Fibrillin expression is regulated by abscisic acid response regulators and is involved in abscisic acid-mediated photoprotection

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Fibrillins are lipid-binding proteins of plastids that are induced under abiotic stress conditions. In response to environmental stress, plants generate abscisic acid (ABA) as an endogenous signal. We show that ABA treatment and fibrillin accumulation enhance the tolerance of photosystem II toward light stress-triggered photoinhibition in Arabidopsis. ABA induces fibrillin accumulation, and the ABA response regulators ABI1 and ABI2 regulate fibrillin expression. The abundance of fibrillin transcripts was specifically reduced in the ABA-insensitive abi1 mutant but not in the abi2 mutant. However, leaves of abi2 revealed in comparison to WT and abi1 enhanced fibrillin levels, pointing to a posttranscriptional control mechanism. Protein interaction analysis identified the protein phosphatase ABI2 to target the preprotein of fibrillin. Interaction was abrogated either by deleting the signal peptide of prefibrillin or by the single amino acid exchange present in the phosphatase-deficient abi2 protein. Thus, ABI1 and ABI2 seem to control fibrillin expression that is involved in mediating ABA-induced photoprotection.

ABI1 | ABI2 | abiotic stress | photoinhibition | light stress

Fibrillins are plastid-associated lipid-binding proteins that are ubiquitous in plants (1, 2). They have been primarily characterized from chloroplasts of tomato and pepper fruits and are known to accumulate during abiotic stress in plastids e.g., inflicted by high light, cold, and drought (3–5), and also during pathogen infection (6). The family of fibrillin-like proteins is unique to plants, and the members contain a hydrophobic domain that associates with or anchors within lipids (7). Fibrillins associate with stromal lamellae of thylakoids and fibrillic carotenoid-containing structures of chromoplasts (3, 8). A model for the fibrillic structures predicts a layer of fibrillin shielding polar lipids and carotenoids (1). In potato, the fibrillin C40.4 protein is specifically associated with the photosystem II (PSII) light-harvesting complex with a presumptive role in the modulation of photosynthetic efficiency (9). A posttranslational control of fibrillin accumulation by abscisic acid (ABA) was indicated by studies on ABA-deficient tomato that failed to accumulate fibrillin despite normal transcript levels (5). Although fibrillin is induced in response to abiotic stress, its role in stress responses and the molecular mechanism regulating its accumulation are still elusive.

ABA plays a major role in regulating plant growth and development and mediating adaptations to environmental stress such as cold, drought, and salinity (10). ABA regulates ion channel activities involved in osmoregulation and stomatal closure and influences gene expression at the transcriptional and posttranscriptional levels (11, 12). Key regulators of diverse ABA-mediated responses are ABI1 and ABI2. Both proteins are members of a larger family of plant protein phosphatases 2C (PP2Cs), several of which act negatively in a partially redundant manner on ABA responses (13–15). The Arabidopsis mutants abi1-1 (abi1) and abi2-1 (abi2) show a dominant ABA-insensitive phenotype conferred by an identical amino acid exchange within the catalytic domain that results in a strongly reduced protein phosphatase activity of abil (ABI1Gly180Asp) and abil (ABI2Gly168Asp) (16–19). Little is known about the substrates and cellular regulators of ABI1 and ABI2. Protein microinjection analysis revealed a competition of ABI1 and abil for common binding sites and an action downstream of Ca2+ in the ABA signal pathway (20). An interacting protein of ABI1 is the homeodomain transcription factor AtHb6 (21). AtHb6 is up-regulated by ABA and constitutes part of a negative feedback loop involved in the adjustment of plant’s sensitivity to ABA. Furthermore, ABI1 and ABI2 physically interact with a SNF1-like protein kinase that forms a calcium-responsive negative regulatory circuit together with the calcium binding protein CaBP5 (22). The central role of ABI1 in ABA signal transduction is reflected by the finding that >90% of ≈1,300 identified ABA-responsive genes are deregulated in abi1 (23). The massive ABA-evoked readjustment of gene expression is integrated into the plant’s adaptive responses to environmental challenges (10, 24).

In this study, we establish a molecular link between the ABA signal pathway and fibrillin accumulation in Arabidopsis. Regulation of fibrillin expression involves the two ABA response regulators ABI1 and ABI2 that act at the transcriptional and posttranscriptional level, respectively. Fibrillin and ABA enhance the tolerance of PSII toward light stress-triggered photoinhibition.

