The *Greening after Extended Darkness1* Is an N-End Rule Pathway Mutant with High Tolerance to Submergence and Starvation[1][OPEN]

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Plants respond to reductions in internal oxygen concentrations with adaptive mechanisms (for example, modifications of metabolism to cope with reduced supply of ATP). These responses are, at the transcriptional level, mediated by the group VII Ethylene Response Factor transcription factors, which have stability that is regulated by the N-end rule pathway of protein degradation. N-end rule pathway mutants are characterized by a constitutive expression of hypoxia response genes and abscisic acid hypersensitivity. Here, we identify a novel *proteolysis6* (*prt6*) mutant allele, named *greening after extended darkness1* (*ged1*), which was previously discovered in a screen for *genomes uncoupled*-like mutants and shows the ability to withstand long periods of darkness at the seedling stage. Interestingly, this ethyl methanesulfonate-derived mutant shows unusual chromosomal rearrangement instead of a point mutation. Furthermore, the sensitivity of N-end rule pathway mutants *ged1* and *prt6-1* to submergence was studied in more detail to understand previously contradicting experiments on this topic. Finally, it was shown that mutants for the N-end rule pathway are generally more tolerant to starvation conditions, such as prolonged darkness or submergence, which was partially associated with carbohydrate conservation.

During their life cycle, plant cells encounter periods of oxygen deficiency, generally termed as hypoxia stress. This stress depends on the ratio between oxygen consumption and availability. In plants, oxygen diffusion can be constrained by anatomical and morphological features (i.e. densely packed cells [e.g. meristems] and a low surface-to-volume ratio [e.g. fruits and seeds]) or external conditions that hamper gas diffusion, such as complete submergence of plants or waterlogging of soils (root flooding). Because of the requirement of oxygen for biosynthetic processes and more importantly, mitochondrial respiration, a shortage in oxygen leads to an energy deficit in hypoxic cells. To cope with this stress, plants reprogram their metabolism involving down-regulation of energy-demanding processes (e.g. protein biosynthesis) and up-regulation of genes important for glycolysis, fermentation, and protection against reactive oxygen species (Bailey-Serres and Voesenek, 2008; Branco-Price et al., 2008; Mustroph et al., 2009; Voesenek and Bailey-Serres, 2013).

The transcriptional regulation of flooding-adaptive genes operates through a specific signal transduction pathway triggered by a decline in the cellular oxygen concentration. This oxygen-sensing mechanism is mediated by the oxygen- and nitric oxide-dependent degradation of subgroup VII Ethylene Response Factor (VII-ERF) transcription factors through the N-end rule pathway of proteolysis (Gibbs et al., 2011, 2014; Licausi et al., 2011). Stabilization of VII-ERFs (among them, RELATED TO AP2 2 [RAP2.2] and RAP2.12) during hypoxia activates the transcription of downstream hypoxia-responsive genes, including those involved in fermentation and sugar consumption (Gibbs et al., 2011; Licausi et al., 2011). During reoxygenation or normoxia, these transcription factors are modified in the presence of oxygen at the N-terminal Cys, most likely through the action of Plant Cys Oxidase (PCO1/PCO2; Weits et al., 2014), making them accessible to protein degradation through the N-end rule pathway through arginyl-tRNA:protein arginyltransferase (ATE1/ATE2) and the E3 ubiquitin protein ligase PROTEOLYSIS6 (PRT6). Mutants for either *ate1/ate2* or *prt6* are disturbed in degradation of VII-ERFs and consequently, accumulate these proteins even under oxygen-rich conditions, therefore exhibiting a constitutive hypoxia response (Gibbs et al., 2011, 2014; Licausi et al., 2011).

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Despite the largely consistent results in the works by Gibbs et al. (2011) and Licausi et al. (2011), there were some variations in the outcome of the submergence/hypoxia survival assays for *ate1/ate2* and *prt6* transfer DNA (T-DNA) insertion null alleles (Sasidharan and Mustroph, 2011). Licausi et al. (2011) submerged 5-week-old soil-grown plants in darkness for 3.5 d and reported a lower survival of the mutants compared with the wild type. Gibbs et al. (2011) treated 7-d-old seedlings grown on Murashige and Skoog (MS) medium supplemented with 1% (w/v) Suc with argon gas in darkness for 9 and 12 h and reported a higher survival of the mutants compared with the wild type. This discrepancy in survival could be caused by variation in plant age and growth conditions and differences in the internal oxygen content of the plants during the argon/submergence treatment, but this has not yet been resolved.

Through the Genevestigator platform (https://www.genevestigator.com/gv/plant.jsp; Hruz et al., 2008), we identified a mutant that is also characterized by a constitutively high expression of hypoxia-responsive genes, similar to *prt6*, *ate1/ate2*, and *pco1/pco2* (Gibbs et al., 2011; Weits et al., 2014). We hypothesized that this mutant, *greening after extended darkness1* (*ged1*), is disturbed in the hypoxic signal transduction pathway. This ethyl methanesulfonate (EMS)-derived *ged1* mutant was discovered in a screen to find *GENOMES UNCOUPLED1* (*gun1*)-like mutants (Gray et al., 2003; Choy et al., 2008). *gun1* mutants are defective in chloroplast retrograde signaling and do not down-regulate the transcription of the ribulose bisphosphate carboxylase small subunit (*RBCS*) and the light harvesting complex after treatment with lincomycin or norflurazone, which causes chloroplast damage (Susek et al., 1993; Gray et al., 2003). The screen identified the *ged1* mutant, which was named like that, because it accumulated more chlorophyll than the wild type when seedlings were grown in darkness for several days followed by illumination for 1 d in contrast to the original *gun1* mutant (Choy et al., 2008). Interestingly, this *ged1* mutant also exhibited enhanced sensitivity to abscisic acid (ABA; Choy et al., 2008). Altered ABA sensitivity has been also described for N-end rule pathway mutants (Holman et al., 2009; Gibbs et al., 2014).

In this work, we first attempted to identify the affected gene in the *ged1* mutant. Following the mapping of the mutation in *ged1* to the gene *PRT6*, thus making it an N-end rule pathway mutant, we also focused our research on determining the survival rates of this novel *prt6* allele under various conditions. Furthermore, we analyzed how primary metabolism is affected by different hypoxic stress treatments in *ged1* and *prt6* and their respective wild-type ecotypes (*Wassilewskaja* [WS] and Columbia-0 [Col-0]) to understand differences in survival of these two accessions. Furthermore, we show that a main consequence of an impaired N-end rule pathway in Arabidopsis (*Arabidopsis thaliana*) is adaptation to starvation rather than up-regulation of sugar consumption and fermentation.

RESULTS

ged1 Displays a Hypoxic Expression Pattern under Normoxia and Possesses a Nonfunctional *PRT6* Gene

The *ged1* mutant has been initially characterized as a mutant that exhibits the ability to green after extended periods of darkness and additionally, shows sensitivity of germination to ABA (Choy et al., 2008). The *ged1* mutant is characterized by a down-regulation of many ABA-responsive genes when grown for 5 d in darkness followed by 2 d in light (Choy et al., 2008) and an up-regulation of many hypoxia-responsive genes (Fig. 1A). Of 408 significantly induced genes (signal log ratio > 1, adjusted $P < 0.05$), 40% were hypoxia responsive (Supplemental Table S1; Branco-Price et al., 2008; Mustroph et al., 2009; Gibbs et al., 2011; Licausi et al., 2011), and 36 of 49 hypoxia core response genes (Mustroph et al., 2009) were among these up-regulated genes. For example, many genes for hypoxic metabolism (*alcohol dehydrogenase*, *pyruvate decarboxylase* [*PDC1*], *Suc synthase1* [*SUS1*], and *SUS4*) as well as genes coding for *HYPOXIA-RESPONSIVE UNKNOWN PROTEIN*s (Mustroph et al., 2010; Lee et al., 2011) were highly expressed in *ged1* under normoxic conditions. However, not all well-known hypoxic genes were among the significantly highly expressed genes in *ged1*, including the hypoxia-responsive *phosphofructokinase3* (*PFK3*) and *PFK6*, *Ala aminotransferase1*, *RESPIRATORY BURST OXIDASE HOMOLOG D*, and *SIMILAR TO RCD ONE5*. In addition, the down-regulated genes in *ged1* were often ABA responsive, which has already been observed previously (Choy et al., 2008); 161 of 540 significantly down-regulated genes were up-regulated in ABA-treated seeds or seedlings from published microarray experiments (Fig. 1C; Supplemental Table S1; Goda et al., 2008; Kim et al., 2011).

In a subsequent analysis, the constitutive expression of hypoxic genes was confirmed on 7-d-old *ged1* and WS wild-type seedlings by PCR analyses (Fig. 1D). To identify the *ged1* mutation, we performed map-based cloning based on the ABA-hypersensitive arrest of seedling establishment. F2 seeds of a cross between *ged1* (background WS) and Col-0 were grown on MS medium containing 0.1 μM ABA. About 16.4% of all F2 seedlings showed the mutant phenotype and did not develop green cotyledons (Fig. 2A). They were rescued by transfer to ABA-free medium and subsequently used for genotyping. ABA hypersensitivity of selected lines was confirmed by analyzing the F3 generation.

The first rough mapping process with 26 loci across all five chromosomes revealed the existence of two cosegregating loci: one on chromosome 3 and one on chromosome 5. Fine mapping of the two regions with additional markers specified the regions on chromosome 3 between 2.6 and 3.3 million bp and on chromosome 5 between 1.0 and 2.7 million bp. All plants that showed the mutant phenotype and were evidentially not segregating in the F3 generation were homozygous for the mutant genotype (WS) on top of chromosome 5. However,

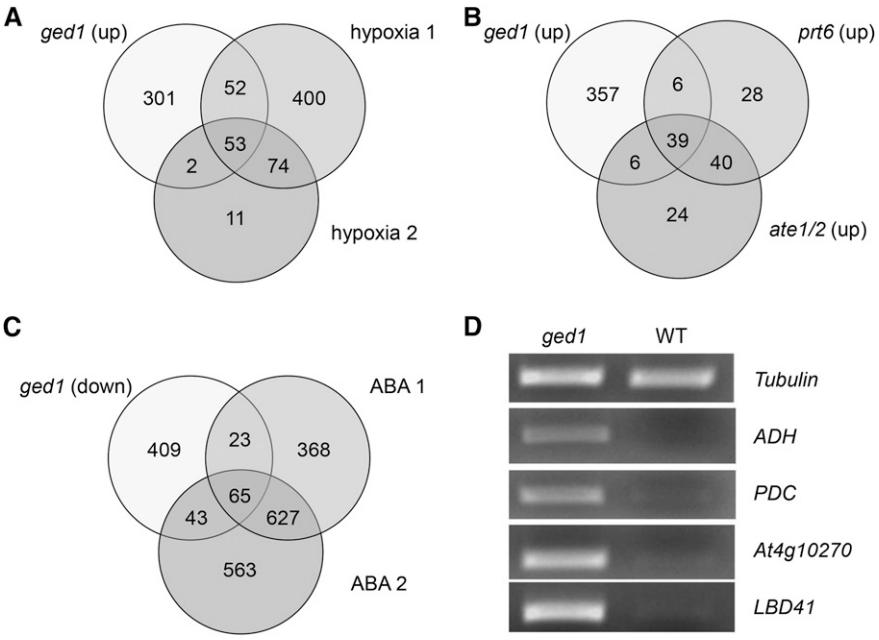

Figure 1. Gene expression of the *ged1* mutant. Differentially expressed genes of *ged1* compared with its wild type (WT; signal log ratio > |1|, adjusted $P < 0.05$) as assessed by microarray analysis were compared with published microarray data by use of the same selection criteria. A, *ged1* up-regulated genes show an overlap with hypoxia-induced genes. Experiments for comparison: hypoxia 1, total RNA of 2-h hypoxia-treated Col-0 plants (Branco-Price et al., 2008); and hypoxia 2, RNA of 2-h hypoxia-treated Col-0 plants (Gibbs et al., 2011). B, *ged1* up-regulated genes show an overlap with the gene expression profile of *prt6* and *ate1/ate2* T-DNA insertion null alleles (Gibbs et al., 2011). C, *ged1* down-regulated genes show an overlap with ABA-responsive genes. Experiments for comparison: ABA 1, RNA of 3-h ABA-treated seedlings (Goda et al., 2008); and ABA 2, RNA of 6-h ABA-treated seedlings (Kim et al., 2011). D, Confirmation of hypoxic gene expression by PCR analysis. Seedlings were harvested after 7 d of growth on solid MS medium supplemented with 1% (w/v) Suc under long-day light conditions; one representative experiment of at least 10 independent experiments is shown. The photo shows the reverse transcription PCR bands separated by agarose gel electrophoresis and after staining with Serva DNA stain G. *ADH, Alcohol dehydrogenase*; *LBD41, LOB DOMAIN-CONTAINING PROTEIN41*.

no recombination event could be detected in the first 2.7 million bp, despite a mapping population of about 1,000 plants. None of the plants with the mutant phenotype were homozygous for the wild-type genotype Col-0 in the second cosegregating region on chromosome 3, but about 20% were heterozygous.

Genotyping of individuals from different F3 generations that were derived from those plants that were heterozygous on chromosome 3 in the F2 generation and showed the mutant phenotype without segregation in the F3 generation revealed an abnormal segregation pattern in the mapped region on chromosome 3, with mostly homozygous WS genotype and few heterozygous and no homozygous Col-0 genotype plants. Therefore, the pattern of genotype distribution between the mapped regions on chromosomes 3 and 5 was analyzed in 91 F2 plants without previous phenotype selection. One representative marker was used for each region (3367348_bp and NGA249 for chromosomes 3 and 5, respectively). No plants with the genotype combination homozygous Col-0 and homozygous WS on both chromosomes were found, and all possible heterozygous/homozygous combinations were less frequent, which was not to be expected by random distribution (Fig. 2B). In contrast, combinations of the same genotype at both chromosome regions were more frequent. For example, the combination of homozygous WS at both loci was detected in 16.5% of all F3 plants.

