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AtNG1 encodes a protein that is required for seed germination

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ABSTRACT

The pentatricopeptide repeat (PPR) family of eukaryotic proteins has numerous members in plants and is important for plant development. In the present study, we cloned a novel PPR gene, designated *AtNG1*, and characterized the *ng1* Arabidopsis mutant. Morphological and structural observation of an *ng1* mutant revealed that its sexual reproduction and seed formation processes are essentially normal. The mature embryonic root of *ng1* is fully developed and has a well-differentiated structure; however, *ng1* seeds cannot germinate, even when supplied with supplemental hormones and nutrition. Further investigation showed that embryo expansion and root cell elongation fails to occur after water imbibitions. Transient gene expression analysis indicated that AtNG1 localizes in mitochondrion. This implies that the deficiency of mitochondrion function might be the reason for the failed seed germination. Thus, our finding confirmed that AtNG1 plays a critical role in the early process of seed germination.

1. Introduction

The pentatricopeptide repeat (PPR) family of eukaryotic proteins, which was first recognized several years ago [1,2], is greatly expanded in plants; most plant species, including Arabidopsis, rice, and grape, have more than 400 members [3,4]. PPR proteins contain up to 26 repeats of a pentatricopeptide motif of ~35 amino acids [3] and are grouped into two major subfamilies, P and PLS, based on the nature of these motifs [1]. They have been found to participate in RNA editing, splicing, cleavage, and translation in mitochondria and plastids, and have been proposed to possess sequence-specific RNA-binding activity that allows them to act as adaptors that bring executors to target RNAs, although structural evidence for this hypothesis is lacking [3].

Although PPR proteins are numerous in plants, few functional redundancies among these proteins have been found [3]. Because many PPR proteins are essential for plant development, a single gene mutation in a PPR gene usually has a strong phenotypic effect. Several PPR genes restore fertility in cytoplasmic male sterility (CMS) lines of crop species, which have mutations in their mitochondrial DNA that prevent the production of functional pollen [5–9]. In Arabidopsis, many members of the PPR family, such as EMB175 [10] and GRP23 [11], are required for embryogenesis or vegetative growth [3]. Systematic studies of EMB genes recently have characterized dozens of PPR proteins, which are required for embryo development [12–16].

Some *PPR* mutants, such as *otp43* and *ppr40* in Arabidopsis and *ogr1* in rice, exhibit defective seed germination [17–19], an essential part of the plant life cycle. Their germination phenotypes are cause by abnormal embryo development (*otp43* and *ogr1*) or enhanced stress sensitivity (*ppr40*). Thus, as studies on light, temperature, saccharides, gibberellins and abscisic acid that regulate seed germination [20,21], studies on PPR proteins offers new clue for the investigation of molecular mechanism of seed germination. Here, we describe a new member of the PPR gene family, *At1g62720*, which is also required for seed germination in Arabidopsis.

Seed germination normally begins with water imbibition, which is followed by embryo expansion and embryo root elongation via cell growth [20–22], and finally by protrusion of the root tip through the seed coat so that it makes contact with the environment. A mutation in *At1g62720* appears to prevent activation of the seed germination process without causing any significant defects in the morphology or development of the mature embryo.

2. Materials and methods

2.1. Plant material

Arabidopsis (Columbia type) seeds were sterilized with 20% bleach for 5 min, washed five times in sterilized water, and plated on half-strength (0.5×) Murashige and Skoog basal medium [23] (MS) with 50 μg/mL hygromycin, as required. They were chilled at 4 °C for 4 days and then placed in 22 °C chambers to grow under a 16-h light/8-h dark cycle. After 2 weeks of growth, plants

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were moved to a greenhouse to continue growing under the same conditions. Arabidopsis transformation was performed via *Agrobacterium*-mediated infiltration [24].

2.2. Genetic analysis and complementation

The sequences flanking the T-DNA insertion sites were isolated by thermal asymmetric interlaced PCR as described previously [25]. The mutants were characterized by PCR amplification of the fragments of interest using primer L14-17 (5′-GTGAA ACAAG GTGGA CGATT AG-3′) in combination with either primer L1402 (5′-GTTGT TGGGA AGATG ATGAA GT-3′) or the T-DNA left-border primer gfpLB3 (5′-AATAG AGTAG ATGCC GACCG GATCT G-3′).

For complementation experiments, a 2814-bp genomic fragment containing *NG1* (from 919 bp upstream of the ATG start codon [nt −918] to 413 bp downstream of the TAA stop codon) was PCR-amplified using Phusion polymerase (Finnzymes) with the primers L14-1 (5′-NNNGG TACCT GGTGT CTGTA GTTTC CATAT CCAG-3′) and L14-2 (5′-NNNGC GGCCG CAAGA GAAGG ACTAA GCAAT GTCGT G-3′). The resulting fragment was cloned into plasmid pART27 to produce pART27-NG1C. After sequence verification, this construct was introduced into heterozygous *ng1* mutant plants by *Agrobacterium*-mediated infiltration [24]. Transformants were obtained by double selection on 0.5× MS–agar plates containing 50 μg/mL each kanamycin and hygromycin. Transformants were analyzed by 1% agarose gel electrophoresis and ethidium bromide staining of PCR-amplified fragments produced using primers L14-6 (5′-NNNCT GCAGT AATGG AAGAA GCCCA TCTTC TTG-3′) and L1402.

2.3. Phenotype analysis

The phenotypes of seeds produced by selfed *ng1* heterozygotes and of F_1 seeds produced in complementation tests were analyzed by genetic experiments and differential interference contrast (DIC) microscopy. Seed germination ratios were analyzed on 0.5× MS medium supplemented as required for selection. Seeds were dissected with hypodermic needles to remove seed coats and cleared in Hoyer's solution (prepared as 30 g gum arabic, 200 g chloral hydrate, 20 g glycerol, and 50 mL distilled water). Tissue morphology and cell development ware studied under a Zeiss Axioskop II microscope equipped with DIC optics. Micrographs were obtained using a Nikon 4500 digital camera. The morphology of other tissues of the mutant plants was also carefully observed. Root cell length was measured using Image J, and statistical analysis was performed using the SPSS software package.

