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REVIEW PAPER

The importance of safeguarding genome integrity in germination and seed longevity

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Abstract

Seeds are important to agriculture and conservation of plant biodiversity. In agriculture, seed germination performance is an important determinant of crop yield, in particular under adverse climatic conditions. Deterioration in seed quality is associated with the accumulation of cellular damage to macromolecules including lipids, protein, and DNA. Mechanisms that mitigate the deleterious cellular damage incurred in the quiescent state and in cycles of desiccation–hydration are crucial for the maintenance of seed viability and germination vigour. In early-imbibing seeds, damage to the embryo genome must be repaired prior to initiation of cell division to minimize growth inhibition and mutation of genetic information. Here we review recent advances that have established molecular links between genome integrity and seed quality. These studies identified that maintenance of genome integrity is particularly important to the seed stage of the plant lifecycle, revealing new insight into the physiological roles of plant DNA repair and recombination mechanisms. The high conservation of DNA repair and recombination factors across plant species underlines their potential as promising targets for the improvement of crop performance and development of molecular markers for prediction of seed vigour.

Key words: Ageing, DNA repair, double-strand break, germination, longevity, recombination, seed vigour, viability.

Introduction

Seeds are important from both ecological and agricultural perspectives, providing a highly effective strategy for survival through harsh environmental conditions and allowing successful transmission of genetic information from the mother plant to the next generation. Seeds are classified on the basis of their ability to withstand desiccation as either orthodox (desiccation tolerant) or recalcitrant (desiccation intolerant), although intermediates exist between these two extremes (Ellis *et al.*, 1991; Roberts, 1973). Orthodox seeds, which include the majority of crop plants, are common in temperate regions characterized by large seasonal temperature fluctuations. The desiccated state enables long-term survival of orthodox seeds, although there is considerable variation in seed longevity (the lifespan of seed in dry storage). Seeds may retain viability for decades or even centuries, as illustrated by seeds of the sacred lotus (*Nelumbo nucifera*) that retained germinative capacity after ~1300 years (Shen-Miller *et al.*, 1995). Long-term storage of desiccation-tolerant seeds in the quiescent state is associated with cellular deterioration. This damage arises from the combination of transitions between desiccation and rehydration, coupled with low cellular repair activities in the desiccated state. Together, these lead to an accumulation of damage to macromolecules, including proteins, membrane lipids, and DNA. Accumulating genetic evidence is now revealing the importance of repair mechanisms to safeguard seed viability and germination performance. In this review we summarize current knowledge of the key mechanisms that maintain plant genome stability, and highlight progress

Abbreviations: AP, apurinic; ATM, ataxia telangiectasia mutated; ATR, ATM and RAD3-related; BER, base excision repair; DSB, double-strand break; HR, homologous recombination; NHEJ, non-homologous end-joining; PARP, poly (ADP-ribose) polymerase; QTL, quantitative trait locus; ROS, reactive oxygen species; SSB, single-strand break.

following recent advances in our understanding of the roles of DNA damage and repair in seed vigour and longevity.

Importance of repair mechanisms to seed vigour and longevity

Orthodox seeds survive reduction in water content to as low as 5–15% of the final seed mass. To promote survival in the dry state, seeds have evolved effective mechanisms that protect cellular macromolecules and cellular structures against the adverse effects of desiccation and rehydration. During maturation, drying-protective mechanisms include the synthesis of sugars (e.g. raffinose), heat shock proteins, and late embryogenesis abundant proteins that reduce the damage upon dehydration (Bewley and Black, 1994). A decrease in nuclear size and chromatin condensation during seed maturation that persists through desiccation and germination may also help maintain genome stability (van Zanten *et al.*, 2011). In the seed, metabolism is reduced to very low levels and the embryo enters a state of quiescence, although remains responsive to environmental signals that result in transcriptional and post-transcriptional modifications (Finch-Savage *et al.*, 2007; Holdsworth, Finch-Savage *et al.*, 2008). Cellular macromolecules are protected by the reduced mobility of water and the capability of the cytoplasm to form a 'glassy' state, which also reduces damage by freezing (Bewley and Black, 1994). However, although the incidence of damage may be reduced, so is the capacity for repair, and damage accumulated during storage has the potential to limit the lifespan of seeds. Cellular damage is greatly exacerbated by adverse seed storage conditions and the consequent requirement for an extended repair period may underlie the delay to germination that is characteristic of low-vigour seeds (Powell and Matthews, 2012).

