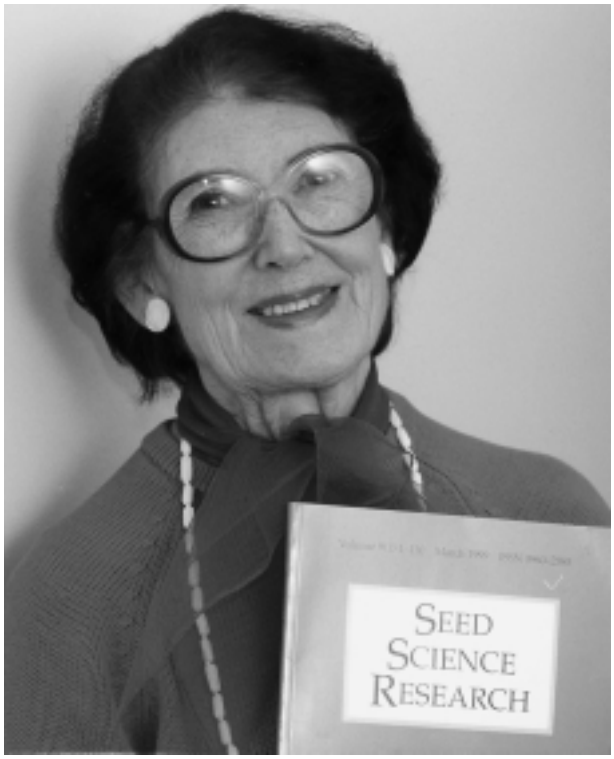
PERSONAL VIEW

An Addiction to Seeds

Daphne J. Osborne*

Oxford Research Unit, The Open University, Foxcombe Hall, Boars Hill, Oxford OX1 5HR UK



Daphne J. Osborne

When I was asked to write my own retrospective view of research on seeds and their germination, and after I had said I would, I sought to pinpoint the circumstance that actually directed my interests to what I now see as one of the most intriguing aspects of plant development. But first, perhaps, I should say how chance made me a scientist at all.

At my old school, in Kent, I was good at history. So, it was decided that I should be an historian. However, all my family were keen students of living things and so was I. My father was a research chemist and a Government adviser to the then British India. When he came home on leave and discovered the school's decision, he said 'you, like me, do history for pleasure but science is for a career'. So I changed from history to science! It was a near thing, and I shall always be so grateful to him. I was then at the Perse Girls School in Cambridge, and my first biology lesson was the earthworm. I listened to the bristles as it crawled over the edge of a vertical piece of paper. That was fine. Now you will dissect your earthworm! No indeed, I would not! Miss Hemmings, the biology mistress was quite wonderful, and it was she, in fact, who determined my early career in biology. For every zoology class I used to go to the Cambridge Botanic Garden just behind the school and for every Botany class I was present. When she told us of plant hormones and the research of Frits Went, I knew then that was what I wanted to do, too.

I did my first degree in Botany and Chemistry and an M.Sc. on the Krebs cycle in fungi at King's College in London with that splendid eccentric Thomas Bennet-Clark. This was followed by a Ph.D. with R.L. Wain at Wye College where I synthesized many potential growth regulators and studied their effects upon plant development and cell growth. Then the real excitement started! I won a fellowship to the California Institute of Technology to work with Frits Went in his famous Phytotron. He was a quite exceptional man with more ideas per minute than anyone else I can remember. Of course, many of the ideas were far-fetched, but many turned out to be right. For a while I stayed with Linus Pauling and his family in their lovely house in the canyon above Pasadena and listened, fascinated, at the seminars he ran in their beautiful swimming pool. All the senior scientists at Caltech were already famous, and most of the graduate students I knew then became famous. At that stage, Fred Addicott would have given me a fellowship to work with him on abscission at UCLA, but by then I was engaged to an X-ray crystallographer in Oxford and Geoffrey Blackman, the UK discoverer with Templeton of the uses of synthetic plant hormones as selective herbicides, had offered me a post there in his Agricultural Research Council Unit in the Department of Agricultural Science. So, I went to Oxford and stayed there until

