Plant Signaling & Behavior 6:12, 1881-1885; December 2011; ©2011 Landes Bioscience

The occurrence of a thylakoid-localized small zinc finger protein in land plants

Yan Lu

Department of Biological Sciences; Western Michigan University; Kalamazoo, MI USA

Keywords: zinc finger, photosystem II, PSII repair and reassembly, non-photochemical quenching, photoinhibitory quenching, complementation, adaptation

Previous studies showed that LOW QUANTUM YIELD OF PHOTOSYSTEM II 1 (LQY1), a small thylakoid zinc finger protein was involved in maintenance and repair of Photosystem II (PSII). Here the author provides additional evidence for the role of LQY1 in PSII maintenance and repair and further commentary on the occurrence of LQY1 protein among land plants. After exposure to high light, *Arabidopsis thaliana* mutants lacking functional *LQY1* gene (At1g75690) are more photoinhibited than wild-type control plants; display higher total non-photochemical quenching and photoinhibitory quenching. These results are consistent with the initial observation that *lqy1* mutants have lower PSII efficiency than wild-type plants after high light treatment. The low-PSII-efficiency phenotype can be suppressed upon complementation of *lqy1* mutants with the *LQY1* gene from wild-type plants. This further demonstrates that LQY1 is important in maintaining the activity of photosystem II in Arabidopsis. LQY1 homologs are present in land plants but are absent from sequenced genomes of green algae and cyanobacteria, which may reflect plant adaptation to excess light stress during the transition to land.

Sunlight is essential for plant's growth and development. However, too much exposure can cause damage to the plants. Under high light conditions, photosystem II (PSII) undergoes rapid damage and repair cycle to degrade and replace photodamaged reaction center proteins.[1] This is a dynamic process that involves dozens of proteins and hundreds of cofactors.[2] Recently, it was found that a thylakoid membrane zinc finger protein, LOW QUANTUM YIELD OF PSII 1 (LQY1), has protein disulfide isomerase activity and it interacts with PSII core monomer and CP43-less monomer (a marker for ongoing PSII repair).[3] LQY1 is primarily localized in thylakoid grana margin and stroma lamellae, where critical steps of PSII repair and reassembly occur.[3] Under high light conditions, the *lqy1*-deficient mutants have faster degradation and synthesis of reaction center protein D1, less PSII-light harvesting complex II (LHCII), lower PSII efficiency, than wild type.[3] These results suggest that LQY1 may act in maintenance and repair of photodamaged PSII complexes.[3]

In this paper, the author reports the non-photochemical quenching (*NPQ*) kinetics of *lqy1* mutants, the characterization of *lqy1* complementation lines, and phylogenetic analysis of LQY1 and homologs in other land plants. The results further demonstrate that *lqy1* mutants are more prone to photoinhibition, and that the low-PSII-efficiency phenotype in *lqy1* mutants is indeed caused by mutations in the *LQY1* gene. Phylogenetic analysis of LQY1 and homologs suggests that LQY1 might be important to plant adaptation to excess light during the transition from water to land.

The *lqy1* Mutants are More Prone to Photoinhibition

The initial characterization of *lqy1* mutants showed that *lqy1* mutants have much higher minimum fluorescence (F_0) and much lower maximum PSII efficiency (F_v/F_m) than wild-type plants, after exposure to high light.[3] This suggests that *lqy1* mutants are more photoinhibited than wild-type plants under high light. To further test this hypothesis, the author monitored the induction and relaxation of *NPQ* before and after 3 h high light (1,500–1,700 μmol photons $m^{-2}s^{-1}$) treatment. Before high light treatment, *lqy1* mutants had similar, but not identical, induction and relaxation kinetics to wild-type plants (**Fig. 1A**). Induction of *NPQ* occurred during the 12 min exposure to actinic light (531 μmol photons m^{-2} s^{-1}) and relaxation of *NPQ* occurred after actinic light was switched off. It is interesting that both *lqy1* mutants had slightly higher *NPQ* values than wild-type plants during the first 6 min of actinic exposure (Student's t-test, $p < 0.05$). However, this trend was not observed in the last 6 min of actinic exposure.