Seed quality and longevity during storage

Germination is initiated by water uptake and completed by radicle emergence through the seed coat, coordinated by the action of plant hormone signalling pathways (Holdsworth, Finch-Savage *et al.*, 2008; Weitbrecht *et al.*, 2011). Seed germination performance is a critical determinant of crop yields because young seedlings represent the most vulnerable stage of the plant lifecycle and are highly susceptible to pathogens, pests, and abiotic stresses. High-vigour seeds, which exhibit rapid and synchronous germination resilient to environmental stresses, establish robust seedlings. Seed vigour is a complex genetic trait influenced by many factors, including the parental environment during seed development, time of harvest, and storage conditions (Rajjou and Debeaujon, 2008; Rajjou *et al.*, 2012). Seed longevity is determined by the rate at which the germination potential deteriorates over time (seed ageing). Decrease in seed quality is manifest as a decline in the rapidity and uniformity of germination, in which a progressively increasing delay (lag phase) to radicle emergence eventually culminates in the loss of viability. Equations using models based on time, humidity, and temperature can predict loss of seed viability with great accuracy (Ellis and Roberts, 1980). Within specified limits of moisture content and temperature (Ellis *et al.*, 1990), these equations also apply to controlled deterioration procedures, in which seeds are exposed to elevated temperatures and relative humidities to simulate natural ageing (Powell and Matthews, 1984). The germination of low-vigour seed is more sensitive to adverse environmental conditions and stresses, which negatively impact on final yields through reduced emergence, and slower and weaker establishment of low-vigour seedlings (Ellis, 1992; Rajjou *et al.*, 2012). Significant natural variation in longevity within species in combination with quantitative trait loci (QTL) analysis has provided significant insight into genetic factors underlying this trait (Clerkx *et al.*, 2004; Nguyen *et al.*, 2012). These and other post-genomic era studies are now revealing the importance of repair processes and mechanisms that safeguard the seed against deterioration and promote seed longevity (Rajjou *et al.*, 2012).

Roles of reactive oxygen species in seed ageing

Oxidative stress, resulting from the production of reactive oxygen species (ROS), is a major determinant of mutagenesis and cellular ageing and is the principal cause of cellular deterioration in aged seeds (Bailly, 2004; Kranner *et al.*, 2010; McDonald, 1999). Maturation drying and environmental stresses including temperature and ultraviolet light generate ROS, resulting in the accumulation of oxidized macromolecules during storage and imbibition. In particular, desiccation and rehydration are associated with the generation of high levels of oxidative stress even though the embryo is a relatively hypoxic environment; partial oxygen pressures in embryonic tissues range from 10% atmospheric levels to below 1% depending on the species (Considine and Foyer, 2014). ROS damage to macromolecules may be caused by a loss in cellular compartmentalization, whilst low levels of metabolism in the quiescent state result in low activity of repair pathways (Kranner *et al.*, 2010). Protection from ROS-induced damage can increase resistance to seed ageing, as recently illustrated in *Arabidopsis* by overexpression of metallothioneins from the sacred lotus, which resulted in enhanced seed longevity (Zhou *et al.*, 2012). Catalase function is also important in recovery after seed ageing in sunflower, consistent with hydrogen peroxide levels limiting germination in low-quality seeds (Kibinza *et al.*, 2011). Although potent agents of cellular deterioration, ROS can also function as signalling molecules in the control of dormancy and germination and can modulate hormone levels in the seed (Bailly, 2004; El-Maarouf-Bouteau *et al.*, 2013; Kranner *et al.*, 2010).

Protein damage in seed ageing

Severe deterioration of cellular proteins in the seed results from both reduced cellular maintenance in the quiescent state and cycle(s) of desiccation and rehydration (Ogé *et al.*, 2008). Various forms of protein damage occur, but oxidation and, in particular, carbonylation of abundant seed storage proteins and core metabolic enzymes are the predominant targets in

Arabidopsis seeds during germination (Arc *et al.*, 2011; Job *et al.*, 2005). Oxidation of storage proteins may promote reserve mobilization through destabilization of protein complexes and may function as a protective sink for oxidative ROS molecules in germination (Arc *et al.*, 2011). Protein oxidation also results in the production of methionine sulfoxide residues. Enzymatic reversion of this damage is catalysed by methionine sulfoxide reductase and levels of this enzyme correlate with seed longevity in varieties of *Medicago truncatula* (Chatelain *et al.*, 2013). Significantly, methionine sulfoxide reductase activity has also been implicated as a lifespan determinant in other organisms, indicative of the conservation of mechanisms to counter the effects of ageing in evolution (Moskovitz *et al.*, 2001). Another form of protein damage common to phylogenetically diverse organisms is the spontaneous conversion of aspartate residues to isoaspartyl residues, which is associated with environmental stress and ageing. This modification results in protein mis-folding that can be reversed by highly conserved protein L-isoaspartyl methyltransferase 1 enzymes. Levels of these enzymes are a key determinant of *Arabidopsis* seed longevity and germination vigour and are found at particularly high levels in sacred lotus seeds (Ogé *et al.*, 2008).

