Functional Modeling Identifies Paralogous Solanesyl-diphosphate Synthases That Assemble the Side Chain of Plastoquinone-9 in Plastids*\\([S]\)

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**Background:** Plastid isoforms of solanesyl-diphosphate synthase catalyze the elongation of the prenyl side chain of plastoquinone-9.

**Results:** Corresponding mutants display lower levels of plastoquinone-9 and plastochromanol-8 and display intact levels of vitamin E.

**Conclusion:** Plastochromanol-8 originates from a subfraction of non-photoactive plastoquinol-9 and is not essential for seed longevity.

**Significance:** Viable plastoquinone-9 mutants are invaluable tools for understanding plastid metabolism.

It is a little known fact that plastoquinone-9, a vital redox cofactor of photosynthesis, doubles as a precursor for the biosynthesis of a vitamin E analog called plastochromanol-8, the physiological significance of which has remained elusive. Gene network reconstruction, GFP fusion experiments, and targeted metabolite profiling of insertion mutants indicated that *Arabidopsis* possesses two paralogous solanesyl-diphosphate synthases, AtSPS1 (At1g78510) and AtSPS2 (At1g17050), that assemble the side chain of plastoquinone-9 in plastids. Similar paralogous pairs were detected throughout terrestrial plant lineages but were not distinguished in the literature and genomic databases from mitochondrial homologs involved in the biosynthesis of ubiquinone. The leaves of the atps2 knock-out were devoid of plastochromanol-8 and displayed severe losses of both non-photoactive and photoactive plastoquinone-9, resulting in near complete photoinhibition at high light intensity. Such a photoinhibition was paralleled by significant damage to photosystem II but not to photosystem I. In contrast, in the atps1 knock-out, a small loss of plastochromanol-8, restricted to the non-photoactive pool, was sufficient to eliminate half of the plastochromanol-8 content of the leaves. Taken together, these results demonstrate that plastochromanol-8 originates from a subfraction of the non-photoactive pool of plastoquinone-9. In contrast to other plastochromanol-8 biosynthetic mutants, neither the single atsp knock-outs nor the atsp1 atsp2 double knock-out displayed any defects in tocopherols accumulation or germination.

Plastoquinone-9 (2,3-dimethyl-6-solanesyl-1,4-benzoquinone) is a vital redox cofactor for oxygenic photoautotrophs. It is required during photosynthesis for electron transfer and proton translocation in thylakoid membranes, as well as for respiration in cyanobacteria and as a necessary oxidant for the desaturation of the carotenoid precursor phytoene (1–3). Furthermore, it is via the redox state of plastoquinone-9 in thylakoid membranes that plants and cyanobacteria monitor the balance of photosystem II and photosystem I activity and adjust the expression of some plastid and nuclear genes (4, 5).

Plastoquinone-9 is a bipartite molecule made up of a redox active benzoquinone ring attached to a solanesyl (C\(_{20}\)) chain (Fig. 1). Plants derive the benzenoid moiety from homogentisate, which is prenylated and decarboxylated in the inner envelope of chloroplasts, yielding 2-methyl-6-solanesyl-1,4-benzoquinol (6, 7). The latter is then methylated to give plastoquinol-9 (Fig. 1). Analogy with the assembly of the polyprenyl side chain of ubiquinone in proteobacteria and mitochondria indicates that the biosynthesis of the plastoquinone-9 solanesyl moiety proceeds from the *trans*-long chain prenyl-diphosphate synthase-catalyzed elongation of a C\(_{15}\)–C\(_{20}\) allylic diphosphate precursor. The corresponding activity has therefore been tentatively attributed in *Arabidopsis* and rice to plastid-targeted solanesyl-diphosphate synthases, products of the *At1g17050* and *Os05g0582300* genes, respectively (8–10), although there is no direct evidence that such enzymes indeed participate in plastoquinone-9 biosynthesis. A peculiarity of the biosynthetic pathway of plastoquinone-9 in photosynthetic eukaryotes is...
that it intertwines with that of tocopherols (Fig. 1). First, the hydroxychroman moiety of tocopherols also originates in part from homogentisate (6). Second, the methyltransferase that decorates the benzoquinol ring intermediate also functions in the biosynthesis of tocopherols (11). Lastly, plastoquinol-9 itself can serve as a substrate for tocopherol cyclase to yield plastochromanol-8 in plastoglobules (12, 13). Although it has received considerably less attention than tocopherols, plastochromanol-8 is thought to function as an antioxidant in plants. This seems a valid possibility, because plastochromanol-8 contains the same hydroxychroman ring as $\gamma$-tocopherol (Fig. 1), and hydroxyplastochromanol-8, formed as a result of singlet oxygen scavenging by plastochromanol-8, has been shown to accumulate in leaves during high light stress and aging (14). In particular, there is evidence that the seeds of an Arabidopsis mutant unable to synthesize either plastochromanol-8 or tocopherols exhibit increased lipid oxidation during desiccation and quiescence, resulting in lower seed longevity compared with those of another mutant that is devoid of tocopherols only (15). However, it is unclear to what extent plastochromanol-8 contributes to the antioxidant arsenal of plant tissues when tocopherols are present, as would normally happen in nature. In fact, studying the specific roles of plastoquinone-9 and plastochromanol-8 by means of straightforward loss-of-function strategies has invariably proved vexing. Not only is the biosynthesis of plastoquinone-9 and plastochromanol-8 seemingly impossible to disconnect from that of tocopherols, but all of the plastochromanol-8 biosynthetic mutants identified to date, namely those corresponding to 4-hydroxyphenylpyruvate dioxygenase, homogentisate solanesyl transferase, and 2-methyl-6-solanesyl-1,4-benzoquinol methyltransferase (Fig. 1), are albino and seedling-lethal (3, 11, 16).

In this study, we demonstrated that Arabidopsis thaliana possesses two solanesyl-diphosphate synthases involved in the assembly of the plastoquinone-9 side chain. We then examined the impact of a deficit of plastoquinone-9, plastoquinol-9, and plastochromanol-8 in the leaves and seeds of cognate mutants, the tocopherol biosynthetic capabilities of which are intact.