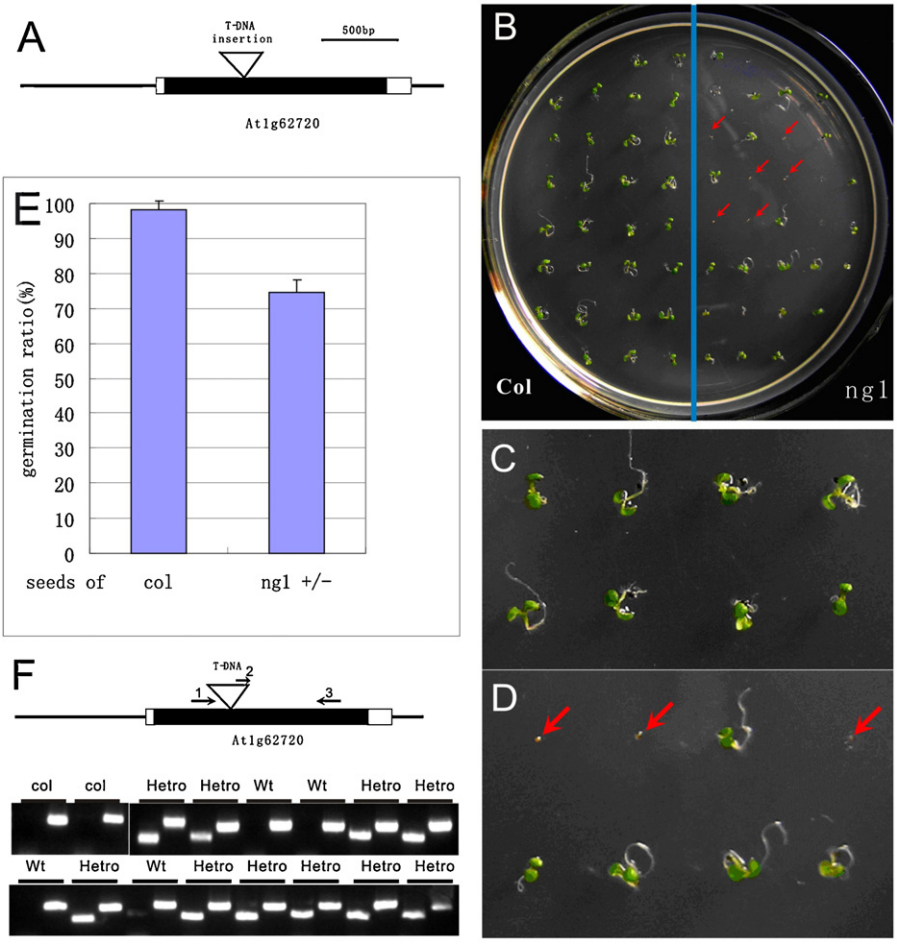

Fig. 1. Identification of the *ng1* mutant. (A) The structure indication of At1g62720 and insertion site of the T-DNA. Black rectangle: open reading frame; white rectangle: UTR region. (B) Comparison of seed germination in wild-type (col, left) and ng1 heterozygous mutant (ng1, right) after 4 days on half strength MS medium. A part of mutant seeds don't germinate (red arrows). (C) and (D) magnifications of (B). (C) Seed germination of wild type. (D) Seed germination of *ng1* heterozygous mutant. Red arrows show non-germinated seeds. (E) Germination ratios of seeds after 4 days on half strength MS medium. About three quarters of heterozygous mutant seeds (ng1+/−) germinated, the ratio is significantly lower than that of wild type control (col). Error bars indicate SD of three independent experiments (n = 280–350). (F) Germinated offspring of *ng1* heterozygous mutant are characterized by PCR. They were found to be heterozygous (hetro) or without T-DNA insertion (wt). Samples of wild type were used as control (col). Black arrows show primer-pairing sites. Left bands of each sample are products of primer 2 and 3. Right bands of each sample are products of primer 1 and 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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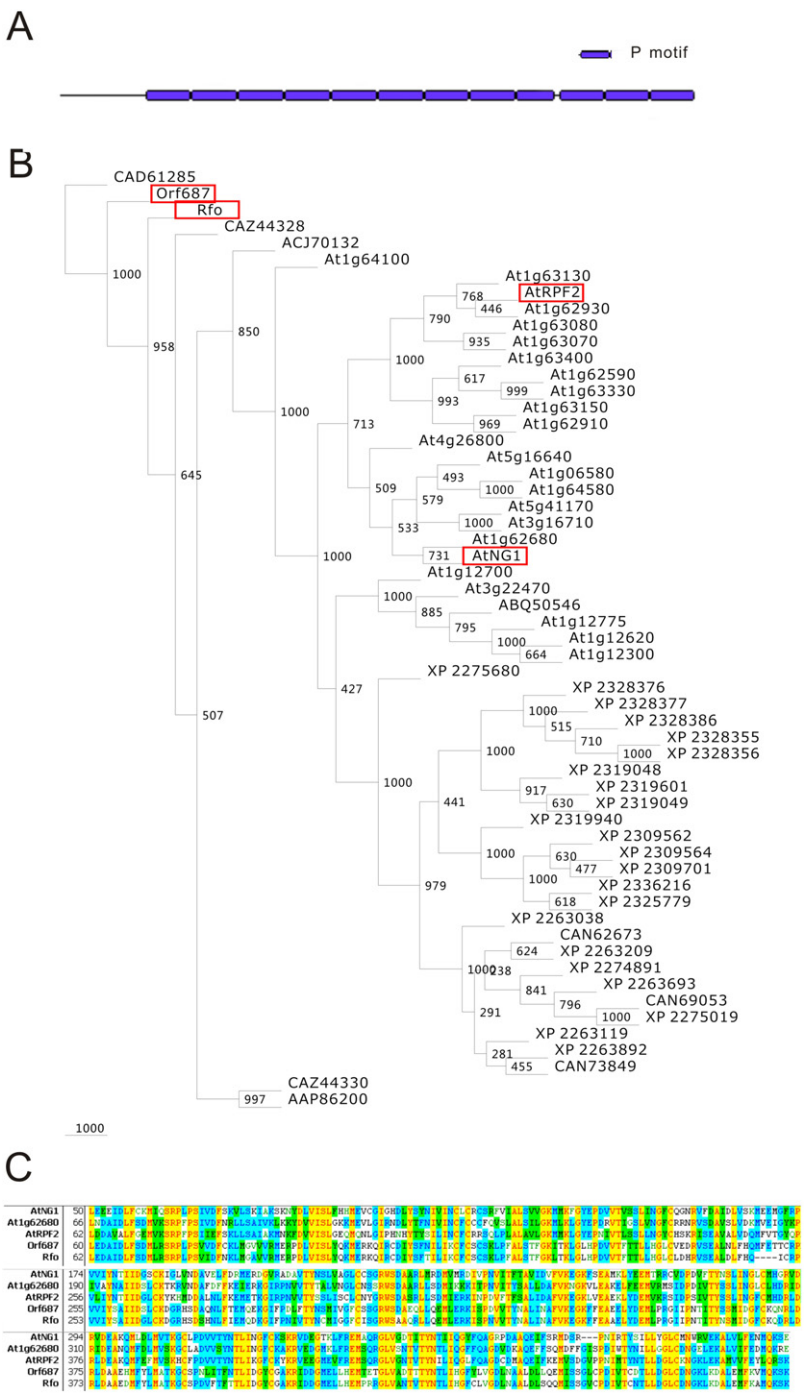