RNA damage

mRNA turnover has important roles both pre- and post-imbibition in the control of germination (Holdsworth, Bentsink *et al.*, 2008). RNA is particularly vulnerable to ROS oxidation as a consequence of its single-stranded structure, cytoplasmic location, and lack of repair mechanisms. RNA damage can block translation, which acts as a signal within the cell to target the mRNA for degradation (Bazin *et al.*, 2011). Oxidation of specific mRNAs, mainly encoding genes with putative functions in cell signalling, was identified during after-ripening of sunflower seeds (Bazin *et al.*, 2011). Targeted oxidation led to the accumulation of 7,8-dihydro-8-oxoguanine in 24 specific transcripts, including those encoding factors that may regulate dormancy and signalling. In a subsequent study, specific oxidation of mRNAs was also identified during after-ripening in wheat, although a distinct subset of genes were targeted to those identified in sunflower (Gao *et al.*, 2013). Oxidative damage provides a conserved mechanism whereby changes in abundance of these mRNAs can be controlled non-enzymatically in the quiescent seed (Bazin et al, 2011; El-Maarouf-Bouteau *et al.* 2013).

Membrane damage and disruption of organelles

Early cytological studies identified disruption to mitochondria in aged seeds that correlated with a loss in seed vigour (Berjak and Villiers, 1972*b*); a reduction in nucleotide levels was subsequently observed (Standard *et al.*, 1983). Lipid peroxidation is associated with this loss of membrane integrity and a decline in seed vigour and viability (Bewley *et al.*, 2013). Antioxidants have important roles in limiting these harmful effects of ROS in the seed. *Arabidopsis* mutants deficient in tocopherol (vitamin E) synthesis, a lipophilic antioxidant that combats lipid peroxidation, are hypersensitive to accelerated ageing (Sattler *et al.*, 2004). Although high-quality seeds did not display an altered germination relative to wild-type lines, seedlings displayed a range of growth defects including abnormal cotyledon expansion and white patches on cotyledons consistent with lipid peroxidation damage. Tocopherol therefore appears to counter lipid peroxidation throughout the seed and seedling establishment stages of the plant lifecycle. Subsequently Nguyen *et al.* (2012) reported co-localization of a QTL for *Arabidopsis* seed longevity in the vitamin E locus, whilst rice lines with elevated tocopherol demonstrated resistance to accelerated ageing and gave rise to high-vigour seedlings (Hwang *et al.*, 2014).

DNA damage in seeds

Studies as early as the 1930s implicated genome integrity as an important determinant of seed quality (Navashin and Shkvarnikov, 1933). DNA damage accumulates in seeds even in the absence of external stresses because of the inherent instability of DNA in the cellular environment (Lindahl, 1993). Damage arises as a consequence of endogenous ROS, metabolic by-products, and breaks induced during DNA replication. Maintenance of genome integrity is critically important to prevent mutation prior to re-initiation of cell-cycle activity in the embryonic meristems. In plants, effective DNA damage responses are particularly important because mutations arising in vegetative meristems can be passed onto the next generation of plants. This is because germ cells differentiate from vegetative meristems at a relatively late stage in plant development. DNA damage can form a wide variety of different products, including DNA double-strand breaks (DSBs), single-strand breaks (SSBs), and damage to bases. These DNA lesions can be mutagenic and cause stalling of the transcriptional and DNA replication complexes, limiting growth and development. Damage to one strand of the DNA duplex is common but less harmful than DNA DSBs and inter-strand crosslinks. DNA damage arises during storage and imbibition and reduces seed and seedling vigour. Repair of damage to the embryo genome is an early event in imbibing seeds, observed as high levels of *de novo* DNA synthesis several hours before cells enter S-phase and activation of the cell cycle (Elder and Osborne, 1993). Loss of seed vigour in aged seeds is accompanied by prolonged DNA repair synthesis and a delay in the onset of replicative DNA synthesis (Burgass and Powell, 1984; Elder and Osborne, 1993). To survive, organisms have evolved effective DNA damage detection, signalling, and repair mechanisms and considerable progress has been made in our understanding of DNA repair and recombination pathways in higher plants over the last two decades (Knoll *et al.*, 2014; Waterworth *et al.*, 2011). The principal eukaryotic mechanisms of DNA repair are largely conserved in higher plants and there is increasing evidence that these have important roles in seed physiology.

Single-stranded DNA damage in seeds

Forms of DNA damage affecting only one strand of the DNA duplex can be repaired using sequence information provided

by the complementary strand. Repair is initiated by the recognition and excision of the damaged region and completed by DNA synthesis and ligation. These pathways are highly conserved through evolution and include base excision repair (BER) and nucleotide excision repair pathways (Britt, 1996; Sancar *et al.*, 2004).