The difference in *NPQ* kinetics between *lqy1* mutants and wild-type plants are much more pronounced after treating the plants under high light for 3 h (**Fig. 1B**). Throughout the 12 min actinic exposure, the *lqy1* mutants had much higher *NPQ* values than the wild-type control plants (Student's t-test, $p < 0.05$). After the actinic light was turned off, *NPQ* in l*qy1*

Short communication to: Lu Y, Hall DA, Last RL. A small zinc finger thylakoid protein plays a role in maintenance of photosystem II in *Arabidopsis thaliana*. Plant Cell 23: 1861-75; PMID: 21586683; DOI: 10.1105/tpc.111.085456.

Correspondence to: Yan Lu; Email: yan.1.lu@wmich.edu
Submitted: 06/30/11; Revised: 09/06/11; Accepted: 09/07/11
DOI: 10.4161/psb.6.12.18022

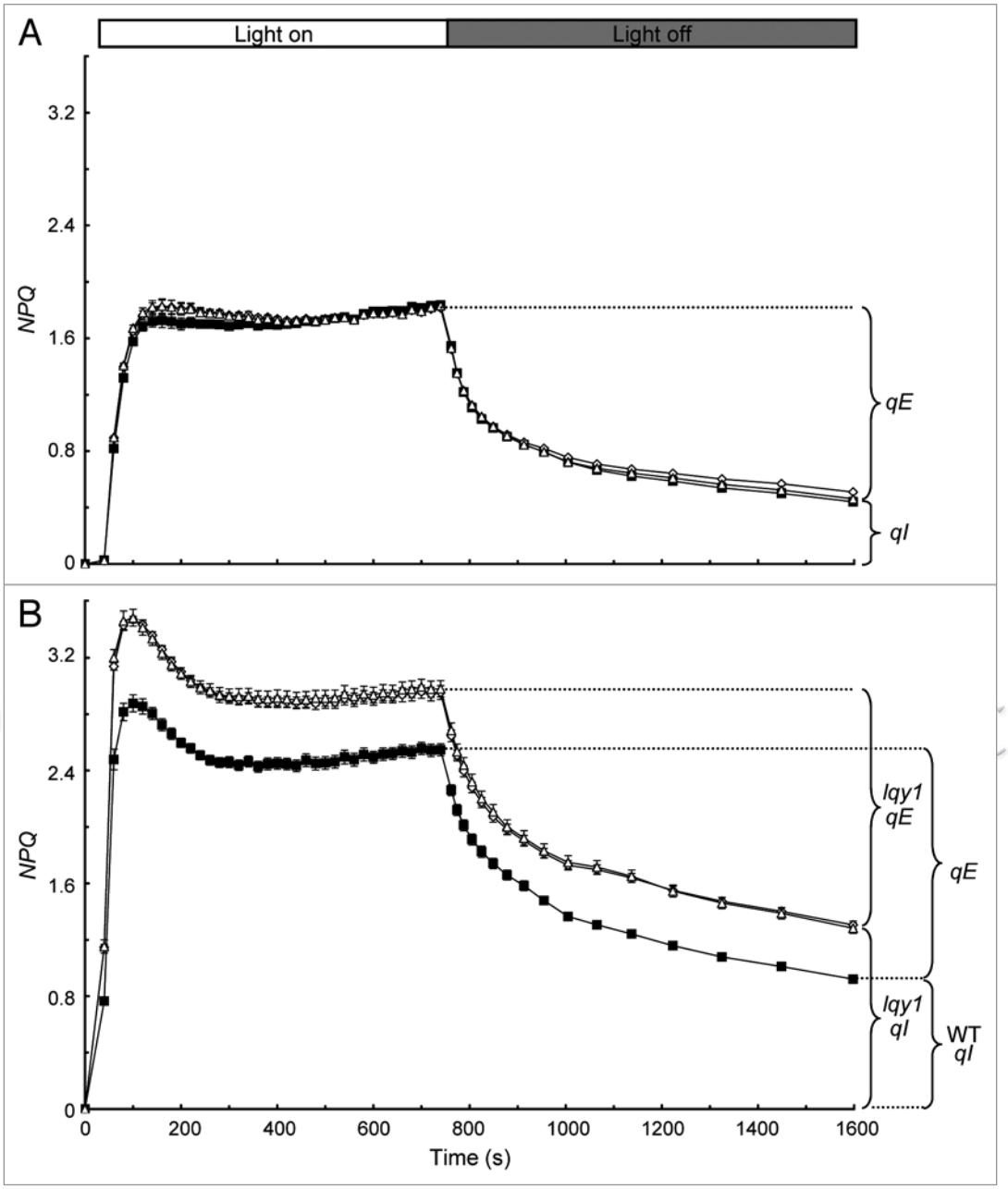

Figure 1. Time courses for induction and relaxation of *NPQ* before (A) and after (B) 3 h high light treatment. Measurements of chlorophyll fluorescence parameters were done on 4-week-old plants after 20 min of dark adaptation. Forty seconds after initial determination of F_0 and F_m, actinic light (531 μmol photons m^{-2} s^{-1}) was switched on for 715 sec. After termination of actinic light, relaxation of *NPQ* (the *qE* component of *NPQ* actually) was monitored for 840 sec. The remainder of *NPQ* primarily represents the *qI* component of *NPQ*. *NPQ* values in (B) were calculated with F_m determined before the high light (1,500–1,700 μmol photons m^{-2} s^{-1}) treatment. Data for Col wild-type (closed squares), *lqy1-1* (open diamonds) and *lqy1–2* (open triangles) plants are presented as mean ± SE (n = 8).

mutants and wild-type plants relaxed at similar rate. This is consistent with the similar energy-dependent quenching (*qE*) in *lqy1* mutants and wild-type plants, as reported previously in reference 3. The remainder of *NPQ* mostly represents photoinhibitory quenching (*qI*).[4,5] The *lqy1* mutants demonstrated a much larger portion of *NPQ* that was not relaxed during the 14 min dark period (**Fig. 1B**), consistent with reportedly higher *qI* in *lqy1* mutants.[3]

