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RESEARCH PAPER

The nuclear protein Poly(ADP-ribose) polymerase 3 (AtPARP3) is required for seed storability in *Arabidopsis thaliana*

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ABSTRACT

The deterioration of seeds during prolonged storage results in a reduction of viability and germination rate. DNA damage is one of the major cellular defects associated with seed deterioration. It is provoked by the formation of reactive oxygen species (ROS) even in the quiescent state of the desiccated seed. In contrast to other stages of seed life, DNA repair during storage is hindered through the low seed water content; thereby DNA lesions can accumulate. To allow subsequent seedling development, DNA repair has thus to be initiated immediately upon imbibition. Poly(ADP-ribose) polymerases (PARPs) are important components in the DNA damage response in humans. *Arabidopsis thaliana* contains three homologues to the human HsPARP1 protein. Of these three, only *AtPARP3* was very highly expressed in seeds. Histochemical GUS staining of embryos and endosperm layers revealed strong promoter activity of *AtPARP3* during all steps of germination. This coincided with high ROS activity and indicated a role of the nuclear-localised AtPARP3 in DNA repair during germination. Accordingly, stored *parp3-1* mutant seeds lacking *AtPARP3* expression displayed a delay in germination as compared to Col-0 wild-type seeds. A controlled deterioration test showed that the mutant seeds were hypersensitive to unfavourable storage conditions. The results demonstrate that AtPARP3 is an important component of seed storability and viability.

INTRODUCTION

Seed viability is an important agronomic trait; only viable seeds can produce healthy and productive offspring. Reduced seed viability as a result of seed deterioration is associated with the loss of membrane integrity, DNA damage and retarded metabolic activity (Priestley 1986). All these processes are linked to the action of reactive oxygen species (ROS). The production of ROS, and ensuing DNA damage, occur at different stages of a seed's life: during desiccation, storage and germination (Weitbrecht *et al.* 2011). During desiccation, ROS formation mainly results from respiration. At this stage of seed development DNA repair pathways are still active until the water content is limited (Bailly *et al.* 2008). Due to the low water content in dry seeds, metabolic activity is highly restricted; the seed remains in a quiescent stage. During this stage, metabolic ROS production is very unlikely. Here, non-enzymatic processes such as lipid peroxidation and Amadori and Maillard reactions lead to the formation of ROS (Sun & Leopold 1995; Kranner *et al.* 2010). The operation of enzymatic DNA repair mechanisms is also limited. Hence, DNA damage can accumulate in stored seeds, especially when seeds are stored under unfavourable conditions, such as high temperature and moisture (Weitbrecht *et al.* 2011). Prolonged seed storage can be simulated using artificial ageing experiments. Such controlled deterioration tests are a well-described means to study seed viability, longevity and storability (Tesnier *et al.* 2002; Rajjou *et al.* 2008).

The germination process starts with the uptake of water into the seed. This is associated with high levels of oxidative stress due to the restart of respiration and metabolic activity (Waterworth *et al.* 2011). The DNA repair machinery now has to cope with damage accumulated during storage and that arising from the start of metabolic activity. Double strand breaks are considered to be the most critical DNA lesions since they can cause chromosomal fragmentation and rearrangement (Waterworth *et al.* 2011; Ventura *et al.* 2012). In eukaryotes, DNA double strand breaks are repaired through two different pathways: homologous recombination (HR) and non-homologous-end-joining (NHEJ). In plants, homologues to components of animal HR and NHEJ have been identified (Waterworth *et al.* 2011).

In humans poly(ADP-ribose) polymerases (PARPs) have been described as important components of the DNA damage response. Activated upon DNA strand breaks, HsPARP1 uses NAD^+ as a substrate to transfer ADP-ribose moieties onto nuclear proteins involved in the DNA strand break response. Thereby poly(ADP-ribose) chains are formed, recruiting proteins involved in DNA repair. Human PARP1 has been described to be involved in both DNA single and double strand break responses (De Vos *et al.* 2012; Pines *et al.* 2013). It is the founding member of the PARP family, comprising 17 members, all showing structural homology to the catalytic domain of HsPARP1. Apart from HsPARP1, only HsPARP2 and HsPARP3 show DNA-dependent activity (De Vos *et al.* 2012; Pines *et al.* 2013). Human PARP2 is also involved in DNA

Table 1. Primers used in this study.