Nucleotide excision repair

Nucleotide excision repair pathways repair a diverse array of lesions and detect modifications that result in conformational changes to the DNA duplex or through blocks to RNA polymerase progression. Following detection of DNA damage, a 24–32 base oligonucleotide containing the damage product is removed by the action of two endonucleases before DNA synthesis and ligation complete the repair process (Sancar *et al.*, 2004). The activity of this pathway has not to date been the subject of detailed investigation in seeds.

Base excision repair

Oxidative base damage is a prevalent form of DNA damage incurred in response to oxidative stress. Base adducts may be miscoded, or block replication and transcription. Damaged bases can be removed from the genome by BER, which is initiated by excision of the damaged base by a DNA glycosylase enzyme specific for the particular base adduct (Caldecott, 2001; Fromme *et al.*, 2004). This results in an abasic site that is subsequently removed to create a DNA SSB or nick. DNA synthesis and ligation completes the repair, with a common pathway acting on the products of base excision as well as naturally occurring SSBs. In *Arabidopsis*, as in other organisms, the single nucleotide gap may be filled in by a short-patch repair pathway or, alternatively, a long-patch repair pathway will replace an additional 2–10 nucleotides 3′ to the abasic site (Córdoba-Cañero *et al.*, 2009; Sancar *et al.*, 2004). Recent studies have identified the major endonuclease activity in *Arabidopsis* BER as the apurinic endonuclease-redox protein and that DNA LIGASE 1 performs the ligation step (Córdoba-Cañero *et al.*, 2011).

Seed ageing increases levels of DNA base damage

A number of early studies established a correlation between genome integrity and seed quality. Accumulation of DNA SSBs accompanied loss of viability in aged *Secale cereale* (rye) seeds (Cheah and Osborne, 1978). DNA is naturally unstable in aqueous solution, and although the reduced water content of orthodox seeds provides some protection against water-derived radicles, DNA damage still occurs. Base damage accumulated over two years of natural ageing of maize seeds in the desiccated state at 20°C was 6-fold less than published values for DNA in aqueous solution (Dandoy *et al.*, 1987). The number of abasic sites (apurinic or AP) sites accumulated in the quiescent embryo was estimated as 38 per million nucleotides. Upon seed imbibition, the number of AP sites was found to increase 4-fold, equating to over a million AP sites per cell (Dandoy *et al.*, 1987). This increase presumably reflects DNA glycosylase activity in seeds combined with increased base loss upon hydration in imbibition. Evidence for base damage induced during seed imbibition was provided by an analysis of 7,8-dihydro-8-oxoguanine (8-oxoG), a major oxidative form of base damage. 8-oxoG is potentially mutagenic and in plants is removed by either one of two DNA glycosylases, termed OGG1 and FPG (Córdoba-Cañero *et al.*, 2014). Accelerated seed ageing at increased temperature and humidity increased 8-oxoG levels in dry wild-type *Arabidopsis* seeds and levels were further increased by 24 h imbibition (Chen *et al.*, 2012). 8-oxoG levels were greatly reduced in transgenic lines overexpressing AtOGG1, most markedly after 24 h imbibition, consistent with increased DNA repair capacity in these plants. In both *Arabidopsis* and *M. truncatula*, *OGG1* transcript levels increase during imbibition, with upregulation of *FPG* also observed in *M. truncatula* (Chen *et al.*, 2012; Macovei *et al.*, 2011). These studies are consistent with a model whereby substantial levels of oxidative genome damage are generated upon seed imbibition (Bailly, 2004), adding further to the abasic sites accumulated during quiescence.

BER repair pathways and seed longevity

High levels of oxidative base damage accumulated in seeds require effective repair pathways during imbibition. Analysis of *Arabidopsis arp* mutant lines deficient in the main BER endonuclease activity displayed pronounced hypersensitivity to seed ageing compared to wild-type lines (Córdoba-Cañero *et al.*, 2014). Similar levels of hypersensitivity were also observed in *zdp* mutants, which lack the ability to remove the 3′ phosphate produced by FPG lyase activity. This provides genetic evidence that BER is important to seed recovery after ageing. Additionally, transgenic *Arabidopsis* seeds overexpressing *AtOGG1* exhibited around 50% lower levels of 8-oxoG in quiescent seeds compared with wild-type lines. Elevated levels of *AtOGG1* expression correlated with a significant increase in seed resilience to controlled deterioration and improvement in germination vigour under abiotic stresses including heat, salt, and oxidative stress (methyl viologen). This study illustrates the potential for genetic enhancement of seed vigour through modification of DNA repair enzyme levels or activities and that base damage can limit germination (Chen *et al.*, 2012).