Fig. 2. The relationship of AtNG1 with other PPRs. (A) Predicted protein motifs in AtNG1. Rectangles mean 12 P motifs found in AtNG1. (B) Phylogenic tree of AtNG1 showing 56 homologous proteins. Three proteins characterized before. AtNG1 are marked by rectangles. (C) The similarity in protein sequences between AtNG1, At1g62680, AtRPF2, Rfo and Orf687.

2.4. RNA isolation and RT-PCR analysis

Total RNA was isolated from Arabidopsis (Columbia) plants using Trizol reagent (Invitrogen). Total RNA (1 μg) was digested with RNase-free DNase (TaKaRa) and reverse-transcribed to cDNA using Reverse Transcriptase XL (TaKaRa). One microliter of the synthesized cDNA was used as a template for PCR amplification of *NG1* with the primers L1402 and L14-7 (5′-AGCCG AGCTG CATCA CTCC-3′) using 35 amplification cycles. The PCR products were resolved on a 1% agarose gel and stained with ethidium bromide.

2.5. Construction of an NG1-β-glucuronidase (GUS) fusion construct and GUS reporter activity assays of transgenic plants

The genomic fragment containing the 918 bp upstream of the *NG1* ATG start codon (nt −918 to 0) was amplified by PCR using the primer pairs L14-1 (5′-NNNGG TACCT GGTGT CTGTA GTTTC CATAT CCAG-3′) and L14-4 (5′-NNNCT GCAGT TGAAT TATTA ACCTC GTTTT ATTCG-3′). The resulting product was cloned into the KpnI and PstI sites (underlined) of the vector pCambia in the correct orientation to produce pNG1-GUS containing an *NG1* promoter–*GUS* reporter gene fusion.

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After pNG1-GUS was introduced into Arabidopsis, transgenic plants were selected on MS agar plates containing 50 μg/mL hygromycin and treated with GUS staining buffer (950 mM sodium phosphate buffer, pH 7.0, 0.1% Triton X-100, 2 mM ferricyanide, 2 mM ferrocyanide, and 2 mM 5-bromo-4-chloro-3-indolyl glucuronide [Sigma–Aldrich]) for 3 days at 37 °C.

2.6. Transient expression assay and sub-cellular localization of AtNG1

All constructs in our expression assay were based on the pCambia1300 plasmid. Full length ORF of AtNG1 was PCR amplified and fused with red fluorescent protein (RFP [26]). The N-terminal peptide from F1-ATPase δ subunit [27] was fused with eGFP (mtGFP) and used for labeling of mitochondrion. Both were driven by the cauliflower mosaic virus (CaMV) 35S promoter. Tested plasmids were coated onto gold particle and transferred into onion cells by particle bombardment [28]. Protein expression and localization were then characterized on microscopes after 10 h culture in a dark incubator.

2.7. Bioinformatic analysis

We performed a BLAST search of the NCBI database with the NG1 protein sequence and aligned and clustered the returned homologous sequences using the ClustalW 2.0.10 program [29] with default parameters. The consensus sequence of the pentatricopeptide domains was calculated using the Omiga 2.0 program.

3. Results

3.1. A PPR gene mutation completely blocks seed germination in Arabidopsis

We previously created an Arabidopsis mutant library containing several mutants with mutations in genes for PPR proteins. When we analyzed the effect of one of these mutant genes on plant development, we found that homozygous seedling was totally lacking, and the process of sexual reproduction, including gamete development and fertilization, was essentially normal in heterozygous plant (Fig. S1 and Table S1). The heterozygous mutant, which contained a T-DNA insertion in *At1g62720* (Fig. 1A), also exhibited no significant phenotypic abnormalities in embryogenesis (Figs. S2 and S3). The seeds harvested from heterozygous plants were not morphologically distinguishable from the control (Fig. S4). However, when these seeds were incubated on half-strength MS germination medium to characterize the segregation ratio, nearly one-quarter of them completely failed to germinate (n = 280–350, 3 repeats; Fig. 1B–E) because the embryonic root was unable to pierce the seed coat (Fig. 1B and D). This mutant was thus designated *ng1* for "non-germination 1".

After a 4-day germination period in half-strength MS medium, the germination ratio of *ng1*-heterozygous offspring was 74.64%, in accordance with a segregation ratio of 3:1 (χ^2 test, $P > 0.26$) and significantly lower than that of the wild-type control (98.71%, $P < 0.001$) (Fig. 1E). PCR characterization of the *At1g62720* locus of more than 200 of the germinated seedlings showed that all were either heterozygous or lacked the T-DNA insertion (Fig. 1F), suggesting that all homozygous mutant seeds were unable to germinate. Because no homozygous mutants were available, further analyses were all performed using heterozygous plants or seeds from them.

