Journal of Experimental Botany, Vol. 63, No. 11, pp. 4107–4121, 2012
doi:10.1093/jxb/ers093 Advance Access publication 2 April, 2012

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Overexpression of AtOGG1, a DNA glycosylase/AP lyase, enhances seed longevity and abiotic stress tolerance in *Arabidopsis*

Huhui Chen[1], Pu Chu[1], Yuliang Zhou[1], Yin Li[1], Jun Liu[2], Yu Ding[3], Edward W.T. Tsang[4], Liwen Jiang[3], Keqiang Wu[5] and Shangzhi Huang[1,*]

[1] State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resource, School of Life Sciences, Sun Yat-Sen University, Guangzhou, 510275, China
[2] Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, China
[3] School of Life Sciences, Centre for Cell and Developmental Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China
[4] Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK S7N 0W9, Canada
[5] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China

* To whom correspondence should be addressed. E-mail: huangsz@mail.sysu.edu.cn

Received 24 November 2011; Revised 2 March 2012; Accepted 5 March 2012

Abstract

Reactive oxygen species (ROS) are toxic by-products generated continuously during seed desiccation, storage, and germination, resulting in seed deterioration and therefore decreased seed longevity. The toxicity of ROS is due to their indiscriminate reactivity with almost any constituent of the cell, such as lipids, proteins, and DNA. The damage to the genome induced by ROS has been recognized as an important cause of seed deterioration. A prominent DNA lesion induced by ROS is 7,8-dihydro-8-oxoguanine (8-oxo-G), which can form base pairs with adenine instead of cytosine during DNA replication and leads to GC→TA transversions. In *Arabidopsis*, AtOGG1 is a DNA glycosylase/apurinic/apyrimidinic (AP) lyase that is involved in base excision repair for eliminating 8-oxo-G from DNA. In this study, the functions of AtOGG1 were elaborated. The transcript of *AtOGG1* was detected in seeds, and it was strongly up-regulated during seed desiccation and imbibition. Analysis of transformed *Arabidopsis* protoplasts demonstrated that AtOGG1–yellow fluorescent protein fusion protein localized to the nucleus. Overexpression of *AtOGG1* in *Arabidopsis* enhanced seed resistance to controlled deterioration treatment. In addition, the content of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) in transgenic seeds was reduced compared to wild-type seeds, indicating a DNA damage-repair function of AtOGG1 *in vivo*. Furthermore, transgenic seeds exhibited increased germination ability under abiotic stresses such as methyl viologen, NaCl, mannitol, and high temperatures. Taken together, our results demonstrated that overexpression of *AtOGG1* in *Arabidopsis* enhances seed longevity and abiotic stress tolerance.

Key words: abiotic stress, *AtOGG1*, controlled deterioration treatment, DNA damage and repair, 8-oxo-G, seed longevity.

Introduction

Reactive oxygen species (ROS) are generated continuously as by-products of aerobic metabolism or as a consequence of exposure to abiotic stresses such as heat, drought, salinity, and redox-active compounds (Tsang et al., 1991; Bowler et al., 1992; Park et al., 1992; Foyer et al., 1994; Alscher et al., 1997). Although recent research has suggested that ROS act as important signalling molecules participating in regulating plant responses to abiotic and

Abbreviations: AP, apurinic/apyrimidinic; AtOGG1, *Arabidopsis thaliana* OGG1; BER, base excision repair; CDT, controlled deterioration treatment; hOGG1, human OGG1; 8-oxo-dG, 8-hydroxy-2′-deoxyguanosine; 8-oxo-G, 7,8-dihydro-8-oxoguanine; Man, mannitol; mRFP, monomeric red fluorescent protein; MS medium, Murashige and Skoog medium; MV, methyl viologen; ORF, open reading frame; RH, relative humidity; RNAi, RNA interference; ROS, reactive oxygen species; YFP, yellow fluorescent protein; yOGG1, yeast OGG1.

biotic stress (Apel and Hirt, 2004; Foyer and Shigeoka, 2011; Maruta et al., 2012). ROS are ubiquitous and highly reactive oxidizing agents that act as an important source of stress for cells. Most macromolecules, including lipids, proteins, and nucleic acids, can be oxidized by ROS, resulting in cell disruption and organism lesions (Pacifici and Davies, 1991; Smirnoff, 1993; Alscher et al., 1997; Mittler, 2002; Nishimura, 2002; Apel and Hirt, 2004). Therefore, oxidative stress induced by ROS has been suggested to be an important causative agent of mutagenesis, aging, and pathogenesis (Farr and Kogoma, 1991; Pacifici and Davies, 1991; Michaels and Miller, 1992; Apel and Hirt, 2004). Furthermore, ROS are often considered to be a main cause of seed deterioration associated with loss of seed vigor and viability (McDonald, 1999; Bailly et al., 2008; El-Maarouf-Bouteau et al., 2011). To eliminate ROS, cells develop a number of ROS scavengers such as superoxide dismutase, peroxidase (Tsang et al., 1991; Bowler et al., 1992; Bailly et al., 1996; Apel and Hirt, 2004), and vitamins (Packer et al., 1979; Sattler et al., 2004). Several studies have reported enhanced seed longevity through elimination of ROS by overaccumulated ROS scavengers in transgenic seeds (Lee et al., 2010; Zhou et al., 2012). In living cells, a major target of ROS is the electron-rich bases of DNA, resulting in a diverse range of genotoxic modifications (Lindahl, 1993; Tuteja et al., 2009). DNA oxidization induced by ROS is thought to be the major source of DNA damage during seed storage and seed germination (Dandoy et al., 1987; Bray and West, 2005). Under normal storage conditions, the loss of seed viability is often associated with the accumulation of DNA breaks and chromosome aberration, establishing a link between DNA damage and reduced germination potential during senescence of the embryo in dry seeds (Cheah and Osborne, 1978; Osborne, 1982; Waterworth et al., 2011).

ROS induce a variety of lesions in DNA, including oxidized bases, apurinic/apyrimidinic (AP) sites and DNA strand breaks (Klungland et al., 1999). Among all the DNA lesions induced by ROS, 7,8-dihydro-8-oxoguanine (8-oxo-G) is a dominant one as a result of ROS-induced hydroxylation of the C-8 position of guanine (Kasai and Nishimura, 1984; Yoshida et al., 2002). During DNA replication, the 8-oxo-G can pair with both cytosine and adenine with almost equal efficiency, giving rise to GC→TA transversions and inducing mutagenesis (Wood et al., 1990; Kouchakdjian et al., 1991; Moriya et al., 1991; Shibutani et al., 1991; Moriya, 1993). In DNA, the 8-oxo-G combines with a deoxyribose in deoxyguanosine, gives rise to 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) (Fig. 1A) (Yoshida et al., 2002). To cope with the damage induced by ROS, cells develop a variety of protective measures including DNA repair enzymes (Ohtsubo et al., 1998; Dany and Tissier, 2001; Garcia-Ortiz et al., 2001; Waterworth et al., 2010) and protein repair enzymes (Petropoulos and Friguet, 2006; Oge et al., 2008). Extensive studies have revealed a base excision repair (BER) system involving excision of 8-oxo-G from DNA to eliminate its mutagenic effects (Fig. 1B) (Britt, 1996; Wood, 1996). First, the DNA N-glycosyl bond is excised by DNA glycosylases to liberate the free 8-oxo-G base and generate an AP site (Michaels and Miller, 1992; Wood, 1996). Then, the AP site is hydrolysed by an AP endonuclease at the phosphodiester bond 5′ or 3′ and retains a nick (Vonarx et al., 1998; McCullough et al., 1999). The completion of repair is mediated by a DNA polymerase and ligase after the terminal sugar-phosphate moiety of the retained nick is removed by an exonuclease (DNA deoxyribophosphodiesterase) (Franklin

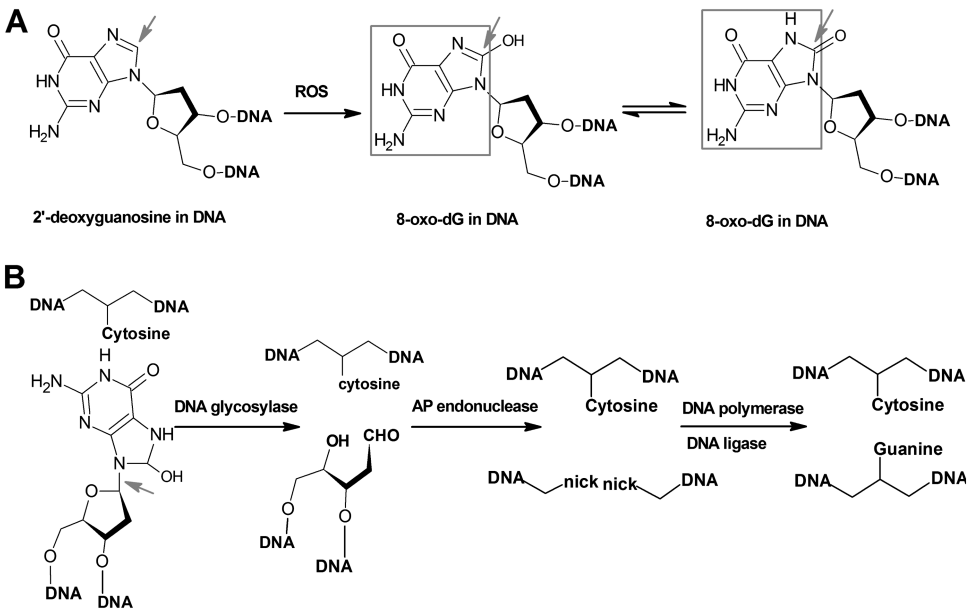

Fig. 1. Schematic diagram of 8-oxo-dG formation and enzymatic repair by the base excision repair (BER) pathway. (A) The guanine of 2'-deoxyguanosine in DNA is hydroxylated by ROS and generates 8-hydroxy-2'-deoxyguanosine (8-oxo-dG), which has two isomers. The grey rectangle indicates the 8-oxo-G in 8-oxo-dG. The grey arrows point to the 8-position carbon atom of guanine. (B) The BER pathway for the repair of 8-oxo-G-mediated mispairs in DNA. The grey arrow indicates the enzyme reaction position.

and Lindahl, 1988; Sandigursky and Franklin, 1992). Studies in *Escherichia coli* have revealed two DNA glycosylases, MutM (or Fpg) and MutY, which participate in the BER system to eliminate the mutation induced by 8-oxo-G (Boiteux et al., 1990; Tchou et al., 1991; Michaels and Miller, 1992). MutM shows a clear preference for excision of 8-oxo-G from the 8-oxo-G:C to that of 8-oxo-G:A pairs in DNA (Tchou et al., 1991; Boiteux et al., 1992; Castaing et al., 1993). In contrast, MutY recognizes 8-oxo-G:A and catalyses the excision of adenine, providing another pathway to repair DNA damage induced by 8-oxo-G (Michaels and Miller, 1992; Michaels et al., 1992; McGoldrick et al., 1995). Inactivation of either *mutM* or *mutY* causes a high frequency of spontaneous GC→TA mutations, and a *mutY mutM* double mutant has a 20-fold higher mutation rate than either mutator alone (Michaels et al., 1992). In eukaryotes, the functional homologue of MutM was first identified from *Saccharomyces cerevisiae* by complementation of the phenotype of *mutY mutM* double mutant and designated OGG1 (Nash et al., 1996; van der Kemp et al., 1996). Since then, human and other mammal OGG1 homologues have also been isolated and characterized (Arai et al., 1997; Radicella and Boiteux, 1997; Radicella et al., 1997; Roldan-Arjona et al., 1997; Rosenquist et al., 1997).