Results

Identification of Prefibrillin as an Interacting Partner of ABI2. The key regulatory functions exerted by ABI1 and ABI2 in diverse ABA responses include regulation of gene expression, ion channel activity, and growth and development. Hence, both protein phosphatases are promising starting points for the elucidation of the integrative network of ABA signaling. We used the yeast-two-hybrid system to identify proteins interacting with ABI2. Fusion of ABI2 to the GAL4 DNA-binding domain did not reveal significant transcriptional autoactivation (Fig. 1A) in contrast to previous results with ABI1 in yeast (21). Screening of Arabidopsis cDNA libraries for interactors of ABI2 in yeast resulted in the identification of six clones that showed lacz activation and histidine autotrophy in dependence on the expression of the cDNA fusion protein. DNA sequence analysis revealed that two cDNA fusions were identical and encoded the fibrillin precursor protein (At4g04200), of which the last three carboxyl-terminal amino acid residues were missing. The preprotein encompasses 318 amino acid residues with a transit peptide of 55 amino acid residues (25). Fibrillin shares high structural similarity with a second gene of Arabidopsis, fibrillin2 (At4g22240, 76% amino acid identity), and members of the protein family of other species such as the homologue of rapeseed (AAK57564, 79%), tobacco (T03635, 72%), and tomato (CAA75688, 90% of amino acid identity). ABA induces fibrillin accumulation, and abi1 (ABI1Gly180Asp) and abi2 (ABI2Gly168Asp) (16–19). Little is known about the substrates and cellular regulators of ABI1 and ABI2. Protein microinjection analysis revealed a competition of ABI1 and abil for common binding sites and an action downstream of Ca2+ in the ABA signal pathway (20). An interacting protein of ABI1 is the homeodomain transcription factor AtHb6 (21). AtHb6 is up-regulated by ABA and constitutes part of a negative feedback loop involved in the adjustment of plant’s sensitivity to ABA. Furthermore, ABI1 and ABI2 physically interact with a SNF1-like protein kinase that forms a calcium-responsive negative regulatory circuit together with the calcium binding protein CaBP5 (22). The central role of ABI1 in ABA signal transduction is reflected by the finding that >90% of ≈1,300 identified ABA-responsive genes are deregulated in abi1 (23). The massive ABA-evoked readjustment of gene expression is integrated into the plant’s adaptive responses to environmental challenges (10, 24).

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Abbreviations: ABA, abscisic acid; FD, fibrillin-down-regulated lines; FU, fibrillin-up-regulated lines; PP2C, protein phosphatases 2C; PSII, photosystem II.

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64%), potato (T07825, 64%), cucumber (T10179, 64%), and pepper (56663, 61%).

To further characterize the specificity and structural requirements of the interaction, different variants of ABI1 and ABI2 were fused to the binding domain and were analyzed in yeast (Fig. 1A). Truncation of the amino-terminal domain of ABI2 (ΔNABI2, amino acids 95–423), or the single point mutation in abi2, which results in catalytic deficiency, abolished the interaction. In addition, all variants of ABI1 tested failed to show significant interaction with fibrillin. Furthermore, deletion of the transit peptide of prefibrillin abrogated the interaction with ABI2 indicating that the ABI2 fusion protein binds to the preprotein rather than to the mature fibrillin.

The interaction was examined in vitro by both affinity chromatography and pulldown assays with purified preparations of ABI2 and prefibrillin. The fibrillin precursor protein was linked to the GST, and the resulting fusion protein (FIB-GST) was tested for binding to ABI2 tethered onto resin material by virtue of a carboxyl-terminal histidine tag. Subsequent chromatography of FIB-GST over the ABI2-coated resin resulted in efficient retention of the fusion protein but not of the GST control (Fig. 1B). Elution of ABI2 and other bound proteins yielded 83% of applied FIB-GST, whereas 24% of applied GST was recovered in that fraction. In the second experiment, the discrimination of ABI2 versus abi2 was analyzed in the interaction with prefibrillin by using a pulldown assay. ABI2- and abi2-coated beads were incubated with radiolabeled FIB-GST and GST, and subsequently the binding efficiency was determined. The majority of applied FIB-GST (78%) was associated with the immobilized ABI2, whereas only 17% of the preprotein was recovered with abi2-coated beads (Fig. 1C). The recovery of FIB-GST with abi2-coated beads is close to the values for the negative controls that consisted of the combinations GST with ABI2 or abi2 and yielded 10% and 14%, respectively. Taken together, the results strongly argue for a specific binding of ABI2 to the fibrillin preprotein in vitro and in vivo.