DNA double-strand break repair: a matter of life or death

DNA DSBs, representing chromosomal breaks, are potentially extremely cytotoxic if unrepaired, resulting in chromosome fragmentation and loss of genetic information. To survive, cells must possess effective DNA damage detection and repair mechanisms to mitigate the harmful effects of DSBs and to minimize growth inhibition. In eukaryotes DSBs are repaired by two major conserved pathways: homologous recombination (HR) or non-homologous end-joining (NHEJ) (Sancar *et al.*, 2004).

Non-homologous end-joining

NHEJ is the predominant DSB repair mechanism in most plant tissues (Bleuyard *et al.*, 2005; West *et al.*, 2002). In NHEJ pathways the DSB is repaired by end-to-end-joining, largely independent of DNA sequence. The well-characterized and highly conserved canonical NHEJ pathway is mediated by the KU70–KU80 complex, which binds and stabilizes broken DNA ends, whilst ligation is performed by the LIG4–XRCC4 complex (Friesner and Britt, 2003; Gallego *et al.*, 2003; West *et al.*, 2000; West *et al.*, 2002). DNA ends arising from damage typically require enzymatic processing to form suitable substrates for end-joining by DNA ligase enzymes, and processing may involve the MRE11–RAD50–NBS1 complex in plants (Heacock *et al.*, 2004). In recent years genetic analyses have revealed that higher plants also have the capacity to repair DSBs by a number of alternative KU-independent NHEJ mechanisms (Charbonnel *et al.*, 2011; Charbonnel *et al.*, 2010). Flexibility between components of repair pathways may represent an effective strategy to cope with a wide range of DNA damage products.

Homologous recombination

HR uses an intact copy of the damaged region as a template, which permits error-free break repair. A homologous template is used for repair in a pathway mediated by the Rad51 recombinase protein in conjunction with accessory proteins (Sancar *et al.*, 2004). A key step in HR is the resection of the DSB to form long regions of single-stranded DNA that are used in homology searching. Initiation of this resection is supressed during the G1 phase of the cell cycle in yeast and mammals (Buis *et al.*, 2012; Cannavo and Cejka, 2014). HR may have only a minor role in early imbibition, if similar regulation occurs in plants, because cells in the quiescent embryo are typically in G1. However, HR may play an increased role during DNA replication that occurs later in germination (Barroco *et al.*, 2005; Masubelele *et al.*, 2005).

DNA double-strand breaks and chromosome aberrations

The DSB is one of the most life-threatening DNA damage lesions for the cell. Chromosome fragmentation has the potential for the loss of large amounts of genetic information, leading to aneuploid cells that lack the normal chromosomal complement. A body of pre-genomic era studies identified a strong correlation between chromosomal abnormalities and loss of seed vigour and viability during ageing (Abdalla and Roberts, 1969; Dourado and Roberts, 1984*a*; Dourado and Roberts, 1984*b*; Roberts, 1972). Chromosomal aberrations arise from mis-repair of DNA DSBs, including anaphase bridges (fused chromosomes) and chromosomal fragments. These studies concluded that even high-quality seeds have a background level of chromosome breaks and that the frequency of chromosomal abnormalities is closely related to viability of the seed lot. Around 4–8% of aberrant cells were observed in seed lots undergoing viability loss in *Pisum sativum* (pea), *Hordeum vulgare* (barley), and *Phaseolus vulgaris* (bean), whilst *Lactuca sativa* (lettuce) seed reportedly displayed 90% abnormal anaphase in cells at 50% viability (Villiers, 1974). This is indicative that very high levels of chromosomal breaks accumulate in orthodox seeds and that there is a strong relationship between seed vigour, viability, and chromosomal break frequency.