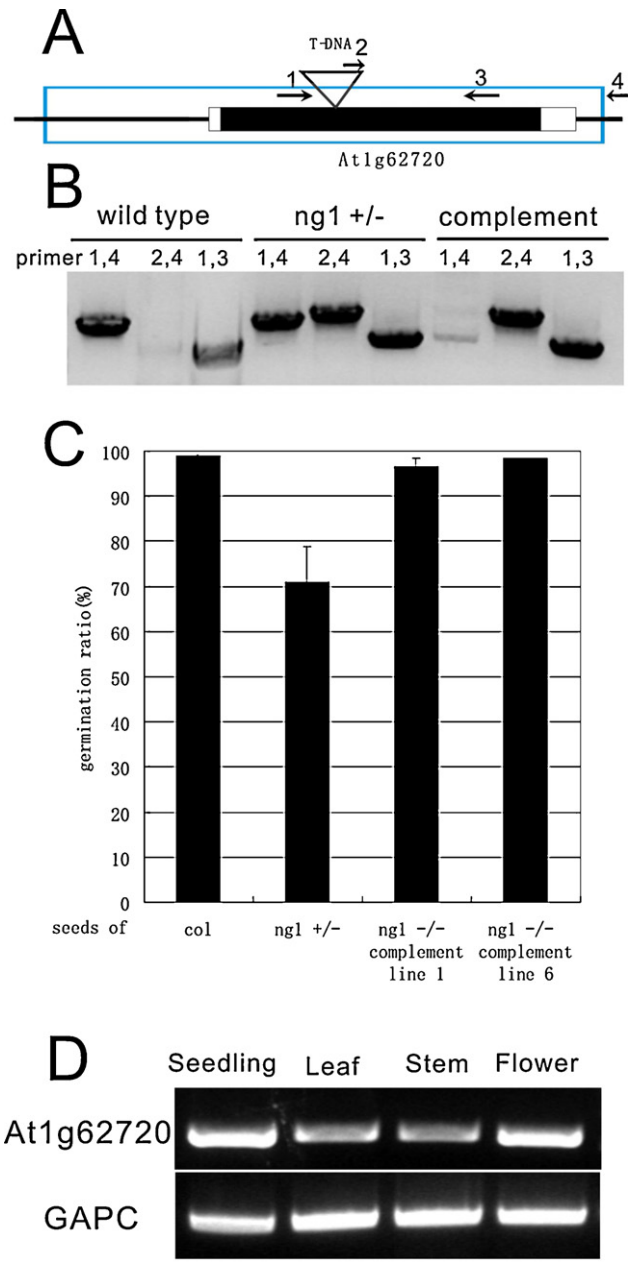

Fig. 3. Complementation test of *ng1* mutant and RT-PCR for AtNG1 expression pattern. (A) The genomic region (bigger rectangle) used for complementation test. Black arrows indicate the sites of primers used for PCR characterization. (B) PCR characterization of complementation lines. Wild type and *ng1* heterozygous mutant (ng1+/−) were used as controls. Left bands: products of primer 1 and 4; middle bands: products of primer 2 and 4 (for the demonstration of heterozygous or homozygous mutant of *ng1*). Right bands: products of primer 1 and 3 (for demonstration for copies of complement DNA in background of *ng1*−/−). (C) Germination ratios of complementation lines. They are the same as that of wild type control (col) but significantly different from the germination ratios of heterozygous *ng1* mutant offspring (*ng1*+/−). Error bars indicate SD of three independent experiments (n = 200–350). (D) RT-PCR for expression pattern of *AtNG1*. *AtGAPC* was used as control. Electrophoresis of DNA bands was operated after 35 cycles of PCR amplification.

3.2. Characterization of AtNG1

The coding region of *AtNG1*, which harbors only one exon, encodes a protein of 485 amino acids containing 12 PPR motifs (Fig. 2A). The PPR motifs are conserved in length, thus placing NG1 in the P subfamily. The *ng1* mutant contains an insertion in codon 188 that might truncate the protein before the fourth PPR motif.

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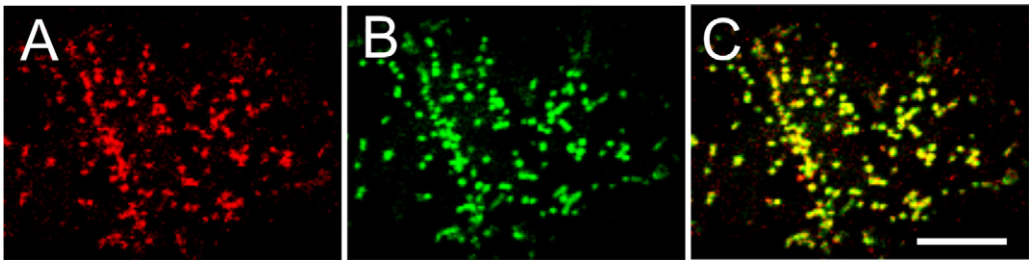

Fig. 4. Sub-cellular localization of AtNG1 in onion cells. (A) Localization of AtNG1:eGFP. (B) Localization of mitochondrion marker mtGFP. (C) Merged images of A and B, showing co-localization of AtNG1:eGFP and mtGFP. The bar = 10 μm.

A BLASTx search of the NCBI protein database using the AtNG1 sequence returned 56 highly homologous proteins ($<e^{-90}$; Fig. 2B). Three of these proteins had been characterized previously. Among them RFO [30] or ORF687 [31] restores pollen fertility in radish, and Arabidopsis RNA processing factor 2 (AtRPF2) participates in processing of mitochondrial RNAs [32]. Although AtNG1 possesses many homologs in Arabidopsis (for example, At1g62680, Fig. 2C), single mutation of this gene caused severe abnormality, suggesting its biological role is not substitutable.