PCR screening and RT-PCR	*parp3-1*_for	AAAGCCTGAAACGATGACGG
	*parp3-1*_rev	AAGGCACAGTTATACAAGAGTCCAT
	Salk_LBa	GGTTCACGTAGTGGCCATCG
	Salk_LBb	GCGTGGACCGCTTGCTGCAACT
PARP3-EYFP	PARP3-EYFP_for	AAAAAACCCGGGATGAAGGTTCACGAGACAAGATCT
	PARP3-EYFP_rev	AAAAAACCCGGGCTCTGGTTCGACATCGACTATCTC
Pr*PARP1*-GUS	Pr*PARP1*-GUS_for	AAAAAAGGATCCTCTCTGCTTCTCCTTCTTCTTGAG
	Pr*PARP1*-GUS_rev	AAAAAAGGATCCTTCTCCGGTAAGAGACAATTACACA
Pr*PARP2*-GUS	Pr*PARP2*-GUS_for	AAAAAAGGATCCGTAAATGGTTTCACAACTTGGTTCC
	Pr*PARP2*-GUS_rev	AAAAAAGGATCCTTTCGTCTTCTTCTTCAGGAGAA
Pr*PARP3*-GUS	Pr*PARP3*-GUS_for	AAAAAACCCGGGAGCATTGTCTCTATCAACCCC
	Pr*PARP3*-GUS_rev	AAAAAACCCGGGTGAGCAAACTCTTTGAACTGTATGA

single strand break repair (Pines *et al.* 2013). Action of HsPARP3 was studied only recently; it was found to act as a component of NHEJ. One possible interaction partner of HsPARP3 is the Ku70/Ku80 complex (Rouleau *et al.* 2007). Additionally, an interaction with the XRCC4/Lig4 complex has been described (Rulten *et al.* 2011).

In the genome of *Arabidopsis thaliana* three *PARP* genes have been identified, *AtPARP1*, *AtPARP2* and *AtPARP3* (Lepiniec *et al.* 1995; Babiychuk *et al.* 1998; Hunt *et al.* 2004). Protein sequences of AtPARP1 and AtPARP2 show strong homology to the catalytic domain of HsPARP1 (Lepiniec *et al.* 1995; Babiychuk *et al.* 1998). Similar to HsPARPs, AtPARP1 and AtPARP2 have been described to interact with DNA and are involved in DNA damage and strand break responses and in cell cycle progression (Amor *et al.* 1998; Babiychuk *et al.* 2001; Doucet-Chabeaud *et al.* 2001; Storozhenko *et al.* 2001; Jia *et al.* 2013). *AtPARP3* is expressed in seed tissue, but has not been characterised functionally (Hunt *et al.* 2007).

To further elucidate the role of AtPARP3 during seed germination, histochemical staining methods were employed to study *AtPARP3* promoter activity and ROS accumulation, and seed germination of a *parp3* mutant was scored. Our results indicate that AtPARP3 is an important component for the maintenance of seed viability during storage.

MATERIAL AND METHODS

Phylogenetic analysis

To identify PARP proteins in *Populus trichocarpa*, *Oryza sativa* and *Physcomitrella patens* BLAST searches were performed using the NCBI database (http://blast.ncbi.nlm.nih.gov). Phylogenetic analysis was performed using Clustal W2 (www.ebi.ac.uk./Tools/msa/clustalw2/). A phylogenetic tree was generated using TreeView software (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

Plant material and identification of homozygous *parp3* mutant plants

A T-DNA insertion line for *PARP3* (SALK_108092) was obtained from the European Arabidopsis Stock Centre (NASC; Alonso *et al.* 2003; Ülker *et al.* 2008). To validate the T-DNA insertion by PCR, the left border primer SALK_LBa and gene-specific primers spanning the predicted insertion site were used (Table 1). The amplicon was sequenced using the SALK_LBb primer. To confirm gene knockout, RNA was extracted from dry seeds using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, Traufkirchen, Germany). cDNA synthesis was performed using Superscript II reverse transcriptase (Life Technologies, Carlsbad, CA, USA) according to manufacturer's instructions. RT-PCR was done with gene-specific primers spanning the T-DNA insertion site (Table 1). *AtACT2* served as a positive control.