After microarray data of *prt6* and *ate1/ate2* T-DNA insertion null alleles were published (Gibbs et al., 2011; Licausi et al., 2011), we became aware of similarities with these mutants and *ged1* regarding the regulation of hypoxia-responsive genes and ABA sensitivity (Fig. 1B; Supplemental Table S1). Additionally, all three genes were close to the mapped regions on chromosome 3 (*ATE2*) or chromosome 5 (*ATE1* and *PRT6*). Therefore, crosses of these genotypes with *ged1* were produced, and the F1 generation was examined for germination on ABA-containing medium. Seedlings of a cross between *ged1* and *prt6-1* but not between *ged1* and *ate1/ate2* showed ABA hypersensitivity during germination (Fig. 2C). Hence, we concluded that *ged1* bears a nonfunctional *PRT6* gene.

To identify the position of the mutation and confirm our hypothesis, we PCR amplified and sequenced a nearly 10,000-bp-long genomic region around the *PRT6* gene in about 2,000-bp sections. However, one primer combination spanning a certain region of the

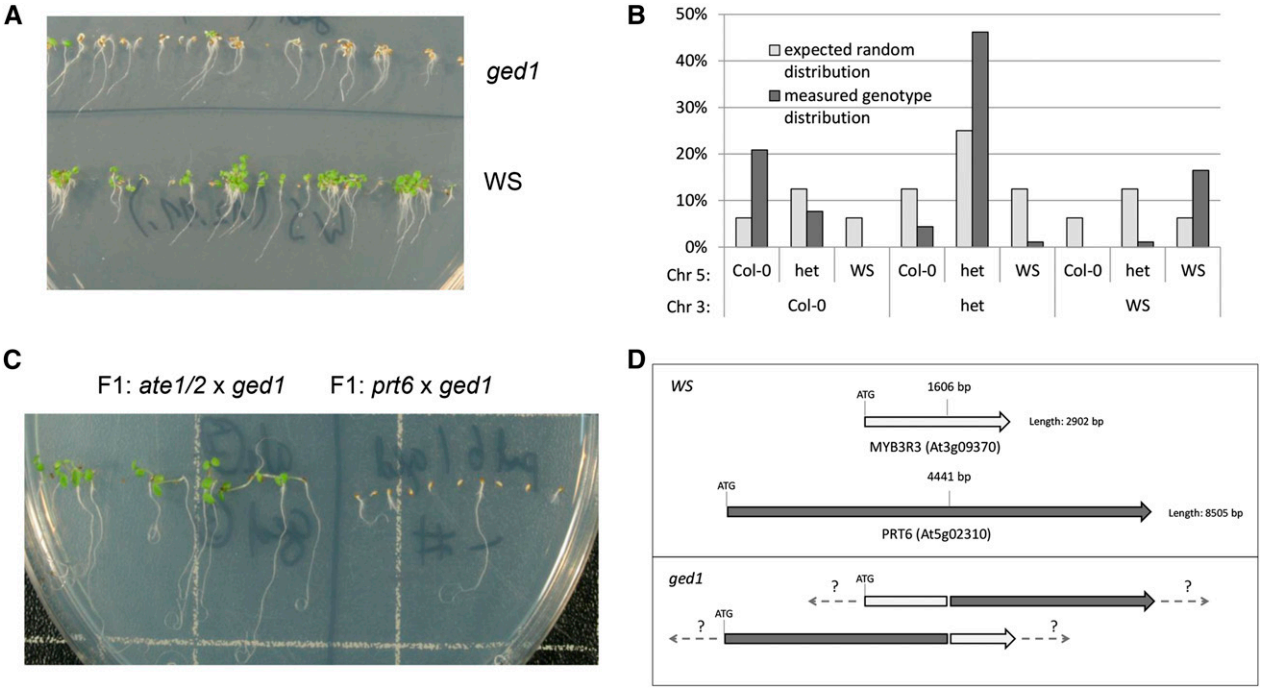

Figure 2. Identification of the mutated gene in *ged1*. A, *ged1* mutants are sensitive to 0.1 μM ABA during germination compared with the WS wild type. The picture was taken after 7 d of germination on MS medium supplemented with 0.1 μM ABA. B, Genotyping of 91 F2 plants from the cross *ged1* (WS) × Col-0. Two ecotype-specific polymorphisms in the mapped regions (one on chromosome 3 and one on chromosome 5) were used for genotyping, and the percentage of genotype distribution was recorded (light gray) and compared with the theoretical distribution (dark gray). het, Heterozygous. C, ABA sensitivity during germination of the F1 generation of a cross between *ged1* and *ate1/ate2* or *ged1* and *prt6-1*. The picture was taken after 7 d of germination on MS medium supplemented with 0.1 μM ABA. D, Schematic presentation of the chromosome rearrangement detected in *ged1*. Parts of chromosomes 3 and 5 were exchanged, and thereby, the genes *PRT6* and *MYB3R3* were destroyed.

PRT6 gene did not yield a PCR product in genomic DNA of *ged1* but worked well in the WS wild type. To identify the cause of this PCR problem, the region was specified with more primer pairs, and finally, thermal asymmetric interlaced (TAIL) PCRs starting from both sites of this region were performed. This revealed that the first part of *PRT6* directly merges into the second part of the gene *MYB DOMAIN PROTEIN 3R-3* (*MYB3R3*; At3g09370), which is located within the cosegregating region on chromosome 3. In the other direction, the second part of *PRT6* directly merges into the first part of *MYB3R3* (Fig. 2D; Supplemental Figs. S1 and S2). These results suggest that one part of *PRT6* moved to chromosome 3 and one part of *MYB3R3* moved to chromosome 5.

Two approaches were followed to estimate the extent of chromosome exchange. (1) Regions around *PRT6* and *MYB3R3* were sequenced, and primers creating overlapping DNA sequences were used to determine if it is possible to yield PCR products of roughly 4,000 bp upstream and downstream of the genes. (2) Because the recombination rate on top of chromosome 5 was very low, F2 plants with the mutant phenotype (homozygous WS on top of chromosome 5) that showed recombination events (heterozygous/WS) near to the cosegregating region on chromosome 3 were analyzed. All plants that were homozygous WS within at least 2.8 and 3.3 million bp on chromosome 3 but heterozygous outside of this region showed normal seed yield and a normal segregation pattern (i.e. in the F3 generation, homozygous Col-0 plants were detected with mapping markers in the heterozygous parts of the respective F2 plants). However, when plants were heterozygous within 2.8 and 3.3 million bp, they had small siliques with low seed yield, and homozygous Col-0 plants could not be detected in the F3 generation. We, therefore, assume that the DNA exchange on chromosome 3 should be restricted to these 0.5 million bp.

The combination of both approaches did not, however, result in the detection of a second point of chromosome exchange. Potentially, a large chromosome part could have been transferred. Furthermore, this chromosome transfer explains the unusual non-Mendelian segregation of the F2 generation (Fig. 2B), making several combinations of loci between Col-0 and *ged1* lethal because of missing chromosome fragments.

In addition to *PRT6*, the other two N-end rule pathway genes *ATE1* and *ATE2* were also sequenced in *ged1* to exclude additional mutations. The sequencing resulted in WS wild-type-like *ATE1* and *ATE2* genes in the mutant *ged1*. Surprisingly, a single-nucleotide insertion in both WS ecotype and *ged1* compared with the Col-0 reference

sequence was observed for *ATE2*, leading to a premature stop codon and a truncated, presumably nonfunctional ATE2 protein (Supplemental Figs. S3–S5). It is, therefore, concluded that WS only contains one functional *ATE* gene. This is further supported by the analysis of the F2 generation of our WS × *ate1/ate2* cross. Here, 25 plants showed ABA-sensitive germination, and 65 plants did not. This 1:3 segregation rate hints at only one functional *ATE* gene.

Survival of *prt6* Mutants under Submergence

Previous work describing the submergence/hypoxia tolerance of *prt6* revealed partially contrasting results (Gibbs et al., 2011; Licausi et al., 2011). Because we discovered that *ged1* is another allele of a *prt6* mutant, we determined the submergence tolerance of *ged1*. The submergence assay was done as described by Vashisht et al. (2011) under illumination or darkness. This assay showed that *ged1* is highly tolerant to submergence in darkness or submergence under short-day light conditions compared with its wild type (median lethal time [LT_{50}] = 14.0 versus 11.8 d and 26.3 versus 18.6 d under darkness and light, respectively; Fig. 3; Table I). In contrast, LT_{50} values for air dark conditions of *ged1* and the wild type were only marginally different (LT_{50} = 12.4 versus 11.7 d). Remarkably, *ged1* mutants survived the compound stress of submergence (dark) better than darkness alone. Although 50% of the *ged1* plants survived 14 d of darkness during submergence, only 10% survived 14 d of darkness in air. In both cases, no wild-type plants survived.

Because *ged1* is a *prt6* knockout (KO) mutant, our results for survival of submergence in darkness are contradictory to the published work by Licausi et al. (2011), who examined a *prt6* T-DNA insertion line (named here as *prt6-6*; Supplemental Table S2) in a WS background and found it to be more intolerant than the corresponding wild type to 3.5 d of submergence in darkness. We repeated our experiments with *prt6-1* (Garzón et al., 2007; Graciet et al., 2009; Supplemental Table S2) and its wild-type Col-0. In these experiments, *prt6-1* is clearly more tolerant than Col-0 to both darkness (in air) and submergence (under dark and short-day light conditions; LT_{50} = 10.0 versus 6.1 d and 22.2 versus 16.2 d under submerged darkness and submerged light, respectively; Fig. 3; Table I). In contrast to the results by Licausi et al. (2011), 100% of the *prt6-1* and *ged1* plants survived 8 d of submergence in darkness in our experimental setup.

A comparison of both ecotypes revealed that the WS wild type is more tolerant than Col-0 under all treatment conditions. Interestingly, *prt6-1* had a higher LT_{50} and therefore, a better survival when treated with darkness in air compared with darkness under submergence, whereas the opposite was observed in *ged1*, indicating important ecotype differences as previously observed (Vashisht et al., 2011).

To understand the contrasting results between both experiments, we looked more deeply into the growth and treatment conditions (Licausi et al., 2011; Vashisht et al., 2011). One major difference between both survival assays is the relative humidity levels of the recovery period. Although in our conditions, the relative humidity during the recovery phase was maintained at 70% (Vashisht et al., 2011), it was much lower (presumably lower than 30%) in the other experimental setup (F. Licausi, personal communication). This difference could have caused the lower overall survivability of plants in the study by Licausi et al. (2011) as well as the difference in sensitivity in the *prt6-6* T-DNA insertion line compared with the wild type. To test this hypothesis, we performed an experiment, in which *prt6* mutants were allowed to recover after submergence at either a low humidity (about 20%) or a high humidity (about 100%). Under the low-humidity recovery conditions, only a few plants survived 5 d of submergence, but all plants survived and looked much healthier when recovery was performed at high humidity (Supplemental Fig. S6). Furthermore, more wild-type plants than *prt6* mutant plants survived when recovered at low humidity (such as in Licausi et al., 2011), whereas more *prt6* mutant plants than wild types survived during high-humidity recovery

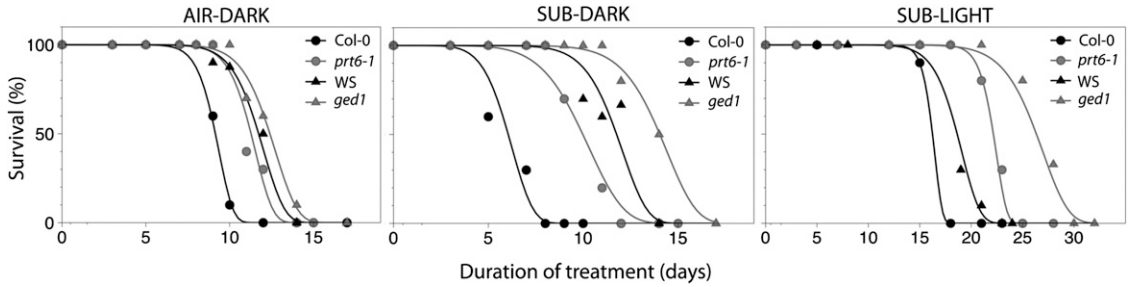

Figure 3. *ged1* and *prt6* are more tolerant to submergence than their respective wild types. Soil-grown 5-week-old plants were submerged for the indicated time points in complete darkness (SUB-DARK) or under a day-night regime (SUB-LIGHT) and assessed for survival exactly as described in Vashisht et al. (2011). Additionally, plants were treated with darkness in air for comparison (AIR-DARK). LT_{50} values and survival estimates were calculated according to the Weibull regression model as described in Vashisht et al. (2011). LT_{50} values ± SE are presented in Table I.

Table I. *Survival scores (LT_{50} values in days) for submergence tolerance*

Four-week-old plants of different genotypes were screened for submergence survival as described in Vashisht et al. (2011). Submergence was performed in complete darkness or a short-day rhythm, and after selected time points, plants were removed from the treatment and evaluated for submergence survival after 14 d of recovery. From these survival curves, LT_{50} values were calculated as previously described. AIR-DARK, Darkness in air; SUB-DARK, submerged in complete darkness; SUB-LIGHT, submerged under a day-night regime.

Treatment	Col-0 Wild Type	*prt6-1*	WS Wild Type	*ged1*
AIR-DARK	9.15 ± 0.25	11.28 ± 0.28	11.73 ± 0.33	12.40 ± 0.33
SUB-DARK	6.05 ± 0.29	9.98 ± 0.38	11.76 ± 0.32	14.04 ± 0.56
SUB-LIGHT	16.24 ± 0.65	22.16 ± 0.35	18.64 ± 0.53	26.34 ± 0.87

(as in our experiments; Fig. 3). These observations suggest that root vitality might be impaired under submergence, leading to dehydration and subsequent death of leaves in the low-humidity recovery phase. The intolerance of *prt6* mutants to low-humidity recovery could be caused by an earlier death of the root under submergence compared with the wild type and/or differences in the tolerance to water deficiency stress. More vital-looking leaves directly after desubmergence as well as better recovery under high humidity indicate better shoot survival of *prt6* mutants during submergence compared with the respective wild type (Supplemental Fig. S6).

