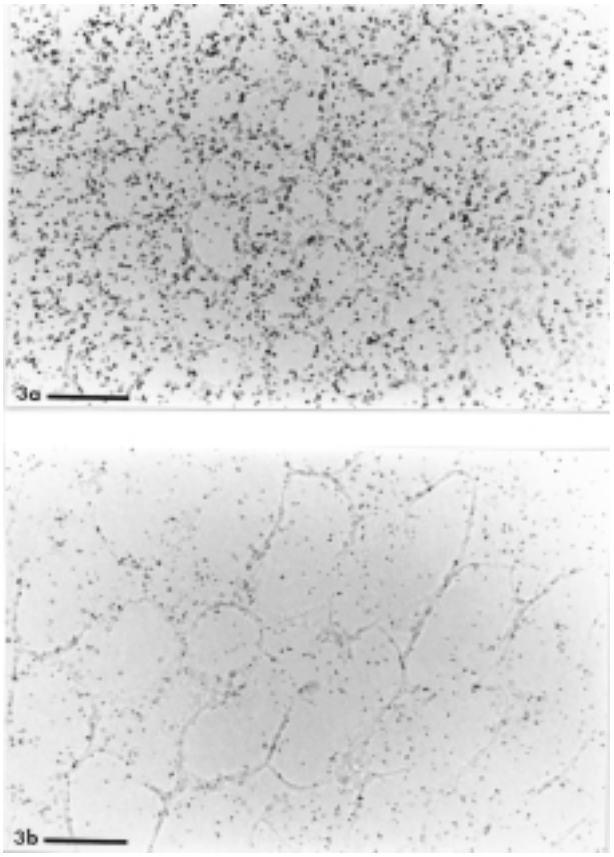

Figure 3. Autoradiographs illustrating incorporation of [^{3}H]leucine into hypocotyl tips of embryos of 80 DAFS shed fruit: (a) root meristem region; (b) adjacent hypocotyl parenchyma. Scale bars = 10 µm.

DNA degradation and accumulation of low molecular weight fragments, compared with tips extracted immediately on excision from 80 DAFS fruit (results not shown).

The question of whether repair of fragmented DNA still operates following dehydration was addressed by first drying the excised hypocotyl tips in an air stream (as before) to 22 and 46% water loss, then introducing a standard DNA damage by immediately irradiating the tips from a γ-source (750 Gy) and at once imbibing them for 2 h in H_2O to permit repair. From the scans of gels following the extraction and electrophoretic fractionation of the DNA, it can be seen that whereas the fresh non-dehydrated tips can restore the low molecular weight DNA to an overall higher value, no repair occurs in the tips that were dehydrated by 22%, and DNA fragmentation is accentuated further when the 46% dehydrated tips are imbibed. From these results we conclude that DNA repair processes are severely curtailed or inactivated by a water loss of 22% or more (Fig. 4).

Nucleosome determination of cell death in hypocotyl tips

Isolation of DNA from dehydrated hypocotyl tips and determination of histone H3 end groups by ELISA-based immunodetection showed no nucleosomes present in hypocotyl tips freshly excised from 80 DAFS fruits, but an increased number as the extent of dehydration increased (Table 5). We conclude that subjecting hypocotyl tips to as little as 10% water loss results in detectable numbers of dying cells.

Discussion

Whereas the embryos of mature *A. marina* fruits held for several days in a humid environment are known not to change in *overall* water content (Pammenter *et al.*, 1984, 1997), direct measurement of the fresh weight of hypocotyl tips excised from freshly harvested fruits at 70 and 80 DAFS and 80 DAFS held for 24 h at high humidity, were as follows: 1032 ± 15 mg; 1203 ± 49 mg and 1431 ± 72 mg, respectively. Clearly, even though there is no net change in fresh weight of the embryo as a whole, there must be a redistribution of water to the region of the multiple root primordia at the hypocotyl tip. This has also been indicated from ultrastructural studies in which vacuolation of root primordial cells occurs in wet-stored, recalcitrant seeds (Pammenter *et al.*, 1994; Smith and Berjak, 1995) and in the differential moisture distribution found in embryos of *Araucaria hunsteinii* before and during drying (Pritchard *et al.*, 1995).