### MATERIALS AND METHODS

**Chemicals and Reagents**—Ubiquinone-10 was from Sigma-Aldrich. $\alpha$-Tocopherol was from Acros Organics, and $\delta$-tocopherol and $\gamma$-tocopherol were from Matreya, LLC. Ubiquinone-9, plastoquinone-9, and plastochromanol-8 standards were extracted from Candida utilis, Synechocystis sp. PCC 6803, and the seeds of Camelina sativa, respectively, and HPLC-purified. Quinol standards were synthesized chemically from the reduction of their corresponding quinone forms using sodium borohydride. Calibration solutions were quantified spectrophotometrically using the molar extinction coefficients of 14,600 M$^{-1}$cm$^{-1}$ at 275 nm for ubiquinone, 15,200 M$^{-1}$cm$^{-1}$ at 255 nm for plastoquinone-9, 3260 M$^{-1}$cm$^{-1}$ at 292 nm for $\alpha$-tocopherol, 3510 M$^{-1}$cm$^{-1}$ at 298 nm for $\gamma$-tocopherol, and 3810 M$^{-1}$cm$^{-1}$ at 298 nm for $\delta$-tocopherol (17) and the absorbance value $E_{1cm}(1\%)$ at 296 nm = 55.5 for plastochromanol-8 (18). High fidelity PCR amplifications were performed with Phusion polymerase (Finnzymes), and PCR genotyping experiments were performed with GoTaq polymerase.
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(Promega). All DNA constructs were verified by sequencing. Unless mentioned otherwise, all other reagents were from Fisher Scientific.

**Plant Material and Growth Conditions—Arabidopsis**

T-DNA insertion mutant SALK_126948 (At1g78510; AtSPS1) and SALK_064292 (At1g17050; AtSPS2) were obtained from the Arabidopsis Biological Resource Center at the Ohio State University (19). Seeds were allowed to germinate *in vitro* on Murashige and Skoog solid medium and transferred to potting mix in a growth chamber at 22 °C in 16-h days (110 μE m⁻² s⁻¹) for 4 weeks. The double knock-out was obtained from the F2 segregating progeny resulting from the cross between the individual SALK_126948 and SALK_064292 homozygous T-DNA mutants. For the measurements of chlorophyll fluorescence and metabolite quantification during acclimation to high light, plants were grown directly on soil under moderate light intensity (110–120 μE m⁻² s⁻¹) for 3 weeks and then switched to high light intensity (600–800 μE m⁻² s⁻¹) for 2 weeks and then switched to high light intensity (600–800 μE m⁻² s⁻¹). For germination assays, *Arabidopsis* seeds stored previously at 4 °C with silica gel as a desiccant were subjected to accelerated aging for 72 h at 40 °C and 100% relative hygrometry and then vernalized for 5 additional days at 4 °C to break dormancy as described previously (20). Unaged controls were vernalized directly. Seeds were then subjected to accelerated aging for 72 h at 4 °C to break dormancy as described previously (20). Unaged controls were vernalized directly. Seeds were then surface-sterilized with sodium hypochlorite and plated on Murashige and Skoog medium containing sucrose (10 g/liter). Plates were placed in 16-h days (110 μE m⁻² s⁻¹) at 22 °C, and germination was scored after 3 weeks by the emergence of the radical root.

**Plant Genotyping and RT-PCR Analyses—Arabidopsis**

Plants were genotyped using a combination of primers: LP1, 5’-TCTGTATCAAGCTTTGAGCTT-3’; RPI, 5’-ATGATCTCATGTTAAGCTTGCAGTG-3’; T-DNA-specific LBb1, 5’-GCGTGAC-ATGATCTCATG-3’; RP1, 5’-ATAAACAATAATTAT-3’, SALK_126948); LP2, 5’-GCGTGAC-ATGATCTCATG-3’; SALK_064292) for the double knock-out. The quick check assay was developed by cloning in the pGEM-T Easy (Promega), and sequenced from the T7 and SP6 priming sites of the vector. For RT-PCR analysis, total RNA prepared from 500 ng of total RNA using the following gene-isolation system (Promega). PCR was performed on cDNAs synthesized using M Supelco Discovery C-18 column (250 × 4.6 mm, Sigma-Aldrich) thermostatted at 30 °C and developed in isocratic mode at a flow rate of 1.5 ml min⁻¹ with methanol. Plastoquinol-9 and ubiquinone-10 were detected spectrophotometrically at 255 and 275 nm, respectively. Plastoquinol-9, plastochroman-8, and tocopherols were detected fluorometrically (290 and 330 nm for excitation and emission, respectively). Retention times were 5.3 min (β-tocopherol), 5.9 min (γ-tocopherol), 6.6 min (α-tocopherol), 14.3 min (plastoquinol-9), 28.4 min (plastoquinol-8), 40.7 min (plastoquinol-9), and 45.1 min (ubiquinone-10). Compounds were quantified according to their corresponding external calibration standards, and data were corrected for recovery of the ubiquinone-10 internal standard. Ubiquinone-9 was quantified as described previously (23).

**Analysis of Photosystems—Imaging of maximum quantum efficiency of photosystem II on whole Arabidopsis plants** was performed using a FluorCam 700 MF system (Photon Systems Instruments) using the quenching analysis effect settings. Pulsed of actinic light and continuous illumination were generated by two arrays, each made up of 345 620 nm-LEDs. Plants were dark-adapted for 20 min before each experiment. Fluorescence parameters were calculated as follows: 

\[ F_v/F_m = (F_m - F_s)/F_m \]

where \( F_v \) is the calculated variable fluorescence, \( F_m \) is the maximal fluorescence measured immediately after the saturating pulse, and \( F_s \) is the fluorescence decrease from \( F_m \) to \( F_s \) (the steady state fluorescence measured after 3 s of continuous illumination); non-photochemical quenching (NPQ) = \( (F_m - F_m^v)/F_m \), where \( F_m^v \) is the maximal fluorescence measured after a 3-s adaptation to actinic light; photo-inhibition = \( 1 - F_v/F_m \) for actinic light, where \( F_v^v \) and \( F_m^v \) are the variable and maximal fluorescence, respectively, measured after 2, 24, and 48 h of exposure to actinic light at 800 μE m⁻² s⁻¹ followed by 20 min of recovery in the dark.
kinetics of photosystem I photoxidation were measured on detached leaves using a JTS-10 LED spectrometer (Bio-Logic Scientific Instruments) in absorbance detection mode. P700 was oxidized using 10-ms actinic flashes of far-red LEDs and a 705-nm interference filter. Base-line absorbances were obtained without actinic illumination and subtracted. Chloroplast preparation and separation of proteins by SDS-PAGE for the immunodetection of subunits D1 and PsA were performed as described previously (24). Anti-D1 and anti-PsaA antibodies were from Agrisera and Dr. J. D. Rochaix’s laboratory (University of Geneva), respectively. Immunoreactive proteins were visualized using the SuperSignal West Pico HRP detection kit (Thermo Scientific) according to the manufacturer recommendations.