Prolonged Submergence Survival of *prt6* Mutants Is Associated with Carbohydrate Conservation

Gene expression analyses of *ged1* and other *prt6* mutants show a constitutive up-regulation of genes involved in fermentation and sugar breakdown (Choy et al., 2008; Gibbs et al., 2011). However, a higher percentage of *ged1* survived during submergence in the dark than in darkness only (Fig. 3). This contradicted our intuitive assumption that carbohydrate resources would be wasted during submergence because of fermentative metabolism, which has been previously suggested (Licausi et al., 2011). Therefore, we addressed the question of how far the difference in tolerance might be reflected by a difference in primary metabolism in: (1) the two ecotypes Col-0 and WS that showed huge differences in tolerance, and (2) the wild type compared with the respective *prt6* knockout mutants.

Hence, 5-week-old soil-grown plants (8-h-d/16h-night cycle) were submerged in darkness for 8 and 24 h at the end of the light period, so that the plants went into the stress treatment with a maximal amount of carbohydrate reserves. The sugar content, including Glc, Suc, and starch, was similar at the start of the stress treatments in all tested genotypes (Fig. 4A; Supplemental Table S3B). Fru levels were significantly elevated in the *ged1* and *prt6-1* compared with their respective wild types at the start of the stress treatment but only contribute 0.6% to 1.8% to the total sugar content.

After 8 h of submergence in darkness, Glc and starch levels significantly decreased in all genotypes compared with the starting level, whereas the changes in the other two sugars were not significant (Fig. 4B; Supplemental Table S3A). No significant differences between the genotypes were observed; 8 h of darkness in air also significantly decreased all sugars analyzed, with the exception of Suc and Fru in Col-0, and again, no clear differences between genotypes were visible. Submergence versus air treatment did not show a difference in sugar levels within each genotype, except for a comparatively small but significant elevation in Fru levels after submergence in both *prt6* mutants (Supplemental Table S3B). ATP and ADP levels also did not differ significantly among treatments and genotypes (Supplemental Fig. S7).

Similarly, 24 h of submergence in darkness led to lower sugar levels in all genotypes, except for Fru (Supplemental Table S3A). However, higher amounts of starch remained in both *prt6* mutants compared with their respective wild types and air treatment (Fig. 4D; Supplemental Table S3B). The difference in starch content between 24 h of submergence and air treatment was about 6-fold in *prt6-1* and 10-fold in *ged1*. The *prt6-1* T-DNA insertion line additionally showed significantly higher Suc and Fru contents compared with the Col-0 wild type. In this regard, no significant difference was measured between WS and *ged1*. Interestingly, no difference between all genotypes was observable after 24 h of darkness in air. Adenylates again showed no significant differences, such as after 8 h of submergence (Supplemental Fig. S7).

In addition to submergence, we also treated the plants with a pure nitrogen atmosphere in darkness (anoxia) for 8 h. This treatment resulted in highly decreased starch degradation compared with air dark treatment without any noticeable difference between genotypes and a high accumulation of Glc, whereas Suc values approximated the detection limit. An ecotype-specific difference was observable for the Glc levels after anoxia treatment, with higher levels in Col-0 than WS genotypes (Fig. 4C; Supplemental Table S3B).

prt6 Mutants Are Tolerant to Starvation Conditions

In the first description of the *ged1* mutant, its ability to show increased greening after extended darkness

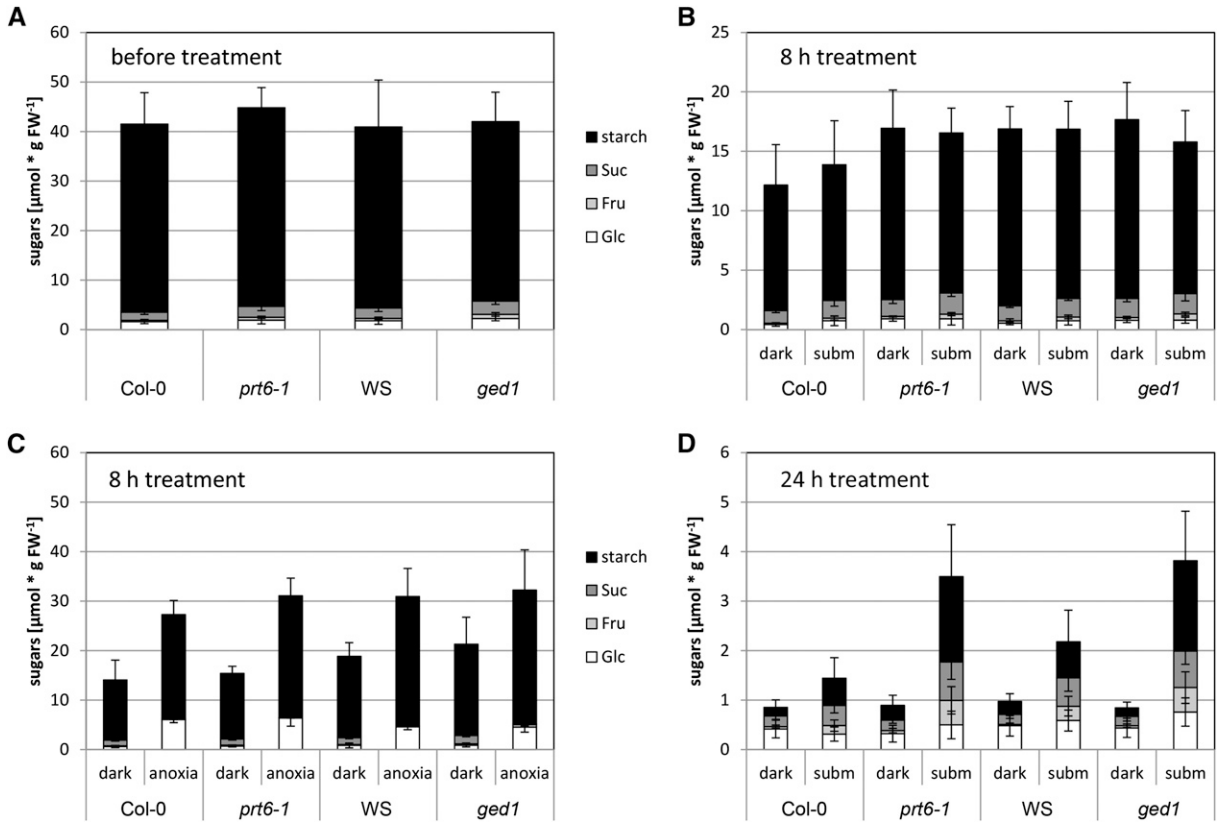

Figure 4. Carbohydrate content of different genotypes before and after stress treatment. Soil-grown 5-week-old plants were submerged for 8 (B) or 24 h (D) in complete darkness or treated with darkness in air for the same time. The sugar content before starting the treatments was also recorded (A). In addition, similar plants were treated with a nitrogen atmosphere for 8 h in complete darkness (C; 8 h of anoxia). After treatment, leaves were harvested, and soluble sugars (Glc, Fru, and Suc) as well as starch were extracted and enzymatically measured. Values are means ± SD of six to nine samples from three independent experiments. Statistics for the values are presented in Supplemental Table S3. FW, Fresh weight; subm, submerged.

was discovered (Choy et al., 2008). Here, we wanted to confirm this finding and extend it to an independent *prt6* T-DNA insertion line. In contrast to WS genotypes, the Col-0 genotypes were not able to green after 10 d of dark germination. For these genotypes, 8 d of dark germination were applied. For both *prt6* alleles, however, we could clearly show the better ability to green after extended darkness compared with the respective wild type (Fig. 5). During our analyses, it was also confirmed that Suc supplementation of the medium interrupted this phenotypic difference (Supplemental Fig. S8).

In addition to germination under darkness, we tested another starvation condition. It had been previously shown that *ate* mutants exhibit delayed leaf senescence (Yoshida et al., 2002; Holman et al., 2009). When 7-d-old light-grown seedlings were transferred to darkness for 9 to 12 d, the wild-type plants did not survive this treatment, whereas *prt6-1* and *ged1* were still able to recover from this treatment (Fig. 5).

Leaf Respiration Rates Are Not Different in *prt6* Mutants

The observations above (Figs. 4D and 5; Supplemental Fig. S6) led us to assume the presence of lower respiration rates in *ged1* and *prt6-1*. However, neither rosette leaves after 24 h of submergence nor seedlings after normal growth or short-term starvation (additional 6 h of darkness treatment shortly after the night phase) showed a difference in oxygen consumption between *prt6* mutants and the respective wild types under normoxic conditions (Fig. 6). In planta measurements of respiration rates during the whole stress period would be required to answer this question.

DISCUSSION

The *ged1* Mutant Is a Novel *prt6* Allele

A phenotype of the mutant *ged1* (Choy et al., 2008) was an increased expression of hypoxic genes under normal oxygen conditions (Fig. 1), showing the importance of this mutation in hypoxia signaling. The mapping process revealed a mutation in the previously characterized gene *PRT6* (*At5g02310*), a component of the N-end rule pathway of protein degradation that is important for hypoxic signal transduction (Gibbs et al., 2011; Licausi et al., 2011). Interestingly, the mutation was not a simple nucleotide exchange

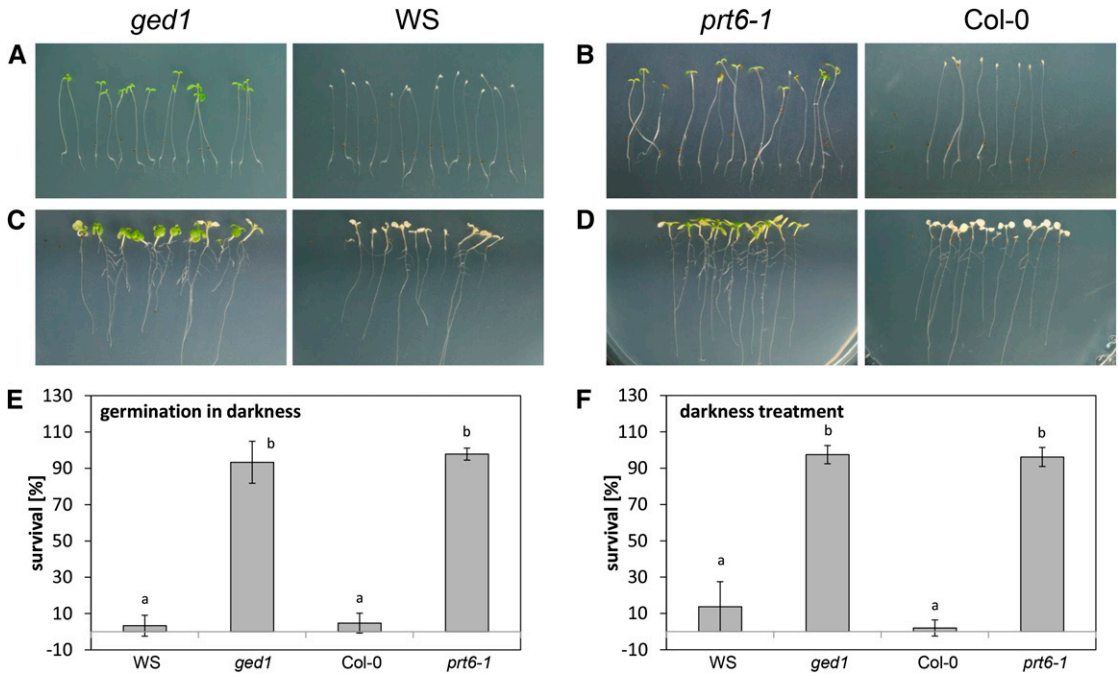

Figure 5. Survival of seedlings under dark germination and darkness treatment. Seeds of Arabidopsis lines (Col-0, *prt6-1*, WS, and *ged1*) were either germinated in darkness for 10 (A) or 8 d (B) and then allowed to recover for another 7 d, or seeds were germinated under long-day conditions for 7 d and then subjected to a darkness treatment for 12 d (C and D) followed by a 7-d recovery. E and F, Quantification of survival experiments. Number of surviving seedlings after treatment and recovery was related to the total number of seedlings. Values are means ± SD of three to six independent experiments. Different letters show significant differences as determined by Tukey's honestly significant difference mean separation test ($P < 0.05$).

caused by the EMS treatment but an exchange of larger chromosomal sequences between chromosomes 3 and 5. This chromosome exchange occurred between two syntenic regions on these chromosomes containing 108 anchors (chromosome 3: *At3g09300–At3g12320* and chromosome 5: *At5g02100–At5g06980*), including *ATE1* (*At5g05700*), *ATE2* (*At3g11240*), and the transcription factors *MYB3R3* (*At3g09370*) and *MYB3R5* (*At5g02320*; Supplemental Fig. S9; Lee et al., 2013; http://chibba.agtec.uga.edu). *MYB3R3* is the gene where the chromosome exchange in *ged1* occurred on chromosome 3, whereas its syntenic gene *MYB3R5* is a direct neighbor of *PRT6*. One can only speculate that the EMS reagent caused a strand break and chromosome rearrangement because of an alignment of closely related but not homologous regions during cell division.

Although a chromosome exchange because of EMS treatment is not common, this effect has been reported several times for animals and plants (Moutschen et al., 1964; Natarajan and Upadhya, 1964; Cattanach et al., 1968; Sega, 1984). Such mutations have not been discovered often in Arabidopsis because of the experimental difficulties in detecting them.

The affected gene *PRT6* was confirmed by the analysis of a cross between *prt6-1* and *ged1*, showing the same ABA-sensitive germination phenotype in the F1 generation as in both parental lines (Fig. 2). Furthermore, the various phenotypes of *prt6* T-DNA insertion null alleles and *ged1* are similar (for example, the ABA-sensitive germination [Choy et al., 2008; Holman et al., 2009], the ability to green after extended darkness [Choy et al., 2008; Fig. 5], and the high expression of hypoxic core genes [Choy et al., 2008; Gibbs et al., 2011]).