Importance of double-strand break repair in early germination

Gamma irradiation causes an array of cellular damage including DNA SSBs, DSBs, and slow seed germination. *Arabidopsis* NHEJ mutants *ku70* and *atlig4* displayed an even greater delay to germination relative to irradiated wild-type controls (Friesner and Britt, 2003). As these mutants are defective in DSB repair, the conclusion from this observation is that the prolonged presence of DSBs are sufficient to delay germination. Germination of seeds deficient in *ku70* also exhibited hypersensitivity to the genotoxin methyl methanesulfonate, which induces methylation damage to bases (Riha *et al.*, 2002). Excision of damaged bases has the potential to generate DSBs through conversion of a SSB into a DSB, typically by replication past a SSB. Given that hypersensitivity was observed only at the seed stage of the plant lifecycle, this is indicative of a higher requirement for KU70-dependent DNA repair specifically in germination (Riha *et al.*, 2002). DNA ligase enzymes catalyse the final DNA end-joining step in almost all DNA repair pathways and Lig4 functions in the canonical NHEJ pathway. Plants also possess an additional unique DNA ligase termed LIG6, which has an N-terminal motif shared with mammalian Artemis, a protein responsible for processing hairpin structures at DNA ends (Pannicke *et al.*, 2004; Waterworth *et al.*, 2010). Mutant *lig6* plants display hypersensitivity to X-rays, consistent with a role in DSB repair, although the low level of redundancy with LIG4 suggests that these ligases have largely different substrates. *Arabidopsis lig6*, *lig4*, and *lig4 lig6* mutant seeds displayed hypersensitivity to accelerated ageing, establishing a genetic link between DSBs and seed longevity. Mutant *lig6* seeds in particular showed reduced vigour when germinated under cold stress or oxidative stresses (Waterworth *et al.*, 2010). The additive nature of the *lig4* and *lig6* mutations establishes distinct roles for each ligase in seed longevity and germination. A QTL for seed longevity was subsequently identified to co-localize with a chromosomal region containing *AtLIG4* (Nguyen *et al.*, 2012). Furthermore, evidence from work in maize points to roles for HR in repair events in seed imbibition. Phenotypic analysis of irradiated kernels of maize *rad51* mutant lines, a HR pathway component, also revealed a severely delayed germination relative to wild type (Matsuoka *et al.*, 2007).

Telomeres

Eukaryotic linear chromosomal ends are protected from recognition as broken DNA ends by highly conserved nucleoprotein telomeric cap structures. Deprotected telomeres

trigger a DNA damage response and can be substrates for recombination pathways, resulting in chromosomal fusions and rearrangements and cell death (Amiard *et al.*, 2014; Watson and Riha, 2010). Plant telomeres are usually formed of tandem TTTAGG repeats and vary in length between species. Genome instability results from shortening of telomeres beyond a minimum length, around 250 nucleotides in the case of *Arabidopsis* (Heacock *et al.*, 2007). Studies with dry wheat grain established that fragmentation of wheat telomeric sequences occurred with seed ageing and preceded loss of viability (Bucholc and Buchowicz, 1992; Bucholc and Buchowicz, 1995). Telomere loss in the quiescent state could result from nucleolytic degradation or accumulation of genomic lesions leading to deletional recombination events within telomeres (Watson and Riha, 2010). Telomere deprotection in the *ku70* mutant line may provide an explanation for the hypersensitivity of these mutants specifically at the seed stage of the plant lifecycle (Riha *et al.*, 2002). Telomerase is a nucleoprotein complex that plays a key role in telomere maintenance and extends telomere length after DNA replication. Telomerase activity, which correlates with DNA replication activity, was absent in the dry seed in *Melandrium album* (synonym *Silene latifolia*; white campion) (Riha *et al.*, 1998), whereas aged wheat seed restored telomeres within 90 min imbibition (Bucholc and Buchowicz, 1992). Telomere loss upon seed deterioration could therefore represent a possible source of DSBs that contribute to the observed increase in chromosomal defects upon seed ageing.

Combinatorial effects of damage in seeds

Seed deterioration arises as a consequence of combinatorial damage to multiple cellular components, which together impact on seed vigour. Loss of membrane integrity causes loss of cellular compartmentalization, resulting in impaired mitochondrial function and increased ROS damage (Bewley *et al.*, 2013). Damage to nucleic acids will result in an inhibition of transcription and translation, exacerbating the loss in enzyme activities resulting from protein oxidation and misfolding, including protein components of repair pathways. For example, both DNA ligase and DNA polymerase activities decline as seeds approach loss of viability (Coello and Vázquez-Ramos, 1996; Elder *et al.*, 1987; Vázquez *et al.*, 1991) and compromised DNA repair capacity results in a progressively impaired ability to cope with ageing (Gutiérrez *et al.*, 1993). Eventually seeds reach an 'exhaustion' phase where they lose viability (Kranner *et al.*, 2010). This highlights the additive nature of cellular damage upon seed ageing and the requirement for multiple damage detection and repair pathways to operate in early imbibition to maintain seed vigour (Bewley and Black, 1994).