RESULTS

Modeling of Gene Co-expression Network Predicts That Arabidopsis Possesses Two Solanesyl-Diphosphate Synthases That Functionally Intersect in Plastids—BLASTp searches of Arabidopsis genomic databases using either Escherichia coli octaprenyl-diphosphate synthase (IspB) or Saccharomyces cerevisiae hexaprenyl-diphosphate synthase (Coq1) as queries, both of which assemble the side chain of ubiquinone in their respective hosts (25, 26), detected three proteins that bear the molecular attributes of trans-long chain diphosphate synthases, At1g78510, At1g17050, and At2g34630. As previous reports indicate that all three of these proteins harbor solanesyl-diphosphate synthase activity (9, 27, 28), we shall call them hereafter, for purpose of clarity, AtSPS1, AtSPS2, and AtSPS3, respectively. Modeling of a high stringency network made up of the top 100 genes (0.45% of the 22,263 expressed loci in the ATTED-II database) that co-express with AtSPS1, AtSPS2, and AtSPS3 generated six gene clusters (Fig. 2). Three of those clusters corresponded to genes that uniquely co-express with AtSPS1, AtSPS2, or AtSPS3, two to genes that co-express either with AtSPS1 and AtSPS2 or with AtSPS1 and AtSPS3, and one to genes that co-express with all three AtSPS members (Fig. 2). (Supplemental Data File 1 provides a list of these genes including their correlation rank and functional annotations.) Subcellular localization data compiled from the SUBA and TAIR databases were then overlaid on each cluster (Fig. 2). Remarkably, AtSPS1 and AtSPS2 emerged from such a reconstruction as top co-expressors of each other, number 2 and number 4, respectively (supplemental data File 1), sharing more than half of their co-expressing genes (Fig. 2). The co-expressors of AtSPS1 and AtSPS2 also displayed strikingly similar patterns of subcellular distribution, including a marked prevalence for genes that encode for plastid-targeted proteins (Fig. 2). In sharp contrast, 93% of the genes that co-expressed with AtSPS3 clustered uniquely with this enzyme (Fig. 2). Over one-third of those were found to encode for proteins targeted to the mitochondrion (Fig. 2), agreeing with the role of AtSPS3 as a ubiquinone biosynthetic enzyme in this organelle (23). Taken together, these data imply that AtSPS1 and AtSPS2 belong to the same functional network. The latter is not solely distinct from the AtSPS3 functional network but is also intimately linked to plastids. Although such a model fits with the demonstration that AtSPS2 is targeted to plastids, it conflicts with the previous assumption that AtSPS1 localizes to the endoplasmic reticulum (8, 9).

AtSPS1 Is Targeted Exclusively to Plastids—To reexamine the subcellular localization of AtSPS1, a 3089-bp genomic fragment comprising the AtSPS1 sequence (minus its stop codon)
as well as its 5′-untranslated region and native promoter was cloned in-frame to the 5′-end of GFP. Confocal laser scanning microscopy of the transiently expressed AtSPS1-GFP construct in tobacco leaf tissues showed a pattern of green pseudocolor that co-localized with the autofluorescence of chlorophyll in plastids (Fig. 3, A–C). Untransformed plastids confirmed that the fluorescence attributed to GFP was not merely due to an overflow of the red pseudocolor into the green pseudocolor (Fig. 3, D–F). To confirm these findings, the AtSPS1-GFP construct was introduced into Arabidopsis for stable expression. Imaging of the leaves of T1 transgenics demonstrated that again the fluorescence of GFP was localized in plastids (Fig. 3, D–F). A comparison with the leaves of wild type showed that fluorescence detected outside of plastids. These data demonstrate that it is either transiently or stably expressed under the control of its native promoter, the AtSPS1 protein is targeted to plastids. In neither case was GFP-associated fluorescence detected outside of plastids.

**Duplication of Solanesyl-diphosphate Synthase of Plastids Is Widespread in Land Plants**—Database mining of fully sequenced genomes pointed to cyanobacterial trans-long chain prenyl-diphosphate synthases as the closest prokaryotic relatives of AtSPS1 and AtSPS2. However, unlike metazoa and fungi, cyanobacteria have only one gene coding for this class of enzymes, whereas without exception plants have several. For instance, the green alga *Chlamydomonas reinhardtii*, the red alga *Cyanidioschloris merolae*, barley, grape, tomato, and...
cucumber have two, the lycopod Selaginella moellendorffii, the moss Physcomitrella patens, corn, rice, and soybean have three like Arabidopsis, and the grass species Brachypodium distachyon have four. A maximum likelihood phylogeny showed that the plant species mentioned above generally have one of their AtSPS homologs that regroups within a strictly eukaryotic clade and one or more of the others that are monophyletic with the cyanobacterial subfamily (Fig. 4). AtSPS1, AtSPS2, and the plastid-encoded C. merolae1 homolog, as well as tomato (SISPS) and rice (OsSPS2) solanesyl-diphosphate synthases, both of which are targeted to plastids (10, 31), belong to this cyanobacterial lineage (Fig. 4). Importantly, the species that possess more than two trans-long chain prenyl-diphosphate synthases owe these extra enzymes to duplicates within the cyanobacterial/plastid subclade (Fig. 4). The only exception is Brachypodium, which has duplicates in both the eukaryotic and cyanobacterial/plastid clades (Fig. 4). It thus appears that the occurrence of duplicated plastid isoforms of solanesyl-diphosphate synthase is not specific to Arabidopsis.

AtSPS1 and AtSPS2 Contribute to the Biosynthesis of Plastoquinone-9 and Plastochromanol-8—A T-DNA mutant corresponding to an insertion located in the first exon of AtSPS1 (SALK_126948) was identified using the T-DNA Express gene mapping tool and confirmed by DNA genotyping (Fig. 5, A and B). RT-PCR analysis using a primer pair designed to amplify a cDNA region spanning from the end of the second exon to the beginning of the sixth exon confirmed that the atsp1 locus was null (Fig. 5, A and C). A tandem T-DNA insertion was similarly identified in the sixth exon of AtSPS2 (SALK_064292), but RT-PCR amplification of a cDNA region located upstream of the predicted insertion revealed that the cognate locus was not null (Fig. 5, D–F). Sequencing of a PCR fragment encompassing the junction between the T-DNA border and the last exon located the insertion precisely at the second nucleotide position of the AtSPS2 stop codon (Fig. 5D). Conceptual translation of the cognate mRNA resulted in a chimeric protein formed from the in-frame C-terminal fusion between AtSPS2 and a T-DNA encoded polypeptide.