3.3. Complementation testing and expression pattern analysis

To confirm that the germination-blocked phenotype of the *ng1* mutant is indeed the result of a T-DNA insertion within *AtNG1*, we performed a genetic complementation test. The 2814-bp genomic DNA fragment extending from 918 nucleotide upstream of *AtNG1* to 413 nucleotide downstream of the TAA stop codon was amplified and cloned into pART27 (Fig. 3A). *Agrobacterium*-mediated infiltration was used to transform the resulting construct into

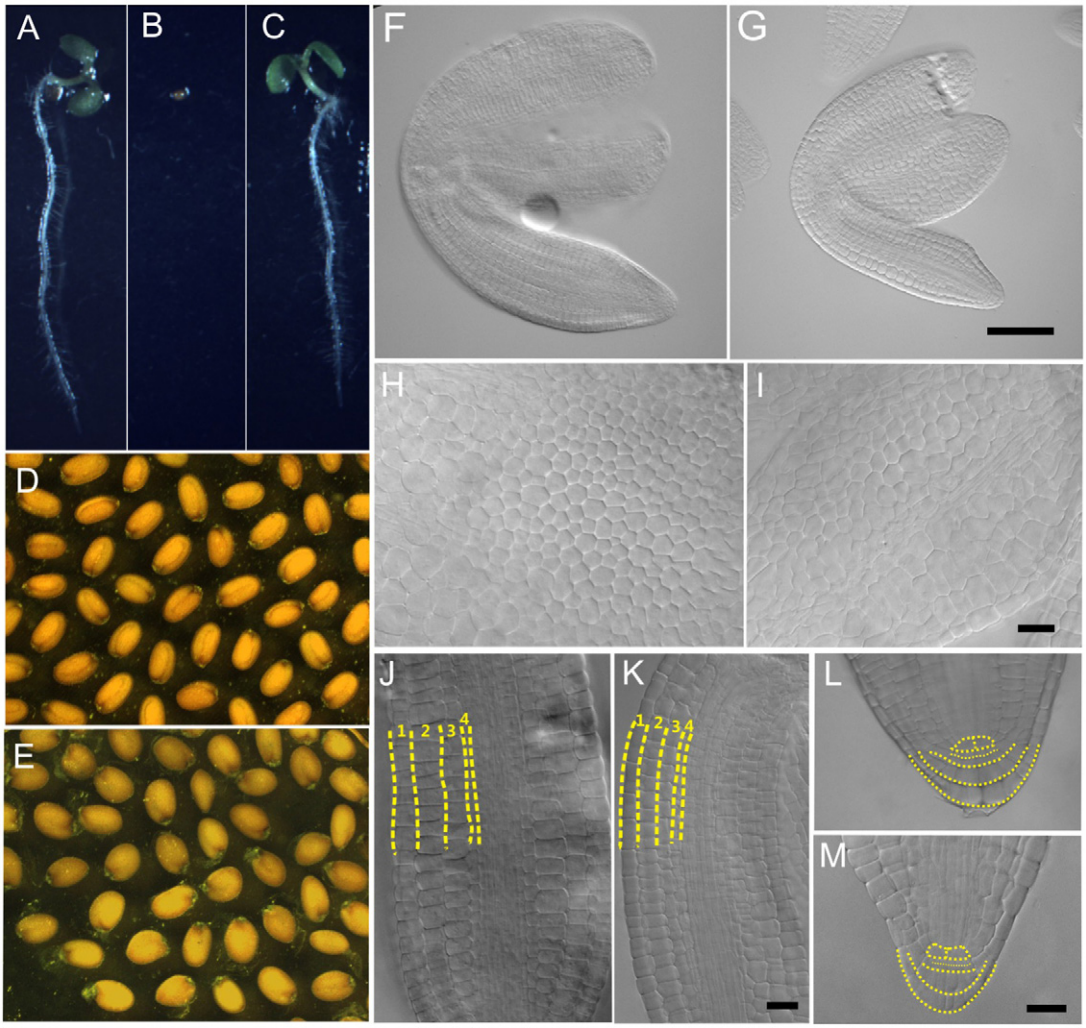

Fig. 5. Morphology and cell development of the ng1 mutant. (A)–(C) Seed germination after 6 days on medium. These samples are wild type control (A), non-germinated seed (B) and germinated seeds (C) of *ng1* heterozygous offspring. (D) and (E) Morphology of wild type seeds after water imbibition (D) and non-germinated seeds of *ng1* mutant after germination for 4 days on medium. (F)–(M) Morphology and cell development observed via whole mount clearing. These samples are seed (F), cotyledon (H), root (J) and root tip (L) of wild type seeds (col) after water imbibition; seed (G), cotyledon (I), root (K) and root tip (M) of *ng1* non-germinated seeds after 4 days on medium. The dotted lines indicate different cell layers in root. The bar = 100 μm in (F) and (G) while 20 μm in (H)–(M).