*Correspondence
Fax: 011–44 (1865) 326322

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Geoffrey Blackman retired in 1969. His department was a Mecca for plant physiologists from all over the world. He, too, was a great eccentric. I remember his wife (sculptress and potter, also famous) buying him a bright blue Aston Martin for his birthday one year and how he was known in Oxford as Flash Blackman for his smart suits and the two black servants from St. Helena who cared for them in their large house on Boars Hill. They are now all buried together in the same grave in the nearby Sunningwell churchyard. Most of the time in his department, I was concerned with hormonal controls of cell growth, cell senescence and abscission. But even before Geoffrey Blackman retired, I had begun to view seeds with more than a little interest.

The conversion of the delicate growing embryo in the ovule into a desiccation tolerant, partly differentiated plantlet within a dry seed, which can then survive alive in the dry state, perhaps for centuries, is an extraordinary event. Where animals succeed in their environment by positive analysis and rapid decision making, plants have succeeded *par excellence* by adaptation and selection on a strictly biochemical basis and in the absence of any known conscious action. In this sense, the almost universal formation of the dry seed and its revival on the addition of water is nothing short of an evolutionary miracle.

Oxford days

My entry into the world of seed research came about in a much more prosaic way than a desire to understand the miracle! I wanted to use seeds to answer questions that I was not able to answer from the study of pea stems, *Avena* coleoptiles or the senescence of leafy tissues. I wanted to explore molecular mechanisms of how hormones controlled cell growth, cell differentiation and in particular, cell ageing.

At about the same time as I became disenchanted with the way we were all tackling these problems, James Bonner came to Oxford as the 1964 Eastman Visiting Professor. At that time, he believed that hormones must act by binding directly to some part of the nuclear chromatin, thereby changing gene expression by modifying RNA transcription. Dorothy Tuan, in his laboratory at Caltech had just isolated chromatin from the tiny buds on dormant potatoes and from buds in which dormancy had just been broken by treatment of the tubers with ethylene chlorohydrin. In the presence of added polymerase from *E. coli* and the four nucleotide triphosphates (ATP labelled with ^{14}C), she found much higher transcription levels in the samples containing chromatin from awakening buds. This implied that the chromatin of dormant buds was therefore repressed and that of sprouting buds had been de-repressed by the synthetic hormone treatment (Tuan and Bonner, 1964). The language of that time is also of interest, because reference was always made to 'DNA-dependent RNA synthesis', as if there was some other RNA synthesis with which we could be confused.

To test the idea that a hormone might directly de-repress the chromatin, J. Bonner and I in Oxford, actually did the experiments with gibberellin and pea stem chromatin from the nuclei of dwarf peas to show whether or not a hormone could directly interact with the chromatin to increase transcription. We confirmed a difference between control chromatin and chromatin to which we added GA_3. In the States, a colleague of his (Anne Matthysse) did similar experiments, exposing nuclei of pea stems to indole-3-acetic acid. But our differences were always small and non-hormonal substances could also cause similar results.

A very nice simple idea, but it was not the answer to the question of how hormones regulated cell growth and gene expression in plants (Bonner, 1965). But I was impressed by the potato bud dormancy experiments. In a way, these buds resembled the embryos of seeds, except that they were more highly hydrated and certainly much more inconvenient to work with.

My conversion to seeds finally came from reading the work of Moscow's Alexander Spirin, from talking to Abraham Marcus from Pennsylvania and meeting David Chen in Israel. All three convinced me (as it turned out, wrongly) that the embryos of seeds do not immediately start to transcribe new messages from their nuclear genome in the early hours of germination – new protein synthesis depending, instead, upon the mRNA's stored within the dry seed on ribonucleoprotein particles or 'informosomes', and translated only when water became available. Leon Dure at the University of Georgia, was at this time embarking on his exploration into germination of cotton seeds and had come to the conclusion '*that during the initial stages of cotton-embryo germination, protein synthesis is catalyzed by both mRNA and rRNA which comes already formed in the mature seed, and that mRNA is not rapidly degraded and re-synthesized during this period*' (Dure and Waters, 1965).