DNA damage responses

Activation of signalling kinases

The response to DNA damage in plants is coordinated by the phosphoinositide-3-kinase-related protein kinases ataxia telangiectasia mutated (ATM) and ATM and RAD3-related (ATR). ATM controls a rapid and dynamic transcriptional response to DSBs, including many genes involved in DNA repair and DNA damage signalling (Culligan *et al.*, 2006). This characteristic DNA DSB transcriptional response has been observed 3 h into imbibition, even in high-quality seeds, consistent with DSB detection and ATM activation as a very early and programmed component of normal seed imbibition (Waterworth *et al.*, 2010). The ATM transcriptional response is also upregulated in meristematic tissues, presumably reflective of a requirement for increased DSB repair in DNA replication (Yadav *et al.*, 2009) However, imbibing seeds display a response in the absence of DNA replication that is indicative of high levels of genotoxic stress in early-imbibing seeds and consistent with the previous identification of chromosomal breaks (section *DNA DSB repair: a matter of life or death*). Seeds, therefore, represent a developmental stage of the plant lifecycle that is associated with particularly high levels of genotoxic stress. This is not unique to orthodox seeds; exceptionally high genotoxic stress is also associated with other desiccation-tolerant organisms including the highly radioresistant bacterium *Deinococcus radiodurans* (Mattimore and Battista, 1996), suggesting that a high DNA repair capacity is an evolutionary adaptation to survival in the desiccated state.

Poly (ADP-ribose) polymerase in germination

Poly (ADP-ribose) polymerase (PARP) proteins mediate the transfer of ADP-ribose groups to nuclear-localized proteins, creating long polyADP ribose polymers (Smith, 2001). This protein-modification activity plays a number of cellular roles in higher plants including DNA repair, and PARP activity is strongly induced by DNA breaks (Briggs and Bent, 2011). PARP activity is inhibited by nicotinamide, a naturally occurring NAD derivative present in seeds. *Arabidopsis nic-2* mutants display reduced levels of nicotiminidase, an enzyme that metabolizes nicotinamide and relieves inhibition of PARP activities. Increased nicotinamide in *nic-2* mutants correlated with reduced levels of seed PARP ribosylation and germination hypersensitivity to the alkylating reagent methyl methanesulfonate (Hunt *et al.*, 2007). This is consistent with nicotinamide-mediated inhibition of PARP activities slowing DNA repair and inhibiting germination. In a subsequent analysis of different *Arabidopsis* accessions, poly(ADP-ribosyl)ation levels correlated with germination hypersensitivity to the genotoxin methyl methanesulfonate, revealing significant variation in the sensitivity of accessions to genotoxic stress (Hunt and Gray, 2009).

The cell cycle

Cellular responses to DSBs are integrated with progression of the cell cycle and programmed cell death (Fulcher and Sablowski, 2009). Cells in embryos of dry seed are mostly in the G1 stage of the cell cycle (Bewley and Black 1994) and, in *Arabidopsis* seeds, cell-cycle activation occurs late in germination around the time of radicle protrusion (Barroco *et al.*, 2005; Masubelele *et al.*, 2005). DNA repair processes occur in

the earliest phases of germination, measured as ^{3}H-thymidine incorporation into DNA within 30 min imbibition in rye and *Avena fatua* (oat) (Elder and Osborne, 1993). In these cereals, DNA replication takes place several hours into germination prior to radicle emergence (Elder and Osborne, 1993). In *Arabidopsis*, activation of the cell cycle plays an important role in driving germination, and mutants deficient in specific D-type cyclins display reduced germination vigour (Masubelele *et al.*, 2005). Collectively, this indicates that activation of cell-cycle activity contributes to seed vigour and that the delay in germination of deteriorated seeds is accompanied by an extended lag phase of DNA repair and delayed resumption of DNA replication.

Programmed cell death

Programmed cell death of damaged cells in meristematic regions provides an extremely effective method of removing compromised genomes whilst maintaining meristem structure and function (Fulcher and Sablowski, 2009; Furukawa *et al.*, 2010). DNA laddering characteristic of plant programmed cell death occurs in *Phaseolus vulgaris* (pea) and *Helianthus annuus* (sunflower) seeds coincident with a loss of viability (El-Maarouf-Bouteau *et al.*, 2011; Kranner *et al.*, 2011). Sunflower seeds showed extensive cell death throughout the embryonic axis after ageing, coincident with a loss of viability. Seed ageing was associated with an increase in DNA polymorphisms, illustrating an increased mutational load associated with the decline in seed quality (El-Maarouf-Bouteau *et al.*, 2011). Ageing-induced cell death was also observed in *Zea mays* (maize) seeds and associated with a dramatic loss in cellular organization (Berjak and Villiers, 1972*a*; Berjak and Villiers, 1972*b*).

Agricultural and ecological importance of genome maintenance

In the natural environment seeds lie dormant in the soil until conditions are suitable for germination. Seed integrate sensing of seasonal changes (temporal sensing) with spatial sensing of local conditions to co-ordinate dormancy and germination (Footitt *et al.*, 2011). Over time, seeds experience wet–dry cycling in the seed bank that could potentially influence genome maintenance. Commercially controlled hydration is exploited in seed priming technologies for the enhancement of seed vigour in crop species.