**Gene Expression of Fibrillin Is Controlled by ABA and Depends on ABI1.**

Fibrillins are inducible proteins, and their expression is up-regulated by drought, cold, and oxidative and salt stress (3–6). These stress conditions involve ABA as a signal, and, hence, we examined whether ABA is able to regulate fibrillin gene expression. Exposure of *Arabidopsis* seedlings to ABA (30 μM) resulted in moderate enhancement of transcript levels within 3 h and with induction levels of ~3-fold (Fig. 2A). Analysis of the dose–response dependence showed detectable up-regulation at 1 μM ABA reaching saturation at ~10 μM ABA. Interestingly, analysis of ABA-insensitive *abi1* and *abi2* plants revealed a clear dependence of fibrillin transcript levels on *abi1* but not *abi2* (Fig. 2B). In the *abi1* mutant background, the mRNA abundance of fibrillin was lowered by a factor of >10 in comparison with WT and the *abi2* mutant. The basal transcript levels present in WT and *abi2* plants were recovered in *abi1* in the presence of ~100 μM exogenous ABA.

The observed difference in fibrillin transcript levels could reflect ABA-stimulated promoter activity or enhanced mRNA stability. To discern the action of ABA, a fibrillin promoter-luciferase construct was transfected into *Arabidopsis* protoplasts for transient analysis. ABA-responsive reporter expression was enhanced 2- to 3-fold in the presence of ~30 μM ABA (Fig. 2C). Exposure of transfected protoplasts to light stimulated the fibrillin promoter as well, and a combined treatment of light and ABA further activated luciferase expression (Fig. 2D). However, coexpression of dominant-acting *abi1* efficiently blocked ABA-mediated reporter induction in agreement with an ABA-inducible and ABI1-dependent gene expression of fibrillin.

**Functional Analysis of Fibrillin.** The emerging interaction of the ABA response pathway with fibrillin expression prompted us to examine
a possible link between fibrillin, light stress, and ABA. A photoprotective role of ABA has been postulated for chloroplasts under cold conditions (26). Furthermore, fibrillin is known to accumulate during high-light conditions (5), and fibrillin affects photosynthetic efficiency (9). Taken together, the findings provided a hint that fibrillin and ABA might be involved in the plant response toward light stress. Therefore, we analyzed the effect of ABA and fibrillin on light-triggered photoinhibition of PSII by using plants with deregulated fibrillin accumulation. Arabidopsis plants of ecotype RLD with elevated or lowered levels of fibrillin were successfully generated by up- and down-regulation of fibrillin transcript abundance (Fig. 3). Two fibrillin-up-regulated lines (FU) and two fibrillin-down-regulated lines (FD) were randomly selected. Analysis revealed a 6-fold and >20-fold enhanced level of fibrillin transcripts in FU6 and FU7, respectively, whereas the level was lowered by a factor of 2 and 5 in the FD2 and FD7 compared with the control (Fig. 3A). ABA challenge (30 μM for 24 h) increased the fibrillin mRNA level in control plants but not in FD and FU lines. RT-PCR analysis discriminative for fibrillin and the homologous fibrillin2 revealed the down-regulation of only fibrillin transcripts (Fig. 3B). Immunoblot analysis with a fibrillin-specific antibody confirmed that altered transcript levels resulted in correspondingly altered protein levels (Fig. 3C).

The contribution of fibrillin and ABA toward photoprotection of PSII was examined by high-fluence irradiation of leaves in the absence or presence of ABA. Maximal photochemical efficiency of PSII \( (F_v/F_m) \) was determined after light stress and subsequent recovery period in the dark to allow for reversible adaptation processes. A light stress-dependent reduction of \( F_v/F_m \) values is interpreted as photoinhibition of PSII (27). Photoinhibition in leaves of Arabidopsis clearly depended on the duration of light stress and already was observed after 30 min of high-intensity illumination. A light stress of 1 h followed by the recovery period resulted in a drop of maximal photochemical PSII activity by 35% in leaves of RLD (Fig. 4A; see also Table 1, which is published as supporting information on the PNAS web site). In the analysis, the transgenic lines with deregulated fibrillin expression differed considerably in the degree of photoinhibition. Leaves of fibrillin-overexpressing lines revealed enhanced phototolerance, whereas those of fibrillin-down-regulated plants were more photoinhibited than RLD. The rate of photoinhibition after 1 h of light stress corresponded to \( \approx 0.15 \min^{-1} \) and \( 0.11 \min^{-1} \) for PSII in FU6 and FU7, respectively, whereas the values were 2- to 3-fold higher in leaves of FD2 and FD10 corresponding to \( 0.33 \min^{-1} \) and \( 0.37 \min^{-1} \), respectively (\( P < 0.01 \)). Leaves of RLD revealed an intermediate rate of \( 0.22 \min^{-1} \), which was still 2-fold higher than the inactivation rate of the FU7 line.