The OGG1 protein is a bifunctional DNA glycosylase/AP lyase which excises 8-oxo-G and cleaves DNA at the 3′-side of the resulting AP site via a β-elimination reaction (Girard et al., 1997; Sandigursky et al., 1997). Both yeast OGG1 (yOGG1) and human OGG1 (hOGG1) proteins display a preference for 8-oxo-G excision from DNA duplexes containing 8-oxo-G:C, and the cleavage efficiencies are 8-oxo-G:C > 8-oxo-G:T >> 8-oxo-G:G and 8-oxo-G:A (Girard et al., 1997; Rosenquist et al., 1997). The hOGG1 is expressed as four splice variants (types 1a, 1b, 1c, and 2) by alternative splicing, leading to different intracellular localization (Aburatani et al., 1997; Takao et al., 1998). The three tagged isoforms, types 1b, 1c, and 2, are localized in the mitochondria, while type 1a is mainly found in the nucleus and to a lesser extent in the mitochondria (Takao et al., 1998; Nishioka et al., 1999). Expression of hOGG1 in a DNA repair-deficient *E. coli mutM mutY* strain or a *yOGG1* mutant partially suppresses the spontaneous mutation phenotype (Radicella et al., 1997; Roldan-Arjona et al., 1997). The hOGG1 protein is involved in many diseases such as carcinogenesis and aging, and can be inhibited by NO (Jaiswal et al., 2001; Osterod et al., 2001; Shinmura and Yokota, 2001). In mice, OGG1 has an important role in repairing genomic damage caused by oxidative stress under ischemic conditions thereby protecting neurons from damage by ROS (Liu et al., 2011). Further studies in Eker rats revealed that deficiency in tuberin is associated with reduced expression of OGG1 and the accumulation of significant levels of 8-oxo-dG, implying that OGG1 may be regulated by tuberin (Habib et al., 2010).

The functional homologue of hOGG1 was also identified in *Arabidopsis* (Dany and Tissier, 2001; Garcia-Ortiz et al., 2001). *Arabidopsis thaliana* OGG1 (AtOGG1) is also a bifunctional DNA glycosylase/AP lyase with significant sequence identity to yeast and human OGG1 proteins (Garcia-Ortiz et al., 2001). In contrast to the hOGG1, AtOGG1 does not appear to undergo alternative splicing since there is only one isoform (Dany and Tissier, 2001). It has been reported that AtOGG1 contains a putative nucleus-localization signal peptide (Garcia-Ortiz et al., 2001), but the subcellular localization of AtOGG1 remains to be clearly established. *In vitro* experiments indicate that AtOGG1 prefers to cleave 8-oxo-G:C mispair in duplex DNA (Morales-Ruiz et al., 2003) to form a Schiff base with 8-oxo-G in the presence of $NaBH_4$ (Garcia-Ortiz et al., 2001), and is a feature of all bifunctional DNA glycosylases/AP lyases. Furthermore, expression of *AtOGG1* in an *E. coli* strain deficient in 8-oxo-G repair can partially revert its spontaneous mutant phenotype (Dany and Tissier, 2001; Garcia-Ortiz et al., 2001). Comparing the *in vitro* substrate specificities of AtOGG1 and AtMMH *in vitro* revealed that AtOGG1 was more active in excising 8-oxo-G from an oligonucleotide while AtMMH appeared to preferentially recognize depurinated DNA as a substrate, suggesting that the two enzymes have been retained in plants during evolution for their specialized activities (Murphy and George, 2005). The transcripts of AtOGG1 were found in a variety of plant tissues including roots, stems, leaves, and flowers (Garcia-Ortiz et al., 2001).

Compared to bacteria, yeast, and mammals, the physiological function of plant OGG1 is still unclear. In this study we show that *AtOGG1* was highly expressed in developing and imbibing seeds and that AtOGG1–yellow fluorescent protein (YFP) fusion protein was localized in the nucleus. Overexpression of *AtOGG1* in *Arabidopsis* seeds conferred enhanced seed resistance to controlled deterioration treatment (CDT). In addition, we found that the amount of 8-oxo-dG was remarkably low in transgenic seeds compared to wild-type seeds with or without CDT, indicating a significant role for AtOGG1 in seed longevity. Furthermore, transgenic seeds also displayed improved germination performance under abiotic stresses including high temperatures, high salinity, osmotic stresses, and especially under oxidative stresses imposed by methyl viologen (MV).

Materials and methods

Plant materials and growth conditions

A. thaliana (ecotype Columbia-0) plants were grown routinely in a greenhouse under 22±1 °C with a light/dark regime of 16 h light/8 h dark. Sterilized seeds were sown on Petri dishes containing half-strength Murashige and Skoog (MS) medium (Duchefa, Haarlem, The Netherlands) and then stratified at 4 °C for 2 days. After stratification, seeds were germinated under the same growth conditions as above and seedlings were transferred to soil after 2 weeks. In all experiments seeds were harvested from wild-type and transgenic plants grown under identical conditions.

Cloning of AtOGG1 *and generation of* AtOGG1 *overexpression and RNA-interference-silenced lines*

The full-length cDNA sequence of *AtOGG1* was obtained from GenBank (accession no. NM_102020). The primer pair 5′-ACGGCGATGAAGAGACCTC-3′ and 5′-GATTCCTCGTAGCTTAGGTAGAC-3′ was used for gene cloning. Using cDNAs

generated from *Arabidopsis* leaves, a fragment containing the open reading frame (ORF) of *AtOGG1* was amplified by PCR and subcloned into the pGEM T-Easy vector (Promega, Madison, WI, USA).

To construct the plant-transforming vector, the ORF of *AtOGG1* was amplified using the primer pair 5′-TCCCCCGGGATGAAGAGACCTCGACCTAC-3′ and 5′-CGAGCTCTCATGGCTTCAACGTATCAC-3′. The PCR products were digested by SmaI and SacI (indicated by underlining in the forward and reverse primers above) and subcloned into the binary plasmid pBI121 (Clontech, Palo Alto, CA, USA) by replacing the β-glucuronidase (*GUS*) gene, to generate the plant-transforming vector pBI121-AtOGG1. The plant-transformation vector containing *AtOGG1* under the control of the cauliflower mosaic virus 35S promoter was electroporated into *Agrobacterium tumefaciens* strain EHA105. Transformation of *A. thaliana* was conducted by the floral dip method (Clough and Bent, 1998). T0 seeds were harvested and germinated on a sterile medium containing 50 μg ml^{-1} kanamycin to select the transformants. The heterozygous transgenic plants were further characterized by a 3:1 segregation with respect to kanamycin resistance. Similarly, T3 homozygous seeds were obtained and confirmed by resistance.

To obtain AtOGG1-silenced lines, RNA interference (RNAi) vector pFGC5941 (TAIR) was used for generation of the silencing constructs. A 378 bp region of AtOGG1 was amplified by PCR to generate inverted repeat products using two primer pairs: 5′-CATGCCATGGATGGATGAAGAGACCTCGAC-3′ and 5′-GGGATTTAAATGAAATCAGACCATAGCTCAG-3′ (the first pair, forward and reverse), and 5′-TCCCCCGGGATGGATGAAGAGACCTCGAC-3′ and 5′-CGGGATCCGAAATCAGACCATAGCTCAG-3′ (the second pair, forward and reverse). The products were subcloned into pFGC5941 in a two-step cloning procedure that orients the fragments as inverted repeats separated by an intron from *Petunia hybrida* chalcone synthase A gene. The RNAi construct was then electroporated into strain EHA105 and used to transform *Arabidopsis* as described above. Transformed plants were selected by basta resistance. The transcript and protein levels of AtOGG1 in basta-resistant plants were determined by real-time PCR and Western blotting respectively.

Subcellular localization assay

To investigate the subcellular location of AtOGG1, vector pA7-YFP, a pre-made vector in pUC18 containing a YFP gene (Voelker et al., 2006), was used to study transient gene expression. Using the primer pair 5′-GCGTCGACATGAAGAGACCTCGACCTAC-3′ and 5′-TCCCCCGGGTGGCTTCAACGTATCAC-3′, SalI and SmaI sites (indicated by underlining in the primer pair above) were introduced into the ORF of *AtOGG1* and the resulting DNA fragment was cloned into pA7-YFP via the SalI and SmaI sites to generate the vector AtOGG1–YFP. The recombinant construct was electroporated into protoplasts of *Arabidopsis* suspension-cultured cells. For the colocalization assay, *Arabidopsis* protoplasts were co-electroporated with AtOGG1–YFP, and the DNA of a single organelle marker as indicated. Mitotracker (Invitrogen, Carlsbad, CA, USA) was used to stain mitochondria. The subcellular locations of fusion proteins were examined by confocal laser scanning microscopy 12–16 h after electroporation. Transient expression analysis was carried out essentially as previously described (Miao and Jiang, 2007).

RNA extraction and real-time PCR

Total RNA was extracted from dry mature and imbibing seeds of transgenic and wild-type plants using the Universal Plant RNA Extraction Kit (BioTeke, Beijing, China). Purified RNA was digested with RNase-free DNase I (Takara, Dalian, China) to eliminate DNA contamination. RNA quality and quantity were determined by electrophoresis and spectrophotometry. First-strand cDNA was synthesized from the total RNA with the PrimeScript 1st Strand cDNA Synthesis Kit (Takara). Real-time PCR was performed on an IQ5 Multicolor Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) using the SYBR Green Real-time PCR Master Mix (Toyobo, Shanghai, China). *AtActin2* was used as an internal reference. Gene-specific primers (forward and reverse, respectively): for *AtOGG1* were 5′-TACAGAGCCAAATACATAACAG-3′ and 5′-TGCTACCTTCGGACCAAC-3′; for *AtMMH* (AT1G52500) were 5′-AGCCAGAATCCACCCGTT-3′ and 5′-GTCCTTCCACCAGCAGTAATG-3′; for *AtARP* (AT2G41460) were 5′-TATCAACAACAGCAAGCGAA-3′ and 5′-TTCTTGAACAGTCTCGCCTC-3′; for *AtLIG1* (AT1G08130) were 5′-GCGGTTAGGGTTCTCAGGT-3′ and 5′-TCCACACACCGCCACTTAG-3′; for *AtPARP2* (AT2G31320) were 5′-CGTATTCTGCGTCCTGTATTGT-3′ and 5′-CGTCTCTGATATCTGTCAGTCCAC-3′; for *AtRAD51* (AT5G20850) were 5′-TGAGGGAACATTCAGGCCAC-3′ and 5′-AGAGAGCGGTAGCACTATCG-3′; and for *AtActin2* (AT3G18780) were 5′-ATTACCCGATGGGCAAGTCA-3′ and 5′-TGCTCATACGGTCAGCGATA-3′. The conditions for real-time PCR were as follows: 95 °C for 5 min, 40 thermal cycles of 95 °C for 10 s, 58 °C for 10 s, and 72 °C for 20 s, followed by 75 thermal cycles with ramping at a rate of 0.5 °C between 58 and 95 °C to check fusion curves and verify the specificity of the PCR amplification. The amount of cDNA was calculated using the comparative C_T method (Schmittgen and Livak, 2008) using Bio-Rad iQ5 2.0 Standard Edition Optical System software. Data represent three biological replicates each consisting of three technical replicates.