Autoradiographic evidence from the incorporation of [methyl-^{3}H]thymidine into nuclear DNA in root meristems of *A. marina* embryos has shown that S-phase DNA synthesis continues almost to the time of fruit shedding (80 DAFS). It ceases during shedding but is resumed again actively in the root meristems within 48 h of fruits being shed (Table 1). This resumption of the level of incorporation in the number of cells showing DNA replication takes place whether or not the fruits (hand-picked or shed) have access to extraneous water. Provided the 80-DAFS fruit are protected from desiccation, the root meristems in the hypocotyl tips remain fully capable of regaining DNA replicative synthesis. This suggests that DNA replication is not completely arrested, as in the maturation of orthodox seeds, but instead, it is the duration of the cell cycle interphase that is temporarily extended over the time of shedding. We have not yet ascertained whether an extension occurs in G_1 or G_2, or in both stages of the cycle. Since the nuclear DNA of *A. marina* axes fails to stain by conventional Fuelgen or aceto-carmine techniques, the stage at which this extended interphase occurs

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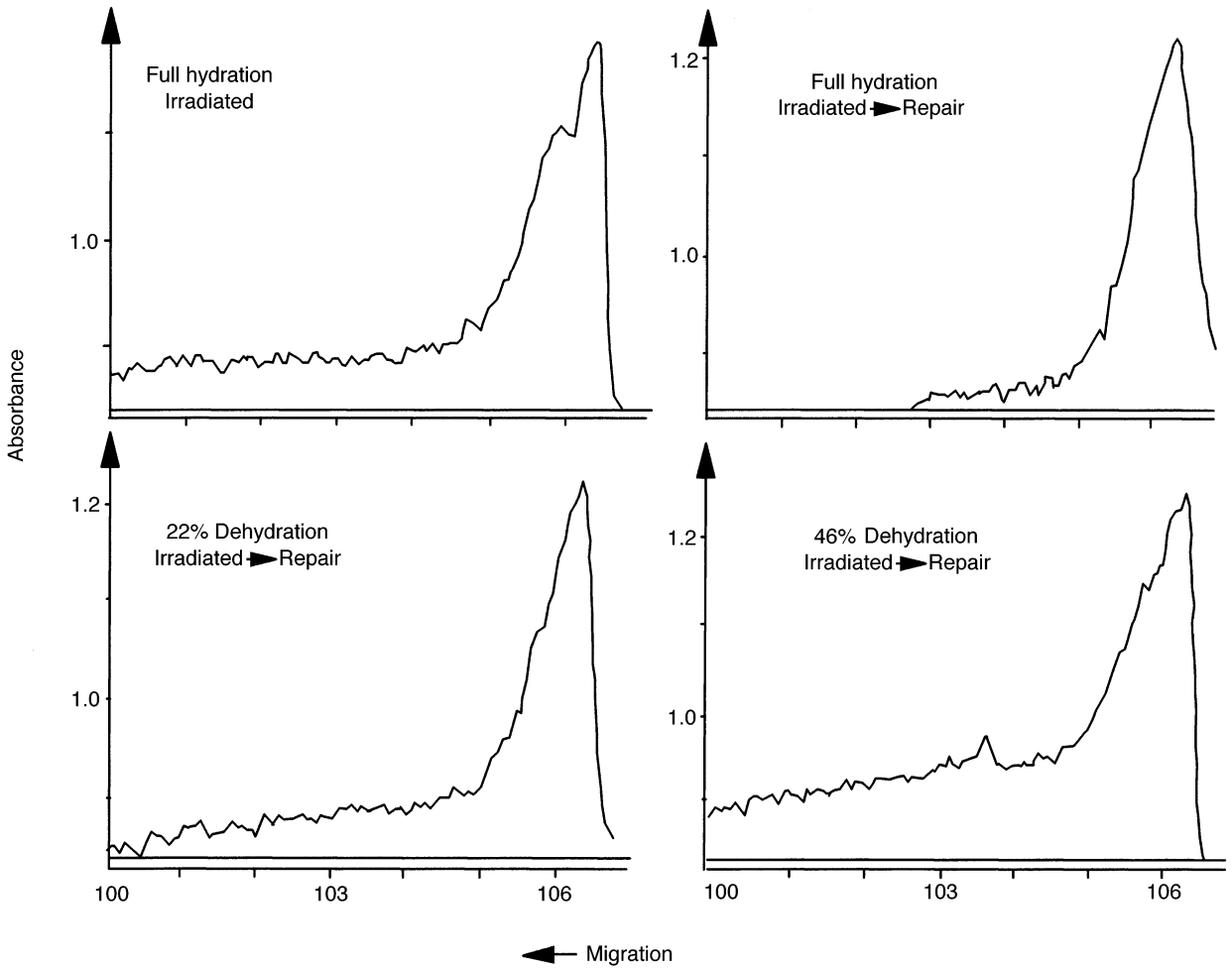

Figure 4. Scans of mobility distribution of DNA. Electrophoretic fractionations of DNA extracted from hand picked 80 DAFS hypocotyl tips. Comparison of DNA repair efficiency (during 2 h imbibition) following different levels of dehydration and subsequent exposure to a 750 Gy γ-irradiation dose.

cannot be determined by the usual visible-light scanning microdensitometry, and an alternative quantitative methodology must be used. *Avicennia marina* is an extreme example of a desiccation-sensitive (recalcitrant) seed but for the relatively desiccation-tolerant, intermediate seeds of *Azadirachta indica* (neem) hand-harvested at maturity, analysis by flow cytometry has shown that 90% of the embryo cells are arrested with 2C DNA (G_1), as in most orthodox seeds (Sacandé *et al.*, 1997).