Analysis of the sequences of the other N-end rule pathway genes, *ATE1* and *ATE2*, revealed that the WS ecotype only has one functional ATE gene, *ATE1*, which we showed by sequencing and analysis of the F2 generation of the WS × *ate1/ate2* cross. Our finding is further supported by a previously identified mutant, *delayed leaf senescence1*, which shows delayed leaf senescence and has only a mutated *ATE1* gene in the WS background (Yoshida et al., 2002). This delayed leaf senescence is similar to the phenotype of *prt6* mutants that we show here (Fig. 5), and the mutant confirms that *ATE2* had to be nonfunctional in this ecotype. In Col-0, both genes have to be knocked out to show a similar severe phenotype (Graciet et al., 2009; Holman et al., 2009).

Other than *PRT6*, *ged1* also contains a nonfunctional *MYB3R3* gene, which is, to our knowledge, uncharacterized until now. However, we do not expect a phenotype from the mutation of *MYB3R3*, because Arabidopsis has the closely related ortholog *MYB3R5*, which is still intact in *ged1* and shares about 51% identity at the amino acid level with MYB3R3. It is most likely that the defect in *MYB3R3* can be replaced by *MYB3R5*.

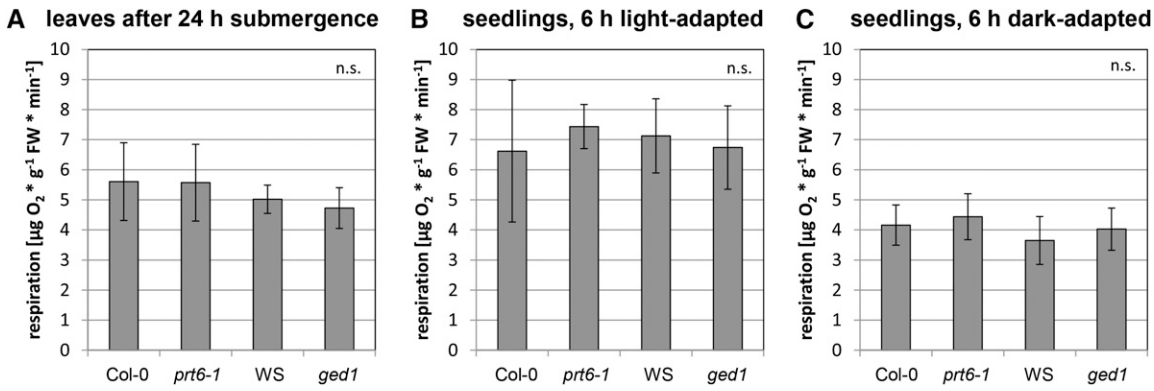

Figure 6. Aerobic respiration rates of Arabidopsis plants. Rates of oxygen consumption (in micrograms of O_2 per gram of fresh weight per minute) in Arabidopsis lines (Col-0, *prt6-1*, WS, and *ged1*) measured in water. A, Oxygen consumption of rosette leaves of 4-week-old Arabidopsis plants. B and C, Oxygen consumption of 7-d-old seedlings that were pretreated with 6 h of illumination (B) or 6 h of darkness (C) before measurements. Values are means ± SD of six to eight samples. FW, Fresh weight; n.s., no significant differences between genotypes as assessed by the Tukey's honestly significant difference mean separation test ($P < 0.05$).

Hypoxic Survival of *prt6* Mutants

After the identification of *ged1* as another *prt6* allele, we investigated its sensitivity toward oxygen deficiency stress because of contradicting reports on the tolerance of *prt6* T-DNA insertion null alleles to hypoxia and submergence (Gibbs et al., 2011; Licausi et al., 2011). Hypoxia survival experiments with Arabidopsis are usually carried out under rather controlled environmental conditions (for example, compared with field experiments). However, a wide range of different hypoxia survival experiments exists in the literature, and especially, submergence survival experiments vary greatly in the length of survival without any obvious reasons (for example, Banti et al., 2010; Licausi et al., 2011; Vashisht et al., 2011; Mithran et al., 2013). In case of submergence in darkness, for example, different publications report great differences in the length of survival (i.e. Col-0 has an LT_{50} of about 8 d with 100% survival after 5 d of submergence [Vashisht et al., 2011], whereas only about 70% of plants from the same genotype survived 3.5 d of submergence in another experiment [Licausi et al., 2011]). Furthermore, factors like developmental stage, sugar supplementation, oxygen concentrations, hypoxic pretreatment, illumination, and genotypic background have been shown to greatly influence the length of survival and have an impact on the measured tolerance or intolerance of transgenic plants (Ellis et al., 1999; Hunt et al., 2002; Ismond et al., 2003; Loreti et al., 2005; Banti et al., 2008; Vashisht et al., 2011).

In our experiments, we report that two *prt6* mutant alleles show better submergence survival compared with their respective wild types under two different illumination conditions (Fig. 3; Table I). These results confirm the findings by Gibbs et al. (2011) showing higher hypoxia tolerance of the *prt6-1* T-DNA insertion line. With respect to the contradicting results of other studies (Licausi et al., 2011; Weits et al., 2014), we propose that the air humidity during the recovery period is a crucial factor for the length of survival of submergence in darkness, which was shown here for treatment of the different genotypes for 5 and 7 d of submergence in darkness (Supplemental Fig. S6). The humidity during recovery not only influenced overall survivability of plants but also, caused opposite behavior of *prt6* mutants versus wild-type plants. These observations hint at different reasons for plant death under different treatment conditions. Root damage caused by submergence itself impairs water uptake and leads to dehydration and wilting of leaves during low-humidity recovery, which seems to be the ultimate cause of death under this condition. Differences in tolerance to low-humidity recovery could possibly be influenced by the length of root survival and/or differences in the adaptation to water deficiency stress (e.g. stomatal closure; Gibbs et al., 2014). Tolerance to submergence followed by high-humidity recovery, however, seems to be rather independent of these tolerance factors. Together with our analysis of carbohydrate levels in shoots of *prt6* mutants after submergence in darkness (as discussed below; Fig. 4), one could argue that they survive submergence followed by high-humidity recovery better because of a more efficient carbohydrate usage in leaves and delayed damages related to carbohydrate starvation. However, at the same time, this observation might suggest a reduced carbohydrate supply to the roots, leading to faster death of this organ and therefore, faster death during low-humidity recovery. More experiments are required to fully understand these survival differences.

A very important aspect of the submergence in darkness experiments by Vashisht et al. (2011) is that the LT_{50} values are compared with survival of darkness in air by the calculation of a hazard ratio, which is a measure of the difference in the respective length of survival. Interestingly, this shows that the majority of

86 examined ecotypes survived darkness in air longer than under submergence (positive hazard ratio) but that some ecotypes are able to survive darkness during submergence as long as or even longer than in air under similar growth and recovery conditions (negative hazard ratio). We show here that the WS wild type of *ged1* is one of those ecotypes that survives darkness in air as long as during submergence (Table I), similar to the WS-0 and WS-2 ecotypes (Vashisht et al., 2011) that are genotypically only slightly different from the wild type of *ged1*. Amazingly, *ged1* exceeds survival of darkness during submergence compared with its wild type by more than 2 d and is the most tolerant genotype that has ever been measured in these survival experiments. However, *ged1* survives prolonged darkness only about as long as the wild type in air, resulting in a negative hazard ratio. In contrast, the Col-0 wild type and *prt6-1* have positive hazard ratios, with a clearly higher LT_{50} value of *prt6-1* than Col-0 both in air and during submergence (Table I). The reason for the similar or better survival of dark submergence than darkness alone for *ged1*, WS, and the ecotypes that share a negative hazard ratio is, however, still unknown. The sugar content in leaves does not differ between the ecotypes after 24 h of darkness (Fig. 4D) as well as the aerobic dark respiration rates (Fig. 6), but other factors could be involved, such as the efficient usage of other energy sources (e.g. lipids).

Different causes of death might have occurred in the experiments presented here (Fig. 3), among them death from sugar starvation, death from root damages, death from reactive oxygen species during desubmergence, and other factors. This hypothesis is being underlined by our starch and sugar measurements after 24 h of submergence in darkness, which reveal a higher total sugar content in *prt6-1* compared with the WS ecotype (Fig. 4D), whereas WS survived submergence better than *prt6-1* (Fig. 3). This indicates a different cause of death in *prt6-1* than leaf carbohydrate starvation. In contrast, the higher total sugar content of *ged1* compared with WS gives an explanation for the negative hazard ratio and its superior survival rate. This indicates that not only do different hypoxia survival experiments measure different causes of death, but also, in the very same survival experiment, different causes of death are measured in different genotypic backgrounds. In this regard, it is interesting that a positive effect of hypoxia pretreatment has been shown for the C24 ecotype in previous publications (Ellis et al., 1999; Hunt et al., 2002; Ismond et al., 2003). The same ecotype has a high negative hazard ratio in the publication by Vashisht et al. (2011) and also, shows the highest LT_{50} of survival of submergence in darkness among 86 tested accessions.

The importance of carbohydrates for survival of oxygen deficiency stress has been also shown in experiments with anoxia or severe hypoxia, such as those carried out, for example, by Gibbs et al. (2011). It has been shown that survival of Col-0 seedlings can be prolonged from less than 1 to 3 d simply by a triplication of Suc supplementation (90 mM compared with 30 mM; Banti et al., 2008). Similar results were published for Col-0 *glabra1* (Loreti et al., 2005), indicating that carbohydrate starvation greatly contributes to the cause of death in these survival experiments. In this context, our sugar measurements after 8 h of nitrogen gas treatment in darkness reveal a high accumulation of Glc together with reduced starch consumption compared with air treatment (Fig. 4C). This suggests that the first energy-consuming step for the degradation of Glc through glycolysis by hexokinases is hampered because of a bioenergetic collapse of the cells. In contrast, Suc levels that were at a concentration of about 2 μmol g^{-1} of fresh weight at the start of the treatment approximated the detection limit after 8 h of stress treatment, confirming observations that Suc but not Glc supplementation results in prolonged anoxia survival of Col-0 seedlings and is associated with a higher fermentation rate (Banti et al., 2008). Similar observations have been reported for tomato (*Solanum lycopersicum*) roots (Germain et al., 1997). Most likely, energy-efficient cleavage of Suc by Suc synthases is favored under anoxic conditions rather than starch and Glc consumption (Bailey-Serres and Voesenek, 2008).

The Submergence Tolerance of *prt6* Mutants Is Associated with Sugar Conservation

When oxidative phosphorylation is hampered, a reduction of energy-consuming processes is inevitable. Several evidences for a down-regulation of energy consumption in response to hypoxia exist. In both plants and animals, this happens by a down-regulation of translation (Branco-Price et al., 2008; Mustroph et al., 2009; Wheaton and Chandel, 2011). Furthermore, down-regulation of other biosynthetic processes (for example, storage metabolism; Geigenberger, 2003) and growth processes (for example, root growth of Arabidopsis; van Dongen et al., 2009) have been observed.

In our experiments, treatment with darkness expectedly reduced sugar and starch levels in Arabidopsis leaves (Fig. 4). However, higher total sugar levels were detected after 24 h of submergence in darkness compared with air in darkness in rosette leaves of all genotypes, indicating that our submergence treatment triggered a reduction in carbohydrate consumption. No differences in total sugar levels were observable after 8 h, which could be because of the remaining air inclusions inside the leaves or slowly decreasing oxygen partial pressures in the water column or soil that become anoxic only after 24 h (Vashisht et al., 2011). Interestingly, the reduction in carbohydrate consumption after 24 h was significantly stronger in *prt6* mutants than the respective wild type (Fig. 4D). Remarkably, the difference in starch content between 24-h submergence and air treatment was about 6-fold in *prt6-1* and 10-fold in *ged1*, whereas no significant difference in starch content between both treatments could be observed in the wild types (Supplemental Table S3). In contrast, no differences in sugar or starch levels were

observable between the genotypes after darkness in air treatment.

The lower sugar consumption rate in *prt6* mutants under submergence compared with wild-type plants is not readily expected, because the potential of sugar consumption is expected to be much higher because of a higher expression of genes involved in fermentative metabolism in the mutants, at least at the start of the treatment (Fig. 1; Choy et al., 2008; Gibbs et al., 2011). Interestingly, hypoxia-inducible *SUS* genes *SUS1/SUS4* but not *PFK* genes *PFK3/PFK6* are among the highly expressed genes in *prt6* mutants (Supplemental Table S1; Choy et al., 2008; Gibbs et al., 2011), which could influence the glycolytic rate under submergence. A generally high fermentation capacity does not always result in an increased rate of fermentation and a higher submergence survival. Although PDC overexpression lines of Arabidopsis showed enhanced hypoxia tolerance (Shiao et al., 2002; Ismond et al., 2003), experiments with other plant species showed the opposite effect (Tadege et al., 1998; Rahman et al., 2001). Furthermore, rice (*Oryza sativa*) plants expressing the transcription factor *Submergence 1A* exhibited enhanced expression of fermentative genes but lower fermentation rates and higher submergence survival than other genotypes (Fukao et al., 2006). It is, therefore, concluded that high expression of fermentative genes in *prt6* mutants does not necessarily lead to higher sugar consumption and fermentation rates under submergence. Rather, the carbohydrate status hints at a lower fermentation rate in those mutants under submergence. Despite the lower carbohydrate consumption in *prt6* mutants (Fig. 4D), the adenylate content after 24 h of submergence was similar in all genotypes (Supplemental Fig. S7), possibly indicating a more efficient energy usage in *prt6* mutants or the use of other energy sources.