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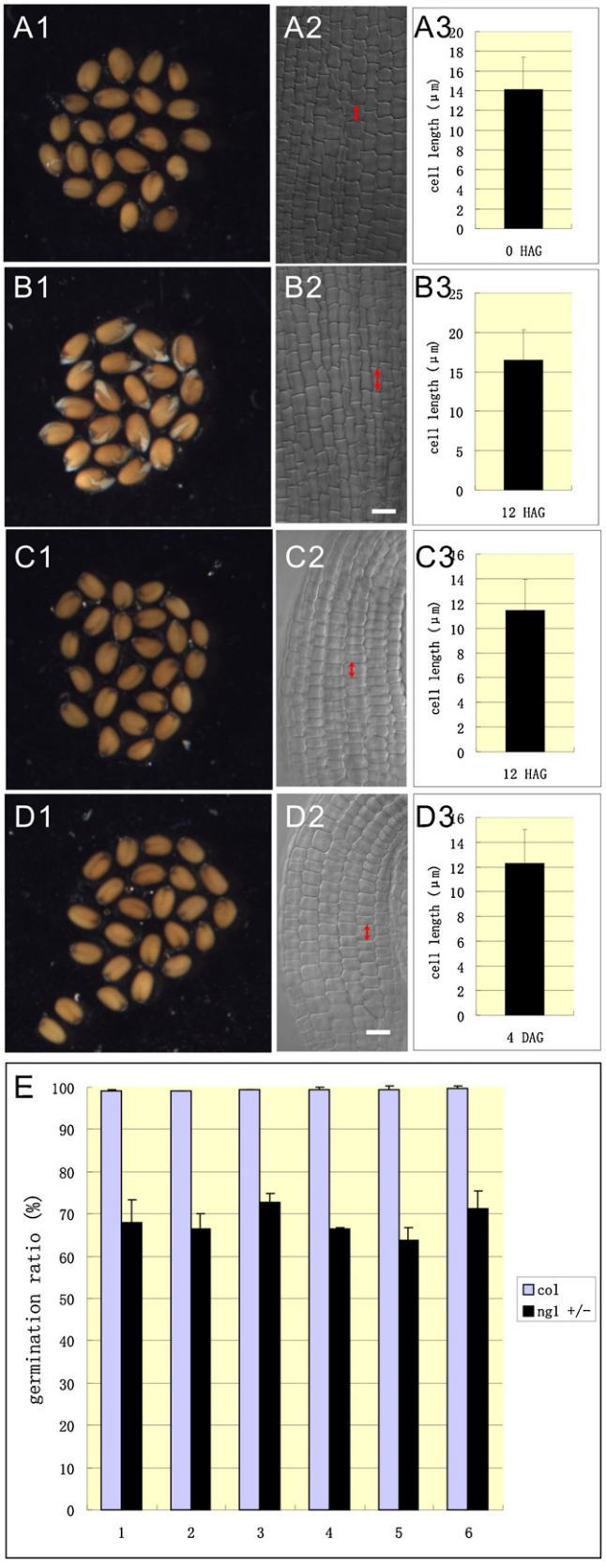

Fig. 6. Characterization of cytological factors for seed germination. (A)–(D) Morphology and measurement of the root epidermal cells in wild type (col) seeds after 0 h (A) or 12 h (B) germination, and *ng1* non-germinated seeds after 12 h (C) or 4 days (D) germination on half strength MS medium. Morphology of seeds is shown in (A1), (B1), (C1) and (D1). Morphology of seed epidermal cells is shown in (A2), (B2), (C2) and (D2). Bar = 20 μm in (A2), (B2), (C2) and (D2). Lengths of epidermal cells are measured by Image J and shown in (A3), (B3), (C3) and (D3). C3 or D3 are significantly different to A3 and B3 ($P < 0.01$). Error bars indicate SD of three independent repeats ($n = 100$–150). (E) Seed germination ratios on different media. These germination media are 1/2 MS containing 1% sucrose (1), 1% glucose (2), 1% maltose (3), 1% sucrose + 1 μM NAA (4), 1% sucrose + 10 μM GA_3 (5), and 1% sucrose + 1 μM 6-BA (6). Error bars indicate SD of three independent experiments ($n = 350$–600).

heterozygous *ng1* mutants. Seven transgenic lines were obtained by antibiotic selection and characterized by gel electrophoresis of PCR-amplified fragments of their genomes (Fig. 3B). Analysis of their germination ratios on 0.5× MS medium revealed that the level of seed germination was restored to that of the wild-type control ($P > 0.15$; Fig. 3C), confirming that loss of function of *AtNG1* is the cause of the germination-blocked *ng1* phenotype.

We then investigated the expression pattern of the *AtNG1* gene using reverse transcription–PCR analysis of total RNA isolated from various organs, including shoots, stems, leaves, and germinated seedlings. As shown in Fig. 3D, 35 cycles of PCR amplification yielded detectable levels of fragments of the expected size for all of these tissues. Next, we used pNG1::GUS and pNG1::green fluorescent protein (GFP) reporter systems to monitor the activity of the *AtNG1* gene promoter in various tissues. When the 918-bp promoter region of *AtNG1* was used to drive the GUS reporter gene, no or very little reporter signal was detected in more than 20 lines, possibly due to very weak expression.

By the aid of analytical tools, iPSORT and TargetP, we predicted that AtNG1 might be located in mitochondrion. To verify this prediction, we used mitochondrion marker, mtGFP [27], for labeling of this organelle. By the simultaneous expression of AtNG1:RFP and mtGFP in onion cells, we found these two proteins were co-localized, demonstrating that AtNG1 was indeed localized in mitochondrion (Fig. 4A–C).

3.4. Microscopic observation and analysis of possible factors involved in the seed germination defect

We further investigated the *ng1* heterozygous offspring for possible clues to the germination-blocking mechanism in these plants. The heterozygous *ng1* seedlings (Fig. 5A–C) and non-germinated *ng1* seeds appeared morphologically similar to those of the wild-type control, and the non-germinated *ng1* seeds were also similar in size to the non-germinated wild-type seeds (Fig. 5D and E). After removing the seed coats, we investigated homozygous *ng1* and wild-type seed cell and tissue development using whole-mount microscopy with clearing (Fig. 5F–M). The *ng1* and wild-type samples did not exhibit any significant differences in root tip structure or in the organization of the different root cell layers, suggesting that the *ng1* embryonic root develops normally. Thus, the failure of homozygous *ng1* seeds to germinate is not attributable to a defect in root structure.

When germination conditions were extended until the embryonic roots of the wild-type plants were protruding from the seed coats, the embryonic roots of the homozygous *ng1* mutant remained inside the seeds (Fig. 6A–D). Because cell extension in the radicle is critical for seed germination [22], we measured the lengths of the root epidermal cells. After 12 h in germination medium, the root epidermal cells of wild-type plants, but not of homozygous *ng1* seeds, were elongated ($P < 0.001$; Fig. 6A–D), indicating that radicle cell elongation had not yet been triggered in the mutant.

We then examined the *ng1* mutant for a correctable physiological defect by altering the seed germination conditions. Generally, the germination process is regulated by hormones (e.g., gibberellins and auxins) and saccharides (e.g., glucose) [20,21]. Gibberellins often act together with cytokinins to promote germination [33], and auxins regulate the activities of expansions, which increase

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the extensibility of radicle cell walls and thus promote root elongation [22]. Therefore, we examined whether hormone or saccharide supplementation of the germination medium would promote the germination of heterozygous *ng1* offspring. However, the defect in seed germination was not reversed by any of the tested factors ($P > 0.19$; Fig. 6E). Thus, the reason for the failure of germination in the *ng1* mutant remains unknown at this time.