Seed priming

Seed vigour in many species can be improved by commercial pre-germination priming treatments in which seeds are dried back following controlled hydration. Seedling field emergence for many commercial species can be increased 5–10% by vigour enhancement through seed priming, which is thought to facilitate cellular repair processes without the completion of germination (Burgass and Powell, 1984). DNA repair synthesis has been observed during priming, suggesting that completion of DNA repair could represent a contributory factor to rapid germination and initiation of DNA replication (Ashraf and Bray, 1993; Bray *et al.*, 1989; Burgass and Powell, 1984; Redfearn and Osborne, 1997; Thornton *et al.*, 1993; vanPijlen *et al.*, 1996). A naturally occurring priming-like repair activity was identified in seeds of *Artemisia* desert species, where partial hydration during the night by dew correlated with a significant decrease in DNA fragmentation (Huang *et al.*, 2008).

Dormancy

Dormancy is a block to germination that persists in the imbibed state. It represents an adaptive mechanism for a seed to germinate under environmental conditions that favour seedling establishment. Dormancy in species and ecotypes adapted to different habitats can be broken by different responses to specific environmental signals, commonly including light and temperature (Finch-Savage and Leubner-Metzger, 2006; Graeber *et al.*, 2012; Holdsworth, Bentsink *et al.*, 2008). Lettuce seed stored in the dormant and hydrated state incurred little chromosome damage and retained germination vigour for extended periods in comparison to dry stored seed (Villiers, 1974), whilst DNA repair synthesis was observed in hydrated dormant oat embryos (Elder and Osborne, 1993). These studies are indicative that genome repair mechanisms are likely to be active in hydrated dormant seed during wet–dry cycling in the soil seed bank. Dormancy and seed longevity could represent distinct adaptive mechanisms to prolong embryo viability in varying climates. These two traits are negatively correlated in *Arabidopsis*, such that seeds with the capacity for high dormancy typically deteriorate more rapidly in dry storage relative to low dormancy seeds (Nguyen *et al.*, 2012). This is consistent with the hypothesis that adaptation of *Arabidopsis* ecotypes to dry climatic conditions is facilitated by greater tolerance of seeds to long periods of desiccation, presumably by increased cellular protection and repair activities. Seeds in wetter climatic zones experience longer periods of hydration, permissive of cellular repair activities, but greater levels of dormancy would be required to control the timing of germination (Nguyen *et al.*, 2012).

Conclusions

Seed vigour is a major determinant of crop yield, especially under adverse environmental conditions that can severely compromise seedling establishment. Much of the developing world has limited access to controlled seed storage facilities, and seed deterioration in storage is exacerbated in hot, humid climates. Improvement of seed vigour and longevity is therefore a clear strategy to maintain and improve crop yields and mitigate the detrimental effects resulting from climate change. Genetic approaches now highlight the importance of cellular repair mechanisms in the maintenance of seed viability and germination vigour. Repair of the genome early in imbibition is essential to prevent both transmission of mutations and

growth inhibition. In particular, DSBs are a potentially lethal form of DNA damage and cytological analyses established a strong correlation between the accumulation of chromosomal breaks with seed quality. Recent studies now provide genetic evidence that repair of these DSBs is important in recovery from seed ageing. There is an increasing awareness that maintenance of genome integrity is particularly important to the seed stage of the plant lifecycle, revealing new insight into the physiological roles of plant DNA repair and recombination mechanisms.

Understanding the underlying molecular factors that determine seed vigour will facilitate the development of improved crop varieties with enhanced germination and storability characteristics through breeding or biotechnological approaches. Upregulation of BER provides proof of principle that enhancement of seed longevity and improved germination performance can be attained through understanding the molecular basis of seed vigour (Chen *et al.*, 2012). Considerable genetic variation has been identified in seed longevity within cultivars, which could be exploited in breeding programmes to improve seed longevity and germination performance.

New research is also opening up the possibility for novel approaches for the prediction of seed quality. Evaluation of seed lot vigour is important to the seed industry and necessary for optimization of seed technology treatments such as priming, but is widely dependent on germination testing. Consequently, there is a requirement for improved methods of seed lot vigour prediction based on sensitive molecular markers for seed quality. The DNA damage response fulfils the criteria for ideal marker development for seed vigour: it is highly conserved across plant species, activated in the earliest stages of germination, quantitative, and amenable to high-throughput analysis. Seed vigour is an excellent predictor for seedling establishment and crop yield, increasing the potential benefits of quantitative and sensitive molecular markers in evaluation of seed lot quality (Powell and Matthews, 2012; Rajjou *et al.*, 2012). These tools will be important in the preservation of plant biodiversity in seed banks, where evaluation and prolonged maintenance of seed viability is essential for the conservation of plant germplasm resources, and for seed improvement in agriculture.

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