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1 **Participation of the stress-responsive CDSP32 thioredoxin in the modulation of**
2 **chloroplast ATP-synthase activity in *Solanum tuberosum***

3
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22 **Keywords:** ATP-synthase, saline-alkaline stress, photosynthesis, redox regulation, *Solanum*
23 *tuberosum*, structure modeling, thioredoxin.

1 **Abstract**

2 Plant thioredoxins (TRXs) are involved in numerous metabolic and signalling pathways, such as light-
3 dependent regulation of photosynthesis. The atypical TRX CDSP32, chloroplastic drought-induced
4 stress protein of 32 kDa, includes two TRX-fold domains and participates in responses to oxidative
5 stress as an electron donor to other thiol reductases. Here, we further characterized potato lines modified
6 for *CDSP32* expression to clarify the physiological roles of the TRX. Upon high salt treatments,
7 modified lines displayed changes in the abundance and redox status of CDSP32 antioxidant partners,
8 and exhibited sensitivity to combined saline-alkaline stress. In non-stressed plants overexpressing
9 CDSP32, a lower abundance of photosystem II subunits and ATP-synthase γ subunit was noticed. The
10 CDSP32 co-suppressed line showed altered chlorophyll *a* fluorescence induction and impaired
11 regulation of the transthylakoid membrane potential during dark/light and light/dark transitions. These
12 data, in agreement with the previously reported interaction between CDSP32 and ATP-synthase γ
13 subunit, suggest that CDSP32 affects the redox regulation of ATP-synthase activity. Consistently,
14 modeling of protein complex 3-D structure indicates that CDSP32 could constitute a suitable partner of
15 ATP-synthase γ subunit. We discuss the roles of the TRX in the regulation of both photosynthetic
16 activity and enzymatic antioxidant network in relation with environmental conditions.

17

18 **Introduction**

19 Redox post-translational modifications (PTM) in proteins lead to switches modifying enzymatic activity,
20 protein conformation or subcellular distribution. In the presence of reactive oxygen species (ROS),
21 cysteine (Cys) can be oxidized to various forms such as sulfenic acid or disulfide bond, while methionine
22 (Met) can be oxidized to Met sulfoxide (Davies, 2005; Delaunay et al. 2002; Tada et al. 2008). Of note,
23 most redox PTMs in Cys and Met are reversible thanks to the action of thiol reductases (TRs), in which
24 thiol deprotonation of a catalytic cysteine generates a nucleophilic reactive thiolate form. By modulating
25 Cys redox status in interacting partners, TRs play key roles in cell redox homeostasis maintenance and
26 ROS-related signalling transduction pathways (Lu and Holmgren, 2014).

27 Plant TRs form complex multigene families including thioredoxins (TRXs) (Meyer et al, 2012;
28 Geigenberger et al. 2017) and glutaredoxins (GRXs), which are closely related to TRXs and use
29 glutathione (GSH) as an electron donor (Rouhier et al. 2008). TRXs are ubiquitous small disulfide
30 reductases carrying a two-Cys active site, generally WCGPC. Cytosolic TRXs get reducing power from
31 NADPH-TRX reductases and are involved in various processes such as mobilization of seed reserves
32 upon germination or biotic stress responses (Sweat and Wolpert, 2007; Hägglund et al. 2016). Plastidial
33 TRX isoforms are mainly supplied with electrons from the photosystem I (PSI)/ferredoxin/ferredoxin-
34 TRX-reductase (FTR) pathway and play, in concert with the NADPH-dependent TRX reductase system
35 termed NTRC, essential roles in the regulation of photosynthetic metabolism notably upon dark/light
36 and light/dark transitions (Serrato et al. 2004; Schürmann and Buchanan, 2008; Courteille et al, 2013;
37 Nikkanen et al. 2016; Yoshida et al. 2018; Lampl et al. 2022). Thiol peroxidases such as peroxiredoxins

1 (PRXs) also fulfil signalling roles via the control of peroxide concentration or direct thiol oxidation in
2 protein partners (Rhee and Woo, 2011; Cerveau et al. 2016a; Liebthal et al. 2018). Other TRX targets
3 like methionine sulfoxide (MetO) reductases (MSRs) maintain protein redox status thanks to redox-
4 active cysteines that reduce peptide-bound MetO (Tarrago et al. 2009; Laugier et al. 2010). By
5 modifying the Cys redox status in a large set of partners (Montrichard et al. 2009), plant TRXs
6 participate in multiple metabolic, developmental and stress-related processes (Gelhaye et al. 2005;
7 Vieira Dos Santos and Rey, 2006; Geigenberger et al. 2017; Montillet et al. 2021).