CDT

CDT was conducted according to Tesnier et al. (2002) and Oge et al. (2008) with minor modifications. Dry mature seeds were harvested and stored at 4 °C for 15 days before CDT. Each step of CDT was performed in airtight tube carriers containing appropriate saturated solution of salts to obtain stable relative humidity (RH). The carriers were placed in a dark incubator at appropriate temperature for various numbers of days. Temperature and RH were monitored with a Testo 610 controller placed inside the tube carriers. Seeds were placed in microcentrifuge tubes with lids removed and equilibrated for 3 days at 85% RH (15 °C) in the presence of an appropriate saturated solution of KCl. Untreated controls were immediately dried for 3 days at 33% RH (20 °C) in the presence of an appropriate saturated solution of $MgCl_2$. For the controlled treatment, a saturated solution of KCl was used to obtain 82% RH at 40 °C to equilibrate the seeds to 15–20% moisture content. Seeds were treated under these storage conditions for 1–7 days. After that, seeds were dried for 3 days at 33% RH (20 °C). Then seeds were surface sterilized and stored at 4 °C for 2 days before sowing. Four sets of 100 seeds were used for each genotype and germination was monitored with a microscope every day and radicle protrusion of the seeds was scored until a plateau was reached, indicating completion of seed germination. Cotyledon expansion was scored 10 days after sowing and was expressed as a percentage of the seeds that had germinated. Seed moisture content was measured by weighing the seeds before and after drying at 105 °C for 24 h, and relative moisture content was expressed as percentage fresh weight for each RH. This study was replicated for three times and similar results were obtained.

Abiotic stress treatments

For chemical-induced stress treatments, four replicates of 100 dry mature seeds of each genotype were germinated in Petri dishes containing half-strength MS medium with 5–200 μM MV and 75–200 mM NaCl, or 150–600 mM mannitol (Man). The thermotolerance assay was conducted according to (Yokotani et al., 2008) with minor modifications. Briefly, four replicates of 100 dry mature seeds of each genotype were placed in microcentrifuge tubes with the lid removed and equilibrated for 3 days at 85% RH

(15 °C) before heat stress. After equilibration, the tubes were first immersed in water at 50, 51, and 52 °C for 30 min, and then immediately dipped in water at room temperature to eliminate the heat stress. Seeds were surface sterilized and sown on half-strength MS medium. Germination and cotyledon expansion percentages were obtained as described for CDT.

DNA extraction and quantification of 8-oxo-dG

Total genomic DNA was extracted from seeds of each genotype with or without CDT using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Purified DNA was digested with proteinase K (10 mg ml^{-1}, Sigma-Aldrich) for 2 h at 37 °C according to the manufacturer's instructions and subsequently deproteinized using chloroform/isoamyl alcohol. DNA (5 µg) was hydrolysed by incubating with S1 nuclease (10 units; Takara) for 3 h at 37 °C followed by treatment with alkaline phosphatase (10 units; Takara) for 2 h at 37 °C. Free deoxynucleosides were isolated by filtering the whole reaction mixture using an Amicon Ultra-0.5 (Millipore, Billerica, MA, USA) filtration unit. Detection and quantification of 8-oxo-dG in the purified deoxynucleosides were carried out using the DNA Damage ELISA Kit (Stressgen, Enzo, San Diego, CA, USA) according to the manufacturer's instructions.

Protein expression and Western blotting

The full-length cDNA of AtOGG1 was cloned into the pET14b vector (Novagen, San Diego, CA, USA) and expressed in *E. coli* strain BL21 (DE3) as described by Chu et al. (2011). The recombinant proteins were purified following the manufacturer's protocol (Novagen). Polyclonal antiserum was raised in mouse and used in Western blotting. Western blot analysis was performed as described by Chu et al. (2011) with minor modifications. Protein samples were separated by SDS/PAGE and the gels were transferred onto nitrocellulose membranes (Schleicher and Schuell, Keene, NH, USA). The primary antibody was the polyclonal antiserum described above at 1:2000 dilution, and the secondary antibody was goat anti-mouse immunoglobulin horseradish peroxidase antibody (Sigma-Aldrich, St Louis, MO, USA) at 1:5000 dilution. ECL-Plus Western Blotting Detection Reagents (Invitrogen) were used for detection, according to the supplier's instructions.

Tetrazolium assay

Seed viability was estimated using tetrazolium staining. *Arabidopsis* seeds were incubated in a 1% (w/v) aqueous solution of 2,3,5-triphenyltetrazolium chloride (Alfa, Ward Hill, MA, USA) at 30 °C in darkness for 2 days as described by Wharton (1955). Tetrazolium salts were metabolically reduced to highly coloured end products called formazans by NADH-dependent reductases of the endoplasmic reticulum.

Results

Expression analysis of AtOGG1 *during seed development and germination*

A previous study has indicated that *AtOGG1* was found in various tissues including roots, stems, leaves, and especially flowers (Garcia-Ortiz et al., 2001). However, the expression profile of *AtOGG1* in seeds is still unclear. To address this issue, the expression of *AtOGG1* was analysed during seed development and germination using real-time PCR. In the developing siliques, the expression of *AtOGG1* was detected from 5 to 20 days after pollination, with the highest transcript levels observed 15 days after pollination (Fig. 2). During seed germination, the expression of *AtOGG1* was strongly increased compared to that of dry seeds (20 days after pollination), and high levels of expression for 24 h after imbibition (Fig. 2).

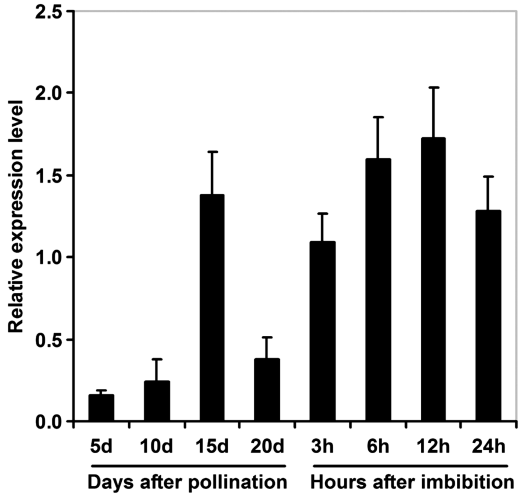

Fig. 2. Real-time PCR analysis of *AtOGG1* transcripts in developing siliques and imbibing seeds. Data represent three biological replicates each consisting of three technical replicates (n=9). Error bars represent the standard error of the means.

AtOGG1–YFP fusion protein was localized in the nucleus

The full-length cDNA of *AtOGG1* was obtained by RT-PCR. A putative nucleus localization signal peptide (KRPRP) was identified in the N-terminus (Garcia-Ortiz et al., 2001). We re-evaluated the possible targeting signals using the PSORT program (version WoLF, http://psort.hgc.jp/), and signals for nucleus, mitochondria, and chloroplast were predicted. To investigate the intracellular localization of AtOGG1 in plant cells, YFP was fused to the C-terminus of AtOGG1 to construct an expression vector, AtOGG1–YFP, for transient expression in protoplasts prepared from *Arabidopsis* suspension-cultured cells. We co-expressed AtOGG1–YFP in *Arabidopsis* protoplasts with a fluorescent marker protein that would mark the nucleus [SV40-monomeric red fluorescent protein (mRFP); Kalderon et al., 1984]. The fluorescence of AtOGG1–YFP fusion protein colocalized with the nucleus marker SV40 (Fig. 3A), which demonstrated a clear localization of AtOGG1 in the nucleus. It has been reported that the three isoforms of hOGG1 were localized in the mitochondria, while only type 1a was found in the nucleus (Takao et al., 1998; Nishioka et al., 1999). In addition to the nuclear genome, plant cells possess mitochondria and chloroplast DNA which can be the targets of oxidative damage by ROS and should be repaired. To further document the localization of AtOGG1, we co-expressed the AtOGG1–YFP with fluorescent marker proteins for chloroplasts (RecA-mRFP; Kohler et al., 1997) and mitochondria (F1 ATPase-mRFP; Jin et al., 2003), or stained the mitochondria with Mitotracker. Essentially, our results showed no fluorescent signal in the chloroplasts (Fig. 3B) or mitochondria (Fig. 3C, 3D).

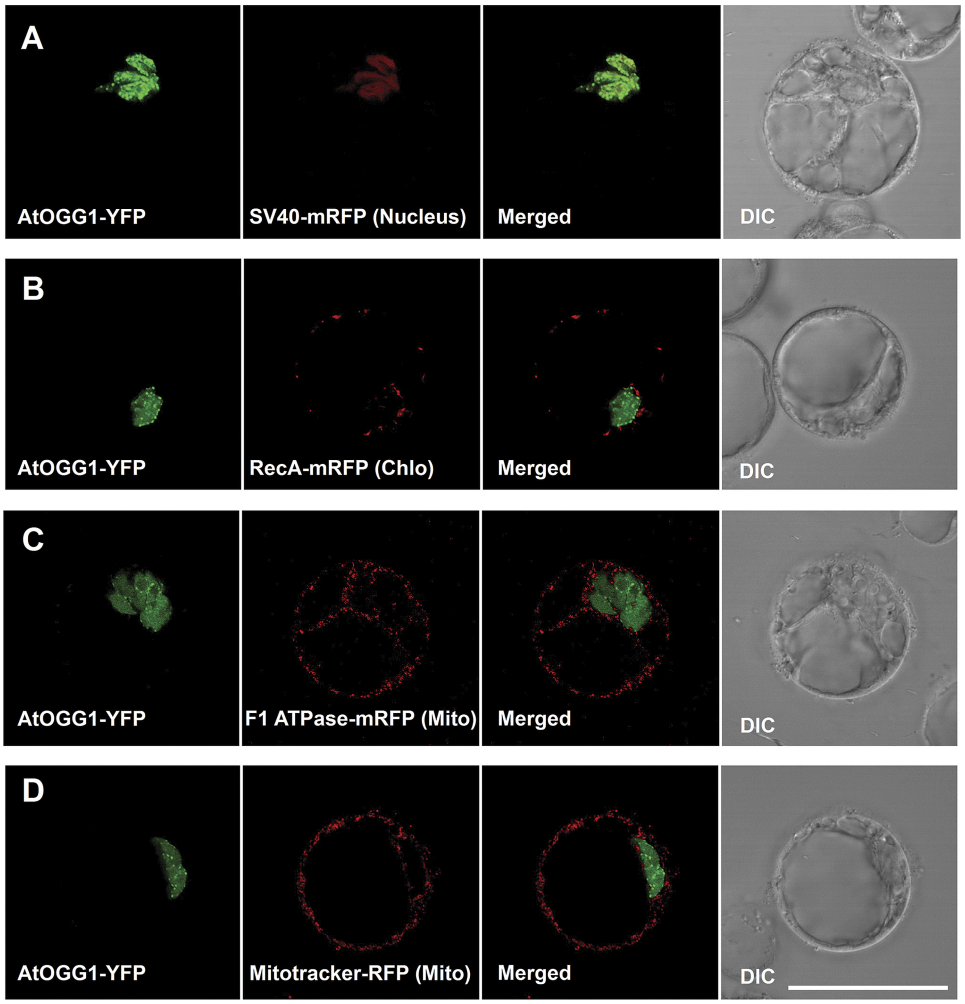

Fig. 3. Subcellular localization of AtOGG1–YFP in *Arabidopsis* protoplasts. (A–C) *Arabidopsis* protoplasts were co-electroporated with AtOGG1–YFP and the DNA of a single organelle marker as indicated. (D) *Arabidopsis* protoplasts transfected with AtOGG1–YFP were stained with Mitotracker which was used to indicate the location of mitochondria. After 13–16 h of expression, the protoplasts were observed by confocal laser scanning microscope. Chlo, chloroplast; DIC, differential interference contrast; Mito, mitochondria. Scale bar 50 μm.