HPLC analyses of prenylated benzoquinones and tocochromanols showed that the content in total plastoquinone-9, quinone (oxidized) + quinol (reduced) forms, was decreased by 11 and 57% in the leaves of the atsp1 and atsp2 mutants, respectively, compared with that of wild type plants (Fig. 6A). The level of plastochromanol-8, the product of plastoquinol-9 cyclization, was decreased by about 35% in the atsp1 plants as compared with wild type controls (Fig. 6A). Plastochromanol-8 was not detected in the leaves of the atsp2 mutant (Fig. 6A). For each atsp mutant, these differences in plastoquinone-9 and plastochromanol-8 levels still strictly co-segregated with the T-DNA insertion after three back-crosses. Total tocopherol levels were either undistinguishable or, owing to a ~20% increase in \( \alpha \)-tocopherol content, slightly higher than those of the wild type in the atsp1 mutant and atsp2 mutant, respectively (Fig. 6B). No statistically significant differences were observed in the levels of ubiquinone-9 between the mutants and the wild type control (Fig. 6C). Dry seeds of the atsp1 and atsp2 mutants accumulated 11 and 36% less plastochromanol-8 than their wild type counterparts, respectively (Fig. 6D). Although atsp2 seeds contained about 40% more \( \alpha \)-tocopherol than wild type and atsp1 seeds, such an increase was counterbalanced by a similar decrease in \( \delta \)-tocopherol, resulting in no statistically significant difference in total tocopherol accumulation (Fig. 6E). The plastoquinone-9 and ubiquinone-9 levels of dry seeds were below the detection threshold. The atsp1 atsp2 double homozygous mutant displayed a prominent albino phenotype (Fig. 7, A and B) and was devoid of plastochromanol-9 and plastochromanol-8 (Fig. 7C). As observed for the atsp1 and atsp2 single mutants, it did how-
ever retain the ability to accumulate tocopherols to wild type levels (Fig. 7C). The atsps1 atsps2 double knock-out survived in vitro on an external carbon source and under low illumination but never developed beyond a couple of leaves. These plants died quickly when transferred to soil, and we therefore deemed the atsps1 atsps2 knock-out seedling-lethal. Taken together, these data indicate that AtSPS1 and AtSPS2 are involved in the biosynthesis of plastoquinone-9. Moreover, because plastoquinone-9 and plastochromanol-8 was undetectable in the atsps2 double homozygous mutant, it can also be deduced that the chimeric ATSPS2 mRNA in SALK_064292 mutant (atsps2) is not functional. Most importantly, the deficit or absence of plastochromanol-8 in the atsps1, atsps2, and atsps1 atsps2 knock-outs was not paralleled by that of tocopherols, as is the case for other plastochromanol-8 biosynthetic mutants (15, 12, 32). We will come back to this point later.

The Photoactive and Non-photoactive Pool Sizes of Plastoquinone-9 Are Differently Altered in the atsps1 and atsps2 Mutants—Because the quantities of plastoquinone-9 and plastochromanol-8 were altered to markedly differing extents in each atsps mutant, we sought to determine whether such deficiencies were related to the size of the photoactive pool of plastoquinone or to the non-photoactive one or to both. To that end, we quantified plastoquinone-9 and plastoquinol-9 in the leaves of plants placed in the dark and then exposed to saturating light intensity. In this method, the amount of plastoquinone the redox status of which responds to changes in light regime represents the size of the photoactive pool; it is calculated from the difference between the levels of plastoquinol-9 measured in the light and that measured in the dark. Conversely, the quantity of plastoquinol-9 that does not re-oxidize in the dark and that of plastochromanol-8 that is not reduced in the light represent the non-photoactive pool. These measurements showed that the pool size of photoactive plastoquinone in the atsps1 knock-out was not statistically different from that of the wild type, whereas it was markedly decreased in the atsps2 mutant, representing a mere 43% of the photoactive pool of control plants (Fig. 8A). The quantity of non-photoactive plastoquinone was decreased by 15% as compared with the wild type control in the atsps1 mutant and by up to 60% in the atsps2 knock-out (Fig. 8B). The ratio of plastoquinol-9 to plastoquinone-9 of the atsps1 knock-out was not statistically different from that of the wild type (1.68 ± 0.51 for the WT versus 1.36 ± 0.20 for atsps1; p analysis of variance = 0.27). On the other hand, this ratio was significantly lower in the atsps2 knock-out (0.80 ± 0.19; p analysis of variance = 0.003), indicating that the redox state of non-photoactive plastoquinone had been altered in these plants. It should be noted that the atsps1 knock-out, in which the deficit of plastoquinone-9 has an impact solely on its non-photoactive...
The atsps2 knock-out displayed a severe increase in photoinhibition at high light intensity. Whereas on Murashige and Skoog solid medium containing sucrose and under a moderate light regime, the atsps1 and atsps2 knock-outs were morphologically indistinguishable from the wild type (data not shown), when grown on soil the atsps2 knock-out seemed to develop more slowly than the atsps1 knock-out and the wild type (Fig. 9A). At a high light regime, the atsps2 mutant displayed a remarkably stunted phenotype and pale yellowish leaves, indicative of a strong increase in photosensitivity (Fig. 9B). Analyses of the induction of chlorophyll a fluorescence showed that wild type and atsps plants grown at moderate light intensity (120 \( \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) displayed overall similar photosynthetic performances (Fig. 9C). Maximum quantum efficiencies \( (F_v/F_m) \) of photosystem II were typical of non-stressed Arabidopsis leaves (33), ranging from 0.77 for the wild type to 0.76 and 0.75 for the atsps1 and atsps2 knock-outs, respectively (Fig. 9C and Table 1). Similarly, calculations of the variable fluorescence decrease ratio \( (R_{vd}) \), which correlates linearly with net \( \text{CO}_2 \) assimilation and assesses potential photosynthetic activity (34), did not reveal any defects for either of the atsps mutants (Table 1). Somewhat lower values for NPQ, i.e. the protective dissipation of excess chlorophyll excitation as heat (35), were observed for both mutants (Table 1). Upon exposure to high light intensity (800 \( \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)), however, the fluorescence emission of the atsps2 knock-out differed noticeably from that of the atsps1 mutant and wild type control (Fig. 9, D–F). The \( F_v/F_m, R_{vd} \) and NPQ values of the atsps2 mutant collapsed after only 2 h of strong illumination (Table 1). Already at this point, photoinhibition, i.e. the irreversible loss of photosystem II efficiency, was five times higher in the atsps2 mutant than in the atsps1 and wild type plants (Table 1). By 24 h of high light treatment, the \( F_v/F_m \) value of the atsps2 knock-out had dropped to 0.31, pointing to a critical defect of photosystem II, and by 48 h of treatment all of the fluorescence parameters indicated that photosynthesis was barely operating (Table 1). It is noteworthy that up until 48 h of exposure to high light intensity, NPQ was the sole calculated fluorescence parameter that differed markedly between the atsps1 mutant and the wild type (Table 1). Thus, this observation provides evidence that non-photoactive plastoquinone-9, the pool of which is altered independently of that of its photoactive counterpart in the atsps1 knock-out (Fig. 8B), is involved in NPQ. Immunodetection of the reaction center polypeptide D1 showed that photoinhibition of the atsps2 knock-out at high light intensity was paralleled by a loss of the core of photosystem II complex (Fig. 9G). In contrast, measurements of P700 absorption indicated that the photo-oxidation of...
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FIGURE 9. Phenotypes and analysis of photosystem II of wild type, atsps1, and atsps2 plants. A, 1-month-old plants grown at 110 μE·m⁻²·s⁻¹ for 16-h days. B, 1-month-old plants grown at 500 μE·m⁻²·s⁻¹ for 16-h days. C, imaging of maximum quantum efficiency (Fv/Fm) of photosystem II of wild type, atsps1, and atsps2 plants grown at moderate light intensity (120 μE·m⁻²·s⁻¹). D-F, representative FluorCam 700 MF images acquired after the plants had been exposed to high light intensity (800 μE·m⁻²·s⁻¹) for 2, 24, and 48 h, respectively. Plants were dark-adapted for 20 min prior to illumination with actinic light. G, immunodetection of photosystem II polypeptide D1 in thylakoid membranes prepared from wild type, atsps1, and atsps2 plants grown at moderate light intensity (120 μE·m⁻²·s⁻¹) or exposed to high light regime (800 μE·m⁻²·s⁻¹) for 24 h. Each of the lanes contains 20 μg of proteins.