8 In addition to canonical TRXs, such as cytosolic h, mitochondrial o and plastidial f, m, x, y and z
9 isoforms, which contain only one TRX-fold domain and a typical active site WCGPC, plants harbour
10 TRXs carrying atypical active site motifs or including other domains (Meyer et al. 2012). Among them,
11 the CDSP32 (Chloroplastic Drought-induced Stress Protein of 32 kDa) TRX, initially identified in
12 potato plants subjected to water deficit (Rey et al. 1998), is induced by various environmental constraints
13 (Pruvot et al. 1996 ; Rey et al. 1998 ; Broin et al. 2000). The protein is composed of two TRX-fold
14 domains with the N-terminal one having no redox-active motif, but instead a SXXS motif, and the C-
15 terminal one displaying an atypical redox-active HCGPC motif (Rey et al. 1998). The involvement of
16 CDSP32 in responses to oxidative stress was established based on the phenotype of transgenic lines
17 (Broin et al. 2002; 2003) and on its capacity to reduce various PRXs and MSRs (Rey et al. 2005; Vieira
18 Dos Santos et al. 2007; Tarrago et al. 2010). Accordingly, ectopic expression of mulberry and cotton
19 *CDSP32* genes in *Arabidopsis* was found to trigger recovery following a drought period (Sun et al. 2020)
20 and to confer tolerance to osmotic or oxidative stresses (Elasad et al. 2020).

21 Besides this role in stress response, CDSP32 could function in photosynthesis regulation, because in
22 CDSP32-overexpressing tobacco, Zhang et al. (2020) noticed higher chlorophyll (Chl) content together
23 with a reduced Chl *a/b* ratio, and better maintenance of photosynthetic activity upon Cd treatment,
24 compared to wild-type (WT). They hypothesized that the TRX regulates photosynthetic cyclic electron
25 flow and energy dissipation allowing photoprotection upon stress (Zhang et al. 2021). Most importantly,
26 by performing redox proteomics during a dark/light transition in tobacco, Zimmer et al. (2021) revealed
27 that the redox status of both TRX f and CDSP32 is dependent on the rate of linear electron flow, and
28 proposed that the two TRXs act in concert in the regulation of Calvin-Benson cycle activity.

29 Here, we further characterized potato lines modified for *CDSP32* expression to better delineate the
30 physiological roles of the TRX. We investigated the sensitivity of the modified lines to osmotic and
31 alkaline stress, as well as the effects of such stresses on the abundance and redox status of CDSP32
32 partners and on the abundance of photosynthetic proteins. We also investigated the effects of modified
33 *CDSP32* expression on photosynthetic metabolism, and in particular on the chloroplast ATP-synthase
34 activity, during dark/light and light/dark transitions. Based on the obtained results, we discuss the role
35 of CDSP32 in photosynthesis regulation in relation with its participation in the maintenance of plastidial
36 redox homeostasis.

37

1 **Materials and methods**

2 **Plant material and growth conditions**

3 Potato (*Solanum tuberosum* L. cv Désirée) lines were propagated *in vitro* on Murashige and Skoog
4 medium, and transferred for growth *in vivo* in a phytotron under LED light (VEGELED Horticulture
5 Floodlights Apollo LL300, Colasse SA, Belgium) (PPFD of 185 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 12 h day/12 h night,
6 23/19°C) in the “Phytotec” platform (CEA, DRF, BIAM). In addition to WT, four transgenic lines co-
7 suppressed for the expression of the potato *CDSF32* gene (termed D4), overexpressing it (D10) or
8 overexpressing in the WT background a mutated form of the TRX, where the catalytic Cys is replaced
9 by a Ser (DM19 and DM15), were used (Broin et al. 2002; Rey et al. 2005). Following transfer on soil,
10 control plants were watered with water the first three days, and then with nutritive solution (Coic and
11 Lesaint, 1971). For salt treatments, the nutritive solution was supplemented with 0.125 M NaCl or 0.1
12 M NaHCO₃. The treatments were applied for 21 days. At this stage, plant height was measured and
13 aerial parts weighed, and samples for chlorophyll determination and protein analyses collected.

14

15 **Protein extraction and content determination**

16 Pieces from the terminal leaflet of young expanded leaves were blended in liquid N₂, and the powder
17 was resuspended in 50 mM Tris-HCl, pH 8.0, 1 mM phenylmethylsulfonyl fluoride, and 50 mM β -
18 mercaptoethanol. Following centrifugation (14,000 g, 4°C, 20 min), soluble proteins were recovered by
19 precipitation of the supernatant using two acetone volumes and stored at -20°C. The pellet, containing
20 thylakoids, was resuspended in 50 mM Tris-HCl pH 8.0, 1% SDS, agitated for 2 h at 4°C, then
21 centrifuged (14,000 g, 4°C, 20 min). Membrane proteins were precipitated at -20°C by the addition of
22 four volumes of acetone to the supernatant. Protein concentration was quantified using the “Protein
23 Quantification BCA Assay” kit (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific).