Preincubation of detached leaves with exogenous ABA (25 μM for 4 h) before high fluence exposure changed the light stress-triggered photoinhibition in WT plants. Phototolerance was now improved to a level indistinguishable from the previously more phototolerant FU lines (Fig. 4B and Table 1). Interestingly, the phototolerance of FU lines was not further improved by ABA. ABA did not change the decline of \( F_v/F_m \) values in FD leaves, which were light-stressed up to 1 h (\( P < 0.05 \)); however, longer periods lead to a partial recovery. Fibrillin levels were induced 4-fold and 2-fold within 2 h of exposure to ABA and light, respectively (Fig. 4C). Fibrillin accumulation occurred also in the FD line, however, at a reduced rate (Fig. 4D).

The results are consistent with an ABA-induced and fibrillin-dependent protection of the photosynthetic machinery. To gain insight into the role of ABI2 in that process, leaves of abi2 and ABI2 plants of the ecotype Landsberg (Ler) were analyzed for light-induced inactivation of photochemical PSII activity (Fig. 5A; see also Table 2, which is published as supporting information on the PNAS web site). Light stress up to 2 h led to an almost linear decline of \( F_v/F_m \) values at an inactivation rate of 0.27% min\(^{-1}\) and 0.13% min\(^{-1}\) in control and abi2 leaves, respectively. The effective quan-
Regulation of Fibrillin Expression at the Transcriptional Level by ABA.

The role of ABA in transcriptional regulation of fibrillin has been elusive in several species of Solanaceae (3–6). In Arabidopsis, however, fibrillin expression is unequivocally regulated by ABA. Fibrillin transcript levels increased rapidly and sensitively in the presence of ABA (25 μM) and light (L; 1,400 μmol m⁻² s⁻¹) in RLD (C) and comparison between wild type and FD2 transgenic (D). Fibrillin levels were quantified by the immunosignal that was normalized via the chlorophyll content of extracts to leaf fresh weight. The induction factor is presented and factor 1 corresponds to 0.2 and 0.1 μg fibrillin per gram of fresh weight for RLD and FD2, respectively.

**Discussion**

Abiotic stress conditions inflicted by cold, salt, and drought affect a plant’s water status and trigger ABA signaling (10, 24). The ABA signal mediates reduction of water loss by closing stomata, and this response generates a major dilemma during ongoing photosynthesis (29). CO₂ deficiency due to impaired gas exchange can lead to misdirection of the light-driven electron transport to the acceptor O₂, causing the generation of toxic superoxide radicals and other reactive oxygen species (30, 31). This scenario may lead, in turn, to photooxidation and membrane damage. Thus, protection of chloroplast functionality under these stress conditions is of paramount importance for plants. One of the plastidic proteins induced during abiotic stress is fibrillin. Our analysis revealed that fibrillin accumulation contributes to the protection of PSII against light stress and that fibrillin accumulation depends on the ABA signal pathway.
Genome-comprehensive profiling of *Arabidopsis* transcripts identified coexpression of fibrillin and ABI2 in leaves, reproductive organs, and, at low levels, in seeds and roots (www.arabidopsis.org/info/expression/ATGenExpress.jsp). Histochemical analysis of *Arabidopsis* plantlets expressing a reporter gene under the control of a fibrillin promoter fragment revealed predominant expression in the mesophyll and guard cells (data not shown). Consistent with the fibrillin promoter activity, cell-specific transcript analyses detected coexpression of fibrillin and ABI2 in guard cells and mesophyll (33), lending support for the presence of both components in common cells, a prerequisite for interaction of prefibrillin and ABI2 in planta.