Table 1

| Fluorescence parameters | WT     | atsps1 | atsps2 |
|------------------------|--------|--------|--------|
| 120 μE·m⁻²·s⁻¹         |        |        |        |
| Fv/Fm                  | 0.77 ± 0.01 | 0.76 ± 0.01 | 0.75 ± 0.03 |
| Rm                     | 2.12 ± 0.06 | 2.31 ± 0.03 | 2.11 ± 0.02 |
| NPQ                    | 1.24 ± 0.02 | 1.08 ± 0.02 | 1.12 ± 0.01 |
| 800 μE·m⁻²·s⁻¹ (2 h)   |        |        |        |
| Fv/Fm                  | 0.75 ± 0.02 | 0.74 ± 0.02 | 0.64 ± 0.02 |
| Rm                     | 1.85 ± 0.04 | 1.65 ± 0.05 | 1.23 ± 0.03 |
| NPQ                    | 1.63 ± 0.03 | 1.42 ± 0.03 | 0.97 ± 0.02 |
| Photoinhibition (%)    | 3.0 ± 2.1 | 3.2 ± 2.4 | 15 ± 3.7 |
| 800 μE·m⁻²·s⁻¹ (24 h)  |        |        |        |
| Fv/Fm                  | 0.72 ± 0.04 | 0.70 ± 0.05 | 0.31 ± 0.05 |
| Rm                     | 1.61 ± 0.07 | 1.44 ± 0.05 | 0.32 ± 0.03 |
| NPQ                    | 1.43 ± 0.03 | 0.87 ± 0.03 | 0.52 ± 0.03 |
| Photoinhibition (%)    | 6.5 ± 3.3 | 7.9 ± 3.4 | 59 ± 17.2 |
| 800 μE·m⁻²·s⁻¹ (48 h)  |        |        |        |
| Fv/Fm                  | 0.71 ± 0.03 | 0.62 ± 0.04 | 0.15 ± 0.07 |
| Rm                     | 1.42 ± 0.08 | 1.24 ± 0.07 | 0.21 ± 0.03 |
| NPQ                    | 1.21 ± 0.05 | 0.83 ± 0.05 | 0.41 ± 0.07 |
| Photoinhibition (%)    | 7.9 ± 3.8 | 18.5 ± 5.3 | 80 ± 27.4 |

photosystem I in the atsps2 mutant was only marginally lower than that of the atsps1 knock-out and wild type control, under either a normal or high light regime (Fig. 10A and B). Immunodetection of the photosystem I subunit PsaA did not reveal any significant differences between the atsps mutants and the wild type (Fig. 10C). Altogether, these data indicate that the bulk of the photodamage in the atsps2 knock-out was specific to photosystem II. In a parallel experiment designed to compare the plastoquinone-9 and tocochromanol levels of the wild type and atsps mutants during their acclimation to high light conditions, plants were pregrown under a moderate light regime (110 μE·m⁻²·s⁻¹) with alternating day and night cycles (16-h days) and then exposed to continuous high light intensity (600 μE·m⁻²·s⁻¹) for 48 h. Once switched to high light, the wild type and atsps1 plants both displayed a linear increase in plastoquinone-9 levels, although the rate of accumulation was comparatively half as fast in the mutant (Fig. 11A). Notably, in the wild type plants the accumulation of plastoquinone-9 coincided with an increase in the level of AtSPS1 and AtSPS2 transcripts (Fig. 11A, inset). Meanwhile the quantity of plastoquinone-9 in the atsps2 mutant steadily decreased and after 48 h was only half of its value at the onset of the high light treatment (Fig. 11A). Plastochromanol-8 levels decreased at a rate of 1.9 and 1.5 nmol g⁻¹ fresh weight/24 h in the wild type control and atsps1 mutant, respectively, while remaining below the detection limit in the atsps2 mutant (Fig. 11B). As for tocopherols, the quantity of which is known to increase dramatically in leaves exposed to high light intensities (36), no significant differences in the rate of accumulation were observed between the atsps mutants and the wild type control (Fig. 11C).
segregated according to the ratios expected for two unlinked genes in canonical Mendelian inheritance (Table 2). Such a finding was particularly remarkable in the case of the double atsps1 atsps2 knock-outs, indicating that the absence of plastochromanol-8 had no visible impact on seed longevity and germination. Neither the seedlings of the double atsps1 atsps2 knock-out nor those of its atsps parents exhibited the failure to expand cotyledons that is typically associated with tocochromanol deficiencies (data not shown). None of the aged vte2-1 mutant seeds, which are devoid of tocopherols and display a marked decrease in longevity (20), germinated, thus verifying the efficiency of the aging procedure in our hands (Table 2).