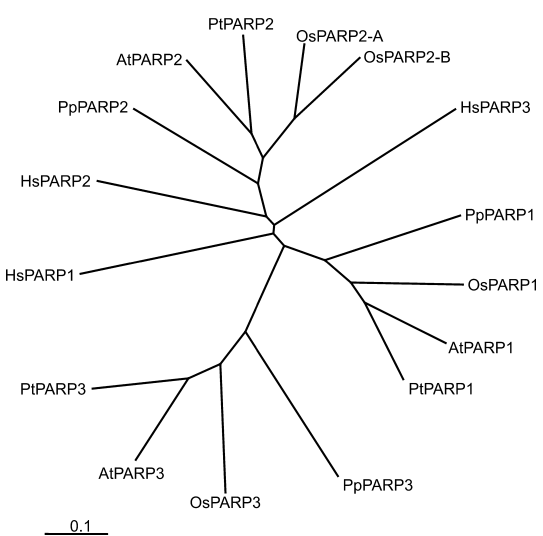

Fig. 1. PARP proteins are found in diverse plant species. A phylogenetic analysis was performed using the distance-based neighbour-joining method with Clustal W2. The tree was visualised with TreeView software. Full-length sequences of the PARP proteins were used for sequence alignment and aligned sequences were used to generate the phylogenetic tree. The scale bar of 0.1 represents 10% sequence divergence. The accession numbers of the sequences available in the NCBI database are as follows: *Populus trichocarpa*: PtPARP1 (XP_002302058), PtPARP2(XP_006375453), PtPARP3 (XP_002313672); *Oryza sativa*: OsPARP1 (NP_001059453), OsPARP2-A (Q5Z8Q9), OsPARP2-B (QOJMY1), OsPARP3 (NP_001047021); *Physcomitrella patens*: PpPARP1 (XP_001769471), PpPARP2 (XP_001782209), PpPARP3 (XP_001763226); *Homo sapiens*: HsPARP1 (NP_001609.2), HsPARP2 (NP_05475), HsPARP3 (NP001003931.2); *Arabidopsis thaliana*: AtPARP1 (NP_850165), AtPARP2 (NP_192148), AtPARP3 (NP_197639).

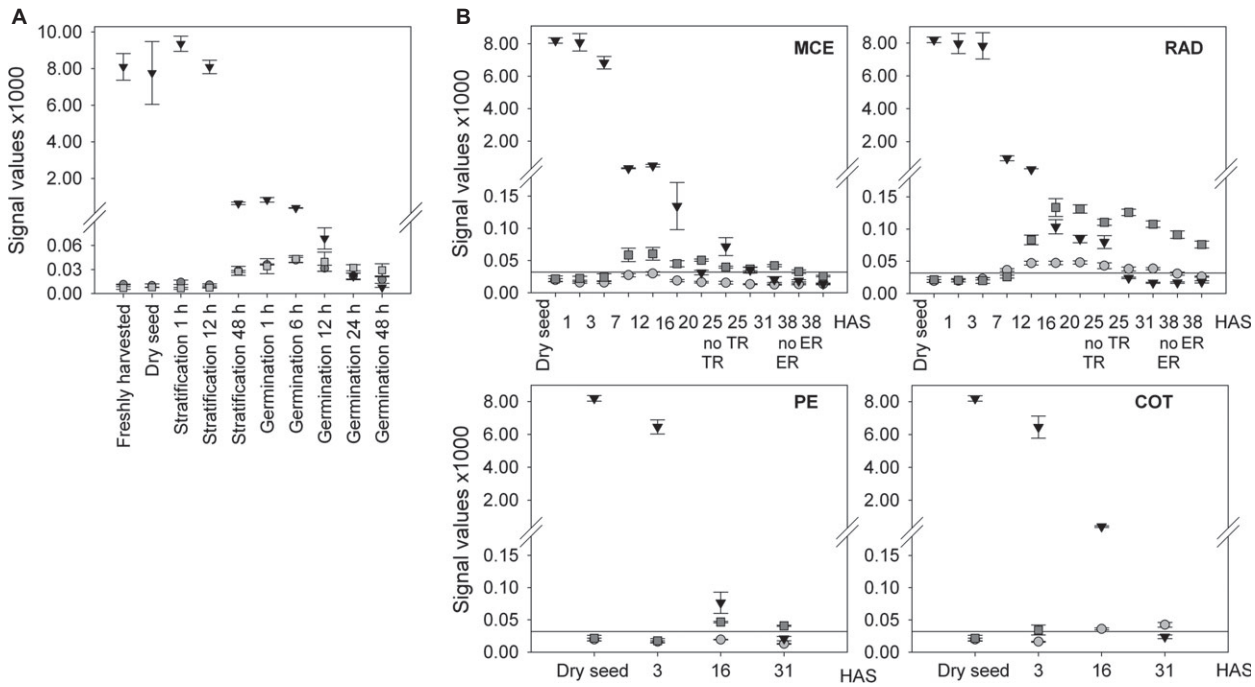

Fig. 2. Publicly available microarray data reveal high *AtPARP3* transcript levels in dry, imbibed and germinating seeds. A: Expression of *AtPARP1* (circles), *AtPARP2* (squares) and *AtPARP3* (triangles) during different stages of seed imbibition and germination. Data were retrieved from an assay described in Narsai *et al.* (2011). Data points marked with asterisks indicate an absent call ($P > 0.06$). B: Expression of *AtPARP1* (circles), *AtPARP2* (squares) and *AtPARP3* (triangles) in the micropylar and chalazal endosperm (MCE), the peripheral endosperm (PE), the radical and hypocotyl (RAD) and the cotyledons (COT). Gene expression was determined at different time points (hours after sowing, HAS) of seed germination. At 25 HAS, gene expression level was determined in both seeds with a ruptured testa (TR) and a non-ruptured testa (no TR). At 38 HAS, transcript level was determined prior to endosperm rupture (ER) and after endosperm rupture (no ER). Data were retrieved from an assay described in Dekkers *et al.* (2013).