The author also monitored the changes in F_v/F_m during *NPQ* induction and relaxation kinetics before and after 3 h high light treatment (**Fig. 2**). Consistent with the initial mutant characterization,[3] the *lqy1* mutants have slightly lower F_v/F_m than wild-type plants before the 3 h high light exposure, and they have

much lower F_v/F_m than wild-type plants after the 3 h high light exposure (**Fig. 2**). The data from the time course of *NPQ* kinetics further demonstrated that the *lqy1* mutants are more prone to high light-induced photoinhibition, and that PSII in *lqy1* mutants is less efficient that wild-type plants under high light.

It is worth mentioning that the *NPQ* induction curves in high light treated samples exhibit a complex shape with a quick increase followed by a partial relaxation to steady-state during actinic light illumination (**Fig. 1B**). Similar behavior has been observed in high light-acclimated *Physcomitrella patens*.[6] This transient relaxation of quenching during the induction of *NPQ* may be attributed to the activation of the Calvin-Benson cycle, which consumes ATP and reduces ΔpH.[6]

The Low-PSII-efficiency Phenotype in *lqy1* Mutants can be Suppressed by Complementation

As mentioned above, PSII in *lqy1* mutants is not as efficient as wild-type plants under high light irradiance. To test whether the low-PSII-efficiency phenotype in *lqy1* mutants is caused by mutations in the *LQY1* gene, wild-type *LQY1* gene or empty vector was introduced into *lqy1-1* and *lqy1–2* mutants. The T2 generation transformants were treated under high light (1,500–1,700 μmol photons m^{-2} s^{-1}) for 3 h and their PSII efficiency analyzed by chlorophyll fluorescence (**Fig. 3**). Complementing *lqy1* mutants with wild-type *LQY1* gene significantly increased F_v/F_m, which was measured in dark-adapted leaves. Similar results were observed in maximum PSII efficiency during actinic exposure, F_v'/F_m'. The four representative complementation lines in either *lqy1-1* or *lqy1–2* background show a wide range of efficiency, from partial complementation to complete complementation. This experiment confirms that mutations in the *LQY1* gene are the cause of the low-PSII-efficiency phenotype in *lqy1* mutants and that LQY1 plays an important role in regulating PSII activity, especially under high light stress conditions.