4. Discussion

We have identified *AtNG1* as a PPR protein gene required for seed germination in Arabidopsis. As discussed in the Introduction, several PPR family members play roles in various stages of plant development [3], and three of them (OTP43, OGR1, and PPR40) are involved in seed germination. In an analysis of the seeds of *otp43* heterozygotes, one-fourth were smaller and darker and only a few, which were determined to be homozygous, were able to germinate. The homozygous *otp43* mutant also showed obviously delayed development. Its flowers and seeds were smaller than those of wild-type plants, and its leaves were curled. The altered germination phenotype in the *otp43* mutant is cause by abnormal embryos [17]. The altered phenotype of *ogr1* mutants includes an opaque endosperm, delayed seed germination, growth retardation, reduced tillering, pollen sterility, and late flowering [18]. The *ppr40* mutant shows enhanced sensitivity to abiotic stressors, including abscisic acid, resulting in slightly delayed seed germination [19].

Because the *otp43*, *ogr1*, and *ppr40* mutants have only minor or moderate defects in seed germination, researchers have been able to obtain and characterize the mutant homozygotes. In contrast, seed germination is completely blocked in the *ng1* mutant, even though seed development appears normal, indicating that the *ng1* mutation critically impairs at least one physiological process essential for seed germination.

In seed germination, water imbibition and the resumption of active metabolism are followed by elongation of the embryo axis and protrusion of the root tip out of the seed coat. Cell elongation is necessary and sufficient for the completion of the protrusion process [22,34]. Although the *ng1* mutant exhibits normal embryonic development, its embryonic root tip never protrudes from the seed coat. Our cytological observations revealed that *ng1* embryonic root cells fail to elongate at all, suggesting that a physiological process essential to root elongation is blocked in this mutant. Thus, AtNG1 may not be involved in the establishment of the seed structure; rather, it appears to play an essential role in seed germination, probably in its initiation.

According to our analysis of protein localization, AtNG1 locates in mitochondria as our prediction based on protein sequence analysis. It has been known that mitochondria provide the cellular ATP to support metabolic activity required for seed germination, and the quality of seed mitochondria is strongly related to germination vigor [22,35–37]. Among the mutants mentioned above, the *otp43* presents undetectable mitochondrial Complex I activity [17], the *ppr40* shows strongly reduced electron transport through mitochondrial Complex III [19], and *ogr1* suffers from the deficiency of mitochondrial electron transport chain and ATP generation [18]. Clearly, functional deficiency of mitochondrion could cause problems in seed germination. Thus, it is reasonable to propose that the failed seed germination of *ng1* might be also due to the deficiency of mitochondrion function, possibly owing to inadequate ATP supply, although necessary structures of seed were fully developed. However, determining exactly how seed germination is affected in the *ng1* mutant requires further detailed analysis of this mutant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.plantsci.2011.07.011.

References

[1] C. Lurin, C. Andrés, S. Aubourg, M. Bellaoui, F. Bitton, C. Bruyère, M. Caboche, C. Debast, J. Gualberto, B. Hoffmann, et al., Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis, Plant Cell 16 (8) (2004) 2089–2103.
[2] I.D. Small, N. Peeters, The PPR motif – a TPR-related motif prevalent in plant organellar proteins, Trends Biochem. Sci. 25 (2) (2000) 46–47.
[3] C. Schmitz-Linneweber, I.D. Small, Pentatricopeptide repeat proteins: a socket set for organelle gene expression, Trends Plant Sci. 13 (12) (2008) 663–670.
[4] N. O'Toole, M. Hattori, C. Andres, K. Iida, C. Lurin, C. Schmitz-Linneweber, M. Sugita, I.D. Small, On the expansion of the pentatricopeptide repeat gene family in plants, Mol. Biol. Evol. 25 (6) (2008) 1120–1128.
[5] S. Desloire, H. Gherbi, W. Laloui, S. Marhadour, V. Clouet, L. Cattolico, C. Falentin, S. Giancola, M. Renard, F. Budar, et al., Identification of the fertility restoration locus, Rfo, in radish, as a member of the pentatricopeptide-repeat protein family, EMBO Rep. 4 (6) (2003) 588–594.
[6] R.R Klein, P.E. Klein, J.E. Mullet, P. Minx, W.L. Rooney, K.F. Schertz, Fertility restorer locus Rf1 of sorghum (Sorghum bicolor L.) encodes a pentatricopeptide repeat protein not present in the colinear region of rice chromosome 12, Theor. Appl. Genet. 111 (6) (2005) 994–1012.
[7] S. Bentolila, A.A. Alfonso, M.R. Hanson, A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants, Proc. Natl. Acad. Sci. U.S.A. 99 (16) (2002) 10887–10892.
[8] Z. Wang, Y. Zou, X. Li, Q. Zhang, L. Chen, H. Wu, D. Su, Y. Chen, J. Guo, D. Luo, et al., Cytoplasmic male sterility of rice with boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing, Plant Cell 18 (3) (2006) 676–687.
[9] H. Akagi, A. Nakamura, Y. Yokozeki-Misono, A. Inagaki, H. Takahashi, K. Mori, T. Fujimura, Positional cloning of the rice Rf-1 gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein, Theor. Appl. Genet. 108 (8) (2004) 1449–1457.
[10] D.A. Cushing, N.R. Forsthoefel, D.R. Gestaut, D.M. Vernon, Arabidopsis emb175 and other ppr knockout mutants reveal essential roles for pentatricopeptide repeat (PPR) proteins in plant embryogenesis, Planta 221 (3) (2005) 424–436.
[11] Y.H. Ding, N.Y. Liu, Z.S. Tang, J. Liu, W.C. Yang, Arabidopsis GLUTAMINE-RICH PROTEIN23 is essential for early embryogenesis and encodes a novel nuclear PPR motif protein that interacts with RNA polymerase II subunit III, Plant Cell 18 (4) (2006) 815–830.
[12] N. Bryant, J. Lloyd, C. Sweeney, F. Myouga, D. Meinke, Identification of nuclear genes encoding chloroplast-localized proteins required for embryo development in Arabidopsis, Plant Physiol. 155 (2011) 1678–1689.
[13] J. McElver, I. Tzafrir, G. Aux, R. Rogers, C. Ashby, et al., Insertional mutagenesis of genes required for seed development in Arabidopsis thaliana, Plant Physiol. 159 (2001) 1751–1763.
[14] D. Meinke, R. Muralla, C. Sweeney, A. Dickerman, Identifying essential genes in Arabidopsis thaliana, Trends Plant Sci. 13 (9) (2008) 483–491.
[15] I. Tzafrir, R. Pena-Muralla, A. Dickerman, M. Berg, R. Rogers, et al., Identification of genes required for embryo development in Arabidopsis, Plant Physiol. 135 (2004) 1206–1220.
[16] D.W. Meinke, L.K. Meinke, T.C. Showalter, A.M. Schissel, L.A. Mueller, I. Tzafrir, A sequence-based map of Arabidopsis genes with mutant phenotypes, Plant Physiol. 13 (2004) 409–418.
[17] A.F. de Longevialle, E.H. Meyer, C. Andrés, N.L. Taylor, C. Lurin, A.H. Millar, I.D. Small, The pentatricopeptide repeat gene OTP43 is required for trans-splicing of the mitochondrial nad1 Intron 1 in Arabidopsis thaliana, Plant Cell 19 (10) (2007) 3256–3265.
[18] S.R. Kim, J.I. Yang, S. Moon, C.H. Ryu, K. An, K.M. Kim, J. Yim, G. An, Rice OGR1 encodes a pentatricopeptide repeat-DYW protein and is essential for RNA editing in mitochondria, Plant J. 59 (5) (2009) 738–749.
[19] L. Zsigmond, G. Rigo, A. Szarka, G. Szekely, K. Otvos, Z. Darula, K.F. Medzihradszky, C. Koncz, Z. Koncz, L. Szabados, Arabidopsis PPR40 connects abiotic stress responses to mitochondrial electron transport, Plant Physiol. 146 (4) (2008) 1721–1737.
[20] W.E. Finch-Savage, G. Leubner-Metzger, Seed dormancy and the control of germination, New Phytol. 171 (3) (2006) 501–523.
[21] M. Santos-Mendoza, B. Dubreucq, S. Baud, F. Parcy, M. Caboche, L. Lepiniec, Deciphering gene regulatory networks that control seed development and maturation in Arabidopsis, Plant J. 54 (2008) 608–620.
[22] J.D. Bewley, Seed germination and dormancy, Plant Cell 9 (7) (1997) 1055–1066.
[23] T. Murashige, F. Skoog, A revised medium for rapid growth and bio assays with tobacco tissue cultures, Physiol. Plantarum 15 (3) (1962) 473–497.