Cells of both root meristems and hypocotyl of *A. marina* embryos remain synthetically active through the shedding period as confirmed by the [^{3}H]leucine incorporation values at 0% water loss (Table 4). Cotyledons continue to respire immediately prior to shedding (Brown *et al.*, 1969) and, in addition to protein synthesis, respiratory activity and ultra-structural organisation in mature *A. marina* embryos have also indicated ongoing metabolism but with the lowest ebb immediately before, and just after, shedding (Pammenter *et al.*, 1984; Farrant *et al.*, 1985; Berjak *et al.*, 1984, 1989; Farrant *et al.*, 1992).

Table 5. Effect of dehydration on nucleosome formation in hypocotyl tips from embryos of picked 80 DAFS fruits.

	Water loss (%)			
	0	10	20	40
Nucleosome Units ml^{-1}	0.88 ± 0.54	6.76 ± 2.14	6.26 ± 0.44	9.36 ± 0.39

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Unlike the early germination stage in embryos of orthodox desiccation-tolerant seeds (Brunori, 1967; Sargent *et al.*, 1981), the substantial incorporation of [^{3}H]leucine into cells of the root meristem and hypocotyl in embryos from newly shed *A. marina* fruit (Table 4) is greatly reduced following a 2 h drying period in which only 18% of the water content is removed. Protein synthesis in hypocotyl cells surrounding the meristems appears somewhat more resistant to dehydration than in those of the meristems themselves. It could not be determined, however, what degree of water loss had occurred in hypocotyl cells compared with those of meristems.

Isolation and electrophoretic fractionation of the DNA from freshly excised 80 DAFS hypocotyl tips and from tips that were progressively dehydrated showed increased fragmentation to low molecular weight with increasing water loss. When such tips were irradiated to induce a standard level of random single and double strand breaks in the nuclear DNA and the tips re-imbibed in water for 2 h to permit DNA repair, tips that had not been dehydrated at all were competent to repair (Fig. 5), whereas tips that had lost 22% water did not repair and those tips that had suffered 46% water loss showed further DNA degradation. These gel scans show the extent to which DNA repair is limited in the hypocotyl tip region of the embryo by removal of as little as 22% of the water.

The accumulation of nucleosomes is a more sensitive measure of specific DNA fragmentation linked to cell disorganisation. With increasing dehydration this measure of irreversible nuclear damage indicates that apoptotic-like symptoms of cell death are initiated by water losses of as little as 10%.

We know already that desiccation tolerance in certain prokaryotic and eukaryotic cells is linked not only to the cycle stage of replication arrest, but also to an altered conformational state of the DNA. Both at late-stage spore formation in *Bacillus subtilis* (Setlow, 1992) and at maturation or during early imbibition of embryos of the orthodox seed, *Secale cereale* (Osborne and Boubriak, 1994), the DNA does not lose structural integrity as a result of cell dehydration. We suggest that in *A. marina*, the short and temporary replication arrest at the time of shedding is, in reality, an extended interphase and is not followed by the necessary suppression of cell cycling to initiate the conformation changes that render DNA stable to water loss in orthodox seeds. We see the very short period of replication arrest (*c.* 24–48 h), coupled with the maintenance of a high level of cytoplasmic hydration, as failing to provide the conditions necessary to permit structural reorganisation of chromatin to a stress-tolerant form. In *B. subtilis*, a specific DNA binding protein synthesised during dehydration is instrumental in retaining an A-form DNA (rather than the usual B-form) in the dry spore (Setlow, 1992). However, in a recalcitrant seed like *A. marina*, sufficient dehydration to cause the acquisition of any similar conformational change may never normally take place and additionally, the recalcitrant seed may not possess the genetic information to synthesise an appropriate DNA-binding protein.

On the basis of our results, we suggest that failure to suppress the cell cycle fully, the probable lack of a stabilized dehydration conformation of DNA and a labile DNA repair are three factors contributing to the lack of desiccation tolerance in embryos of *Avicennia marina* and, perhaps, in those of other highly desiccation intolerant species also.

Acknowledgements

We acknowledge financial support from the University of Natal, Durban and the National Research Foundation (formerly the FRD) , Pretoria, South Africa and the award to D.J. Osborne of a Leverhulme Fellowship.

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Received 10 January 2000
accepted after revision 12 April 2000