Cloning of GUS and EYFP vectors

To generate the pBI101-PrPARPx-GUS vectors, the sequences including the 5′UTR and promoter of the three *AtPARP* genes were cloned from *Arabidopsis* Col-0 genomic DNA. For *AtPARP1* a sequence of 2034 bp and for *AtPARP2* a sequence of 2041 bp upstream of the ATG was PCR-amplified using *BamHI* restriction site-containing primers. To amplify *AtPARP3*, *XmaI* restriction site-containing primers were used, amplifying a sequence of 1961 bp upstream of the ATG (Table 1). All sequences were cloned upstream of the *uidA* gene into the binary vector pBI101.3 (Jefferson *et al.* 1987). *Arabidopsis* Col-0 plants were stably transformed with *Agrobacterium tumefaciens* strain GV3101 using the floral dip method (Clough & Bent 1998). Transformed plants were selected on half-strength MS agar plates containing 50 $mg \cdot l^{-1}$ kanamycin.

For the construction of an EYFP localisation vector, *AtPARP3* cDNA was obtained from 16-day-old *Arabidopsis* Col-0 plants, which were treated for 2 days with 250 mM NaCl to induce *AtPARP3* expression (Ogawa *et al.* 2009). Primers containing *XmaI* restriction sites (Table 1) were used to amplify *AtPARP3* without the stop codon. *AtPARP3* was cloned into the *XmaI* restriction site of the pART7 vector containing *EYFP* ligated into the *BamHI* restriction site of the plasmid (Peiter *et al.* 2007).

Germination and controlled seed deterioration assays

Seeds were collected from mutant and wild-type plants grown side-by-side in a controlled environment greenhouse. To analyse seed germination, seeds were surface-sterilised with 4% NaOCl (33.3% bleach) and 0.02% Triton X-100. Approximately 150 seeds per genotype were sown onto three half-strength MS agar plates (adjusted to pH 5.8 with 2.5 mM MES-KOH). Seeds were stratified for 2 days at 4 °C and plates were placed horizontally into a plant growth cabinet (AR-75; Percival Scientific, Perry, IA, USA) set to 16 h/8 h day/night, 22/18 °C, 65% RH, and a light intensity of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Seed germination was scored under a stereomicroscope (SteREO Discovery V.20; Carl Zeiss, Jena, Germany) at the indicated time points. Experiments were repeated three times. Statistically significant differences were determined using the *t*-test tool of the SigmaPlot10.0 (Systat Software, San Jose, CA, USA) software package.

A controlled deterioration assay was performed as described in Bentsink *et al.* (2000). In brief, seeds were equilibrated for 4 days at 15 °C and 85% RH. Control seeds were re-dried for 3 days at 20 °C and ambient humidity. Seed ageing was performed for 4 days at 40 °C and 85% RH, followed by re-drying as above. Seeds were stored at 4 °C. Germination assays were performed immediately after deterioration.

Histochemical estimation of *PARP* gene expression and ROS activity

To analyse *PARP* gene expression during germination, PrPARP1-GUS, PrPARP2-GUS and PrPARP3-GUS seeds were subjected to a germination assay. At indicated stages of germination, seeds were collected from agar plates and seed coats