Herein, I had thought, lay the opportunity to study hormone action directly upon the cell growth of a germinating embryo in the absence of any newly transcribed information. David Chen came to Oxford in 1969. Together we sought to tackle the question of how does gibberellin enhance germination rates, and we used for this isolated wheat embryos, excised from dry caryopses. Part of the Spirin, Bonner, Marcus, Dure concept was indeed correct. Embryos are rich in messages stored and subsequently

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translated as soon as the seed takes up water, but it was quite incorrect in the view that the transcription process was not also immediately activated.

The move to Cambridge

At about this time (1970) the Unit of Developmental Botany was set up in Cambridge and appointed with me were a number of other seed enthusiasts. We adopted rye (*Secale cereale*) as our model seed because its viability under normal commercial storage lasts for 3–4 years (which is also the life of a Ph.D. student).

There was another reason for choosing rye. The grains closely resemble those of wheat, and Johnston and Stern (1957) had published their mechanical method for isolating the embryos of wheat in quantity, and without harm, from the rest of the caryopsis. John Walters became an expert in converting a sack of rye to a small bottle of intact embryos that could then be stored over $CaCl_2$ at 4°C with only slow deterioration over several years.

In a very beautiful autoradiographic study with rye embryos, Swati Sen showed that silver grains, depicting new RNA molecules, could be detected over nuclei in less than 10 minutes of the addition of water. She and Peter Payne then demonstrated, by sensitive electrophoretic gel fractionation procedures, that all classes of RNAs were synthesized within 20 minutes (Sen *et al.*, 1975). The use of seeds for studying the hormonal control of cell expansion growth at germination was therefore no improvement upon the growing pea stem or the elongating coleoptile. But by then we were all captivated to discover more about why embryos take longer to germinate the longer they are stored and what were the biochemical lesions that led to eventual loss of viability.

We were fortunate that the Unit laboratories were quite close to those of the National Institute of Agricultural Botany in Cambridge, and we soon became good friends with their scientists. Donald Mackay was a special colleague, for he had carried out many seed storage trials and had stocks of rye grain that he had held under controlled environmental conditions over a number of years. He made these priceless stocks available to us most willingly. Using these, Bryan Roberts sought to discover which components of the protein synthesizing system became non-functional as embryos lost viability. It was quite clear that embryos lost the ability to synthesize protein on imbibition the older they became. By using homologous and heterologous *in vitro* protein synthesizing components extracted from fully viable, lowered viability and non-viable embryos and with polyU as a synthetic messenger and phenylalanine as the supplied amino acid, Bryan showed that some of these enzyme participants remained functional (the tRNAs and their synthetases) even after the embryo was dead. But others lost function (aminoacyl tRNA transferase enzymes binding the aminoacyl tRNAs to the aminoacyl sites on the ribosome), whilst the ribosomes themselves exhibited progressive breaks in both the 25S and 18S subunits.

The question was still being debated in the seed world whether new or stored messenger RNA was translated at germination and whether stored messages were degraded with time as the embryo aged. Together, Margaret Gordon from New Zealand and Peter Payne showed that embryos could for a while still translate old stored message coding for storage proteins as soon as cells were re-hydrated. When Marta Dobrzanska came from Warsaw, she demonstrated that stored polyA-rich RNA could be extracted and translated in the rye cell-free protein synthesizing system and that such messages were still present in just dead embryos, though at lower levels than in freshly harvested material. Messages translatable *in vitro* were therefore present in dead seed even though protein synthesis *in vivo* was no longer possible. These apparent differences in the rates at which parts of the protein synthesizing system degenerate was new to our understanding of ageing in the dry state and encouraged us to look far back into antiquity for further evidence from examples of really old seed.