The mechanism of how plants reduce their sugar consumption rate under oxygen deficiency stress, despite the availability of enzymes and carbohydrates, is still unclear. We hypothesized that *prt6* mutants use an as yet unknown mechanism of down-regulation of respiration and sugar consumption at the time when oxygen inside the leaves reaches a certain threshold better than wild-type plants. However, all aerobic respiration rates that were measured after normal growth or treatment with 24 h of submergence or 6 h of prolonged darkness were similar in *prt6* mutants and wild types (Fig. 6). Maybe this higher carbohydrate retention is mediated by an, until now, unidentified protein, which is hypoxia inducible and regulated by the N-end rule pathway. It would, therefore, be highly expressed in *prt6* mutants and could decrease the carbohydrate consumption rate more than in the wild type if oxygen concentrations fall below a certain point or other starvation signals appear. Indeed, other starvation conditions, such as germination under dark conditions or treatment of seedlings with prolonged darkness, also lead to better survival of *prt6* mutants than their respective wild types (Fig. 5). Furthermore, *prt6* mutants show reduced root growth at the seedling stage in air that is associated with a reduced breakdown of oil bodies but can be complemented by Suc supplementation (Holman et al., 2009). Therefore, it could be also speculated that an adaptation takes place at the level of energy supply rather than energy consumption. The identification of signals and factors that trigger this response in *prt6* mutants would give some unique insights into the regulation of primary metabolism during hypoxia.

CONCLUSION

The identification of a novel mutant allele for *PRT6*, *ged1*, reveals a unique link between the N-end rule pathway and resistance to starvation. It could be shown that N-end rule pathway mutants are generally tolerant to submergence-induced starvation as well as starvation in darkness, which was partially associated with carbohydrate conservation under submergence but also, other unidentified factors. This occurred despite the fact that glycolytic and fermentative genes were more highly expressed in these mutants. This suggests that the N-end rule pathway is involved in a quiescence-like response during submergence in Arabidopsis.

MATERIALS AND METHODS

Plant Material

Seeds of the WS ecotype and *ged1* were obtained from John C. Gray (Choy et al., 2008). Briefly, *ged1* is an EMS mutant of a transgenic plant expressing GFP under control of a tobacco (*Nicotiana tabacum*) *RbcS* (Rubisco small subunit) promoter (Choy et al., 2008). The Syngenta Arabidopsis Insertion Library T-DNA insertion line *prt6-1* and the respective Col-0 ecotype were obtained from Julia Bailey-Serres (Gibbs et al., 2011).

Analysis of Gene Expression

Microarray data from selected published experiments were downloaded from the Gene Expression Omnibus database (GSE12401, GSE14578, GSE9719, GSE29941, GSE29187, GSE5700, GSE39384, and GSE28800) and reanalyzed as previously described (Mustroph et al., 2010).

RNA was extracted from 7-d-old Arabidopsis (*Arabidopsis thaliana*) seedlings using TRIsure (Bioline) according to the manufacturer's instructions. Subsequently, 1 μg of total RNA was reverse transcribed with oligo(dT) using RevertAid Reverse Transcriptase (Fermentas) and used for semiquantitative PCR analyses.

Map-Based Cloning

Map-based cloning of the *ged1* mutation was done on the F2 generation of a cross between *ged1* and Col-0. Individual F2 plants were chosen according to their sensitivity of seedling establishment on MS medium containing 1% (w/v) Suc, 1% (w/v) agar, and 0.1 μM ABA. Genomic DNA was extracted from leaf samples after recovery and growth on soil. F3 seeds were harvested to exclude false-positive selections in selected individuals; 26 simple sequence length polymorphism and cleaved amplified polymorphic sequence markers across five Arabidopsis chromosomes were used to map the mutation on a chromosome region. For subsequent fine mapping, several additional primers on chromosomes 3 and 5 were used (Supplemental Table S4).

Because of the lack of recombination events on the affected region on chromosome 5, another strategy was applied to find the mutated gene, namely by crossing of the mutant with already published T-DNA insertion null alleles with the same phenotype (*prt6-1* and *ate1/ate2*; Graciet et al., 2009; Holman et al., 2009; Gibbs et al., 2011; Licausi et al., 2011). The F1 generation of the

crosses was tested for ABA-sensitive germination. After identification of the affected gene as *PRT6*, its genomic sequence was determined from overlapping approximately 2,000-bp-long fragments by sequencing of the PCR products.

Because one region could not be amplified by PCR in *ged1*, a TAIL PCR was performed from the left and right sites of the region according to the method previously described (Liu et al., 1995). For this, subsequent PCRs with the primers prt6_3b fw, prt6_3d fw, and prt6_3c fw were done (Supplemental Table S4) combined with one of the respective random primer pools: AD1 to AD6 (Mustroph et al., 2009). In the reverse direction, the primers prt6_rev3, prt6_rev3n, and prt6_rev3d were used. Respective PCR products of the third PCR were sequenced.

Submergence Survival Experiments

The submergence survival experiments were carried out at the University of Utrecht as described by Vashisht et al. (2011). Briefly, plants were grown on soil until the 10-leaf stage in 9-h-light (200 μmol photons m^{-2} s^{-1})/15-h-dark cycles. Stress treatment was started 2 h after the start of a light cycle; 10 plants per genotype treatment and time point were allowed to recover for 2 weeks in a growth chamber with 70% humidity and light conditions as mentioned above.

For additional survival experiments at Bayreuth University, plants were germinated on MS medium for 1 week and grown on soil for 4 weeks in short-day conditions; subsequently, plants were submerged in tap water at the end of the light period in complete darkness. Five and 7 d later, plants were taken out of the water and directly photographed. For recovery, one-half of the plants were grown for another 14 d at low-humidity levels in the growth chamber (about 20%) in short-day conditions, whereas the other one-half were covered with a light-transmittable lid to increase humidity around the plants to about 100%. Pictures were taken again after 14 d of recovery.

Plant Growth and Treatment for Sugar Measurements

Seeds were incubated in deionized water at 4°C for at least 3 d. Several seeds per genotype were sown in the same pot on sterilized soil. In the case of the submergence treatment, a mesh was spanned over the soil to prevent its disturbance during the sinking process. After 1 week, late-germinated seedlings were removed, so that only one plant per pot remained. Plants were grown for 5 weeks in total at 23°C in a growth chamber with 8-h-light (80 μmol photons m^{-2} s^{-1})/16-h-dark cycles. Stress treatments were started at the end of a light cycle. For submergence treatments, three plants per genotype and time point were put in the same box (40-cm length × 31-cm width) filled with acclimated tap water, and glass weights were used to sink the pots. After 8 and 24 h, rosette leaves were detached at the petiole-leaf blade junction, omitting the two oldest and very small young leaves. A separate pool of rosette leaves was created for each plant, the respective fresh weights were measured, and samples were then immediately frozen in liquid nitrogen. Leaves of submerged plants were gently dabbed with tissue paper before weight measurement. The corresponding air-treated plants were processed similarly and grown and stressed together with the plants for the submergence treatment. For anoxia treatments, two pots per genotype were put in a desiccator, in which nitrogen gas was continuously pumped and allowed to exit under positive pressure, thereby replacing the air. Separate air controls with two plants per genotype that were grown and treated together with the plants used in the anoxia experiment were harvested. All experiments were repeated three times.

Metabolite Extraction and Measurements

Frozen rosette leaves of each plant were ground, mixed with 0.5 mL of 0.83 N perchloric acid, and centrifuged; the supernatant was isolated and neutralized by the addition of 125 μL of 1 M bicine and 75 μL of 4 M KOH. After centrifugation, the supernatant was isolated and used for soluble sugar measurements. For starch measurements, the pellet of the first centrifugation step was washed two times with 80% (v/v) ethanol; 400 μL of 0.2 M KOH was added to the pellet and incubated for 1 h at 95°C. After centrifugation, the supernatant was isolated and neutralized with 80 μL of acetic acid; 50 μL of this extract was mixed with 100 μL of 50 mM sodium acetate buffer, pH 5.0, containing 0.5 units of amyloglucosidase and incubated at 55°C overnight. Sugars were measured with a spectrophotometer at 340 nm based on the conversion of NAD to NADH by Glc-6-P dehydrogenase; 50 μL of extract was mixed with 800 μL of 0.1 M imidazole buffer, pH 6.9, containing 5 mM $MgCl_2$, 1 mM ATP, 0.5 mM NAD, and 1 unit of Glc-6-P dehydrogenase. After stabilization of the absorption, 0.5 units of hexokinase, 0.5 units of phosphoglucose isomerase, and 60 units of invertase were added in subsequent steps to determine Glc, Fru, and Suc, and the respective changes in absorption were measured. Adenylates (ATP and ADP) were measured as previously described (Mustroph et al., 2006).

Measurement of Respiration Rates

For detecting aerobic dark respiration on seedlings, Arabidopsis seeds were surface sterilized by incubation in chlorine gas for 45 min. Seeds were sown on MS medium with 1% (w/v) agarose, pH 5.7, without the addition of Suc and kept at 4°C for 3 d. After 7 d of growth under long-day conditions at 23°C, plants were directly used for the experiments about 6 to 8 h after the start of the day. Another set of seedlings was dark adapted shortly after the start of the day for another 6 to 8 h.

Respiration rates, as the rates of oxygen consumption, were measured from seedlings immersed in tap water with the help of a Clark-type oxygen electrode at a constant temperature of 20°C for about 5 min per plant batch. About 15 to 20 seedlings were used for one batch, and the fresh weight was determined as the basis for calculations.

For measuring respiration of rosette leaves, 5-week-old Arabidopsis plants were used. Seeds were sown on a soil:perlite (1:2) mixture and kept in the dark at 4°C for 4 d. The seeds were then germinated in climate chambers at 20°C with 180 μmol photons m^{-2} s^{-1} (9-h photoperiod) and 70% relative humidity. At the two-leaf stage, individual seedlings were transferred to 70-mL pots and maintained in growth chambers with the same conditions as used for germination. For respiration measurements, eight blocks of plants (each block containing one replica of each accession) were submerged in tap water at 1-h intervals in large 65-L containers at a depth of 10 cm above shoot height in the dark for 24 h (±0.5 h) at 21°C (±1°C).

After 24 h, pots with plants were transferred under water and in dim safe-green light into a water-filled, aluminum foil-wrapped beaker to keep the plants in the dark. A leaf was cut off under water, rapidly transferred into a water-filled glass respiration cuvette (inner volume of 1.5 mL; MicroRespiration System; Unisense), and fixed between two plastic mesh discs above the stirring bar. Then, the cuvette was closed with a glass lid and placed in a thermostat water bath (22°C), and an oxygen microsensor (Unisense) was inserted through a capillary in the lid. After approximately 3 min of acclimation, respiration was measured in the dark as oxygen depletion during 5 to 10 min. Depletion curves were linear and typically started around air saturation (21 kPa of oxygen), with a subsequent decrease of oxygen concentrations over 10 min of less than 1 kPa. We anticipated that oxygen concentrations in leaves of the submerged plants had not reached hypoxic conditions before the measurements (Vashisht et al., 2011). After each measurement, leaves were carefully blotted dry and weighed.

Survival of Darkness Experiments with Seedlings

Seeds were surface sterilized by incubation in chlorine gas for 45 min. Seeds were sown on one-half-strength MS medium with 1% (w/v) agarose, pH 5.7 and kept at 4°C for 4 d. For darkness treatments at the seed stage, plates were put into light for 6 to 8 h (at the start of a 16-h-light [80 μmol photons m^{-2} s^{-1}]/8-h-dark cycle) to induce germination. Plants were then grown vertically in darkness for exactly 8 (Col-0 background) and 10 d (WS background) and then, transferred to the same light conditions again for 7 d. For darkness treatments of seedlings, plants were grown under the same light conditions mentioned above for 7 d and transferred to darkness 6 h after the start of the light cycle for 9 to 12 d depending on the first sight of yellowing leaves. For recovery, plates were transferred back to the same light conditions for another 7 d.

Arabidopsis accession numbers of the genes studied according to The Arabidopsis Information Resource are as follows: PRT6, At5g03210; ATE1, At5g05700; ATE2, At3g11240; and MYB3R3, At3g09370.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Alignment of TAIL PCR results with the genes *PRT6* and *MYB3R3* from the 3′ site.

Supplemental Figure S2. Alignment of TAIL PCR results with the genes *PRT6* and *MYB3R3* from the 5′ site.

Supplemental Figure S3. Alignment of sequencing results for the gene *ATE2* from genomic DNA showing a nucleotide insertion.

Supplemental Figure S4. Alignment of sequencing results for the gene *ATE2* from complementary DNA showing a nucleotide insertion.

Supplemental Figure S5. Alignment of translated protein sequences for *ATE2* from complementary DNA sequences.

Supplemental Figure S6. Submergence survival under different recovery conditions.

Supplemental Figure S7. Adenylate content of different genotypes before and after stress treatment.

Supplemental Figure S8. Survival of seedlings under dark germination with or without Suc addition.

Supplemental Figure S9. Section of an alignment of two syntenic regions on chromosomes 3 and 5 of Arabidopsis.

Supplemental Table S1. Comparison of microarray experiments.

Supplemental Table S2. Overview of available *prt6* mutant alleles

Supplemental Table S3. Statistical analysis of sugar content in Arabidopsis plants from Fig. 4.

Supplemental Table S4. Sequence of primers used in this study.