DISCUSSION

Confusion has long prevailed regarding the identity of the Arabidopsis solanesyl-diphosphate synthase that makes the side chain of plastoquinone-9. The corresponding activity was initially attributed to gene At1g78510 (AtSPS1) and then to gene At1g17050 (AtSPS2), whereas AtSPS1 was reassigned to the biosynthesis of the ubiquinone-9 side chain (8, 9, 27).

Further complicating the picture, a third trans-long chain prenyl-diphosphate synthase, the product of gene At2g34630 (AtSPS3), was recently identified in Arabidopsis and shown to be capable of producing solanesyl diphosphate (28). Nevertheless, direct evidence of function in plants was missing all along, leading to the common belief that each of these three enzymes could function in the biosynthesis of both plastoquinone-9 and ubiquinone-9 (27, 28, 37).

Functional modeling shows that AtSPS1 and AtSPS2 are linked with each other as well as with plastid metabolism, whereas AtSPS3 is a marked outlier. Coinciding with these findings, AtSPS1 and AtSPS2 both displayed canonical plastid pre-
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sequences (Fig. 12), and previous studies have indeed confirmed that AtSPS2 is targeted exclusively to chloroplasts (8, 9, 38, 39). The localization of GFP-fused proteins demonstrates that this strict plastid localization holds true for AtSPS1 as long as the cognate gene is expressed under the control of its native promoter.

Our data show that the atsp1 and atsp2 knock-outs both display lower levels of plastoquinone-9. The fact that the atsp1 atsp2 double knock-out is devoid of plastoquinone-9 and cannot grow photoautotrophically rules out that an additional trans-long chain prenyl-diphosphate synthase contributes to plastoquinone-9 biosynthesis. Importantly, the absence of AtSPS1 and AtSPS2 does not impact the level of ubiquinone-9. This result is supported by the observation that in tomato the trans-long chain prenyl-diphosphate synthases that elongate the allylic diphosphate precursors of ubiquinone-10

TABLE 2
Segregation ratios of the atsp1 × atsp2 F2 population

Three-month-old seeds were subjected to accelerated aging and then vernalized, whereas control seeds were vernalized without prior aging treatment. Aged and control seeds were then allowed to germinate on Murashige and Skoog solid medium containing sucrose in 16-h days (110 µE m−2 s−1) at 22 °C. Germinated plants were scored and genotyped after 3 weeks. Numbers of plants calculated from the Mendelian segregation ratios are shown in parentheses.

| Genotype                        | Control (Expected) | Aged (Expected) |
|---------------------------------|--------------------|-----------------|
| AtSPS1/AtSPS1; AtSPS2/AtSPS2    | 13 (13)            | 15 (17)         |
| AtSPS1/AtSPS1; atsp2/AtSPS2     | 29 (26)            | 32 (33)         |
| atsp1/AtSPS1; AtSPS2/AtSPS2     | 24 (26)            | 28 (33)         |
| atsp1/AtSPS1; atsp2/AtSPS2      | 53 (52)            | 75 (67)         |
| atsp1/atps1; atsp2/AtSPS2       | 21 (26)            | 34 (33)         |
| atsp1/atps1; AtSPS2/AtSPS2      | 24 (26)            | 23 (33)         |
| atps1/atps1; AtSPS2/AtSPS2      | 16 (13)            | 15 (17)         |
| atps1/AtSPS1; atsp2/atps2       | 12 (13)            | 22 (17)         |
| atps1/AtSPS1; atps2/atps2       | 15 (13)            | 18 (17)         |
| Not germinated                  | 1                  | 5               |
| Total                           | 208 (208)          | 267 (267)       |

Aging treatment control

vte2-1/vte2-1

| Plants                        | Number of plants |
|-------------------------------|------------------|
| Control                       | 77               |
| Aged                          | 138              |
| Not germinated                | 3                |
| Aged                          | 138              |
plastochromanol-8 of wild type plants, exemplifies the most extreme case of such an arrangement. Our data show that this low level of cyclization does not result from a side effect of the loss of plastoquinone-9 on tocopherol cyclase activity, for the atsp1 atsp2 double mutant, as well as its atsp parents, accumulates tocopherols to wild type levels. We propose instead that the access of tocopherol cyclase to plastoquinol-9 is restricted severely in plastoglobules, possibly in a fashion similar to the protein crowding that hinders the diffusion of plastochrome-9 molecules in thylakoid membranes (44). In support of such a scenario, there is mounting evidence that plastoglobules are not mere depot sites for lipids as thought initially, but are authentic plastidial subcompartments derived from thylakoid membranes and associated with many enzymes and structural proteins (41, 45, 46).

Although the occurrence of plastochochrome-8 in plant tissues has been known for almost 5 decades, its physiological significance is particularly unclear. On the one hand, the genetic dissection of tocopherol-deficient mutants mitigates oxidative damage to lipids and its subsequent negative effects on germination (15). On the other hand, one could argue that the occurrence of plastochochrome-8 in seeds that are devoid of tocopherols does not a priori occur in wild type plants. It appears instead that the amount of tocopherol in seeds dwarfs that of plastochochrome-8. Thus, calculations from our data and those of others (15, 12) show that the pool size of plastochochrome-8 in Arabidopsis seeds ranges from a mere 8 to 13 mol % of that of tocopherols, with even a report of a value as low as 1 mol % (32). Our data demonstrate that, without loss of tocopherols, plastochochrome-8 is not essential for seed survival and germination and therefore invite the question of whether the cyclization of plastoquinol-9 in seeds could simply represent a side-reaction of the accumulation of tocopherols. Along this line, it is noteworthy that hydroxyplastochochrome-8, the main oxidation product of plastochochrome-8, is undetectable in Arabidopsis seeds (14). Arguably, the situation might be different in other organs. In particular, there is evidence that the levels of plastochochrome-8 and hydroxyplastochochrome-8 in leaves increase substantially during aging and can total up to three-quarters of the molar amount of α-tocopherol (14). It is not possible from our data, however, to verify the existence of a photocytotoxic role of plastochochrome-8, for the leaves of the atsp mutants also display lower levels of plastoquinol-9, itself an antioxidant in thylakoids and a necessary redox cofactor for the biosynthesis of carotenoids (3, 47). Lastly, in connection with the current enigma of how changes in the redox state of plastoquinol-9 modulate gene expression in plastids and the nucleus (5), the atsp1 and atsp2 knock-outs might be invaluable tools to probe the respective contribution of the photoactive and non-photoactive pools of plastoquinol-9 to redox sensing and signaling.