Both the Archaeology Department in Cambridge and the Ashmoleum Museum in Oxford possessed a few grains of wheat taken from the tomb of Tutankhamun at Thebes and some from the grain silos of Fayum. A number of these four and six thousand year old grains were not carbonized, and these were subjected to electron microscopic examination by Neil Hallam who was with us from Australia. Although all cytoplasmic structures were wholly degenerated and only an occasional membrane could be seen, starch grains and cell walls appeared as if laid down yesterday, and most surprisingly, the nuclear chromatin showed the same ultrastructural organization as that of our modern day rye. We attempted an isolation of the DNA and RNA, but it was clear that although the chromatin image was ultrastructurally coherent, all the nucleic acids were now completely fragmented. Neil and I were keen to talk to the famous Gertrude Caton-Thompson who in the 1920s was one of Britain's formidable women explorers and archaeologists and part of Howard Carter's team that discovered and recorded the Tutankhamun tomb. Sadly, she died before we arranged to do so, and it will always be one of my regrets that we never met this splendid, intrepid lady.

I find it most interesting how the early problems in

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research that first capture our imagination recur again and again over the years that follow. My interest in ageing and cell senescence has done just that. Whereas seeds failed me as the means for seeking a primary action of hormones in cell growth, they have certainly not failed me in the search for mechanisms of cell dysfunction as embryos grow old.

We know from Fuelgen staining of nuclear DNA that live and dead embryos of rye contain the same amount of DNA per nucleus. Extraction procedures, however, showed that the proportion of high molecular weight DNA was always greatest in fully viable material, and the amounts of low molecular weight fragments always increased with ageing. Kathryn Cheah showed very elegantly how this loss of DNA integrity was linked to loss of viability in rye and how, despite the extensive fragmentation that took place progressively even after the seed was long dead, the basic nucleosome structure of the DNA in the chromatin fragments was retained. Using fixed and sectioned material and with added terminal deoxynucleotidyl transferase and radioactive dCTP, she also found that the number of 3′OH termini in the nucleus that would accept and extend a CTP polymer increased with loss of viability, showing that single strand breaks in the DNA were central to loss of genomic integrity as the rye embryos aged (Cheah and Osborne, 1978).

The stability of nucleases is well known, so it was not surprising that 7-year dead rye embryos still exhibited high RNAase and DNAase activity when tested, and that these enzymes would continue to cleave the nucleic acids in the embryo into smaller and smaller fragments as long as they retained activity. We had found no nuclease activities in our precious Tutankhamun and Thebes samples however, so we tried hard to obtain some younger 200AD material that had been stored by the besieged Zealots at Masada where the air is desert-dry. We were never able to obtain this seed, but most fortunately other very interesting samples of wheat came our way from much nearer to home.

An old school house was being demolished in Kent, and set into one of the walls was found a bottle of wheat grain grown in 1871. The thoughtful builder who discovered it sent the jar to us in the hope that the contents would prove useful. Inside the jar with the seed was an equally old letter. It said, '*Deposited in the wall of this School Sept. 18th 1872 by Peter Ashenden. I leave this to some querist of a future day, as at this present time there is much curiosity in antiquity, and there is now growing the Mummy Wheat the seed of which is said to have been buried two thousand years. Some one of a future day may like to try this, and I wish you success. P.A.*'.

The material was just what we needed. Although long dead, the extracts of these more than 100-year-old embryos still showed high nuclease activity as well as an absence of any high molecular weight DNA (Osborne, 1974). I wished that Peter Ashenden could have known how we had used his thoughtful posthumous gift.

At about that time, I remember taking part in a Society for Experimental Biology meeting entitled 'Senescence in Seeds' organized by Trevor Villiers, who had done beautiful work on ageing maize kernels with Patricia Berjak in South Africa. I was then quite shocked by the degree of acrimony that was aroused amongst those who objected to this use of 'senescence' and who wanted instead to consider any changes in a dry seed simply as 'ageing'.