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[24] N. Bechtold, G. Pelletier, In planta Agrobacterium-mediated transformation of adult Arabidopsis thaliana plants by vacuum infiltration, Meth. Mol. Biol. (Clifton, NJ) 82 (1998) 259–266.
[25] Y.G. Liu, N. Mitsukawa, T. Oosumi, R.F. Whittier, Efficient isolation and mapping of Arabidopsis thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR, Plant J. 8 (3) (1995) 457–463.
[26] R.E. Campbell, O. Tour, A.E. Palmer, P.A. Steinbach, G.S. Baird, D.A. Zacharias, R.Y. Tsien, A monomeric red fluorescent protein, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 7877–7882.
[27] R. Matsushima, Y. Hamamura, T. Higashiyama, S. Arimura, Sodmergen, N. Tsutsumi, W. Sakamoto, Mitochondrial dynamics in plant male gametophyte visualized by fluorescent live imaging, Plant Cell Physiol. 49 (7) (2008) 1074–1083.
[28] N.S. Yang, J. Burkholder, B. Roberts, B. Martinell, D. McCabe, In vivo and in vitro gene transfer to mammalian somatic cells by particle bombardment, Proc. Natl. Acad. Sci. U.S.A. 87 (24) (1990) 9568–9572.
[29] J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (22) (1994) 4673–4680.
[30] G.G Brown, N. Formanova, H. Jin, R. Wargachuk, C. Dendy, P. Patil, M. Lafores, J. Zhang, W.Y. Cheung, B.S. Landry, The radish Rfo restorer gene of Ogura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats, Plant J. 35 (2) (2003) 262–272.
[31] N. Koizuka, R. Imai, H. Fujimoto, T. Hayakawa, Y. Kimura, J. Kohno-Murase, T. Sakai, S. Kawasaki, J. Imamura, Genetic characterization of a pentatricopeptide repeat protein gene, orf687, that restores fertility in the cytoplasmic male-sterile Kosena radish, Plant J. 34 (4) (2003) 407–415.
[32] J. Christian, F. Joachim, H. Angela, T. Sabine, B. Stefan, RNA PROCESSING FACTOR2 is required for 5′ end processing of nad9 and cox3 mRNAs in mitochondria of Arabidopsis thaliana, Plant Cell 22 (2010) 443–453.
[33] J.D. Bewley, M. Black, Seeds Physiology of Development and Germination, Plenum Press, New York, 1994.
[34] W.E. Finch-Savage, G. Leubner-Metzger, Seed dormancy and the control of germination, New Phytol. 171 (2006) 501–523.
[35] M. Bettey, W.E. Finch-Savage, Respiratory enzyme activities during germination in Brassica seeds of different vigour, Seed Sci. Res. 6 (1996) 165–173.
[36] A. Benamar, C. Tallon, D. Macherel, Membrane integrity and oxidation properties of mitochondria isolated from imbibing pea seeds after priming or accelerated aging, Seed Sci. Res. 13 (2003) 35–45.
[37] J. Catusse, J. Strub, C. Job, A.V. Dorsselaer, D. Job, Proteome-wide characterization of sugarbeet seed vigor and its tissue specific expression, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 10262–10267.

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