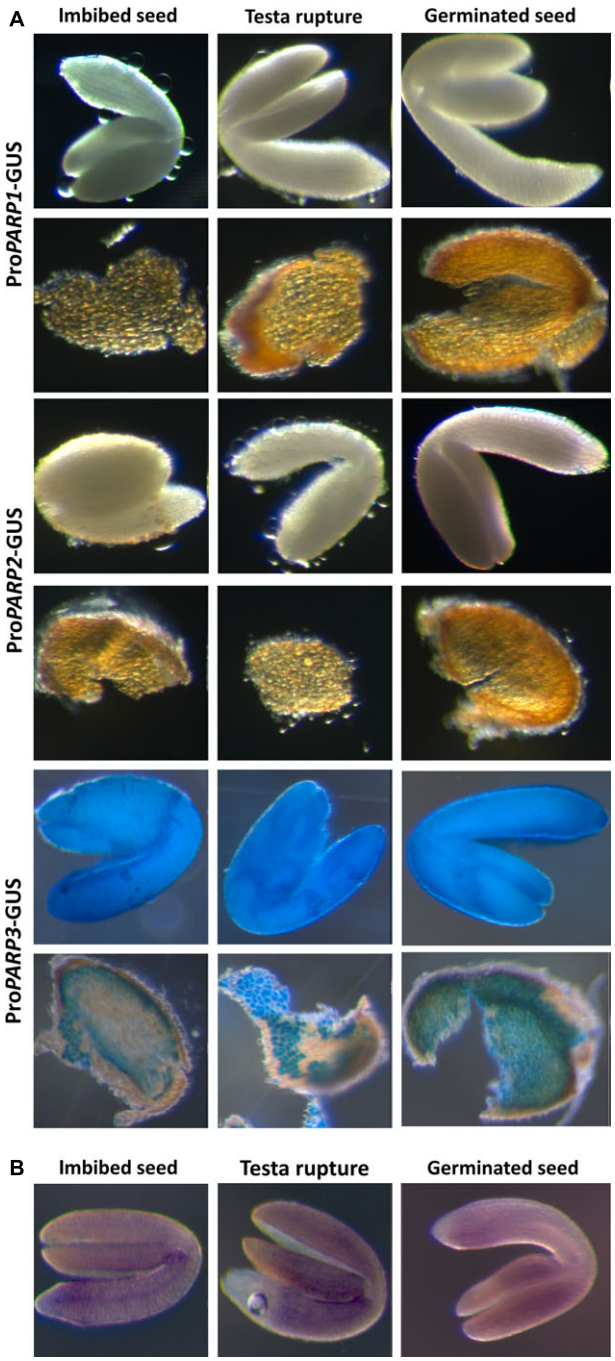

Fig. 3. *AtPARP3* expression and ROS activity coincide during seed germination. A: *AtPARP* promoter activity in embryo and endosperm tissues during different stages of seed germination. Gene expression was visualised with GUS staining. The seed coats were removed before staining. B: ROS activity during different stages of seed germination. ROS activity was visualised with NBT staining. The seed coats were removed before staining.

were removed under a stereomicroscope. Embryos and seed coats were transferred to GUS staining solution (100 mM sodium phosphate buffer (pH 7.0), 10 mM EDTA, 3 mM $K_4[Fe(CN)_6]$, 0.5 mM $K_3[Fe(CN)_6]$, 0.1% Triton X-100, 2 mM X-Gluc in DMSO). The staining solution was vacuum-infiltrated twice for 5 min, and PrPARP3-GUS tissues were further incubated for 30 min at 37 °C. PrPARP1-GUS and PrPARP2-GUS tissues were incubated for at least 16 h. Stained embryos were stored in 80% ethanol. To determine ROS activity in embryos of germinating *Arabidopsis* Col-0 seeds, embryos were incubated in 0.5 $mg \cdot ml^{-1}$ nitroblue tetrazolium (NBT) in 10 mM potassium phosphate buffer (pH 7.8) for 30 min at room temperature in the dark. Subsequently, seeds were stored in 70% glycerol. Pictures of GUS and ROS staining were taken using an Axiocam HRc digital camera (Carl Zeiss) mounted on a SteREO Discovery V.20 stereomicroscope.

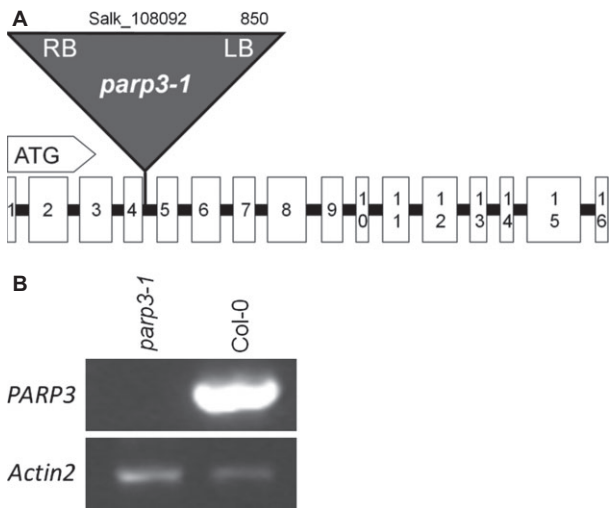

Fig. 4. Identification of a T-DNA insertion line for *AtPARP3*. A: Model of the *AtPARP3* genomic region and the T-DNA insertion. Coding regions are presented as white boxes; introns are shown with a line. The triangle indicates the site of the T-DNA insertion. B: RT-PCR analysis on RNA isolated from seeds showing the absence of the full-length *AtPARP3* transcript in the mutant line. *AtACT2* served as a control.