Moreover, the YFP fluorescence displayed a distinctly punctate pattern within the nucleus. A similar result was observed with the localization of several chloroplast DNA glycosylase/AP lyases (Gutman and Niyogi, 2009). It has been observed that hOGG1 protein rapidly accumulates at sites of laser-induced oxidative DNA damage (Zielinska et al., 2011). The punctate pattern of AtOGG1 fluorescence implies that AtOGG1 may be associated with oxidized genomic DNA.

Isolation and characterization of transgenic plants overexpressing AtOGG1

To further investigate the *in vivo* functions of AtOGG1 in seeds, we generated transgenic *Arabidopsis* plants overexpressing *AtOGG1* under the control of the constitutive cauliflower mosaic virus 35S promoter. Three independent transgenic lines (OE-1, OE-2, and OE-3) showing high expression levels of *AtOGG1* in seeds were selected for further analysis. Real-time PCR analysis demonstrated that the transcript levels of *AtOGG1* in transgenic seeds were 10-fold greater than those of wild-type seeds (Fig. 4A). However, the phenotype of transgenic plants was indistinguishable from wild-type plants under normal growth conditions (data not shown).

In the oxidized DNA induced by ROS, the 8-oxo-G combines with a deoxyribose in the deoxyguanosine, gives rise to 8-oxo-dG (Fig. 1A). 8-oxo-dG is a frequently used biomarker of oxidative DNA damage and can be detected by immunological techniques using the specific anti-8-oxo-dG antibody (Yoshida et al., 2002). Based on the chemical characteristics of 8-oxo-dG and the biological role of AtOGG1 (Dany and Tissier, 2001; Garcia-Ortiz et al., 2001) it is tempting to speculate that high *AtOGG1* expression might be correlated with a low content of 8-oxo-dG. Using the quantification assay of 8-oxo-dG, we found that the levels of 8-oxo-dG in the dry mature seeds from transgenic *Arabidopsis* overexpressing *AtOGG1* was significantly lower than those of wild-type seeds (Fig. 4B). In *Arabidopsis* another DNA glycosylase/AP lyase, AtMMH, which is a structural homologue of yeast MutM, has been reported (Ohtsubo et al., 1998). To further understand the correlation between the levels of 8-oxo-dG and the expression of

AtOGG1, the transcript levels of *AtMMH* were analysed in dry mature seeds of transgenic lines and wild-type seeds. As shown in Fig. 4A, the expression levels of *AtMMH* were similar in both wild-type and transgenic seeds, suggesting that the lower content of 8-oxo-dG in the transgenic seeds was due to the increased *AtOGG1* expression and not *AtMMH*.

Transgenic seeds overexpressing AtOGG1 *exhibited enhanced seed longevity associated with reduced DNA damage*

The *in vivo* functions of AtOGG1 in seeds were evaluated using CDT, which has been widely used for estimating seed storage potential or seed longevity (Bentsink et al., 2000; Tesnier et al., 2002; Oge et al., 2008; Rajjou and Debeaujon, 2008). Storage temperature and seed moisture content are key factors that control seed deterioration and viability loss during storage (Bradford et al., 1993; McDonald, 1999). Dry mature seeds of various lines were submitted to the storage treatment at 40 °C with 82% RH. All untreated seeds displayed nearly 100% germination in 2 days at 22 °C (Fig. 5A), indicating a high viability of the untreated seeds. After CDT for 5 days the germination rates (measured as a percentage of germination) of OE-1, OE-2, and OE-3 ranged between 60 and 75% compared with only 27% from those of the wild type at 7 days when germination of various lines reached the maximum level (Fig. 5B). The viability of seeds that failed to germinate were further estimated by tetrazolium assay and no living seeds was found (see Supplementary Fig. S1).

To further document this finding, CDT was performed for between 1 and 7 days on various lines and percentage germination was monitored 7 days after sowing. In response to varying lengths of CDT both transgenic and wild-type seeds displayed decreased rates of germination after 2 days (Fig. 5C), indicating a loss of seed viability. Similar results have been reported with dry mature seeds from various *Arabidopsis* seed databases (Tesnier et al., 2002; Oge et al., 2008; Rajjou et al., 2008; Sattler et al., 2004; Zhou et al., 2012). In the wild-type seeds CDT for 4–7 days resulted in a dramatic loss of germination potential, whereas only moderately decreased of germination potential was observed in transgenic lines (Fig. 5C). Aged seeds that germinate often suffer from loss of vigour (Berjak and Villiers, 1972; Chu et al., 2011; Zhou et al., 2012). Among the seeds that germinated after CDT, the percentages of seedlings with cotyledon expansion from the transgenic lines were much higher than those of the wild type (Fig. 5D, 5E), implying a better repair and recovery mechanism in the transgenic seeds. These results demonstrated that the transgenic seeds overexpressing *AtOGG1* were more resistant to CDT than the wild-type seeds.

It has been suggested that ROS accumulation in aged seeds leads to the deleterious effects of oxidative stress (McDonald, 1999; Bailly, 2004; Lee et al., 2010). The content of 8-oxo-dG induced by CDT was assessed in dry and imbibing aged seeds of both wild-type and transgenic lines. After CDT treatment for 5 days, dry mature seeds of the wild type showed a substantially higher accumulation of 8-oxo-dG (7 pmol μg^{-1} DNA) than the transgenic seeds (2.5–4 pmol μg^{-1} DNA) (Fig. 5F). After 24 h of imbibition there was an increased level of 8-oxo-dG in both wild-type and transgenic seeds, suggesting possible oxidative activities during seed germination (Fig. 5F). However, the transgenic lines displayed only a slight increase in 8-oxo-dG levels (3–6 pmol μg^{-1} DNA) compared to wild-type seeds (16 pmol μg^{-1} DNA) (Fig. 5F).

Overexpression of AtOGG1 *enhanced seed tolerance to abiotic stresses*

The up-regulated expression of *AtOGG1* during imbibition (Fig. 2A) indicated a role for AtOGG1 in the germination process. To investigate the role of *AtOGG1* during germination, dry mature seeds were subjected to abiotic stress treatments. In the chemically induced stress treatments, MV was used to mimic oxidative stress due to the accumulation of superoxide anions and hydrogen peroxide (Espelund et al., 1995; Haslekas et al., 2003). NaCl and Man were used to mimic osmotic stresses that lead to the accumulation of ROS and ultimately affect seed germination (Price and Hendry, 1991; Borsani et al., 2001). In the presence of 100 μM MV, 175 mM NaCl, or 500 mM Man, germination was complete after 8 days for all genotypes (Fig. 6A, 6C, 6E). With 5 μM MV, 75 mM NaCl, or 150 mM Man, the percentages of germination in the wild-type and transgenic lines were similar (Fig. 6B, 6D, 6F). However, seeds overexpressing *AtOGG1* were substantially more tolerant than the wild-type seeds to the increased concentrations of MV, NaCl, or Man (Fig. 6B, 6D, 6F). In response to 100μM MV the transgenic seeds displayed a significantly higher germination percentage in comparison with wild-type seeds (Fig. 6A, 6B). After 8 days 56–65% of the transgenic seeds germinated, whereas only about 31% of wild-type seeds germinated. Similar results were also observed in the presence of different concentrations of NaCl and Man (Fig. 6C, 6D, 6E, 6F).

Furthermore, an extreme thermotolerance assay was conducted to test the thermotolerance of transgenic seeds. Dry mature seeds from transgenic and wild-type lines were immerged into hot water with different temperatures for 30 min. Although the heat stress treatments slowed down the subsequent germination of all genotypes, seeds from transgenic lines were much more tolerant to heat stress than controls (Fig. 6G). In addition, the cotyledon expansion rates from the germinating transgenic seeds were significantly higher than those of wild-type seeds, and transgenic seedlings were also more vigorous than the wild type (Fig. 6H). Taken together, these data demonstrated that overexpression of AtOGG1 in *Arabidopsis* positively enhanced seed-germination ability under adverse conditions.

Evidence of up-regulation of genes involved in the BER and down-regulation of genes response to DNA damage in germinating seeds of transgenic lines

Previous studies in human and bacteria have revealed that there are several enzymes involved in the course of BER of DNA damaged by 8-oxo-G. After the excision of 8-oxo-G

by DNA glycosylase, both AP endonuclease and DNA ligase are required for the completion of BER (Fig. 1B) (Lu et al., 2001). In *Arabidopsis*, an apurinic endonuclease-redox protein (AtARP) appears to constitute the major AP endonuclease in cell extracts (Cordoba-Canero et al., 2011). Three DNA ligase genes have been identified in *Arabidopsis*, termed *AtLIG1*, *AtLIG4*, and *AtLIG6* (Taylor et al., 1998; West et al., 2000; Waterworth et al., 2010). However, only AtLIG1 has been proved to be required for the completion of BER (Cordoba-Canero et al., 2011). Using real-time PCR, we investigated the expression levels of both *AtARP* and *AtLIG1* in imbibing seeds. Both *AtARP* and *AtLIG1* transcripts showed no significant up-regulation in the dry mature seeds (Fig. 7A). However, after 6 h of imbibition the induction of *AtLIG1* and *AtARP* transcripts in the three transgenic seeds was much higher than those of the wild-type seeds (Fig. 7B). It is tempting to speculate that the higher induction of transcripts of both genes, *AtLIG1* and *AtARP*, may be required to complete the BER pathway initiated by the AtOGG1 in the overexpressing seeds.

Although we have demonstrated that 8-oxo-dG accumulated in germinating seeds after CDT (Fig. 5), it is of interest to examine whether DNA damage can also occur in the imbibing seeds without CDT. To address this purpose, the expression of two DNA damage-response genes, *AtRAD51* and *AtPARP2*, was examined. RAD51 functions as a factor participating in the homologous recombination repair of DNA double-strand breaks and PARP2 is a DNA signalling factor involving in the DNA repair process (Babiychuk et al., 1998; Bray and West, 2005). Both *AtRAD51* and *AtPARP2* have been found to be up-regulated in response to DNA damage and have been used as markers to indicate the occurrence of DNA damage (Waterworth et al., 2010, 2011). In the early germination stage of untreated wild-type seeds, both genes exhibited increased transcript levels (Fig. 7A), suggesting the possibility of DNA damage during seed germination. In contrast, the induction of *AtRAD51* and *AtPARP2* transcripts in the seeds from three transgenic plants imbibed for 6 h were significantly lower than those of the wild-type seeds (Fig. 7B). This suggested that over-expression of *AtOGG1* in transgenic seeds resulted in reduced accumulation of DNA damage in imbibing seeds.