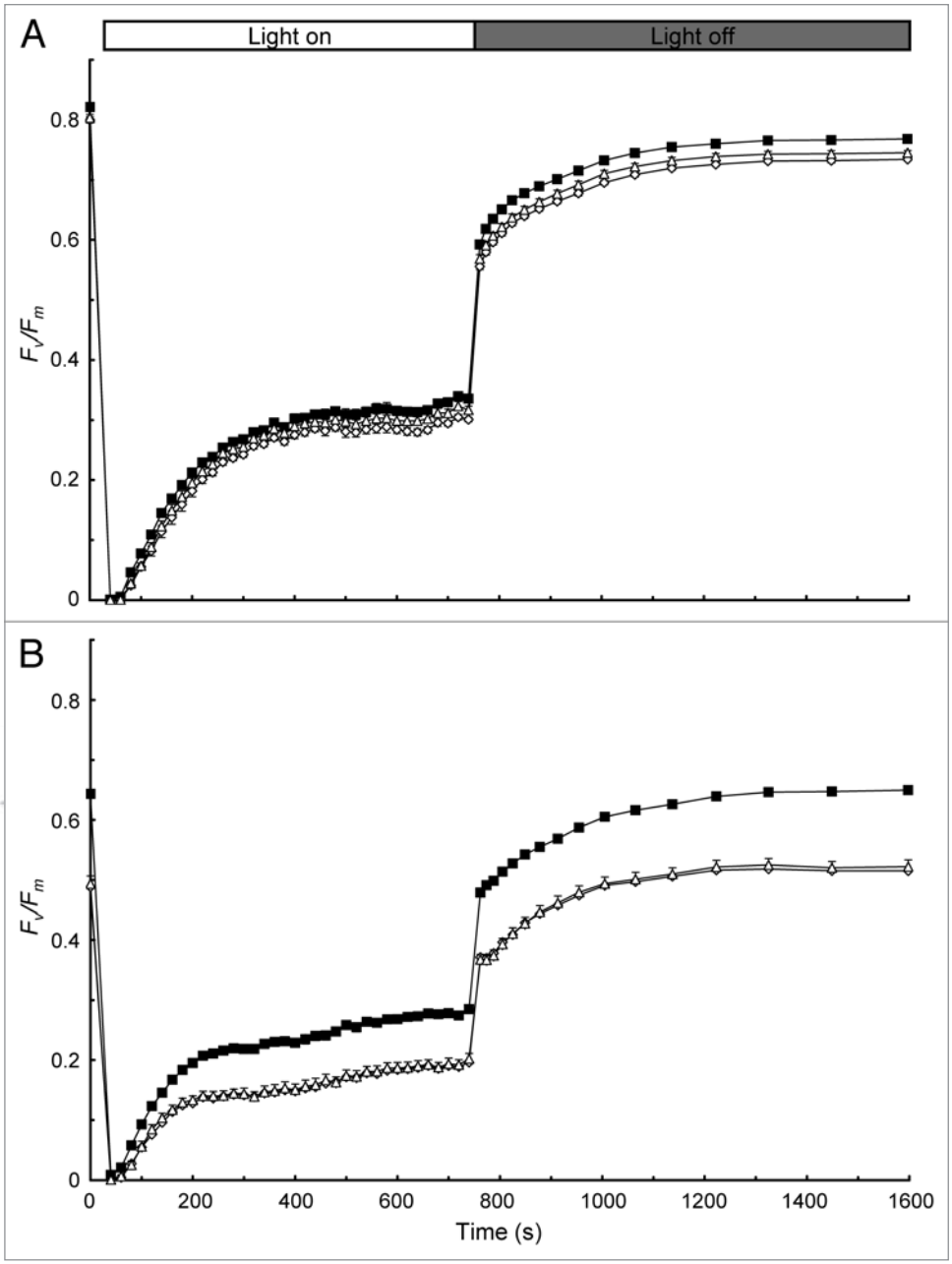

Figure 2. F_v/F_m during the induction and relaxation of *NPQ* before (A) and after (B) 3 h high light treatment. Measurements of chlorophyll fluorescence parameters were done as described in **Figure 1** legend. Data for Col wildtype (closed squares), *lqy1-1* (open diamonds) and *lqy1–2* (open triangles) plants are presented as mean ± SE (n = 8).