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SUPPLEMENTAL DATA

Supplemental Figure S1: Alignment of TAIL-PCR results with the genes *PRT6* and *MYB3R3* showing the chromosomal exchange point in yellow. Similar sequences are shaded with gray color. TAIL-PCR and sequencing was done from the 3' site.

```
part of PRT6       1   TGATGGCTGCTGTTCGCTCCCTGACTTGTCTGGGTCCTGCATGCTCCCTGTTAATGTATC 60
TAIL1A_rev         1   ------------------------------------------------------------ 1
TAIL1B_rev         1   -------------------------------------------------------TGTGGA 6
part of MYB3R3     1   ----------------------------------------TGATATTAACAAGGATGCT 19
Clustal Consensus  1                                                               1

part of PRT6       60  --TCCTTGTTGATAAGCATCAACAAGGA-GCATGCAGAACGTAGACATAGTTACCTCTGT 117
TAIL1A_rev         1   -----CAGAGAGGAAGAAGTAGCTCTCATGAATGCTCATCGAAGCCACGGAAACAAATGG 55
TAIL1B_rev         7   G-TAGCAGAGAGGAAGAAGTAGCTCTCATGAATGCTCATCGAAGCCACGGAAACAAATGG 65
part of MYB3R3     20  TGGACCACAGAGGAAGAAGTAGCTCTCATGAATGCTCATCGAAGCCACGGAAACAAATGG 79
Clustal Consensus  1                                                               1

part of PRT6       118 GGTAATA-----GAAGATTTTTGTATAAGACTGTCTAAATTGTTTTTATGCTCATTATTC 172
TAIL1A_rev         56  GCTGAAATTGCTAAGGT-------ATAAGACTGTCTAAATTGTTTTTATGCTCATTATTC 108
TAIL1B_rev         66  GCTGAAATTGCTAAGGT-------ATAAGACTGTCTAAATTGTTTTTATGCTCATTATTC 118
part of MYB3R3     80  GCTGAAATTGCTAAGGTCTTACCCGGCAGGTATTTTTACTTTTCTTGGCATGCGTCATTG 139
Clustal Consensus  1                          *** *  * *    * * ****  * ** ** **  *  20

part of PRT6       173 AAGTTGTTATCTGTAGTTAGAATTAATAAGTTCCAGGATATGGTAGTTATGCTTATATAT 232
TAIL1A_rev         109 AAGTTGTTATCTGTAGTTAGAATTAATAAGTTCCAGGATATGGTAGTTATGCTTATATAT 168
TAIL1B_rev         119 AAGTTGTTATCTGTAGTTAGAATTAATAAGTTCCAGGATATGGTAGTTATGCTTATATAT 178
part of MYB3R3     140 GAGTTGAGACTTTAGAGAAGGGTTA----GCTTCTGGGTTTAATGAAGGT-TTTACTTAA 194
Clustal Consensus  20   **   ** * * *        *    *        **    * * * ** * **      39

part of PRT6       233 AACTTGCATGCAGGCAAAAATGAAAGCTGAACAGTCAAAGTTCTTGTCTACCTTGAGTTC 292
TAIL1A_rev         169 AACTTGCATGCAGGCAAAAATGAAAGCTGAACAGTCAAAGTTCTTGTCTACCTTGAGTTC 228
TAIL1B_rev         179 AACTTGCATGCAGGCAAAAATGAAAGCTGAACAGTCAAAGTTCTTGTCTACCTTGAGTTC 238
part of MYB3R3     195 AACTTGC-TGCAGG----ACTGATAATGCAATAAA-GAAC--CA--------TTGGAATA 238
Clustal Consensus  39   * * ***  *****  *  *      **  ***    * * * ** * *  *     *  65

part of PRT6       293 CAGCATGGATGATGATGATCCGAGATCTGAATTTGAAACAAGTGATTCTGTGATGGAACA 352
TAIL1A_rev         229 CAGCATGGATGATGATGATCCGAGATCTGAATTTGAAACAAGTGATTCTGTGATGGAACA 288
TAIL1B_rev         239 CAGCATGGATGATGATGATCCGAGATCTGAATTTGAAACAAGTGATTCTGTGATGGAACA 298
part of MYB3R3     239 GTTCATTGAAAAAGA-AGTCAGAATTCTACTTATTGACTGGTAGGTTACCACCACCAACA 297
Clustal Consensus  65   ***  ** ******* ******    * *** *    ** *    **   *        94

part of PRT6       353 TGATTCAGAAATAGCTGTCCGTGAAGTTTGCTCCCTCTGCCATGACCCAGATTCCAAAGA 412
TAIL1A_rev         289 TGATTCAGAAATAGCTGTCCGTGAAGTTTGCTCCCTCTGCCATGACCCAGATTCCAAAGA 348
TAIL1B_rev         299 TGATTCAGAAATAGCTGTCCGTGAAGTTTGCTCCCTCTGCCATGACCCAGATTCCAAAGA 358
part of MYB3R3     298 ACAACAAGGAATGG-TGTTC---------------------------------------- 316
Clustal Consensus  94   ***   *    *** **  * **   ** **  ***   * *  *      * **     119

part of PRT6       413 CCCCGTTTCTTTCTTGATTTTTCTCCAGGTTGGTAGCATGACTGATGCTATAATATGCGA 472
TAIL1A_rev         349 CCCCGTTTCTTTCTTGATTTTTCTCCAGGTTGGTAGCATGACTGATGCTATAATATGTGA 408
TAIL1B_rev         359 CCCCGTTTCTTTCTTGATTTTTCTCCAGGTTGGTAGCATGACTGATGCTATAATATGTGA 418
part of MYB3R3     316 ------------------------------------------------------------ 316
Clustal Consensus  119      ****  *   ** *** * *** *                                134

part of PRT6       473 CTGTTAAGGTTAAATAAACATATACTCCGAGGAAATTTTGTTCTGCATCATTCTTAATGA 532
TAIL1A_rev         409 CTGTTAAGGTTAAATAAACATATACTCCGAGGAAATTTTGTTCTGCATCATTCTTAATGA 468
TAIL1B_rev         419 CTGTTAAGGTTAAATAAACATATACTCCGAGGAAATTTTGTTCTGCATCATTCTCAATGA 478
part of MYB3R3     316 ------------------------------------------------------------ 316
Clustal Consensus  134                                                             134
```

Supplemental Figure S2: Alignment of TAIL-PCR results with the genes *PRT6* and *MYB3R3* showing the chromosomal exchange point in yellow. Similar sequences are shaded with gray color. TAIL-PCR and sequencing was done from the 5' site.

```
part of PRT6       1   CTACTTCTGATGAGAAGCGCAAGGCTAAAGCTAAAGAGAGGCAGGCTGCTATTATGGTAA 60
TAIL2A_fw          1   ----------------------------------------AAGGAGTGCTATAATGGGTA 20
TAIL2B_fw          1   ----------------------------------------AGAAGAGGGCATTTATGGTA 20
part of MYB3R3     1   ------------------------------------------------------------ 1
Clustal Consensus  1                                                                1

part of PRT6       61  GATCCTTTTATTTCAAATCTAGTGCAAAAGTTTGACATGGAACCGGTAGCCATTGATGTC 120
TAIL2A_fw          21  GATCCTTTTATTTCAA-TCTAGTGCAAAAGTTTGACATGGAACCGGTAGCCATTGATGTC 79
TAIL2B_fw          21  GGTCCTTTTATTTCAA-TCTAGTGCAAAAGTTTGACATGGAACCGGTAGCCATTGATGTC 79
part of MYB3R3     1   ------------------------------------------------------------ 1
Clustal Consensus  1                                                                1

part of PRT6       121 TGATCATGCACTTGAACATATGATGGCTGCTGTTCGCTCCCTGAC-TTGTCTGGGTCCTG 179
TAIL2A_fw          80  TGATCATGCACTTGAACATATGATGGCTGCTGTTCGCTCCCTGAC-TTGTCTGGGTCCTG 138
TAIL2B_fw          80  TGATCATGCACTTGAACATATGATGGCTGCTGTTCGCTCCCTGAC-TTGTCTGGGTCCTG 138
part of MYB3R3     1   ----------------------------------------TGATATTAACAAGGA--TG 17
Clustal Consensus  1                                          ***  **  *  **   ** 10

part of PRT6       180 CATGCTCCCTGTTAATGTATCTCCTTGTTGATAAGCATCAACAAGGAGCATGCAGAACGT 239
TAIL2A_fw          139 CATGCTCCCTGTTAATGTATCTCCTTGTTGATAAGCATCAACAAGGAGCATGCAGAACGT 198
TAIL2B_fw          139 CATGCTCCCTGTTAATGTATCTCCTTGTTGATAAGCATCAACAAGGAGCATGCAGAACGT 198
part of MYB3R3     18  CTTGGACCACAGAGGAAGAAGTAGCTCTC-ATGAATGCTCATCGAAGCCACGGA-AACAA 75
Clustal Consensus  11  * **  **          *  *   * *  ** *      *       ** * * ***   30

part of PRT6       240 AGACATAGTTACCTCTGTGGTAATAGAAGATTTTTGTATAAGACTGTCTAAATTGTTTTT 299
TAIL2A_fw          199 AGACATAGTTACCTCTGTGGTAATAGAAGATTTTTGT--------TTTTTACTTTTCTTG 250
TAIL2B_fw          199 AGACATAGTTACCTCTGTGGTAATAGAAGATTTTTGT--------TTTTTACTTTTCTTG 250
part of MYB3R3     76  ATGGGCTGAAATTGCTAAGGTCTTACCCGGCAGGTA---------TTTTTACTTTTCTTG 126
Clustal Consensus  31  *      *  *   **  ***  **   *     * * *         * * ** * **  51

part of PRT6       300 ATGCTCATTATTCAAGTTGTTATCTGTAGTTAGAATTAA----TAAGTTCCAGGATATGG 355
TAIL2A_fw          251 GCATGCGTCATTGGAGTTGAGACTTTAGAGAAGGGTTAGCTTCTGGGTTTAATGAAGGTT 310
TAIL2B_fw          251 GCATGCGTCATTGGAGTTGAGACTTTAGAGAAGGGTTAGCTTCTGGGTTTAATGAAGGTT 310
part of MYB3R3     127 GCATGCGTCATTGGAGTTGAGACTTTAGAGAAGGGTTAGCTTCTGGGTTTAATGAAGGTT 186
Clustal Consensus  51       * * ***  *****  *  *      **  ***     *  ***  * **      75

part of PRT6       356 TAGTTATGCTTATATAT-AACTTGCATGCAGGCAAAAATGAAAGCTGAACA-----     405
TAIL2A_fw          311 TTACTAAACACTTGCGTGAACAAACACGGCGCTATACACAACGACTAAAATAACTG     366
TAIL2B_fw          311 TTACTTAAAACTTGCTGCAGGACTGATCATGCAATAAAGAACCATTGGAATAGTT-     365
part of MYB3R3     187 TTACTTAAAACTTGCTGCAGGACTGATAATGCAATAAAGAACCATTGGAATAGTT-     241
Clustal Consensus  76  *   *       *    *      *    *  * * *  *    *  *             88
```