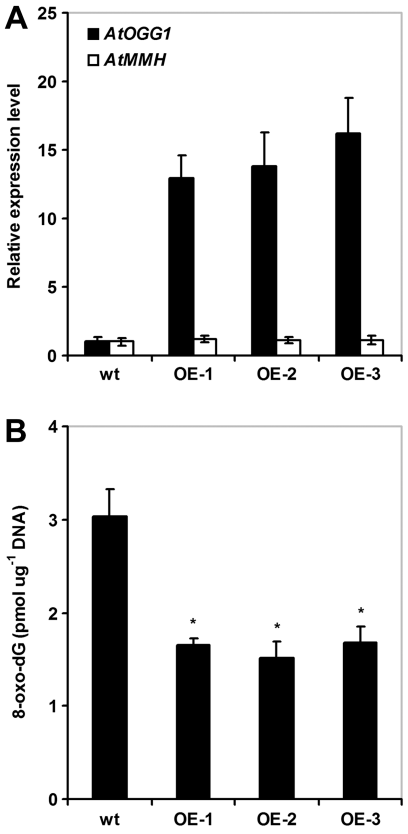

Fig. 4. Genes expression and quantification of 8-oxo-dG in dry mature seeds of wild-type and transgenic plants overexpressing *AtOGG1*. (A) Real-time PCR analysis of *AtOGG1* and *AtMMH* expression in dry mature seeds of transgenic and wild-type (wt) plants. The expression levels of *AtOGG1* and *AtMMH* in wild-type plants were normalized to 1.0. Values represent three biological replicates each consisting of three technical replicates (mean±SD, n=9). (B) Quantification of 8-oxo-dG in dry mature seeds of transgenic and wild-type plants. Values are from three biological replicates each using 5 μg of DNA extracted from 80 mg of seeds (mean±SD). Statistical significance of differences was determined using Student's t test and * indicates that the change in deviance was significant (*P < 0.05).

Discussion

Under normal conditions, oxidized DNA generated by ROS is believed to be the major source of DNA damage during seed storage and germination (Dandoy et al., 1987; Bray and West, 2005). 8-oxo-G, a predominant DNA lesion induced by ROS, mediates mispairing during DNA replication, impairing the genetic resource and necessitating repair by DNA repair enzymes. To combat the adverse effects of ROS on DNA, plants have evolved multiple DNA repair pathways to counteract continuous genome damage (Britt, 1996; Wood, 1996; Lee et al., 2010; Waterworth et al., 2011). The BER pathway is an essential cellular defence mechanism against oxidative DNA damage, especially 8-oxo-G-induced mutagenesis (Lu et al., 2001). AtOGG1 has been identified and enzymatically characterized as a DNA glycosylase/AP lyase involved in the BER pathway (Dany and Tissier, 2001; Garcia-Ortiz et al., 2001). Analysis of the expression pattern of *AtOGG1* has disclosed increased transcript levels at the later development of seeds (15 days after pollination) and during early germination (Fig. 2A). Since seed dessication and imbibition are correlated with high levels of ROS (Waterworth et al., 2011) the increased expression of *AtOGG1* during those processes suggests a role for AtOGG1 in repairing oxidative DNA damage in seeds.

Seed longevity is vital for germplasm conservation and is also an important trait for both ecological and agronomic aspects of crop growth (Oge et al., 2008; Rajjou et al., 2008; Waterworth et al., 2010; Kochanek et al., 2011). However, seeds gradually lose viability during storage (Berjak and

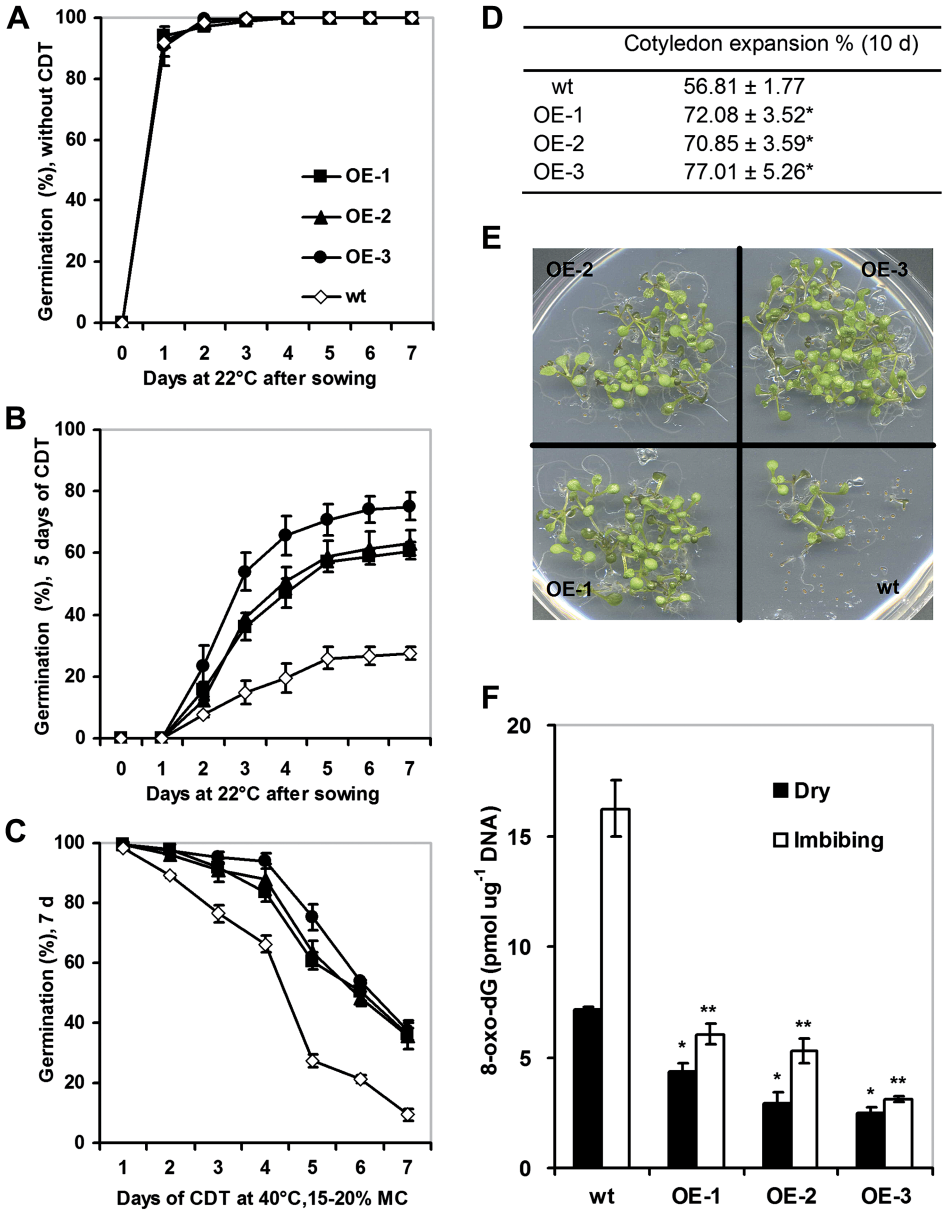

Fig. 5. Characterization of *AtOGG1*-overexpressing lines. Dry mature seeds from transgenic lines overexpressing *AtOGG1* and wild-type (wt) lines were submitted to the controlled deterioration treatment conducted at 15–20% moisture content (MC; 40 °C at 82% RH) for different numbers of days. (A) Germination percentages of seeds without CDT. (B) Germination percentages of seeds submitted to CDT for 5 days. The germination percentages were scored daily until they plateaued on the seventh day. (C) Germination percentages of seeds submitted to CDT for various durations. The germination percentages of these seeds were monitored 7 days after sowing. (D) Percentages of cotyledon expansion of surviving seeds submitted to CDT for 5 days. The data represent the ratio of the seeds with expanded cotyledons to the germinated seeds 10 days after sowing. (E) Phenotypes of 10 day-old seedlings grown on a 9 mm Petri dish containing half-strength MS media from seeds after 5 days of CDT. (F) Quantification of 8-oxo-dG in dry and 24 h-imbibed seeds of transgenic and wild-type plants. Values are from three biological replicates each using 5 μg of DNA extracted from 200 mg of seeds (mean±SD). For (A) and (B) the 0 day time point indicates the beginning of the experiment after seeds were surface-sterilized and stored for 2 days at 4 °C. The symbols used in (B) and (C) to depict the transgenic and wild-type lines are the same as in (A). For (A–D) values are from four technical replicates of 100 seeds each (4 × 100; mean±SD). For (D) and (F) statistical significance of differences was determined using Student's *t* test and * indicates that the change in deviance was significant (*P <0.05, **P <0.01).

Villiers, 1972; Waterworth et al., 2011), and this is greatly accelerated when seeds are subjected to harsh storage conditions (Tesnier et al., 2002; Sattler et al., 2004; Oge et al., 2008). Considering that DNA damage is associated with loss of seed viability during storage (Berjak and Villiers, 1972; Cheah and Osborne, 1978; Waterworth et al., 2011), we investigated the correlation between AtOGG1 and seed longevity. The CDT has been developed under laboratory conditions to mimic poor storage conditions of seeds by exposing seeds to high temperatures and high RH. It has