Localisation of AtPARP3 in mesophyll protoplasts

Mesophyll protoplasts were transformed with the pART7-PARP3-EYFP plasmid as described before (Peiter *et al.* 2005). After transformation, protoplasts were incubated for 20–24 h at 23 °C in the dark. EYFP fluorescence was visualised with confocal microscopy using a LSM 510META (Carl Zeiss).

RESULTS

Phylogenetic analysis

Homologues to the three *Arabidopsis* PARP proteins were present in all other plant species that we examined (Fig. 1). The moss *Physcomitrella patens* and the deciduous tree *Populus trichocarpa* possess one orthologue of each PARP, while the graminaceous crop rice (*Oryza sativa*) possesses one orthologue of PARP1 and PARP3, but two orthologues of PARP2.

Publicly accessible microarray data reveal high *AtPARP3* transcript levels in dry, imbibed and germinating seeds

A survey of publicly available microarray data showed a specific pattern of *PARP* gene expression in seeds. Data obtained by Narsai *et al.* (2011) revealed particularly high *AtPARP3*

expression in freshly harvested and dry seeds. During seed stratification and subsequent germination the expression level of *AtPARP3* decreased. At the time point of 48 h of germination, *AtPARP3* expression was absent from seed tissue (Fig. 2A). *AtPARP1* and *AtPARP2* transcripts were absent until 48 h of stratification. Subsequently, transcript levels slightly increased (Fig. 2A). Data from a high-resolution transcriptome analysis of Dekkers *et al.* (2013) confirmed this pattern of *PARP* gene expression during the germination process (Fig. 2B). *AtPARP1* and *AtPARP2* transcripts were absent in dry seeds and during early phases of imbibition, and remained low in subsequent phases. *AtPARP3* expression level was high in dry seeds, remained at that level during the first hours of imbibition, and decreased thereafter. This expression pattern was similar in micropylar and chalazal endosperm, peripheral endosperm, radicle and hypocotyl, and in cotyledons (Fig. 2B).

Expression of *AtPARP3* and ROS activity coincide in germinating embryos

Promoter-GUS analysis was employed to validate *AtPARP3* gene expression in germinating seeds and to obtain spatial information on promoter activity. During all stages of germination, high levels of promoter activity were visualised throughout the embryo and the endosperm (Fig. 3A). Particularly strong expression was found in the radical tip. This supported the notion of a role for *AtPARP3* during seed germination. *AtPARP1* and *AtPARP2* promoter activity in germinating seeds could not be shown in promoter-GUS analysis, supporting the very low transcript levels in the *in silico* data sets (Fig. 2).

Activity of ROS in embryos of germinating seeds was visualised with NBT staining. Similar to the GUS staining, ROS activity was evenly distributed in the germinating embryo during all stages (Fig. 3B). Hence, during germination *AtPARP3* gene expression and ROS activity coincided.

A knockout line was identified for *AtPARP3*

To analyse the function of *AtPARP3* in seeds, we identified a T-DNA insertion line, *parp3-1*, in the SALK collection. The *AtPARP3* gene consists of 16 exons and 15 introns. The left border of the T-DNA was found to localise in the fourth intron, 850 bp downstream of the ATG (Fig. 4A). According to PCR analysis, the right border is located upstream of the left border, towards the 5′-end of the gene. RT-PCR on RNA from dry seeds confirmed that no *AtPARP3* transcript was present in this T-DNA line (Fig. 4B).

Seeds of *parp3-1* are affected in storability

Seeds of the *parp3-1* mutant and its Col-0 wild type that had been stored for 8 months were subjected to a germination assay. The *parp3-1* mutant showed a clear delay in seed germination compared to the Col-0 seeds (Fig. 5A). To analyse whether this delay was linked to enhanced ABA sensitivity or reduced seed storability, germination of freshly harvested seeds was studied. Under control conditions, freshly harvested *parp3-1* mutant seeds did not show the germination delay (Fig. 5B). Treatment of seeds with 5 μM ABA prolonged the germination process in both genotypes and reduced the germi-