Phylogenetic Analysis of LQY1 Protein and its Homologs in Other Land Plants

LQY1 homologs are found in the sequenced genomes of other land plants, for example, bryophyte moss *Physcomitrella patens*, lycopod *Selaginella moellendorffii*, gymnosperm *Picea stichensis*, monocots *Oryza sativa* and *Sorghum bicolor*, and eudicots (**Fig. 4**). However, LQY1 homologs are not found in either cyanobacteria or algae.[3] This may due to the differences in habitat

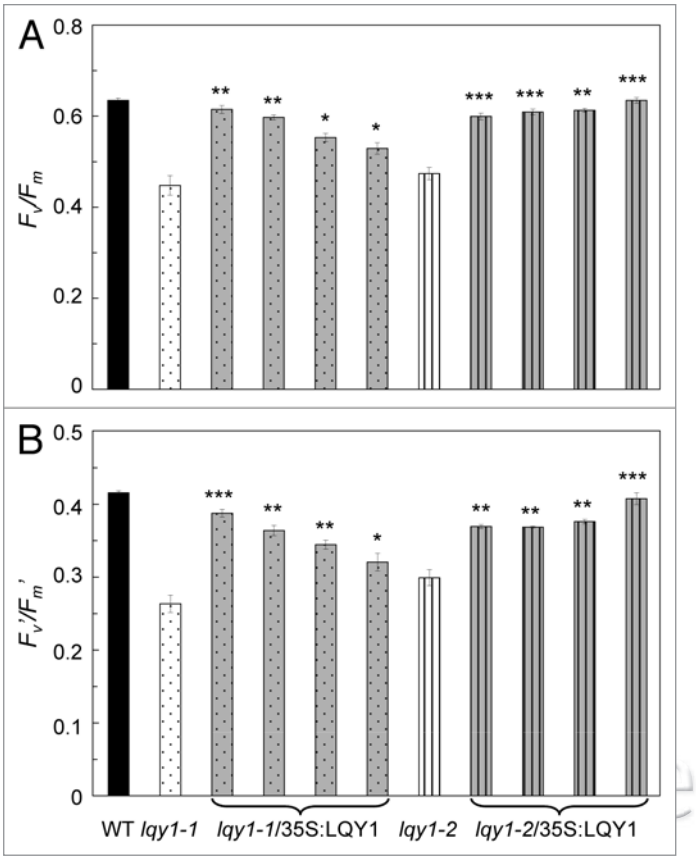

Figure 3. PSII efficiency of *lqy1* complementation lines. F_v/F_m (A) was measured after 20 min of dark adaptation. F_v'/F_m' (B) was measured after 5 min of actinic exposure at 531 μmol photons m^{-2} s^{-1}. Data from T2 generation are presented as mean ± SE (n = 4). Each bar represents a single insertion event at T1 generation. Black bars, wild-type empty vector control; white dotted bars, *lqy1-1* empty vector control; gray dotted bars, representative complementation lines in *lqy1-1* background; white striped bars, *lqy1–2* empty vector control; gray striped bars, representative complementation lines in *lqy1–2* background. The asterisks indicate significant difference between *lqy1* complementation lines and the corresponding *lqy1* mutant (Student's t-test, *p < 0.05; **p < 0.01; ***p < 0.001).

conditions between land plants and their ancestor green algae. For example, both land plants and algae can suffer from photoinhibition when they are exposed to extreme conditions, such as heat, low temperature and excess light.[7] Because the ocean has higher heat capacity than the atmosphere, algae are not exposed to as much heat stress as land plants. The immobile life form of land plants also prevent escape from rapid fluctuation of light intensity by swimming away or deeper, a typical algae behavior.[8] The combination of these conditions requires land plants to use a more efficient system to mitigate the damage of photoinhibition.

The present work provided addition support for the role of LQY1 in maintaining the activity of PSII, especially under high light stress conditions. *NPQ* kinetics show that *lqy1* mutants have much higher total *NPQ* and *qI*, and much lower F_v/F_m than wild-type plants (**Figs. 1 and 2**). Complementation experiments demonstrate that the low-PSII-efficiency phenotype of *lqy1* mutants can be rescued by introducing wild-type *LQY1* gene into the mutant backgrounds (**Fig. 3**). Phylogenetic analysis of LQY1 and homologs suggests that LQY1 might be important during the transition of plants from water to land (**Fig. 4**). Further investigation may lead to the identification of the direct interacting partner(s) of LQY1 and a more detailed understanding of the process and regulation of PSII repair and reassembly.