Of course, no stored seed is completely dry, and enzymes in intimate contact with their substrates frequently function at very low water activity. Much is now understood of water mobility in dry seeds from the concepts of Carl Leopold and his associates on the 'glassy state' that is engendered as a seed loses water at maturation. These findings have helped greatly in our interpretation of the speed at which the degradative changes in loss of viability can take place and which biochemical events determine them (Leopold *et al.*, 1994).

Given that the genomic (and presumably also the mitochondrial and plastid) DNA of an embryo is always under threat, however slowly, one has to ask how does the genome of any harvested seed ever fully recover its integrity? How does an embryo that already has nicks in the DNA of most of its cells restore these fragments to their proper high molecular weight status and uninterrupted coding sequences?

Older seed always takes progressively longer to germinate than seed that is freshly harvested (I will refer to dormancy later), and whereas all live embryos will start to synthesize new protein as soon as the permissible level of hydration in the cell is reached, the rate is always initially lower in older seed. Perhaps most important is the fact that DNA replication events too, are progressively postponed with age. It seemed to me that embryos must have a means of re-annealing, on imbibition of water, those nuclease generated breaks in their DNA incurred whilst the seed was still dry. DNA repair was not a fashionable area of study in plants at that time. Indeed, into the 1970's there were still those who thought that plants did not possess a DNA repair system equivalent to that of animal cells.

In her detailed autoradiographic study of ^{3}H-thymidine incorporation into rye embryos to determine when and in which cells DNA replication first started when water was made available, Swati Sen had always observed a very low level of graining over nuclei very soon after imbibition. We both wondered if this might represent DNA repair, but the levels were too low for certainty. So when Ruth Ben-Ishai at the Technion in Haifa was willing to allow

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seed embryos into her bacterial and animal cell DNA repair laboratories, to run DNA repair experiments in parallel with her own, I was tremendously excited. Using the then very fashionable BND-cellulose affinity columns to separate double stranded (repaired) from single stranded (replicated) DNA, Rivka Sharon and I established without doubt that embryos do repair fragmented DNA (Osborne *et al.*, 1980/81). We showed that rye embryos, irradiated with γ-rays in order to introduce into the DNA known levels of single stranded breaks, could restore DNA integrity quite readily by a dark excision patch repair mechanism similar to that in bacteria and other eukaryotes. Furthermore, it was evident that this non-replicative DNA repair synthesis occurred as one of the earliest events after addition of water and was essentially complete within the first 30–60 minutes.

Back in Oxford

Shortly after this in the early 1980's, Antonio Dell'Aquila from Bari and Jorge Vazquez-Ramos from Mexico City joined us and continued studies on DNA repair. There was no doubt about it! Provided DNA fragmentation had not proceeded too far and provided the enzymes of repair remained functional, even a poor (but still living) embryo could eventually establish as a seedling if protected in its early life from pathogenic infection or other stresses.

But how long did the DNA repair enzymes remain functional in a dry seed? Could loss of activity in one or all of these enzymes be a major cause of the failure to restore genomic integrity? In animal cells mutations in DNA ligase function are linked to the unfortunate condition of Cockayne's syndrome. This particular DNA repair enzyme was of special interest to Alain Sarasin in the CNRS Cancer Research Laboratories in Villejuif, and so once again the rye embryo was taken to a non-plant laboratory for comparative experiments. Together, in Paris, with Antonio Dell'Aquila, Rhoderick Elder and Mario Mezzina we showed quite clearly that DNA ligase fails in parallel with loss of viability, that activity is absent from dead embryos and that the enzyme is newly synthesized in viable embryos as soon as water is supplied (Elder *et al.*, 1987).