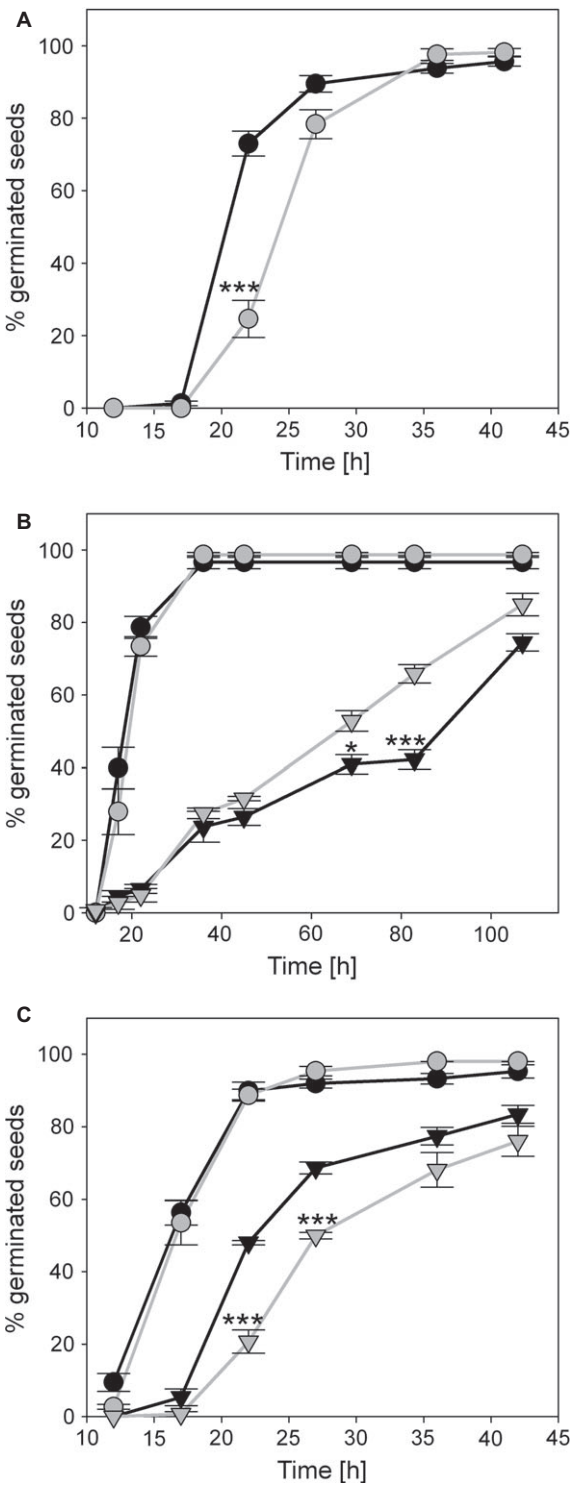

Fig. 5. Stored *parp3-1* mutant seeds show a delay in germination, which is due to accelerated deterioration. A: Germination of stored Col-0 (black) and *parp3-1* (grey) seeds on half-strength MS medium. B: Germination of Col-0 (black) and *parp3-1* (grey) seeds on half-strength MS medium (circles) or on half-strength MS medium supplemented with 5 μM ABA (triangles). C: Germination of freshly harvested Col-0 (black) and *parp3-1* (grey) seeds after equilibration (circles) or after artificial seed ageing (triangles). Data are means ± SE of 150 seeds. The experiments were repeated three times with comparable results. The asterisks mark significant differences (***$P < 0.005$, *$P < 0.05$).

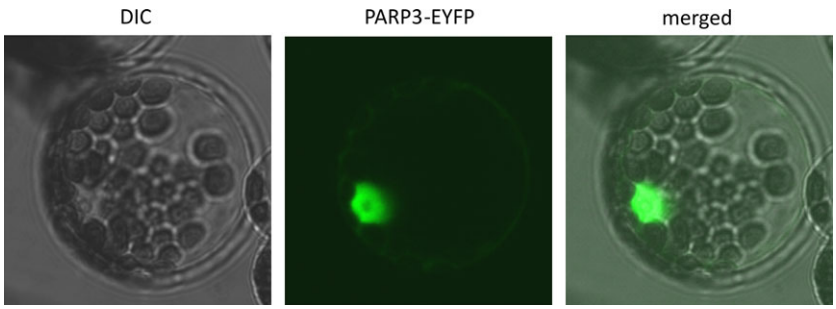

Fig. 6. Confocal image of an *Arabidopsis* mesophyll protoplast transiently expressing a *PARP3-EYFP* fusion construct.

nation rate to approx. 80%. Surprisingly, *parp3-1* mutant seeds even showed slightly accelerated germination compared to Col-0 seeds (Fig. 5B). The freshly harvested seeds underwent a controlled deterioration treatment. Thereby seeds were first equilibrated at relatively low temperature and high humidity, followed by artificial ageing under high temperature and high humidity conditions. Equilibration did not affect the germination of freshly harvested *parp3-1* and Col-0 seeds (Fig. 5C), whereas artificial seed ageing reduced the germination of both genotypes. However, this reduction was more pronounced in the *parp3-1* mutant seeds.