Materials and Methods

Complementation of *lqy1* mutants. A full-length *LQY1* gene containing a 3'-untranslated region was amplified using Col-0 wild type Arabidopsis leaf DNA, Pfu DNA polymerase (Promega) with a forward primer 5'-ACA CAT CTA GAA TGC CAG TTT CAG CTC CAT C-3' (XbaI site underlined) and a reverse primer 5'-ACA CAG GAT CCA AGT ACA AGA AGA AAC ATT T-3' (BamHI site underlined). The resulting PCR product was AT cloned into a pGEM-T vector and sequenced to check for errors. An XbaI/BamHI-digested *LQY1* fragment was subcloned into a binary vector as previously described in reference 9 and 10. The modified binary vector containing the *LQY1* gene was transformed into *lqy1-1* and *lqy1–2* mutants by *Argobacterium tumefaciens*-mediated floral-dip method.[11] Gentamycin-resistant plants were selected at the T1 generation and genotyped to verify transformation.

Chlorophyll fluorescence analysis. *NPQ* kinetics of wild-type and *lqy1* mutant plants were determined as described previously in reference 3. *NPQ* was calculated using the following equation: $NPQ = (F_m - F_m')/F_m'$. For F_v/F_m and F_v'/F_m' measurements of wild-type and *lqy1* complementation lines, initial determination of F_o and F_m was done by the application of a saturation pulse (2,800 μmol photons m^{-2} s^{-1}) after 20 min of dark adaptation, as described previously in reference 12 and 13. After a 40 sec delay, the actinic light (531 μmol photons m^{-2} s^{-1}) was switched on for 360 sec. At the end of actinic exposure, another saturation pulse was applied and F_o' and F_m' determined. Actinic illumination, saturation pulse and measuring light were provided by an array of 44 high-power royal blue (450 nm) LED-lamps equipped with collimating optics. The intensity of actinic illumination was carefully chosen according to the quality of the light source and the performance of Arabidopsis plants at that light intensity.[14] F_v/F_m and F_v'/F_m' were calculated using the following equations: $F_v/F_m = (F_m - F_o)/F_m$; $F_v'/F_m' = (F_m' - F_o')/F_m'$.

Phylogenetic analysis. Multiple alignments of protein sequences were performed by the ClustalX program (www.clustal.org). Phylogenetic reconstructions were performed using PHYLIP 3.68 (evolution.genetics.washington.edu/phylip.html). Protein distance matrixes were calculated with the Jones-Taylor-Thornton model using the PROTDIST program. An unrooted tree was generated with the neighbor-joining method using the NEIGHBOR program. 1,000 trials of bootstrapping were performed and the consensus tree computed. The accession numbers for LQY1 and homologs were as described previously in reference 3.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by U.S. National Science Foundation 2010 Project Grant MCB-0519740 and Western Michigan University startup fund. The author thanks Cheng Peng at Michigan State University for participation in chlorophyll fluorescence analysis on the rescue lines.

References

1. Demmig-Adams B, Adams WW. Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 1992; 43:599-626; DOI:10.1146/annurev.pp.43.060192.003123.
2. Mulo P, Sirpiö S, Suorsa M, Aro EM. Auxiliary proteins involved in the assembly and sustenance of photosystem II. Photosynth Res 2008; 98:489-501; PMID:18618287; DOI:10.1007/s11120-008-9320-3.
3. Lu Y, Hall DA, Last RL. A small zinc finger thylakoid protein plays a role in maintenance of photosystem II in *Arabidopsis thaliana*. Plant Cell 2011; 23:1861-75; PMID:21586683; DOI:10.1105/tpc.111.085456.
4. Müller P, Li XP, Niyogi KK. Non-photochemical quenching. A response to excess light energy. Plant Physiol 2001; 125:1558-66; PMID:11299337; DOI:10.1104/pp.125.4.1558.
5. Krause GH, Jahns P. Pulse amplitude modulated chlorophyll fluorometry and its application in plant science. In: Green BR, Parson WW, Eds. Light-Harvesting Antennas in Photosynthesis. Dordrecht, The Netherlands: Kluwer Academic Publishers 2003; 373-99.
6. Gerotto C, Alboresi A, Giacometti GM, Bassi R, Morosinotto T. Role of PSBS and LHCSR in *Physcomitrella patens* acclimation to high light and low temperature. Plant Cell Environ 2011; 34:922-32; PMID:21332514; DOI:10.1111/j.1365-3040.2011.02294.x.
7. Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI. Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta 2007; 1767:414-21; PMID:17207454; DOI: 10.1016/j.bbabio.2006.11.019.
8. Alboresi A, Gerotto C, Giacometti GM, Bassi R, Morosinotto T. *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. Proc Natl Acad Sci USA 2010; 107:11128-33; PMID:20505121; DOI:10.1073/pnas.1002873107.