**Posttranslational Control of Fibrillin Accumulation by ABI2.** A physical interaction of ABI2 with prefibrillin is supported by *in vivo* analysis in yeast and by *in vitro* experiments. The protein interaction was highly discriminative against the point-mutated abi2 and ABI2 lacking the amino-terminal extension that characterizes the amino-terminal part as a novel interaction domain of plant PP2Cs (34). The interaction of ABI2 with prefibrillin, rather than fibrillin, emphasizes the importance of the signal peptide for binding. The finding is compatible with a cytosolic interaction site. The deregulated fibrillin accumulation in abi2 provides genetic evidence for interaction. Interestingly, fibrillin transcript levels were comparable in the mutant and wild type. However, >4-fold higher levels of fibrillin were observed in abi2. Similarly, the analysis of a tomato deficient in ABA biosynthesis revealed normal fibrillin transcript levels but a failure to accumulate the protein during drought stress (5). Taken together, the observations and the fact that ABA levels are not reduced in the abi2 mutant (35) argue for a posttranscriptional or posttranslational regulation of fibrillin accumulation by both ABA and ABI2. In light of the specific binding of ABI2 to prefibrillin, it seems likely that it is, in fact, a posttranslational control step exerted by ABI2 and regulated by ABA.

The failure of abi2 to interact with prefibrillin and the hyperaccumulation of fibrillin in the mutant suggest a phosphatase-dependent negative control exerted by ABI2. The analysis of *abi2* heterozygous plants revealed intermediate increases of fibrillin levels, also in FD lines, indicating a codominant action of *abi2* on fibrillin accumulation (data not shown). The findings are not compatible with a simple model in which ABI2 targets freely the transit peptide of prefibrillin, e.g., for dephosphorylation. Clearly, the elucidation of the regulatory mechanism requires future analyses.

**Fibrillin and Light Stress.** Our analysis of *Arabidopsis* has unraveled an important role of ABA and fibrillin in establishing tolerance of PSII toward light stress. Plants with deregulated fibrillin expression revealed a clear dependence of photoinhibition on fibrillin levels. The light-triggered reduction of maximal photochemical efficiency of PSII was determined after an extended recovery period, which is interpreted as "irreversible" photoinhibition (27). Pretreatment of leaves with ABA or elevated levels of fibrillin accomplished either by fibrillin overexpression or by the *abi2* mutation resulted in enhanced phototolerance. ABA may act in numerous ways to contribute to photoprotection, e.g., by stimulating the xanthophyll cycle or redirecting gene expression of components contributing to photoprotection (23, 26, 30). However, fibrillin seems to be a major player in this adaptive response. ABA quickly induces fibrillin accumulation in *Arabidopsis* leaves, and preinduced fibrillin levels provide enhanced tolerance. ABA and light both stimulate fibrillin accumulation. Most importantly, preinduced fibrillin levels and ABA do not act additively in providing phototolerance of PSII, suggesting a common response pathway. Analysis in potato identified a specific association of fibrillin with the light-harvesting complex of PSII with a presumptive role in the modulation of photosynthetic efficiency (9). How the modulation is accomplished remains open. The observed photoprotection could involve the scaffold function of fibrillin for lipids and lipophilic substances (1, 7, 36) and reflect a fibrillin-mediated support of the PSII repair system that is a critical component in a plant’s response to light stress (37). As a consequence of this protective role, preinduced fibrillin levels in overexpressor lines and in the *abi2* mutant resulted in higher PSII efficiency under light stress and decreased nonphotochemical quenching compared with control plants.

In conclusion, our data establish previously undescribed links between the ABA response pathway, fibrillin, and light stress by showing that fibrillin accumulation is involved in providing ABA-mediated photoprotection of PSII. The ABA response regulators ABI1 and ABI2 control transcript abundance and accumulation of fibrillin, respectively. Hence, fibrillin gene expression and action seem to be tightly interwoven with steps of ABA signal transduction.