I was always intrigued by a finding of Clifford Bray's from his early work with pea embryo axes. When they had just reached the stage of non-germination on storage and were no longer able to synthesize protein when water was supplied, they still had the capacity for a short while to synthesize small polyribonucleotide fragments that later became degraded. We found a similar sequence of events in our just non-viable rye embryos. The ribonucleotides survived *in vivo* for 6–8 h only before degradation. As far as I know, no one has ever tried to translate these ribonucleotides in a cell free system, so we do not know if any represent messenger RNA's or just nonsense transcribed in the presence of low levels of ATP from a highly fragmented and non-repaired DNA template – a sort of senility effort by dying embryos!

I am certain that I do not have a death wish, but I am also certain that I have retained the desire to know what signals tell a cell to die. In 1989 a most important event happened. The Director of the Institute for Cell Biology and Genetic Engineering in Kiev wrote to me in Plant Sciences in Oxford and asked if I would take a young scientist who had just received a British Council Research Fellowship to join in research into DNA repair of the pollen of Chernobyl birch trees. Five years earlier, such an idea of collaboration between the USSR and UK would have been impossible. But times change. Of course, I said yes. Until today Ivan Boubriak and I have had the most rewarding collaboration on DNA repair not only in pollen but also in seeds. With him and his colleagues in Kiev, we have followed many new ideas together with the most interesting outcomes. Some while after his arrival, the USSR broke up and the Ukraine, his home state, became independent. About this time too South Africa released Nelson Mandela, and my long-term friends in South Africa, the Berjaks, could be freely visited. There I became fully aware of the fascinating performance of 'recalcitrant' seeds so beautifully described by Professor E.H. Roberts in his many publications from Reading. I had already seen *Rhizophora mangle* in the Everglades of Florida and mentally concluded that, from the point of view of its seeds, this mangrove was really a mammal in disguise since the embryo continued to grow from fertilization to germination without apparently arresting growth in any way and continuously increasing in fresh weight.

Pollen (the wind blown pollen of the birch trees at Chernobyl) and recalcitrant seeds of the South African mangrove (*Avicennia marina*) have certain unusual features together with characteristics in common. Although birch pollen is released dry from the catkin and survives without problem in the dry state, as soon as it is exposed to free water, it will at once undergo cell death if it is then dehydrated. In this respect, the imbibed pollen, like *Avicennia*, seems 'recalcitrant'. This contrasts with the embryos of most 'orthodox' seeds, such as rye, which on imbibition remain tolerant to desiccation for some hours until around the time they start the first round of DNA replication prior to cytokinesis. In *Avicennia*, the embryo is continuously replicating DNA except for a single day at the time of fruit shedding, and it remains permanently intolerant of water loss. This suggests that some process, intimate with cell cycling, is highly intolerant of desiccation. But in birch pollen, unlike *Avicennia*, there is no replication

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of DNA on imbibition, only cytokinesis of the one generative nucleus as it proceeds to the pollen tube pore. Most of the cells of a growing plant are not dividing, but nearly all are killed by dehydration. So the question remains, why are some cells desiccation tolerant and others are not?

Desiccation tolerance

We are still not certain what attributes make an embryo desiccation tolerant and which factors make them intolerant. Certainly a functional DNA repair is a requirement for survival (Boubriak *et al.*, 1997). The debate, on whether the accumulation of sugars (close to Ralph Obendorf's heart) or specific proteins (LEAs or dehydrins) are essential, remains unresolved. But one question we have recently answered is, do all embryos die the same way at different levels of hydration or desiccation? It is now clear that they do not. Embryos of rye held dry (8–9% moisture content) will show fragmentations of DNA into the random sized polydeoxyribonucleotides described earlier, and these can be religated if the stored DNA repair enzymes are still functional. However, if the moisture content of the seed is raised to 13–14%, the DNA is cleaved instead at the linker region with histone H1, liberating chromatin fragments with histone H3 termini. The DNA fragments are freed as nucleosome multimers and in animal cells at least, they are not repairable by any ligation process. They are considered therefore to be a terminal stage in the degenerative programme of apoptosis. It remains to be discovered if this is a final death signal in plant cells, too.