Supplemental Figure S3: Alignment of sequencing results for the gene *ATE2* from genomic DNA showing a nucleotide insertion in yellow. Similar sequences are shaded with gray color. The coding sequence is underlined. Several WS ecotypes from NASC were sequenced in addition to the two genotypes used in this study (N1603 to N28828).

```
Col-0 ATE2 gene   1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTATGTGCTACAACA 60
WS-0_SALK         1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
WS-2_SALK         1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
N1603_WS-0        1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
N28825_WS-0       1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
N76303_WS-0       1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
N28827_WS-2       1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
N28828_WS-2       1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
ged ate2 fw2      1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60
WS_ate2_fw2       1   GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 60

Col-0 ATE2 gene   61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTGAAA 120
WS-0_SALK         61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
WS-2_SALK         61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
N1603_WS-0        61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
N28825_WS-0       61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
N76303_WS-0       61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
N28827_WS-2       61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
N28828_WS-2       61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
ged ate2 fw2      61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120
WS_ate2_fw2       61  ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 120

Col-0 ATE2 gene   121 AGGTC-GAGTTTTGAACCAGAGGAATATGAATTATACAAACGGTATCAAATGAAAGTGCA 179
WS-0_SALK         121 AGGTC-GAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 179
WS-2_SALK         121 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATGAAAGTGCA 180
N1603_WS-0        121 AGGTC-GAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 179
N28825_WS-0       121 AGGTC-GAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 179
N76303_WS-0       121 AGGTC-GAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 179
N28827_WS-2       121 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 180
N28828_WS-2       121 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 180
ged ate2 fw2      121 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 180
WS_ate2_fw2       121 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 180

Col-0 ATE2 gene   180 TAATGATAAGCCGGAGAGCATCTCAGAAACTTCATACAAACGGTTTTTGGTAGACACTCC 239
WS-0_SALK         180 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 239
WS-2_SALK         181 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 240
N1603_WS-0        180 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 239
N28825_WS-0       180 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 239
N76303_WS-0       180 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 239
N28827_WS-2       181 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 240
N28828_WS-2       181 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 240
ged ate2 fw2      181 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 240
WS_ate2_fw2       181 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 240

Col-0 ATE2 gene   240 GCTCACTGAAGTTCCTTCTTCAGGTTATGATGATGAAGAAAAGATTCCTCTTTGTGGCTT 299
WS-0_SALK         240 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 299
WS-2_SALK         241 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 300
N1603_WS-0        240 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 299
N28825_WS-0       240 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 299
N76303_WS-0       240 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 299
N28827_WS-2       241 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 300
N28828_WS-2       241 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 300
ged ate2 fw2      241 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 300
WS_ate2_fw2       241 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 300
```

```
Col-0 ATE2 gene   300 TGG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 358
WS-0_SALK         300 TGG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 358
WS-2_SALK         301 TGG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 359
N1603_WS-0        300 TGG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 358
N28825_WS-0       300 TGG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 358
N76303_WS-0       300 GGGTTTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 359
N28827_WS-2       300 -GG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 358
N28828_WS-2       301 GGGGTTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 360
ged ate2 fw2      301 TGG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 359
WS_ate2_fw2       301 TGG-TTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTG 359

Col-0 ATE2 gene   359 ATATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 418
WS-0_SALK         359 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 418
WS-2_SALK         360 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 419
N1603_WS-0        359 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 418
N28825_WS-0       359 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 418
N76303_WS-0       360 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 419
N28827_WS-2       359 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 418
N28828_WS-2       361 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 420
ged ate2 fw2      360 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 419
WS_ate2_fw2       360 ACATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCT 419

Col-0 ATE2 gene   419 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 478
WS-0_SALK         419 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 478
WS-2_SALK         420 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 479
N1603_WS-0        419 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 478
N28825_WS-0       419 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 478
N76303_WS-0       420 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 479
N28827_WS-2       419 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 478
N28828_WS-2       421 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 480
ged ate2 fw2      420 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 479
WS_ate2_fw2       420 TGTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTC 479

Col-0 ATE2 gene   479 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 538
WS-0_SALK         479 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 538
WS-2_SALK         480 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 539
N1603_WS-0        479 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 538
N28825_WS-0       479 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 538
N76303_WS-0       480 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 539
N28827_WS-2       479 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 538
N28828_WS-2       481 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 540
ged ate2 fw2      480 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 539
WS_ate2_fw2       480 ATTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGA 539

Col-0 ATE2 gene   539 GATATAAAGCAGCCTATCGTCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 598
WS-0_SALK         539 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAgtaagttc 598
WS-2_SALK         540 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAgtaagttc 599
N1603_WS-0        539 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 598
N28825_WS-0       539 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 598
N76303_WS-0       540 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 599
N28827_WS-2       539 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 598
N28828_WS-2       541 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 600
ged ate2 fw2      540 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 599
WS_ate2_fw2       540 GATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAGTAAGTTC 599

Col-0 ATE2 gene   599 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCT 658
WS-0_SALK         599 cttaaaccacaaaactaagatcaaattcttgttagttgtggagagcatataactgattcc 658
WS-2_SALK         600 cttaaaccacaaaactaagatcaaattcttgttagttgtggagagcatataactgattcc 659
N1603_WS-0        599 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCC 658
N28825_WS-0       599 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCC 658
N76303_WS-0       600 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCC 659
N28827_WS-2       599 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCC 658
N28828_WS-2       601 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCC 660
ged ate2 fw2      600 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCC 659
WS_ate2_fw2       600 CTTAAACCACAAAACTAAGATCAAATTCTTGTTAGTTGTGGAGAGCATATAACTGATTCC 659
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Col-0 ATE2 gene   659 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 718
WS-0_SALK         659 cttgtattttgttctcagATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 718
WS-2_SALK         660 cttgtattttgttctcagATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 719
N1603_WS-0        659 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 718
N28825_WS-0       659 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 718
N76303_WS-0       660 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 719
N28827_WS-2       659 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 718
N28828_WS-2       661 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 720
ged ate2 fw2      660 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 719
WS_ate2_fw2       660 CTTGTATTTTGTTCTCAGATGGGTTCCATTCGAAGTAGCCAAGCCTCTGCTTGATAAAAA 719

Col-0 ATE2 gene   719 GCCTTATTCCGTCTTGTCCAATATCAGTAAGGTTTCTTCATCATCATCATCACCTCAAGC 778
WS-0_SALK         719 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 778
WS-2_SALK         720 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 779
N1603_WS-0        719 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 778
N28825_WS-0       719 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 778
N76303_WS-0       720 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 779
N28827_WS-2       719 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 778
N28828_WS-2       721 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 780
ged ate2 fw2      720 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 779
WS_ate2_fw2       720 GCCTTATTCCGTCTTGTCCAATATCACTAAGGTTTCTTCATCATCATCATCACCTCAAGC 779
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Supplemental Figure S4: Alignment of sequencing results for the gene *ATE2* from cDNA showing a nucleotide insertion in yellow. Similar sequences are shaded with gray color.

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Col-0_ATE2_cds 1   GTTGGAGAGTTTACTGGTTTTTCTGTTAAGGTCTCCAAGGGTCATATCAACTTCCTTTCA 60