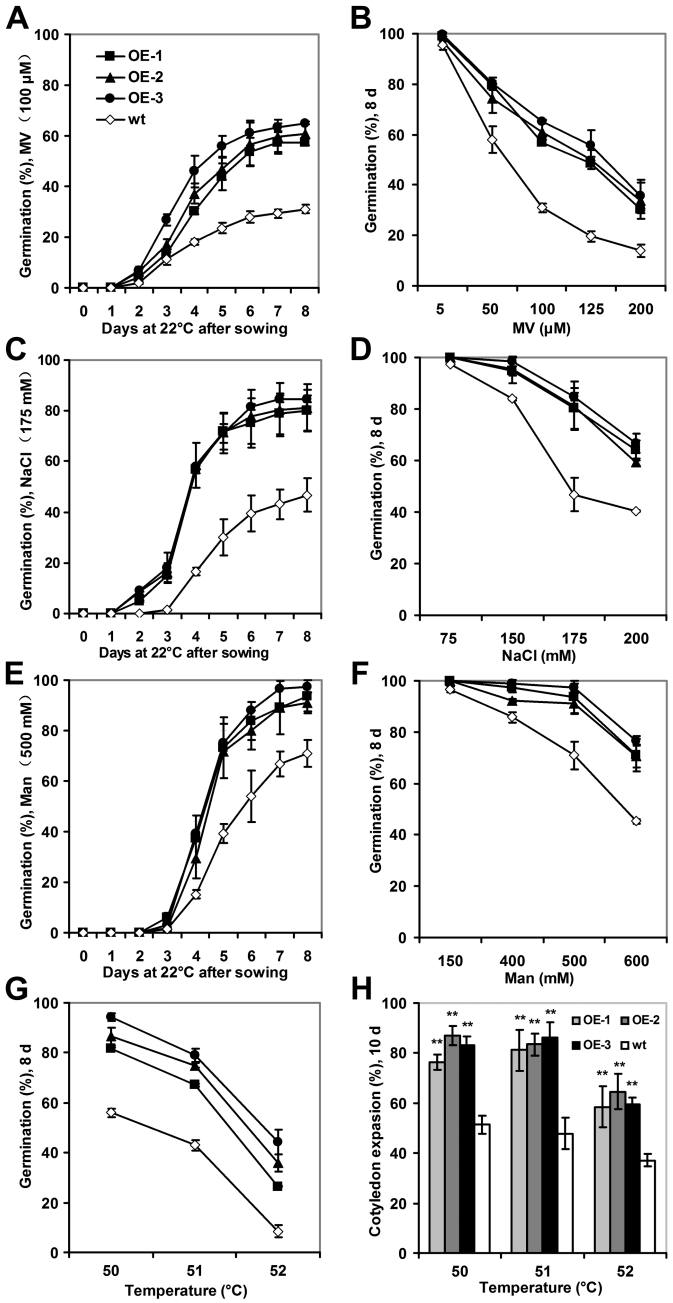

Fig. 6. Transgenic seeds overexpressing *AtOGG1* displayed enhanced tolerance to abiotic stresses. Dry mature seeds from three independent *AtOGG1*-overexpressing transgenic lines and wild-type (wt) lines were germinated in the presence of an imposed stressor as indicated. Germination percentages were monitored daily after sowing until they did not increase, which was the eighth day. (A, C, E) Germination percentages of seeds sown on half-strength MS media supplied with 100 μM MV, 175 mM NaCl, or 500 mM Man, respectively. (B, D, F) Germination percentages of seeds sown on half-strength MS media supplied with different concentrations of MV, NaCl, or Man, respectively. (G) Germination percentages of seeds subjected to heat-stress treatments for 30 min. (H) Percentages of cotyledon expansion of surviving seeds subjected to heat-stress treatments for 30 min. The data represent the ratio of seeds with expanded cotyledons to germinated seeds 10 days after sowing (*$P < 0.05$, **$P < 0.01$; Student's *t* test). For (B, D, F, G) the germination percentages were monitored 8 days after sowing. For (A–G) the symbols used for every line are indicated in (A) and values are from four technical replicates, each with 100 seeds (4× 100) (mean±SD).

been recognized as a good test used to predict the quality and longevity of seedlots. In the present study the CDT assay showed that aged transgenic seeds maintained significantly higher germination percentages than wild-type seeds (Fig. 5B, 5C). In addition, after 5 days of CDT a higher percentage of cotyledon expansion and more vigorous seedlings were observed in transgenic lines in comparison with the wild type (Fig. 5D, 5E). These data demonstrate that over-expression of AtOGG1 confers the transgenic lines with enhanced seed longevity compared to the wild-type lines. Similarly, several studies revealed that seed aging is associated with increased DNA damage in different species (Vijay et al., 2009; El-Maarouf-Bouteau et al., 2011). Recent study by Waterworth et al. (2010) has further confirmed that DNA ligase-mediated DNA repair is a determinant of seed longevity. Enhanced seed longevity through repair of the damage induced by ROS by overexpression of specific enzymes has been reported previously (Prieto-Dapena et al., 2006; Oge et al., 2008; Lee et al., 2010).

Previous studies have demonstrated the function of AtOGG1 in eliminating 8-oxo-G from DNA *in vitro* (Dany and Tissier, 2001; Garcia-Ortiz et al., 2001). Here, quantification of 8-oxo-dG revealed that overexpression of AtOGG1 resulted in reduced oxidative DNA damage in dry and imbibing transgenic seeds (Fig. 4B, 5F). The lower accumulation of 8-oxo-dG in dry mature seeds of transgenic plants is thought to be associated with the overexpression of *AtOGG1* (Fig. 4B), indicating a potential *in vivo* role for AtOGG1 in repairing DNA damage mediated by 8-oxo-G. It has been reported that overexpressing AtOGG1 in a *mutM mutY* mutant could suppress its spontaneous mutator phenotype (Dany and Tissier, 2001; Garcia-Ortiz et al., 2001). Overexpression hOGG1 in human lung cancer cell could reduce the GC→TA transversions induced by 8-oxo-G (Yamane et al., 2003). The repair of oxidative DNA damage by OGG1 has also been directly visualized in the nuclei of live cells (Zielinska et al., 2011). The high level of AtOGG1 in the transgenic plants seemed to initiate an increased level of BER, which was indicated by the increased induction of transcript levels of *AtARP* and *AtLIG1* (Fig. 7B), and thereby decreased the accumulation of 8-oxo-dG in dry mature seeds of transgenic lines. We also showed that the induced transcripts of DNA damage-response genes, *AtRAD51* and *AtPARP2*, significantly decreased after imbibition of the transgenic seeds (Fig. 7B), indicating an improved repair ability in transgenic seeds overexpressing *AtOGG1*. A similar result has also been reported in a study on DNA ligase, which revealed that DNA damage-response genes were induced by the loss of ligase-mediated DNA repair in *atlig6-1* and *atlig4-5 atlig6-1* mutants (Waterworth et al., 2010). The enhanced seed longevity of transgenic lines may be due to the overexpressed AtOGG1-mediated repair by lowering the accumulation of DNA damage in the dry

matured seeds during seed storage and enhanced ability to repair DNA lesions during seed germination.

As sessile organisms, plants are inevitably exposed to environmental stresses. A common effect of many environmental stresses is to cause oxidative damage. The presently observed phenotype of varying tolerance towards MV, NaCl, Man, and high temperature (Fig. 6) in germinating *Arabidopsis* seeds of three transgenic lines revealed a correlation between the accumulation level of AtOGG1 in dry mature seeds and seed germination vigor. During seed germination, repair of accumulated DNA damage is initiated at the earliest stages of imbibition (Osborne et al., 1984; Waterworth et al., 2011) and prior to the initiation of cell division in order to minimize impairment of subsequent seedling development (Waterworth et al., 2010). The reduced induction of 8-oxo-dG in the imbibing seeds of transgenic lines supports the hypothesis that the *AtOGG1* present in the dry seeds could be functional as soon as the imbibed seeds become metabolically active during early germination (Fig. 4A, 5F). A study of DNA ligases has also demonstrated that the accumulation and repair of DNA damage influenced seed germination, and that both genome integrity and the ability to repair damaged DNA affected seed germination (Waterworth et al., 2010).

To further investigate the role of AtOGG1 in seeds, *Arabidopsis* plants with reduced levels of AtOGG1 transcripts and proteins were generated using an RNAi approach (see Supplementary Fig. S2). Although the *AtOGG1*-overexpressing lines displayed enhanced seed viability, AtOGG1-silenced lines showed no difference from the wild type with respect to seed longevity or tolerance to adverse condition (Supplementary Fig. 2D, 2E). The reduction of AtOGG1 protein did not influence the level of 8-oxo-dG in the silenced lines (Supplementary Fig. 2C). Consistent with our observations, Murphy (2005) reported that an AtOGG1-knockout mutant (termed Ogg2) displayed no phenotypic difference from the wild type in growth, development, or reproductive potential under adverse conditions. Furthermore, the double mutant, which lacked both DNA glycosylase/AP lyases, AtOGG1 and AtMMH, also failed to show a difference from the wild type (Murphy, 2005). The similar results were observed in other organisms (Klungland et al., 1999; Friedberg and Meira, 2003). The knockout mice defective in OGG1 showed no indication of pathology but a slower repair of 8-oxo-G (Klungland et al., 1999). In yeast, a yeast strain defective in *ntg1 ntg2 apn1*, although exhibiting a mutant phenotype, did not show any sensitivity to the oxidizing agents H_2O_2 and menadione (Swanson et al., 1999). In *Arabidopsis*, although AtOGG1 and AtMMH displayed substrate specificity, they are functionally redundant (Murphy and George, 2005). In addition, 16 homologues of DNA glycosylase genes have been found in the *Arabidopsis* genome (Murphy, 2005). Recently, research into *Arabidopsis* has reported that both single and triple mutants of three chloroplast DNA glycosylases (AtNTH1, AtNTH2, and AtARP) failed to exhibit a different phenotype from wild-type plants (Gutman and Niyogi, 2009). Seemingly unaffected DNA that has previously been subjected to oxidative stress may be ascribed to the redundant nature of the BER pathway in *Arabidopsis*. This speculation may explain why there is no observed difference in the expression levels of genes involving BER and DNA damage response between the silenced lines and the wild type (Supplementary Fig. 2F). Furthermore, it is likely that there are other DNA repair pathways, such as nucleotide excision repair and recombinational repair, would complement the lack of AtOGG1 (Lu et al., 2001).

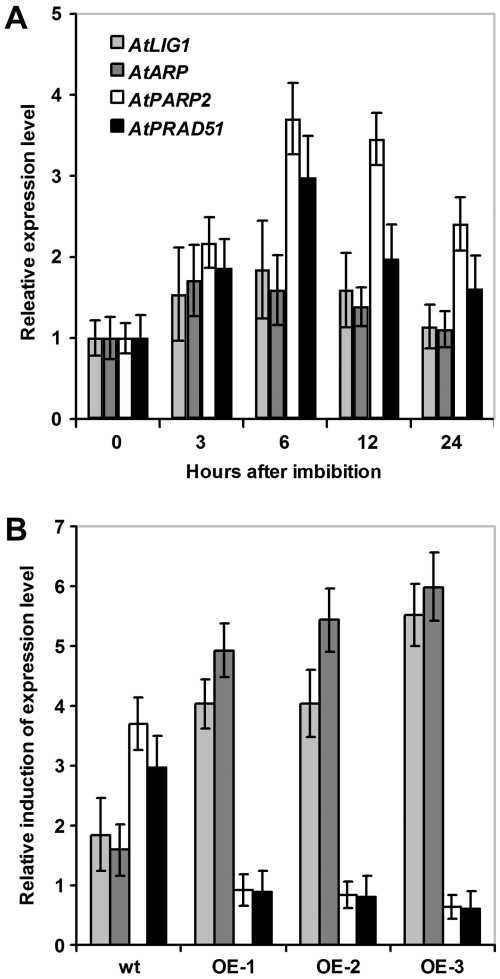

Fig. 7. Analysis of transcript levels of genes involving in the BER and response to DNA damage. (A) Real-time PCR analysis of transcript levels of genes as indicated in imbibing wild-type (wt) seeds. (B) Real-time PCR analysis of the induction of transcript levels of genes as indicated after 6 h of imbibition in wild-type and transgenic seeds, compared with those of dry mature seeds. Genes are indicated by the same symbols in both panels. Data represent three biological replicates and three technical replicates (n=9). Error bars represent the standard error of the means.

Altogether, these data strongly suggest that overexpression of AtOGG1 in *Arabidopsis* enhanced not only seed longevity but also seed tolerance to adverse conditions. Moreover, consistent with previous studies, our work demonstates a close link between seed longevity during dry storage and tolerance to abiotic stress during germination (McDonald, 1999; Clerkx et al., 2004; Oge et al., 2008; Rajjou et al., 2008).

Supplementary material

Supplementary material is available at *JXB* online.

Supplementary Fig. S1. Viability test of the seed samples that failed to germinate.

Supplementary Fig. S2. Characterization of *AtOGG1*-silenced lines.