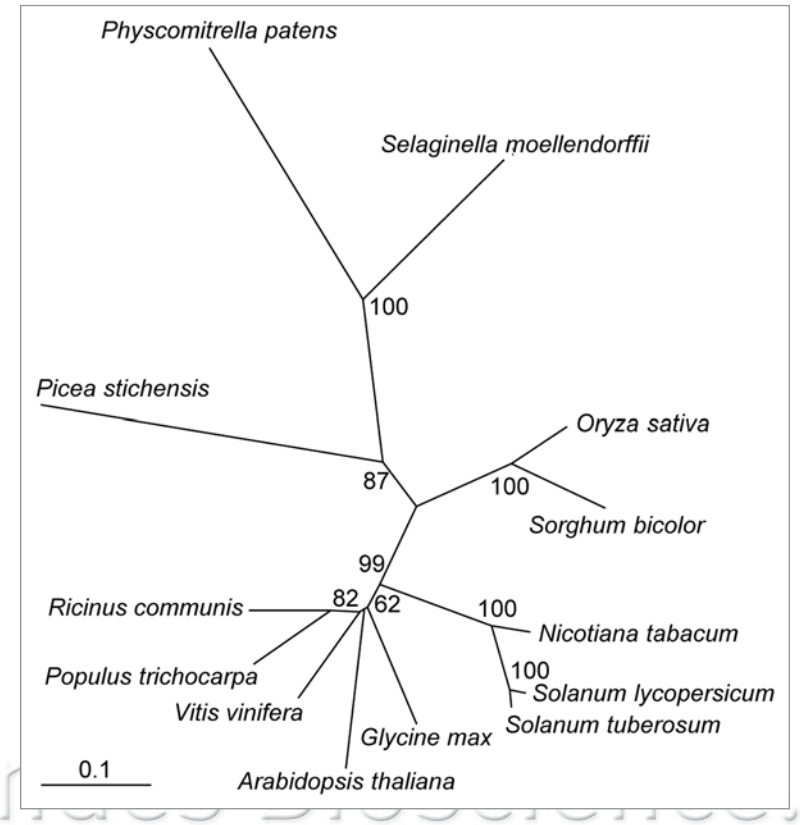

Figure 4. Phylogenetic analysis of LQY1 protein and its homologs in other land plants. Multiple alignments and phylogenetic reconstruments were performed by the ClustalX and PHYLIP 3.68 programs. Bootstrap values (1,000 replicates) above 50% are given on branches. Bar = 0.1 amino acid substitutions.

9. Lu Y, Steichen JM, Yao J, Sharkey TD. The role of cytosolic α-glucan phosphorylase in maltose metabolism and the comparison of amylomaltase in Arabidopsis and *Escherichia coli*. Plant Physiol 2006; 142:878-89; PMID:16980562; DOI:10.1104/pp.106.086850.
10. Fang SC, Fernandez DE. Effect of regulated overexpression of the MADS domain factor AGL15 on flower senescence and fruit maturation. Plant Physiol 2002; 130:78-89; PMID:12226488; DOI:10.1104/pp.004721.
11. Clough SJ, Bent AF. Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 1998; 16:735-43; PMID:10069079; DOI:10.1046/j.1365-313x.1998.00343.x.
12. Lu Y, Savage LJ, Ajjawi I, Imre KM, Yoder DW, Benning C, et al. New connections across pathways and cellular processes: industrialized mutant screening reveals novel associations between diverse phenotypes in Arabidopsis. Plant Physiol 2008; 146:1482-500; PMID:18263779; DOI:10.1104/pp.107.115220.
13. Lu Y, Savage LJ, Last RL. Chloroplast phenomics: systematic phenotypic screening of chloroplast protein mutants in Arabidopsis. In: Jarvis RP, Ed. Methods in Molecular Biology. Iotowa: Humana Press 2011; 775:161-85.
14. Johnson MP, Pérez-Bueno ML, Zia A, Horton P, Ruban AV. The zeaxanthin-independent and zeaxanthin-dependent qE components of nonphotochemical quenching Involve common conformational changes within the photosystem II antenna in Arabidopsis. Plant Physiol 2009; 149:1061-75; PMID:19011000; DOI:10.1104/pp.108.129957.