WS-0 ATE2 prot 1   GTGGGAGAGTTTACTGGTTTTTCTGTTAAGGTCTCCAAGGGTCATATCAACTTCCTTTCA 60
WS-2 ATE2 prot 1   GTGGGAGAGTTTACTGGTTTTTCTGTTAAGGTCTCCAAGGGTCATATCAACTTCCTTTCA 60
ged1 cds ATE2  1   GTGGGAGAGTTTACTGGTTTTTCTGTTAAGGTCTCCAAGGGTCATATCAACTTCCTTTCA 60
WS cds ATE2    1   GTGGGAGAGTTTACTGGTTTTTCTGTTAAGGTCTCCAAGGGTCATATCAACTTCCTTTCA 60

Col-0_ATE2_cds 61  GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTATGTGCTACAACA 120
WS-0 ATE2 prot 61  GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 120
WS-2 ATE2 prot 61  GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 120
ged1 cds ATE2  61  GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 120
WS cds ATE2    61  GCTACCCAAGTTACTTCCTCAGATAGAAATGAAGGTGAAGAAAGCCTTTGTGCTACAACA 120

Col-0_ATE2_cds 121 ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTGAAA 180
WS-0 ATE2 prot 121 ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 180
WS-2 ATE2 prot 121 ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 180
ged1 cds ATE2  121 ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 180
WS cds ATE2    121 ATAAAGTCTTCTTCGAACAAGCTTCATGCAAGAAAGCGGAAGCTGGAGATGCACTTAAAA 180

Col-0_ATE2_cds 181 AGGTC-GAGTTTTGAACCAGAGGAATATGAATTATACAAACGGTATCAAATGAAAGTGCA 239
WS-0 ATE2 prot 181 AGGTC-GAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 239
WS-2 ATE2 prot 181 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATGAAAGTGCA 240
ged1 cds ATE2  181 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 240
WS cds ATE2    181 AGGTCCGAGTTTTGATCCAGGGGAATATGAATTATACAAACGGTATCAGATAAAAGTGCA 240

Col-0_ATE2_cds 240 TAATGATAAGCCGGAGAGCATCTCAGAAACTTCATACAAACGGTTTTTGGTAGACACTCC 299
WS-0 ATE2 prot 240 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 299
WS-2 ATE2 prot 241 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 300
ged1 cds ATE2  241 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 300
WS cds ATE2    241 TAATGATAAGCCGGAGAGTATCTCAGAAACTTCATACAAAAGGTTTTTGGTAGACACTCC 300

Col-0_ATE2_cds 300 GCTCACTGAAGTTCCTTCTTCAGGTTATGATGATGAAGAAAAGATTCCTCTTTGTGGCTT 359
WS-0 ATE2 prot 300 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 359
WS-2 ATE2 prot 301 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 360
ged1 cds ATE2  301 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 360
WS cds ATE2    301 GCTCATTGAAGTTCCTTCTTCAGGTCATGATGATGAAGAAAAGGTTCCTCCTTGTGGCTT 360

Col-0_ATE2_cds 360 TGGTTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTGA 419
WS-0 ATE2 prot 360 TGGTTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTGA 419
WS-2 ATE2 prot 361 TGGTTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTGA 420
ged1 cds ATE2  361 TGGTTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTGA 420
WS cds ATE2    361 TGGTTCTTTCCATCAACAATACCGAGTTGATGACCGTCTAATTGCTGTTGGGGTGATTGA 420

Col-0_ATE2_cds 420 TATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCTT 479
WS-0 ATE2 prot 420 CATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCTT 479
WS-2 ATE2 prot 421 CATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCTT 480
ged1 cds ATE2  421 CATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCTT 480
WS cds ATE2    421 CATTCTTCCTAAATGTTTGTCTAGTAAATACCTGTTCTGGGATCCGGATTTCGCATCCTT 480

Col-0_ATE2_cds 480 GTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTCA 539
WS-0 ATE2 prot 480 GTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTCA 539
WS-2 ATE2 prot 481 GTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTCA 540
ged1 cds ATE2  481 GTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTCA 540
WS cds ATE2    481 GTCTCTTGGAAACTACTCAGCCCTGCAAGAAATCGATTGGGTAAAACAGAACCAAGCTCA 540

Col-0_ATE2_cds 540 TTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGAG 599
WS-0 ATE2 prot 540 TTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGAG 599
WS-2 ATE2 prot 541 TTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGAG 600
ged1 cds ATE2  541 TTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGAG 600
WS cds ATE2    541 TTGCTCTACTCTTGAGTATTACTATCTTGGCTATTACATACATTCCTGTAACAAGATGAG 600

Col-0_ATE2_cds 600 ATATAAAGCAGCCTATCGTCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAATGGGTTCC 659
WS-0 ATE2 prot 600 ATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAATGGGTTCC 659
WS-2 ATE2 prot 601 ATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAATGGGTTCC 660
ged1 cds ATE2  601 ATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAATGGGTTCC 660
WS cds ATE2    601 ATATAAAGCAGCCTATCGCCCGTCTGAGCTTCTATGCCCTCTCCGGTACCAATGGGTTCC 660
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Col-0_ATE2_cds 660 ATTCGAAGTAGCC 672
WS-0 ATE2 prot 660 ATTCGAAGTAGCC 672
WS-2 ATE2 prot 661 ATTCGAAGTAGCC 673
ged1 cds ATE2  661 ATTCGAAGTAGCC 673
WS cds ATE2    661 ATTCGAAGTAGCC 673
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Supplemental Figure S5: Alignment of translated protein sequences for *ATE2* from cDNA sequences presented in Supplemental Figure S4. Similar sequences are shaded with gray color.

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Col_ATE2 prot 1   MSSKKAKTKNEASSSRGGIGGESIVADCGRNKSTCGYCKSSTRFSISHGLWTERLTVNDY 60
ATE2_WS/ged1  1   MSSKKAKTKNEASSSRGGIGGESIVADCGRNKSTCGYCKSSTRFSISHGLWTERLTVNDY 60
WS0 ATE2 prot 1   MSSKKAKTKNEASSSRGGIGGESIVADCGRNKSTCGYCKSSTRFSISHGLWTERLTVNDY 60

Col_ATE2 prot 61  QALLDSGWRRSGCYLYKPEMEKTCCPSYTIRLKASDFVPSKEQQRVRRRIERFLDGELDA 120
ATE2_WS/ged1  61  QALLDRGWRRSGCYLYKPEMEKTCCPSYTIRLKASDFVPSKEQQRVRRRLERFLDGELDA 120
WS0 ATE2 prot 61  QALLDRGWRRSGCYLYKPEMEKTCCPSYTIRLKASDFVPSKEQQRVRRRLERFLDGELDA 120

Col_ATE2 prot 121 KPSEQTEDQDVSFSREVSVSVKKSLGAAKREKNNELEPIMKDLSEQIDNAVQKCIQSGEF 180
ATE2_WS/ged1  121 KPSEQTEDQDVSFSGEVSVLGRKSLGAI-------VEPIMKDLSEQIDNAVQRCIQSGEF 173
WS0 ATE2 prot 121 KPSEQTEDQDVSFSGEVSVLGRKSLGAI-------VEPIMKDLSEQIDNAVQRCIQSGEF 173

Col_ATE2 prot 181 PSNVQIPKASVKKVFSAKRKKLAEGSEDLLYTSNIAFPIVAAMKHTQTLEKGKNVEENRL 240
ATE2_WS/ged1  174 PSNVQIPNASVKKVLSAKRKKLAEGSEELLYTSNIAFPIGAAIKHTQTLEKGINVEENRL 233
WS0 ATE2 prot 174 PSNVQIPNASVKKVLSAKRKKLAEGSEELLYTSNIAFPIGAAIKHTQTLEKGINVEENRL 233

Col_ATE2 prot 241 SPEAVSEKLLSAMNKVGEFTGFSVKVSKGHINFLSATQVTSSDRNEGEESLCATTIKSSS 300
ATE2_WS/ged1  234 SPEAVSEKLLSAMSKVGEFTGFSVKVSKGHINFLSATQVTSSDRNEGEESLCATTIKSSS 293
WS0 ATE2 prot 234 SPEAVSEKLLSAMSKVGEFTGFSVKVSKGHINFLSATQVTSSDRNEGEESLCATTIKSSS 293

Col_ATE2 prot 301 NKLHARKRKLEMHLKRSSFEPEEYELYKRYQMKVHNDKPESISETSYKRFLVDTPLTEVP 360
ATE2_WS/ged1  294 NKLHARKRKLEMHLKRSEF*---------------------------------------- 312
WS0 ATE2 prot 294 NKLHARKRKLEMHLKRSSFDPGEYELYKRYQIKVHNDKPESISETSYKRFLVDTPLIEVP 353

Col_ATE2 prot 361 SSGYDDEEKIPLCGFGSFHQQYRVDDRLIAVGVIDILPKCLSSKYLFWDPDFASLSLGNY 420
ATE2_WS/ged1  312 ------------------------------------------------------------ 312
WS0 ATE2 prot 354 SSGHDDEEKVPPCGFGSFHQQYRVDDRLIAVGVIDILPKCLSSKYLFWDPDFASLSLGNY 413

Col_ATE2 prot 421 SALQEIDWVKQNQAHCSTLEYYYLGYYIHSCNKMRYKAAYRPSELLCPLRYQWVPFEVAK 480
ATE2_WS/ged1  312 ------------------------------------------------------------ 312
WS0 ATE2 prot 414 SALQEIDWVKQNQAHCSTLEYYYLGYYIHSCNKMRYKAAYRPSELLCPLRYQWVPFEVAK 473

Col_ATE2 prot 481 PLLDKKPYSVLSNISKVSSSSSSPQASETLLESTSEHEDMEQGDTNDDDDEMYNSDEDSD 540
ATE2_WS/ged1  312 ------------------------------------------------------------ 312
WS0 ATE2 prot 474 PLLDKKPYSVLSNITKVSSSSSSPQASETLLESTSEHEDMEQGDTNDDDDEMYNSDEDSD 533

Col_ATE2 prot 541 SDSSSSRNRSDITNILISLNGPRLRYKDIPRFKNPVVQKQLESMLVSYRKVVGAELSEIM 600
ATE2_WS/ged1  312 ------------------------------------------------------------ 312
WS0 ATE2 prot 534 SDSSSSRNRSDITNILISLNGPRLRYKDIPRFKNPVVQKQLESMLVSYRKVVGAELSEIM 593

Col_ATE2 prot 601 VYELR 605
ATE2_WS/ged1  312 ----  312
WS0 ATE2 prot 594 VYELR 598
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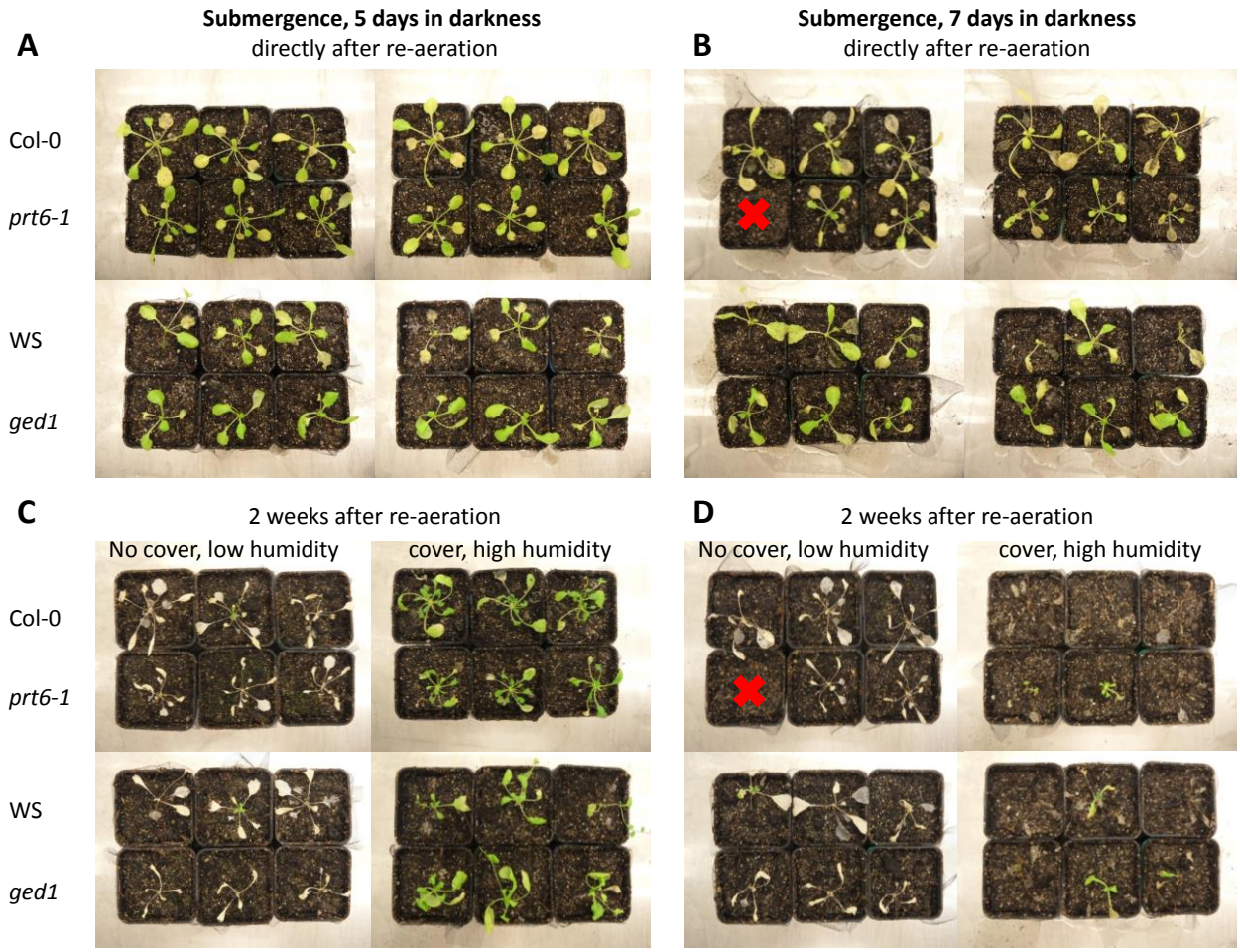
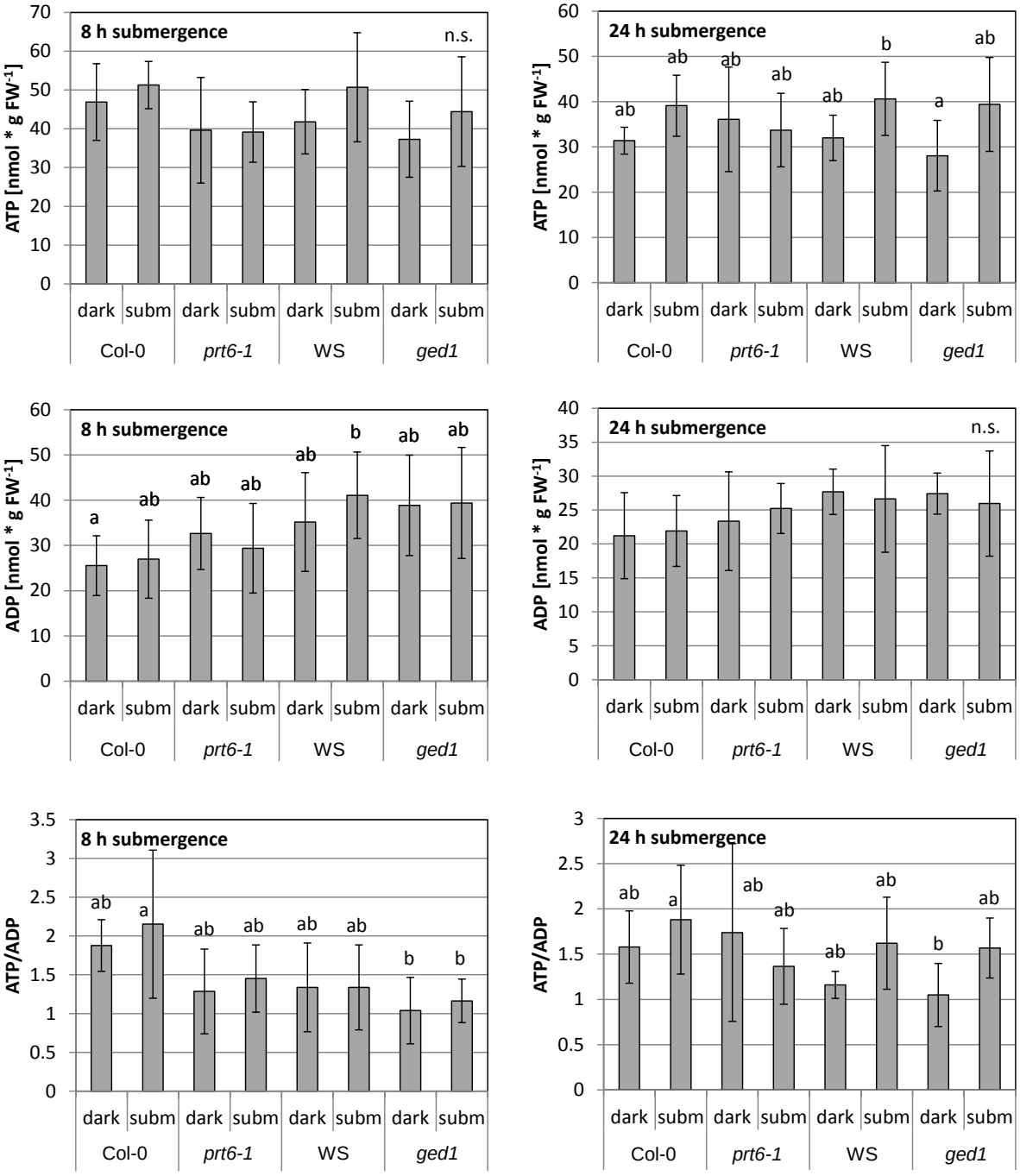
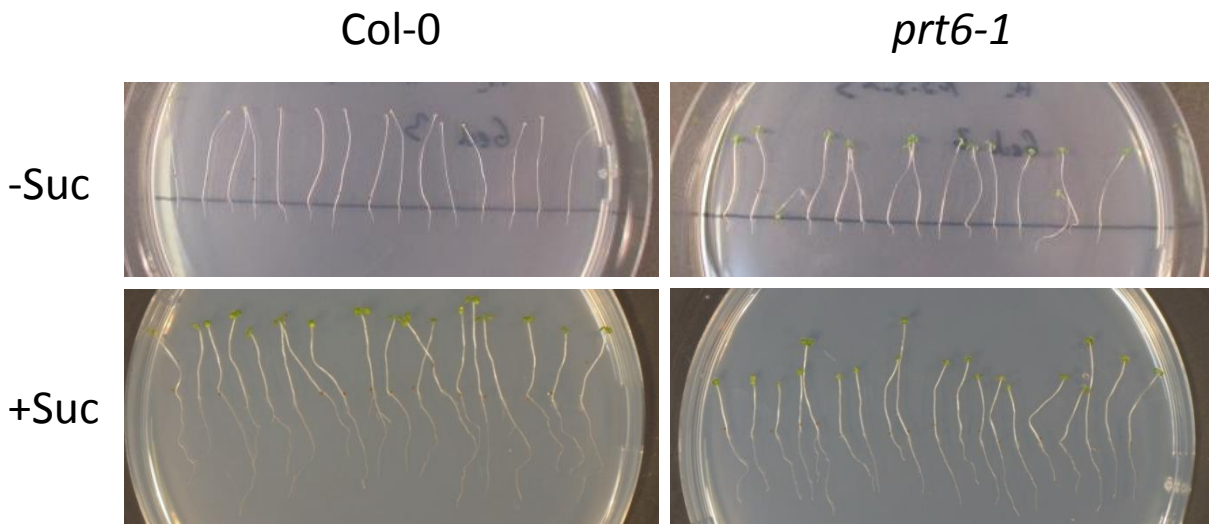
Supplemental Figure S6: Submergence survival under different recovery conditions. Arabidopsis plants were grown on soil for four weeks, and subsequently submerged in darkness for 5 (A, C) or 7 (B, D) days. After submergence, plants were removed from water and directly photographed (A, B), or recovered for 14 days under short-day conditions (C,D). Recovery was done either at about 20% humidity (left) or with a cover leading to about 100% humidity (right). Experiments were performed in the same way twice, and one example is shown.



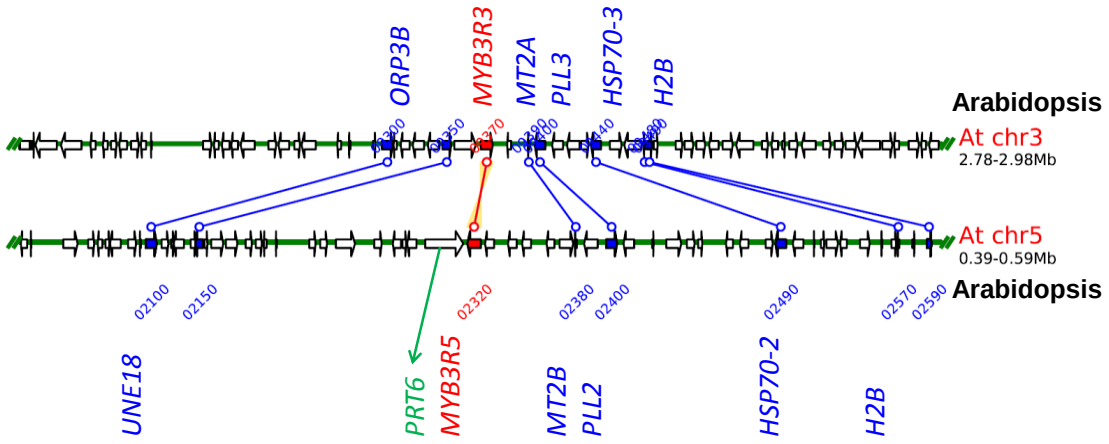
Supplemental Figure S7: Adenylate content of different genotypes before and after stress treatment. Soil-grown 5-week-old plants were submerged for 8 or 24 h in complete darkness or treated with darkness in air for the same time. After treatment, leaves were harvested, metabolites extracted, and ATP and ADP measured. Values are means ± SD of 6 to 9 samples from three independent experiments. Different letters show significant differences as determined by Tukey's honestly significant difference mean-separation test ($P < 0.05$); n.s., no significant differences.



Supplemental Figure S8: Survival of seedlings under dark germination with or without sucrose addition. Seeds of Arabidopsis lines (Col-0, *prt6-1*) were germinated in darkness for 8 days and then allowed to recover for another 7 days under long-day conditions, with (+Suc) or without (-Suc) 1% sucrose in the medium.



Supplemental Figure S9: Section of an alignment of two syntenic regions on chromosome 3 and chromosome 5 of *Arabidopsis thaliana* (taken from http://chibba.agtec.uga.edu, Lee et al. 2012). The *prt6* gene on chromosome 5 is marked with a green arrow, selected similar proteins are marked in blue. The second mutated gene, *MYB3R3*, and its homolog *MYB3R5* is marked in red.



Supplemental Table S1 (Excel file): Comparison of microarray experiments. Gene expression of *ged1* mutant in comparison to its wildtype WS (Choy et al., 2008) revealed higher expression of hypoxia-induced genes (Branco-Price et al., 2008; Mustroph et al., 2009) and N-end-rule pathway mutants (Gibbs et al., 2011; Licausi et al., 2011), and lower expression of ABA-responsive genes (Goda et al., 2008; Kim et al., 2011). SLR, signal-log-ratio; Padj, adjusted P-value.

Supplemental Table S2: Overview over available *prt6* mutant alleles

Name of line	**ID of line**	**Ecotype**	**Kind of mutation**	**Place of insertion (bp from start codon)**	**References**
prt6-1	SAIL 1278_H11	Col-0	T-DNA insertion	ca. 2.360	Garzon et al., 2007, Graciet et al., 2009, Gibbs et al., 2011
prt6-2	GK270G04	Col-0	T-DNA insertion	ca. 7.940	Garzon et al., 2007, Graciet et al., 2009
prt6-4	fn10	Ler	Fast neutron mutation	ca. 4.860	Holman et al., 2009
prt6-5	SALK_051088	Col-0	T-DNA insertion	ca. 2.550	Graciet et al., 2009, Gibbs et al., 2011
prt6-6	FLAG_443C12	WS	T-DNA insertion	ca. 2.930	Licausi et al., 2011
prt6-7	*ged1*	WS	EMS mutation	4.442	This work

Supplemental Table S3 (Excel file): Statistical analysis of sugar content in Arabidopsis plants from **Fig. 4**. Data were analyzed with the Tukey's honestly significant difference mean-separation test, and values that differed at $P < 0.05$ were labelled with different letters. Two types of comparisons were done, (**A**) a comparison of treatments within one genotype, and (**B**) a comparison of genotypes within a treatment.

Supplemental Table S4 (Excel file): Sequence of primers used in this study.