Acknowledgements

This work was supported by grants from Guangdong Agriculture Science and Technology Team Project (2011A02010210), the Natural Science Foundation of Guangdong Province (9151027501000075), the Guangdong Provincial Science and Technology Program (2010D020301003), and the National Natural Science Foundation of China (30370912).

References

Aburatani H, Hippo Y, Ishida T, Takashima R, Matsuba C, Kodama T, Takao M, Yasui A, Yamamoto K, Asano M. 1997. Cloning and characterization of mammalian 8-hydroxyguanine-specific DNA glycosylase/apurinic, apyrimidinic lyase, a functional mutM homologue. *Cancer Research* **57,** 2151–2156.

Alscher RG, Donahue JL, Cramer CL. 1997. Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum* **100,** 224–233.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55,** 373–399.

Arai K, Morishita K, Shinmura K, Kohno T, Kim SR, Nohmi T, Taniwaki M, Ohwada S, Yokota J. 1997. Cloning of a human homolog of the yeast OGG1 gene that is involved in the repair of oxidative DNA damage. *Oncogene* **14,** 2857–2861.

Babiychuk E, Cottrill PB, Storozhenko S, Fuangthong M, Chen Y, O Farrell MK, Van Montagu M, Inzé D, Kushnir S. 1998. Higher plants possess two structurally different poly (ADP-ribose) polymerases. *The Plant Journal* **15,** 635–645.

Bailly C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Science Research* **14,** 93–108.

Bailly C, Benamar A, Corbineau F, Come D. 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiologia Plantarum* **97,** 104–110.

Bailly C, El-Maarouf-Bouteau H, Corbineau F. 2008. From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies* **331,** 806–814.

Bentsink L, Alonso-Blanco C, Vreugdenhil D, Tesnier K, Groot SP, Koornneef M. 2000. Genetic analysis of seed-soluble oligosaccharides in relation to seed storability of *Arabidopsis*. *Plant Physiology* **124,** 1595–1604.

Berjak P, Villiers TA. 1972. Ageing in plant embryos. *New Phytologist* **71,** 1075–1079.

Boiteux S, O'Connor TR, Lederer F, Gouyette A, Laval J. 1990. Homogeneous *Escherichia coli* FPG protein. A DNA glycosylase which excises imidazole ring-opened purines and nicks DNA at apurinic/apyrimidinic sites. *Journal of Biological Chemistry* **265,** 3916–3922.

Boiteux S, Gajewski E, Laval J, Dizdaroglu M. 1992. Substrate specificity of the Escherichia coli Fpg protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry* **31,** 106–110.

Borsani O, Valpuesta V, Botella MA. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiology* **126,** 1024–1030.

Bowler C, Montagu MV, Inze D. 1992. Superoxide dismutase and stress tolerance. *Annual Review of Plant Biology* **43,** 83–116.

Bradford KJ, Tarquis AM, Duran JM. 1993. A population-based threshold model describing the relationship between germination rates and seed deterioration. *Journal of Experimental Botany* **44,** 1225–1234.

Bray CM, West CE. 2005. DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. *New Phytologist* **168,** 511–528.

Britt AB. 1996. DNA damage and repair in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47,** 75–100.

Castaing B, Geiger A, Seliger H, Nehls P, Laval J, Zelwer C, Boiteux S. 1993. Cleavage and binding of a DNA fragment containing a single 8-oxoguanine by wild type and mutant FPG proteins. *Nucleic Acids Res* **21,** 2899–2905.

Cheah KS, Osborne DJ. 1978. DNA lesions occur with loss of viability in embryos of ageing rye seed. *Nature* **272,** 593–599.

Chu P, Chen H, Zhou Y, Li Y, Ding Y, Jiang L, Tsang EWT, Wu K, Huang S. 2011. Proteomic and functional analyses of Nelumbo nucifera annexins involved in seed thermotolerance and germination vigor. *Planta* doi:10.1007/s00425-011-1573-y.

Clerkx EJM, El-Lithy ME, Vierling E, Ruys GJ, Blankestijn-De Vries H, Groot SPC, Vreugdenhil D, Koornneef M. 2004. Analysis of natural allelic variation of *Arabidopsis* seed germination and seed longevity traits between the accessions Landsberg erecta and Shakdara, using a new recombinant inbred line population. *Plant Physiology* **135,** 432–443.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal* **16,** 735–743.

Cordoba-Canero D, Roldan-Arjona T, Ariza RR. 2011. *Arabidopsis* ARP endonuclease functions in a branched base excision DNA repair pathway completed by LIG1. *The Plant Journal* **68,** 693–702.

Dandoy E, Schnys R, Deltour R, Verly WG. 1987. Appearance and repair of apurinic/apyrimidinic sites in DNA during early germination of *Zea mays*. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **181,** 57–60.

Dany AL, Tissier A. 2001. A functional OGG1 homologue from *Arabidopsis thaliana*. *Molecular Genetics and Genomics* **265,** 293–301.

El-Maarouf-Bouteau H, Mazuy C, Corbineau F, Bailly C. 2011. DNA alteration and programmed cell death during ageing of sunflower seed. *Journal of Experimental Botany* **62,** 5003–5011.

Espelund M, Bedout JA, Outlaw WH, Jakobsen KS. 1995. Environmental and hormonal regulation of barley late-embryogenesis-abundant (Lea) mRNAs is via different signal transduction pathways. *Plant, Cell & Environment* **18,** 943–949.

Farr SB, Kogoma T. 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. *Microbiology Reviews* **55,** 561–585.

Foyer CH, Shigeoka S. 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiology* **155,** 93–100.

Foyer CH, Lescure JC, Lefebvre C, Morot-Gaudry JF, Vincentz M, Vaucheret H. 1994. Adaptations of photosynthetic electron transport, carbon assimilation, and carbon partitioning in transgenic *Nicotiana plumbaginifolia* plants to changes in nitrate reductase activity. *Plant Physiology* **104,** 171–178.

Franklin WA, Lindahl T. 1988. DNA deoxyribophosphodiesterase. *EMBO Journal* **7,** 3617–3622.

Friedberg EC, Meira LB. 2003. Database of mouse strains carrying targeted mutations in genes affecting biological responses to DNA damage. Version 5. *DNA Repair (Amsterdam)* **2,** 501–530.

Garcia-Ortiz MV, Ariza RR, Roldan-Arjona T. 2001. An OGG1 orthologue encoding a functional 8-oxoguanine DNA glycosylase/lyase in *Arabidopsis thaliana*. *Plant Molecular Biology* **47,** 795–804.

Girard PM, Guibourt N, Boiteux S. 1997. The Ogg1 protein of *Saccharomyces cerevisiae*: a 7,8-dihydro-8-oxoguanine DNA glycosylase/AP lyase whose lysine 241 is a critical residue for catalytic activity. *Nucleic Acids Research* **25,** 3204–3211.

Gutman BL, Niyogi KK. 2009. Evidence for base excision repair of oxidative DNA damage in chloroplasts of *Arabidopsis thaliana*. *Journal of Biological Chemistry* **284,** 17006–17012.

Habib SL, Bhandari BK, Sadek N, Abboud-Werner SL, Abboud HE. 2010. Novel mechanism of regulation of the DNA repair enzyme OGG1 in tuberin-deficient cells. *Carcinogenesis* **31,** 2022–2030.

Haslekas C, Viken MK, Grini PE, Nygaard V, Nordgard SH, Meza TJ, Aalen RB. 2003. Seed 1-cysteine peroxiredoxin antioxidants are not involved in dormancy, but contribute to inhibition of germination during stress. *Plant Physiology* **133,** 1148–1157.

Jaiswal M, LaRusso NF, Nishioka N, Nakabeppu Y, Gores GJ. 2001. Human Ogg1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide. *Cancer Research* **61,** 6388–6393.

Jin JB, Bae H, Kim SJ, Jin YH, Goh CH, Kim DH, Lee YJ, Tse YC, Jiang L, Hwang I. 2003. The *Arabidopsis* dynamin-like proteins ADL1C and ADL1E play a critical role in mitochondrial morphogenesis. *The Plant Cell* **15,** 2357–2369.

Kalderon D, Roberts BL, Richardson WD, Smith AE. 1984. A short amino acid sequence able to specify nuclear location. *Cell* **39,** 499–509.

Kasai H, Nishimura S. 1984. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Research* **12,** 2137–2145.

Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, Barnes DE. 1999. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proceedings of the National Academy of Sciences. *United States of America* **96,** 13300–13305.

Kochanek J, Steadman KJ, Probert RJ, Adkins SW. 2011. Parental effects modulate seed longevity: exploring parental and offspring phenotypes to elucidate pre-zygotic environmental influences. *New Phytologist* **191,** 223–233.

Kohler RH, Cao J, Zipfel WR, Webb WW, Hanson MR. 1997. Exchange of protein molecules through connections between higher plant plastids. *Science* **276,** 2039–2042.

Kouchakdjian M, Bodepudi V, Shibutani S, Eisenberg M, Johnson F, Grollman AP, Patel DJ. 1991. NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-oxo-7H-dG(syn).dA(anti) alignment at lesion site. *Biochemistry* **30,** 1403–1412.

Lee YP, Baek KH, Lee HS, Kwak SS, Bang JW, Kwon SY. 2010. Tobacco seeds simultaneously over-expressing Cu/Zn-superoxide dismutase and ascorbate peroxidase display enhanced seed longevity and germination rates under stress conditions. *Journal of Experimental Botany* **61,** 2499–2506.

Lindahl T. 1993. Instability and decay of the primary structure of DNA. *Nature* **362,** 709–715.

Liu D, Croteau DL, Souza-Pinto N, Pitta M, Tian J, Wu C, Jiang H, Mustafa K, Keijzers G, Bohr VA, Mattson MP. 2011. Evidence that OGG1 glycosylase protects neurons against oxidative DNA damage and cell death under ischemic conditions. *Journal of Cerebral Blood Flow & Metabolism* **31,** 680–692.

Lu AL, Li X, Gu Y, Wright PM, Chang DY. 2001. Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochemistry and Biophysics* **35,** 141–170.

Maruta T, Noshi M, Tanouchi A, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S. 2012. H_2O_2-triggered retrograde signaling from chloroplasts to nucleus plays a specific role in the response to stress. *Journal of Biological Chemistry* doi: 10.1074/jbc.M111.292847.

McCullough AK, Dodson ML, Lloyd RS. 1999. Initiation of base excision repair: glycosylase mechanisms and structures. *Annual Review of Biochemistry* **68,** 255–285.

McDonald MB. 1999. Seed deterioration: physiology, repair and assessment. *Seed Science and Technology* **27,** 177–237.

McGoldrick JP, Yeh YC, Solomon M, Essigmann JM, Lu AL. 1995. Characterization of a mammalian homolog of the Escherichia coli MutY mismatch repair protein. *Molecular and Cellular Biology* **15,** 989–996.

Miao Y, Jiang L. 2007. Transient expression of fluorescent fusion proteins in protoplasts of suspension cultured cells. *Nature Protocols* **2,** 2348–2353.

Michaels ML, Miller JH. 1992. The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *Journal of Bacteriology* **174,** 6321–6325.

Michaels ML, Cruz C, Grollman AP, Miller JH. 1992. Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. Proceedings of the National Academy of Sciences. *United States of America* **89,** 7022–7025.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* **7,** 405–410.