This raises the very interesting question of why DNA becomes fragmented differently at two different levels of water activity, neither of which, we believe, is permissive to new protein synthesis. Either there must be an activation of a new nuclease activity by the rise in hydration level, or the conformation of the DNA in nuclear chromatin must change to become more accessible to DNAase attack. Considering the options, I favour the concept of conformational change!

We know already from crystallographic studies that the DNA molecule can be reversibly converted from the B-form to an A-form and then to a Z-form on loss of water molecules from the phosphate groups of the helix. Such changes even in limited regions of the supercoiled DNA of dry embryos could provide specific but random sites for the generation of nuclease-mediated breaks (Osborne and Boubriak, 1994). Conformational change might also explain why gene expression at maturation on the mother plant is not the same following rehydration at germination. But these are ideas that we still must test.

Dormancy

That long-ago paper of Tuan and Bonner (1964), showing the onset of transcription in dormant potato tuber buds when they started to sprout, always remained at the back of my mind. It caused me to investigate, with Margaret Macdonald, the initiation of DNA synthesis and replication at potato tuber bud break (Macdonald and Osborne, 1988). But the question I really wanted to answer by the early 1990's was: did the embryos of dormant imbibed seeds retain all their damaged DNA to be repaired only when dormancy was broken, or did the embryo execute a continuous DNA repair throughout the time it remained imbibed and dormant, so that its genome was ready-to-go when the right signal was received? We suspected that they operated a continuous repair because Andrew Cuming, who did his early work in the Cambridge Unit with embryos of dormant and non-dormant wild oats, had shown that for many hours, both protein and membrane synthesis at imbibition were indistinguishable between dormant and non-dormant embryos (Cuming and Osborne, 1978). No differences were discerned until the non-dormant embryos started to grow (germination *sensu stricto*). When Rhoderick Elder joined us after the return to Oxford, he resolved the matter without any doubt. He used the genetically pure lines of dormant and non-dormant wild oat that had been so usefully and carefully segregated by Jim Naylor from the wild types of the Canadian prairie. By irradiating embryos in the dry state, to introduce equal DNA fragmentation in the nuclei, Rhoderick showed that both dormant and non-dormant were equally capable of religating and repairing the introduced single strand breaks as soon as water was supplied (Elder and Osborne, 1993). Dormant wild oat, at least, can repair lesions in DNA whenever the seed is sufficiently hydrated. This raises an interesting question that Yitzchak Gutterman and I have often discussed. Do the long-lived-desert seeds of the Negev repair their DNA each time a dew provides enough water?

Looking back

We now have our laboratories in the Oxford Research Unit of The Open University, and sitting in my office with the autumn sun pouring through the windows and the majestic grounds of the once Earl Berkeley's estate to look out upon, I feel I have been enormously lucky in my scientific career. I have studied the things I wanted to study and have had the best of colleagues with whom to do it. I have never been bored but always challenged.

For investigations into cell ageing, desiccation

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tolerance, dormancy and the controls that initiate cell cycling, the embryos of seeds have proved to be a most valuable, informative and amenable material. Even before these possibilities became fully evident to me, I had already become addicted to seeds. When I joined the great international community of seed scientists, thirty years ago now, it was a tremendously exciting, actively thriving, scientifically adventurous and argumentative group of people. This journal, *Seed Science Research*, and the newly born International Society for Seed Science, are proof of the successful academic thrust and personal drives that have attended this continually expanding interest in seed performance. Now, the commercial pressures of the seed industries to make every seed produce a successful yield has pushed and will continue to push each seed, seedling and seed scientist to the limit.