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REFERENCES

1. Nelson, N., and Yocum, C. F. (2006) Structure and function of photosystems I and II. Annu. Rev. Plant Biol. 57, 521–565
2. Cooley, J. W., and Vermaas, W. F. (2001) Succinate dehydrogenase and other respiratory pathways in thylakoid membranes of Synechocystis sp. strain PCC 6803: capacity comparisons and physiological function. J. Bacteriol. 183, 4251–4258
3. Norris, S. R., Barrette, T. R., and DellaPenna, D. (1995) Genetic dissection of carotenoid synthesis in Arabidopsis defines plastoquinone as an essential component of phytoene desaturation. Plant Cell 7, 2139–2149
4. Mullineaux, C. W., and Emlyn-Jones, D. (2005) State transitions: an example of acclimation to low-light stress. J. Exp. Bot. 56, 389–393
5. Rochea, J. D. (2013) Redox regulation of tocopherol and plastoquinone biosynthesis. J. Biol. Chem. 288, 18317–18323
6. Jun, L., Saiki, R., Tatsumi, K., Nakagawa, T., and Kamewaki, M. (2004) Identification and subcellular localization of two solanesyl diphosphate synthases from Arabidopsis thaliana. Plant Cell Physiol. 45, 1882–1888
7. Hirooka, K., Izumi, Y., An, C. I., Nakazawa, Y., Fukusaki, E., and Kobayashi, A. (2005) Functional analysis of two solanesyl diphosphate synthases from Arabidopsis thaliana. Biosci. Biotechnol. Biochem. 69, 592–601
8. Ohta, K., Sasaki, K., and Yazaki, K. (2010) Two solanesyl diphosphate synthases with different subcellular localizations and their respective physiological roles in Ornica sativa. J. Exp. Bot. 61, 2683–2692
9. Cheng, Z., Sattler, S., Maeda, H., Sakuragi, Y., Bryant, D. A., and DellaPenna, D. (2005) Highly divergent methyltransferases catalyze a conserved reaction in tocopherol and plastoquinone synthesis in cyanobacteria and photosynthetic eukaryotes. Plant Cell 15, 2343–2356
10. Szymańska, R., and Kruk, J. (2010) Plastoquinol is the main prenyl lipid synthesized during acclimation to high light conditions in Arabidopsis and is converted to plastochochrome by tocopherol cyclase. Plant Physiol. 151, 537–545
11. Eugeni Piller, L., Besagni, C., Ksas, B., Rak, C., Glauser, B., Kessler, F., and Havaux, M. (2011) Chloroplast lipid droplet type II NAD(P)H quinone oxidoreductase is essential for prenylquinone metabolism and vitamin K₃ accumulation. Proc. Natl. Acad. Sci. U.S.A. 108, 14354–14359
12. Szymańska, R., and Kruk, J. (2010) Identification of hydroxyl-prenylplastochochrome in Arabidopsis leaves. Acta Biochim. Pol. 57, 105–108
13. Mene-Saffrané, L., Jones, A. D., and DellaPenna, D. (2010) Plastochochrome-8 and tocopherols are essential lipid-soluble antioxidants during seed desiccation and quiescence in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 107, 17815–17820
14. Tian, L., DellaPenna, D., and Dixon, R. A. (2007) The pds2 mutation is a lesion in the Arabidopsis homogentisate solanesyltransferase gene involved in plastoquinone biosynthesis. Planta 226, 1067–1073
15. Dawson, R. M. C., Elliot, D. C., Elliot, W. H., and Jones, K. M. (1986) Data for Biocatalytic Research. (Dawson, R. M. C., ed) 3rd Ed., pp. 132, 138, Oxford University Press, New York
16. Leebrecht, E., Sondergaard, E., and Dam, H. (1990) Occurrence of a plastochochrome in linseed oil. Acta Chem. Scand. 21, 2582
17. Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R., Gadrinab, C., Helle, C., Jeske, A., Koesea, E., Meyers, C. C., Parker, H., Predlin, L., Ansari, Y., Choi, N., Deen, H., Geralt, M., Hazari, N., Hom, E., Karnes, M., Mulholland, C., Nishida, R., Schmidt, M., Guzman, P., Aguilar-Henonin, L., Schmidt, M., Weigel, D., Carter, D. E., Marchand, T., Risseeuw, E., Brogden, D., Zeke, A., Crosby, W. L., Berry, C. C., and Ecker, J. R. (2003) Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science 301, 653–657
18. Sattler, S. E., Gilliland, L. U., Magillanes-Lundback, M., Pollard, M., and DellaPenna, D. (2004) Vitamin E is essential for seed longevity and for pre-
Solanesyl-diphosphate Synthases in Plastids