**Methods**

**Molecular Biological Analysis.** The different GAL4 DNA-binding fusions used in the protein interaction analysis, the *in vitro* binding assays, and primers used for PCR are further detailed in Supporting Methods, which is published as supporting information on the PNAS web site. The analyses were essentially carried out as reported for ABI1 (21). The *Arabidopsis* cDNA library established in pACT prey vector was provided by the DNA Stock Center at *Arabidopsis* Biological Resource Center, (Columbus, OH). *Arabidopsis* cDNA inserts were amplified by PCR with the forward primer (5'-AGATCTGGAAATCCGATCCTC-3') and the reverse primer (5'-TTAAGGCTTGAATCTCTGAGCGGCAGGAG-3') and cloned via the EcoRI and HindIII sites at their 5' ends, respectively, for DNA sequence analysis (ABI310 Genetic Analyzer; Applied Biosystems). For deletion of the transit peptide of prefibrillin (amino acids 1–53) a different forward primer (5'-
CGAATTCATCCGAGCGACGGACATC-3') was used for amplification of a truncated cDNA fragment to replace the 0.7-kb EcoRI-BglII fragment of prefibrillin cDNA in the pACT vector. Fibrillin transcript levels were analyzed by real-time PCR (Carl Roth, Karlsruhe, Germany). Transient gene expression was initiated in protoplasts after 16 h of phenotypic expression in the dark and in the presence of ABA unless otherwise stated. Reporter constructs and conditions were as reported in ref. 21. Briefly, polyethylene glycol-mediated DNA transfections were performed by using plasmid DNA of 35S-GUS (20 μg) and fibrillin promoter-firefly luciferase (LUC) (20 μg) constructs per 5 × 10^6 Arabidopsis mesophyll protoplasts. In some cases, the 35S-abl construct (10 μg) was cotransfected. LUC activities were assayed in a luminometer (Berthold Technologies, Bad Wildbad, Germany) during a 90-s integration period of light emission. The fibrillin promoter-LUC construct was generated by fusing a BamHI-HindIII sites of pBI221 (38). The fibrillin promoter was amplified from BAC T24H24 (DNA Stock Center at Arabidopsis Biological Resource Center) with the forward primer 5'-TTAAGCTTATGAACATCGTCTCAGATC-3' and the reverse primer 5'-AATGGATCCTGGTTTGTTCTCTACAGAAACC-3'. ABA-regulated expression was normalized for glucuronidase activity. The 35S-abl expression cassette was generated by replacing the XbaI-EclI36II GUS gene fragment of pBI221 with the BamHI-EcoI47I abdi cDNA fragment (18).

Transgenic Plants and Physiological Characterization. Arabidopsis plants, accession RLD, were transformed via Agrobacterium tumefaciens strain CS9pGVS3850 (39). Transgenic FU and FD Arabidopsis lines were generated by transformation with pBI121-35S-fibrillin and pBI121-35S-asfibrillin, which were constructed by replacing the GUS gene of pBI121 (38) with the blunted EcoRI-HindIII fibrillin cDNA fragment in sense and antisense orientation into the blunted Smal-EclI36II sites of the vector. For physiological characterization, Arabidopsis plants were grown in pots on perlite/soil mixture at 23°C under long-day conditions with 16-h light (250 μmol·m⁻²·s⁻¹). Detached rosette leaves from 4-week-old plants, fully developed and of comparable size, were floated on half-strength liquid MS medium with adaxial sides facing upward. The temperature was controlled at 23°C (Haake, Karlsruhe, Germany). Incubation was carried out in the presence or absence of ABA, or 0.5 M mannitol at a light intensity of 70 μmol·m⁻²·s⁻¹ for 4 h before exposure to high-light conditions (1,400 μmol·m⁻²·s⁻¹). Light was passed through an infrared-absorbing layer of water. After a variable period of light stress, leaves were kept in darkness (45 min), and maximal photochemical activity of PSI (F_v/F_m) was measured by using a PAM-2000 (Heinz Walz, Effeltrich, Germany) under atmospheric conditions (40). A stress-dependent reduction of F_v/F_m values was interpreted as photoinhibition of PSII (27, 41). For statistical analysis, Student's t test was used.

Immunological Analysis. Proteins of leaf extracts for immunological analysis were obtained and analyzed as described in ref. 42. Protein concentration was determined in extracts to assure loading of equal amounts of protein. Leaves WT, mutant, and transgenic plants were exposed to 2 h of high light conditions before fibrillin analysis unless otherwise indicated. Polyclonal antibodies directed against pepper fibrillin were used (1), and cross reactivity of the antibody to Brassicaceae (43) was confirmed by testing the specific recognition of fibrillins obtained either from pepper fruits and by heterologous expression of Arabidopsis prefibrillin in Escherichia coli. Bound antibody was detected with a secondary anti-rabbit antibody conjugated to horseradish peroxidase by using SuperSignal West Femto (Pierce) as a substrate. Peroxidase activity was measured by light emission recorded by an intensified charge-coupled device camera (ORCAII ERG; Hamamatsu Photonics, Hamamatsu City, Japan) equipped with a Schneider Xenon 0.95/25 objective (Kreuznach, Germany) in a dark box.

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