The *AtPARP3* protein is localised to the nucleus

The localisation of plant PARP3 proteins has not been determined previously. As the observed germination phenotype is in line with a role of the protein in genome stability, it was localised in transiently transformed mesophyll protoplasts using an AtPARP3-EYFP fusion construct. Similar to AtPARP1 and AtPARP2, AtPARP3 was localised in the nucleus, supporting a function in DNA repair (Fig. 6).

DISCUSSION

Recently, AtPARP3 has been hypothesised to be involved in DNA damage repair in stored seeds, but such a role in maintaining viability has not been demonstrated hitherto (Hunt *et al.* 2007; Hunt & Gray 2009). In this study, we showed high *AtPARP3* promoter activity throughout the plant embryo coinciding with ROS activity during germination. Promoter activities of *AtPARP1* and *AtPARP2* were below the threshold of detection with histochemical GUS staining, albeit in publicly available microarray data sets their expression was shown to increase slightly above the background during germination (Figs 2 and 3). This indicated an important and specific role of *AtPARP3* in the repair of ROS-induced DNA damage, which accumulates during seed storage and/or occurs during germination. This hypothesis is further supported by the fact that we identified a nuclear localisation signal in the PARP3 protein sequence from NLSMapper prediction (Kosugi *et al.* 2009). In support of nuclear localisation, the PARP3-EYFP fusion construct was localised in the nucleus of mesophyll cells (Fig. 6).

Interestingly, PARP3 is present in all plant species that we examined (Fig. 1). An expression of this gene during the very early stages of germination, similar to the pattern we observed in *Arabidopsis*, has also been demonstrated for the rice orthologue (Howell *et al.* 2009). This indicates that the role of PARP3 during germination may be widely conserved throughout the plant kingdom.

Like HsPARP1, *AtPARP1* contains two Zn-finger domains, which are responsible for the sensing of DNA single strand nicks (Eustermann *et al.* 2011). According to ClustalW alignment, *AtPARP3* shows 31% sequence identity to AtPARP1, and both proteins share several functional domains, *i.e.* PADR1, BRCT and WGR, as well as the regulatory and catalytic PARP domains. However, *AtPARP3* lacks the two DNA-binding Zn-finger domains of *AtPARP1*. Similarly, HsPARP3, which was attributed a specific role in double strand break repair *via* acceleration of the NHEJ pathway (Rouleau *et al.* 2007; Rulten *et al.* 2011), also lacks Zn-finger domains. It is therefore tempting to infer that AtPARP3 plays an important role in DNA double strand break repair in seeds.

The DNA double strand break repair by HsPARP3 is conferred *via* an interaction with the Ku70/Ku80 heterodimer and recruitment of the XRCC4/DNA ligase4 complex (Rouleau *et al.* 2007; Rulten *et al.* 2011). The Ku70/Ku80 complex may also be an interaction partner for AtPARP3. Seeds of *ku70* mutant *Arabidopsis* plants were shown to exhibit reduced germination potential on MMS, an alkylating agent inducing single strand breaks (Riha *et al.* 2002). During DNA replication these single strand breaks are converted into double strand breaks. These are then repaired by NHEJ before cell cycle progression from G1 to S phase (Riha *et al.* 2002). Moreover, *AtPARP3* possibly also recruits the DNA ligases AtLIG4 and AtLIG6 to sites of DNA double strand breaks. DNA ligase LIG4 has been described to play a specific role in NHEJ in humans (Rulten *et al.* 2011). LIG6 is a plant-specific enzyme. Seeds carrying mutations for either or both genes are affected in germination, similar to *parp3-1* seeds (Waterworth *et al.* 2010).

The stored *parp3-1* seeds showed delayed germination compared to Col-0 seeds (Fig. 5A). It was tested whether this phenotype might be linked to enhanced ABA sensitivity or to reduced seed viability as a consequence of seed storage. Freshly harvested *parp3-1* seeds exhibited accelerated germination as compared to the wild type on 5 μM ABA (Fig. 5B). In contrast, artificial ageing of the *parp3-1* seeds evoked a similar phenotype as seed storage (Fig. 5C). Seed storage and artificial seed ageing are known to provoke DNA damage, which can finally result in programmed cell death. In *parp3-1* mutant seeds, the initiation of DNA double strand break repair is likely to be restricted, which would affect cell cycle progression and hence lead to the observed delay in seed germination and reduced seed viability.

Seed viability strongly depends on DNA integrity. During storage, DNA damage accumulates and has to be repaired during

germination. Structural homology to AtPARP1 and HsPARP3 suggests a role for AtPARP3 in DNA double strand break repair *via* NHEJ. Further research is now necessary to better understand the DNA double strand break response in germinating seeds. In particular, possible interactions between AtPARP3 and components of the NHEJ pathway need to be determined.

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