24

25 **Western blot analysis**

26 Proteins were separated by SDS-PAGE generally in 13%, or when needed in 8, 10, 11 or 15%,
27 acrylamide gels (Laemmli, 1970) in reducing conditions (0.1 M DTT in solubilization buffer) and
28 electroblotted onto 0.45 μm nitrocellulose (Pall Gelman Sciences). Membranes were stained with
29 Ponceau red to ensure homogenous and proper transfer. The list of primary antibodies raised in rabbit
30 used in this work is presented in Table S1. Membranes could be subsequently subjected to two distinct
31 revelation procedures. In the first procedure, bound antibodies were detected using a goat anti-rabbit
32 secondary antibody coupled to a fluorescent molecule (Alexa Fluor 680, Invitrogen) diluted 1:10,000
33 using the “Odyssey Infrared Imager” at 680 nm (Licor, Lincoln, NE, USA). Quantification of band
34 intensity was performed using the software associated with the imager. Then the membrane was
35 incubated with another primary serum and revealed using an anti-rabbit immunoglobulin G coupled to
36 alkaline phosphatase (Sigma). Data were acquired from three or four independent experiments and

1 representative immunoblots are shown. Quantification of band intensities were obtained from biological
2 replicates yielding consistent results.

4 **Chlorophyll content determination**

5 Leaf disks of 0.6 cm diameter were collected on terminal leaflets of young expanded leaves, stored at –
6 80°C before blending in 80% acetone. Following shaking, overnight storage in the dark at 4°C and
7 centrifugation (14,000 g, 20 min), the content in chlorophylls *a* and *b* was measured by
8 spectrophotometry at 647 and 663 nm, and calculated according to Lichtenthaler (1987).

10 **Fluorescence measurements**

11 Chlorophyll fluorescence was measured on leaf fragments using a laboratory-built fluorescence camera.
12 Leaf fragments were harvested from plants incubated in darkness overnight (>12h), and kept on wet
13 Whatman paper in darkness until measurements were performed. Each biological replicate consisted in
14 one leaf fragment harvested from a different plant. Maximal PSII quantum yield [$F_v/F_m = (F_m - F_o)/F_m$]
15 was measured applying a multi-turnover saturating light pulse (200 ms, 3,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to
16 dark-adapted leaves. Fluorescence induction curves were measured during 4 minutes of actinic light at
17 the indicated intensities, and F_m' levels after multi-turnover saturating pulses applied during the actinic
18 illumination. PSII quantum yield [$\Phi_{\text{PSII}} = (F_m' - F)/F_m'$] was calculated with F and F_m' values measured,
19 respectively, right before and after the first and last saturating pulses of the sequence. Non-
20 Photochemical Quenching (NPQ) was calculated as $(F_m - F_m')/F_m'$. Actinic light and saturating pulses
21 were provided by orange-red LEDs, measuring pulses (740 μs) by blue LEDs.

23 **ElectroChromic Shift (ECS) measurements**

24 ECS measurements were performed with a laboratory-built Joliot-Type Spectrophotometer (JTS). Short
25 (15 μs) measuring pulses were provided by a sun-like LED (COB V6HD THRIVE WHT SQ 6500K,
26 Bridgelux) filtered via a 520 nm interference filter (Edmund Optics, 10 nm FWHM). Actinic light was
27 provided by orange-red LEDs. Single-turnover saturating flashes (700 nm, duration 6 ns) were provided
28 by a pulsed Nd:YAG laser pumping an optical parametric oscillator (Surelite II, Continuum). For sub-
29 saturating flashes, the laser light was attenuated using a metal grid. Measurements were performed on
30 intact leaves detached right before measuring from plants incubated in darkness overnight (>12h). Each
31 biological replicate consisted in one leaf detached from a different plant.

32 Electron Transport Rate (ETR) measurements. Dark-adapted leaves were illuminated with the indicated
33 intensities of actinic light for 4 minutes. The photosystem II+photosystem I (PSII+PSI) antenna size and
34 total steady-state ETR were calculated from the slopes of the ECS increase and decay at the onset and
35 offset of the actinic illumination, respectively (for details see Mathiot and Alric, 2021). The absorption
36 changes induced by a single-turnover saturating flash, inducing one charge separation per photosystem
37 (PSII+PSI) were used for normalisation.