venting lipid peroxidation during germination. Plant Cell 16, 1419–1432
21. Christensen, A. C., Lyznik, A., Mohammed, S., Elowsky, C. G., Elo, A., Yule, R., and Mackenzie, S. A. (2005) Dual-domain, dual-targeting organellar protein presequences in Arabidopsis can use non-AUG start codons. Plant Cell 17, 2805–2816
22. Clough, S. J., and Bent, A. F. (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16, 735–743
23. Ducluzeau, A. L., Wamboldt, Y., Elowsky, C. G., Mackenzie, S. A., Schuurink, R. C., and Basset, G. J. (2012) Gene network reconstruction identifies the authentic trans-prenyl-diphosphate synthase that makes the solanesyl moiety of ubiquinone-9 in Arabidopsis. Plant J. 69, 366–375
24. Fristedt, R., Willig, A., Granath, P., Crévecœur, M., Rochaix, J. D., and Lohmann, A., Briesen, I., Porfirova, S., Bréhélin, C., Kessler, F., and Dörman, P. (2010) Intersection of the tocopherol and plastoquinol metabolic pathways at the plastoglobules. Arch. Biochem. Biophys. 425, 389–399
25. Jones, M. O., Perez-Fons, L., Robertson, F. P., Bramley, P. M., and Fraser, C. P. (2013) Functional characterization of long-chain prenyl diphosphate synthases from tomato. Biochem. J. 425, 729–740
26. Zbierak, A. M., Kanwischer, M., Wille, C., Vidi, P. A., Giavalisco, P., Lohmann, A., Briesen, I., Porfirova, S., Bréhélin, C., Kessler, F., and Dörmann, P. (2010) Intersection of the tocopherol and plastidopoietic metabolic pathways at the plastoglobules. Biochem. J. 425, 389–399
27. Hirooka, K., Bamba, T., Fukusaki, E., and Kobayashi, A. (2003) Cloning and kinetic characterization of Arabidopsis thaliana solanesyl-diphosphate synthase. Biochem. J. 370, 679–686
28. Hsieh, F. L., Chang, T. H., Ko, T. P., and Wang, A. H. (2011) Structure and mechanism of an Arabidopsis medium/long-chain-length prenyl pyrophosphate synthase. Plant Physiol. 155, 1079–1090
29. Ashby, M. N., and Edwards, P. A. (1990) Elucidation of the deficiency in two yeast coenzyme Q mutants. Characterization of the structural gene encoding hexaprenyl pyrophosphate synthetase. J. Biol. Chem. 265, 13157–13164
30. Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., and Gascuel, O. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36, W465–W469
31. Martinis, J., Glauser, G., Valimareanu, S., and Kessler, F. (2013) A chloroplast ABC1-like kinase regulates vitamin E metabolism in Arabidopsis. Plant Physiol. 162, 652–662
32. Kirchhoff, H. (2008) Molecular crowding and order in photosynthetic membranes. Trends Plant Sci. 13, 201–207
33. He et al. (2009) Identification of the authentic coenzyme Q10 biosynthetic enzymes. J. Biol. Chem. 284, 37199–37206
34. Newton, L. A., and Staehelin, L. A. (2006) Plastoglobules are lipoprotein subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain bio-synthetic enzymes. Plant Cell 18, 1693–1703
35. Lundquist, P. K., Poliakov, A., Giacomelli, L., Friso, G., Appel, M., McQuinn, R. P., Krasnoff, S. B., Rowland, E., Ponnala, L., Sun, Q., and van Wijk, K. J. (2013) Loss of plastoglobule kinases ABC1K1 and ABC1K3 causes conditional degreening, modified prenyl-lipids, and recruitment of the jasmonic acid pathway. Plant Cell 25, 1818–1839
36. Vidi, P. A., Kanwischer, M., Baginsky, S., Austin, J. R., Csucs, G., Dörmann, P., Kessler, F., and Bréhélin, C. (2006) Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplast plastoglobules lipoprotein particles. J. Biol. Chem. 281, 11225–11234
37. Joliot, P., and Joliot, M. (2008) Molecular crowding and order in photosynthetic membranes. Trends Plant Sci. 13, 201–207
38. Eugeni Piller, L., Abraham, M., Dörmann, P., Kessler, F., and Besagni, C. (2012) Plastid lipid droplets at the crossroads of prenylquinone metabolism. J. Exp. Bot. 63, 1609–1618
39. Ytterberg, A. J., Peltier, J. B., and van Wijk, K. J. (2006) Protein profiling of photosynthetic plastids from tomato. Biochem. J. 402, 420–422
40. Martinis, J., Glauser, G., Valimareanu, S., and Kessler, F. (2013) A chloroplast ABC1-like kinase regulates vitamin E metabolism in Arabidopsis. Plant Physiol. 162, 652–662
41. Gascuel, O. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36, W465–W469
42. Duret, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., and Gascuel, O. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36, W465–W469
43. Jones, M. O., Perez-Fons, L., Robertson, F. P., Bramley, P. M., and Fraser, P. D. (2013) Functional characterization of long-chain prenyl diphosphate synthases from tomato. Biochem. J. 449, 729–740
44. Zbierak, A. M., Kanwischer, M., Wille, C., Vidi, P. A., Giavalisco, P., Lohmann, A., Briesen, I., Porfirova, S., Bréhélin, C., Kessler, F., and Dörmann, P. (2010) Intersection of the tocopherol and plastidopoietic metabolic pathways at the plastoglobules. Biochem. J. 425, 389–399
45. Karamoko, M., Cline, S., Redding, K., Ruiz, N., and Hamel, P. P. (2011) Lumen thiol oxidoreductase 1, a disulfide bond-forming catalyst, is required for the assembly of photosystem II in Arabidopsis. Plant Cell 23, 4462–4475
46. Lichtenhaller, H. K., and Miehé, J. A. (1997) Fluorescence imaging as a diagnostic tool for plant stress. Trends Plant Sci. 2, 316–320
47. Müller, P., Li, X. P., and Niwog, K. K. (2001) Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125, 1558–1566
48. Maeda, H., and DellaPenna, D. (2007) Tocopherol functions in photosynthetic organisms. Curr. Opin. Plant Biol. 10, 260–265
49. Kawamukai, M. (2009) Biosynthesis and bioproduction of coenzyme Q10 by yeasts and other organisms. Biotechnol. Appl. Biochem. 53, 217–226
50. Zybailov, B., Rutschow, H., Friso, G., Rudella, A., Emanuelsson, O., Sun, Q., and van Wijk, K. J. (2008) Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. PLoS One 3, e1994
51. Ferro, M., Brüggière, S., Salvi, D., Seigneurin-Berny, D., Court, M., Moyet, L., Ramus, C., Miras, S., Mellal, M., Le Gall, S., Kieffer-Jaquinos, S., Bruley, C., Garin, J., Joyard, J., Masselon, C., and Rolland, N. (2010) AT-CHLORO, a comprehensive chloroplast proteome database with subplastidal localization and curated information on envelope proteins. Mol. Cell. Proteomics 9, 1063–1084
52. Vidi, P. A., Kanwischer, M., Baginsky, S., Austin, J. R., Csucs, G., Dörmann, P., Kessler, F., and Bréhélin, C. (2006) Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplast plastoglobules lipoprotein particles. J. Biol. Chem. 281, 11225–11234
53. Austin, J. R., 2nd, Frost, E., Vidi, P. A., Kessler, F., and Staehelin, L. A. (2006) Plastoglobules are lipoprotein subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain bio-synthetic enzymes. Plant Cell 18, 1693–1703
54. Zybailov, B., Rutschow, H., Friso, G., Rudella, A., Emanuelsson, O., Sun, Q., and van Wijk, K. J. (2008) Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. PLoS One 3, e1994