Morales-Ruiz T, Birincioglu M, Jaruga P, Rodriguez H, Roldan-Arjona T, Dizdaroglu M. 2003. *Arabidopsis thaliana* Ogg1 protein excises 8-hydroxyguanine and 2,6-diamino4-hydroxy5-formamidopyrimidine from oxidatively damaged DNA containing multiple lesions. *Biochemistry* **42,** 3089–3095.

Moriya M. 1993. Single-stranded shuttle phagemid for mutagenesis studies in mammalian cells: 8-oxoguanine in DNA induces targeted G.C->T.A transversions in simian kidney cells. *Proceedings of the National Academy of Sciences, United States of America* **90,** 1122–1126.

Moriya M, Ou C, Bodepudi V, Johnson F, Takeshita M, Grollman AP. 1991. Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxyguanosine in E. coli. *Mutation Research* **254,** 281–288.

Murphy TM. 2005. What is base excision repair good for?: knockout mutants for FPG and OGG glycosylase genes in Arabidopsis. *Physiologia Plantarum* **123,** 227–232.

Murphy TM, George A. 2005. A comparison of two DNA base excision repair glycosylases from *Arabidopsis thaliana*. *Biochem Biophys Res Commun* **329,** 869–872.

Nash HM, Bruner SD, Scharer OD, Kawate T, Addona TA, Spooner E, Lane WS, Verdine GL. 1996. Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Current Biology* **6,** 968–980.

Nishimura S. 2002. Involvement of mammalian OGG1(MMH) in excision of the 8-hydroxyguanine residue in DNA. *Free Radical Biology and Medicine* **32,** 813–821.

Nishioka K, Ohtsubo T, Oda H, Fujiwara T, Kang D, Sugimachi K, Nakabeppu Y. 1999. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Molecular Biology of the Cell* **10,** 1637–1652.

Oge L, Bourdais G, Bove J, Collet B, Godin B, Granier F, Boutin JP, Job D, Jullien M, Grappin P. 2008. Protein repair L-isoaspartyl methyltransferase 1 is involved in both seed longevity and germination vigor in *Arabidopsis*. *The Plant Cell* **20,** 3022–3037.

Ohtsubo T, Matsuda O, Iba K, Terashima I, Sekiguchi M, Nakabeppu Y. 1998. Molecular cloning of AtMMH, an *Arabidopsis thaliana* ortholog of the *Escherichia coli* mutM gene, and analysis of functional domains of its product. *Molecular and General Genetics* **259,** 577–590.

Osborne DJ. 1982. DNA integrity in plant embryos and the importance of DNA repair. In *Embryonic development, part B, cellular aspects*. Liss, New York, pp 577–592.

Osborne DJ, Dell'Aquila A, Elder RH. 1984. DNA repair in plant cells. An essential event of early embryo germination in seeds. *Folia Biologica (Praha)* **30 Spec No.,** 155–169.

Osterod M, Hollenbach S, Hengstler JG, Barnes DE, Lindahl T, Epe B. 2001. Age-related and tissue-specific accumulation of oxidative DNA base damage in 7,8-dihydro-8-oxoguanine-DNA glycosylase (Ogg1) deficient mice. *Carcinogenesis* **22,** 1459–1463.

Pacifici RE, Davies KJ. 1991. Protein, lipid and DNA repair systems in oxidative stress: the free-radical theory of aging revisited. *Gerontology* **37,** 166–180.

Packer JE, Slater TF, Willson RL. 1979. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* **278,** 737–738.

Park EM, Shigenaga MK, Degan P, Korn TS, Kitzler JW, Wehr CM, Kolachana P, Ames BN. 1992. Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proceedings of the National Academy of Sciences, United States of America* **89,** 3375–3379.

Petropoulos I, Friguet B. 2006. Maintenance of proteins and aging: the role of oxidized protein repair. *Free Radical Research* **40,** 1269–1276.

Price AH, Hendry G. 1991. Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. *Plant, Cell & Environment* **14,** 477–484.

Prieto-Dapena P, Castano R, Almoguera C, Jordano J. 2006. Improved resistance to controlled deterioration in transgenic seeds. *Plant Physiology* **142,** 1102–1112.

Radicella JP, Boiteux S. 1997. Repair of oxidized guanine in mammals: OGG1 genes. *Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales* **191,** 755–763.

Radicella JP, Dherin C, Desmaze C, Fox MS, Boiteux S. 1997. Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences, United States of America* **94,** 8010–8015.

Rajjou L, Debeaujon I. 2008. Seed longevity: survival and maintenance of high germination ability of dry seeds. *Comptes Rendus Biologies* **331,** 796–805.

Rajjou L, Lovigny Y, Groot SP, Belghazi M, Job C, Job D. 2008. Proteome-wide characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. *Plant Physiology* **148,** 620–641.

Roldan-Arjona T, Wei YF, Carter KC, Klungland A, Anselmino C, Wang RP, Augustus M, Lindahl T. 1997. Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proceedings of the National Academy of Sciences, United States of America* **94,** 8016–8020.

Rosenquist TA, Zharkov DO, Grollman AP. 1997. Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proceedings of the National Academy of Sciences, United States of America* **94,** 7429–7434.

Sandigursky M, Franklin WA. 1992. DNA deoxyribophosphodiesterase of Escherichia coli is associated with exonuclease I. *Nucleic Acids Research* **20,** 4699.

Sandigursky M, Yacoub A, Kelley MR, Xu Y, Franklin WA, Deutsch WA. 1997. The yeast 8-oxoguanine DNA glycosylase (Ogg1) contains a DNA deoxyribophosphodiesterase (dRpase) activity. *Nucleic Acids Research* **25,** 4557–4561.

Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M, DellaPenna D. 2004. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *The Plant Cell* **16,** 1419–1432.

Schmittgen TD, Livak KJ. 2008. Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* **3,** 1101–1108.

Shibutani S, Takeshita M, Grollman AP. 1991. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* **349,** 431–434.

Shinmura K, Yokota J. 2001. The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. *Antioxidants and Redox Signaling* **3,** 597–609.

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist* **125,** 27–58.

Swanson RL, Morey NJ, Doetsch PW, Jinks-Robertson S. 1999. Overlapping specificities of base excision repair, nucleotide excision repair, recombination, and translesion synthesis pathways for DNA base damage in *Saccharomyces cerevisiae*. *Molecular And Cellular Biology* **19,** 2929–2935.

Takao M, Aburatani H, Kobayashi K, Yasui A. 1998. Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. *Nucleic Acids Research* **26,** 2917–2922.

Taylor RM, Hamer MJ, Rosamond J, Bray CM. 1998. Molecular cloning and functional analysis of the *Arabidopsis thaliana* DNA ligase I homologue. *The Plant Journal* **14,** 75–81.

Tchou J, Kasai H, Shibutani S, Chung MH, Laval J, Grollman AP, Nishimura S. 1991. 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proceedings of the National Academy of Sciences, United States of America* **88,** 4690–4694.

Tesnier K, Strookman-Donkers HM, Van Pijlen JG, Van der Geest A, Bino RJ, Groot S. 2002. A controlled deterioration test for *Arabidopsis thaliana* reveals genetic variation in seed quality. *Seed Science and Technology* **30,** 149–165.

Tsang EW, Bowler C, Herouart D, Van Camp W, Villarroel R, Genetello C, Van Montagu M, Inze D. 1991. Differential regulation of superoxide dismutases in plants exposed to environmental stress. *The Plant Cell* **3,** 783–792.

Tuteja N, Ahmad P, Panda BB, Tuteja R. 2009. Genotoxic stress in plants: Shedding light on DNA damage, repair and DNA repair helicases. *Mutation Research-Reviews In Mutation Research* **681,** 134–149.

van der Kemp PA, Thomas D, Barbey R, de Oliveira R, Boiteux S. 1996. Cloning and expression in Escherichia coli of the OGG1 gene of Saccharomyces cerevisiae, which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4hydroxy-5-N-methylformamidopyrimidine. *Proceedings of the National Academy of Sciences, United States of America* **93,** 5197–5202.

Vijay D, Dadlani M, Kumar PA, Panguluri SK. 2009. Molecular marker analysis of differentially aged seeds of soybean and safflower. *Plant Molecular Biology Reporter* **27,** 282–291.

Voelker C, Schmidt D, Mueller-Roeber B, Czempinski K. 2006. Members of the *Arabidopsis* AtTPK/KCO family form homomeric vacuolar channels in planta. *The Plant Journal* **48,** 296–306.

Vonarx EJ, Mitchell HL, Karthikeyan R, Chatterjee I, Kunz BA. 1998. DNA repair in higher plants. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **400,** 187–200.

Waterworth WM, Masnavi G, Bhardwaj RM, Jiang Q, Bray CM, West CE. 2010. A plant DNA ligase is an important determinant of seed longevity. *The Plant Journal* **63,** 848–860.

Waterworth WM, Drury GE, Bray CM, West CE. 2011. Repairing breaks in the plant genome: the importance of keeping it together. *New Phytologist* **192,** 805–822.

West CE, Waterworth WM, Jiang Q, Bray CM. 2000. *Arabidopsis* DNA ligase IV is induced by gamma-irradiation and interacts with an *Arabidopsis* homologue of the double strand break repair protein XRCC4. *The Plant Journal* **24,** 67–78.

Wharton MJ. 1955. The use of tetrazolium test for determining the viability of seeds of the genus Brassica. *Proceedings of the International Seed Testing Association* **20,** 81–88.

Wood ML, Dizdaroglu M, Gajewski E, Essigmann JM. 1990. Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry* **29,** 7024–7032.

Wood RD. 1996. DNA repair in eukaryotes. *Annual Review of Biochemistry* **65,** 135–167.

Yamane A, Shinmura K, Sunaga N, Saitoh T, Yamaguchi S, Shinmura Y, Yoshimura K, Murakami H, Nojima Y, Kohno T, Yokota J. 2003. Suppressive activities of OGG1 and MYH proteins against G: C to T: A mutations caused by 8-hydroxyguanine but not by benzo[a]pyrene diol epoxide in human cells in vivo. *Carcinogenesis* **24,** 1031–1037.

Yokotani N, Ichikawa T, Kondou Y, Matsui M, Hirochika H, Iwabuchi M, Oda K. 2008. Expression of rice heat stress transcription factor OsHsfA2e enhances tolerance to environmental stresses in transgenic *Arabidopsis*. *Planta* **227,** 957–967.

Yoshida R, Ogawa Y, Kasai H. 2002. Urinary 8-oxo-7, 8-dihydro-2′-deoxyguanosine values measured by an ELISA correlated well with measurements by high-performance liquid chromatography with electrochemical detection. *Cancer Epidemiology Biomarkers & Prevention* **11,** 1076.

Zhou Y, Chu P, Chen H, Li Y, Liu J, Ding Y, Tsang EW, Jiang L, Wu K, Huang S. 2012. Overexpression of Nelumbo nucifera metallothioneins 2a and 3 enhances seed germination vigor in *Arabidopsis*. *Planta* **235,** 523–537.

Zielinska A, Davies OT, Meldrum RA, Hodges NJ. 2011. Direct visualization of repair of oxidative damage by OGG1 in the nuclei of live cells. *Journal of Biochemical and Molecular Toxicology* **25,** 1–7.