So, have those infant ideas of the molecular biology of seeds changed during the thirty years that I have been viewing and experiencing them? Not greatly, I believe. We certainly have much more knowledge of all aspects of seed development, maturation, survival and germination. Newcomers to the field will be beset by a vast literature of facts to assimilate before deciding where first to direct their own efforts. To how genes are expressed, which ones and when, are still a focus. But what are the other kinds of information that a seed inherits from the mother plant on which it grows? How and when does an embryo know it will be dormant? Do the cells of the different parts of seeds die differently? What determines when the first cell division of germination will take place? I think there is still much to do before we reach a proper understanding of what I still consider to be the evolutionary miracle that produced the seed.

Acknowledgement

To all those I have known and worked with who have made the study of seeds such fun – thank you!

References

Bonner, J. (1965) *The molecular biology of development*. Oxford, Clarendon Press.

Boubriak, I., Kargiolaki, H., Lyne, L. and Osborne, D.J. (1997) The requirement for DNA repair in desiccation tolerance of germinating embryos. *Seed Science Research* **7**, 97–105.

Cheah, K.S.E. and Osborne, D.J. (1978) DNA lesions occur with loss of viability in embryos of ageing rye seed. *Nature* **272,** 593–599.

Cuming, A.C. and Osborne, D.J. (1978) Membrane turnover in imbibed and dormant embryos of the wild oat (*Avena fatua* L.) I. Protein turnover and membrane replacement. *Planta* **139,** 209–217.

Dure, L. and Waters, L. (1965) Long–lived messenger RNA: evidence from cotton seed germination. *Science* **147**, 410–412.

Elder, R.H., Dell'Aquila, A., Mezzina, M., Sarasin, A. and Osborne, D.J. (1987) DNA ligase in repair and replication in the embryo of rye, *Secale cereale*. *Mutation Research* **181**, 61–71.

Elder, R.H. and Osborne, D.J. (1993) Function of DNA synthesis and DNA repair in the survival of embryos during early germination and in dormancy. *Seed Science Research* **3**, 43–53.

Johnston, F.B. and Stern, H. (1957) Mass isolation of viable wheat embryos. *Nature* **179**, 160–161.

Leopold, A.C., Sun, W.Q. and Bernal-Lugo, I. (1994) The glassy state in seeds: analysis and function. *Seed Science Research* **4**, 267–274.

Macdonald, M.M. and Osborne, D.J. (1988) Synthesis of nucleic acids and protein in tuber buds of *Solanum tuberosum* during dormancy and early sprouting. *Physiologia Plantarum* **73**, 392–400.

Osborne, D.J. (1974) Seeds of the past. *New Scientist* **63**, 252–254.

Osborne, D.J., Sharon, R. and Ben-Ishai, R. (1980/81) Studies on DNA integrity and DNA repair in germinating embryos of rye (*Secale cereale*). *Israel Journal of Botany* **29**, 259–272.

Osborne, D.J. and Boubriak, I.I. (1994) DNA and desiccation tolerance. *Seed Science Research* **4**, 175–185.

Sen, S., Payne, P.I. and Osborne, D.J. (1975) Early ribonucleic acid synthesis during the germination of rye (*Secale cereale*) embryos and the relationship to early protein synthesis. *Biochemical Journal* **148**, 381–387.

Tuan, D.Y.H. and Bonner, J. (1964) Dormancy associated with repression of genetic activity. *Plant Physiology* **39**, 768–772.

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Soilborne Diseases of Tropical Crops

Edited by R J Hillocks, Natural Resources Institute, Chatham, Kent, UK, and J M Waller, International Mycological Institute, UK

Soilborne diseases have, until recently, received less attention than plant diseases affecting the shoot and foliage. However, this is not a reflection of their economic importance, but rather of difficulties in investigating and detecting pathogens below soil level. Many soilborne diseases are stress related and it is in the tropics where crop growth is particularly limited by environmental stress, predisposing crops to infection by soilborne pathogens.

There is thus a great need for information on soilborne diseases of crops in the tropics. This book aims to fill this need by providing reviews of relevant research. It covers the major tropical crops and also includes general chapters on principles, ecology and control. Written by leading research workers from countries including the UK, USA, Australia and India, it will be indispensable for plant pathologists working in this area.

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