1 Redox-dependent regulation of the ATP-synthase activity.

2 Activation: absorption changes induced by one sub-saturating flash were recorded in dark-adapted
3 leaves and 2 minutes after a train of n sub-saturating pre-flashes fired at 20 Hz, with $n = 20, 50, 100,$
4 200 and 300 . For each leaf, the loss in amplitude of the slow ECS decay phase induced by n pre-flashes
5 ($-A_n$) was calculated with respect to the amplitude of the slow ECS decay phase in the dark-adapted leaf
6 (A_{Dark}), setting the amplitude of the slow phase after 300 pre-flashes as the zero value. The number of
7 charge separations per PSII+PSI induced by n pre-flashes was calculated by multiplying n for the ratio
8 between the absorption changes induced by a sub-saturating and a saturating flash.

9 De-activation in darkness: absorption changes induced by one sub-saturating single-turnover flash were
10 recorded in dark-adapted leaves and at different times t after a train of 300 sub-saturating pre-flashes
11 fired at 20 Hz, with $t = 2, 4, 6, 8, 10, 15$ and 20 minutes. For each leaf, the recovery in amplitude of the
12 slow ECS decay phase at a time t after the 300 pre-flashes (A_t) was calculated with respect to the
13 amplitude of the slow ECS decay phase in the dark-adapted leaf (A_{Dark}), setting the amplitude of the
14 slow phase at $t = 2$ min as the zero value.

15 For each curve, the $-A_n/A_{\text{Dark}}$ (activation measurements) and A_t/A_{Dark} (de-activation measurements)
16 values calculated for the absorption changes measured at 395, 595, 789 and 985 ms after the flash were
17 averaged.

18

19 **Sequence analysis and 3D structure modelling**

20 Sequences of CDSP32 TRXs and related proteins and of ATP-synthase γ subunit among plant, algae
21 and bacteria phyla were found by searching databases and performing BLAST analyses at NCBI:
22 <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. Prediction of transit peptide was performed at DTU (Technical
23 University of Denmark): <https://services.healthtech.dtu.dk/services/TargetP-2.0/>. Multiple sequence
24 alignments were carried out using Clustal Omega at EMBL-EBI:
25 <https://www.ebi.ac.uk/Tools/msa/clustalo/>. Analysis of conserved protein domain family was performed
26 at NCBI: <https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi> (Wang et al. 2023). The 3D structures
27 of *S. tuberosum* CDSP32 and TRX f were predicted using AlphaFold2 via the software ColabFold
28 (Jumper et al. 2021; Mirdita et al. 2022). The 3D structures of the *S. tuberosum* ATP synthase γ subunit
29 and the complexes between the γ subunit and CDSP32 and TRX f were predicted using a local
30 AlphaFold2 installation (version 2.3.1) (Jumper et al. 2021). All parameters were kept to default values.
31 The quality of each model, presented in supplementary figures, was evaluated using the confidence
32 measures pLDDT (predicted local distance difference test) that indicates the local accuracy, and PAE
33 (predicted aligned error) that assesses the packing between domains or protein chains. Overall
34 confidence in predictions for complexes were based on a combination of all the metrics, including both
35 the predicted template modelling (pTM) score and the interface predicted template modelling (ipTM)
36 score as well as pLDDT and PAE. The 3D structure of *Spinacia oleracea* ATP-synthase and of its
37 γ subunit was obtained from the protein data bank (<https://www.rcsb.org>, PDB 6FKF). Three-

1 dimensional structure or model images (rank_1 model for each AlphaFold2 predicted 3D structure) were
2 generated using PyMOL (PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC). The 3D
3 modelled structures of proteins or complexes do not include transit peptides, therefore the sequence
4 numbering of all proteins starts after the addressing peptides.

5 **Results**

6 **Growth characteristics and chlorophyll content of potato transgenic lines in control conditions**

7 We previously reported the generation of potato lines either lacking the CDSP32 TRX (co-suppressed
8 line D4), or overexpressing the WT protein (line D10) or the Cys-216-Ser active site mutated form in
9 addition to the WT protein (line DM19) (Broin et al. 2002; Rey et al. 2005). In these first assays, we did
10 not notice any obvious growth or development phenotype for these lines in the absence of environmental
11 constraints. As these assays were performed on a limited number of plants and under a different light
12 source (HQI-sodium lamps) than the one used in this work (LED lamps), we first carried out a deeper
13 phenotype characterization in control conditions. WT and transgenic lines displayed similar growth and
14 development features after 3.5 weeks following the transfer from *in vitro* tubes (Fig. 1A). All lines
15 exhibited stem heights in the range of 21 cm and weights of aerial parts close to 30 g (Fig. S1C,D).
16 Based on the somewhat paler leaf colour in plants expressing mutated CDSP32 (DM19), we determined
17 the chlorophyll content of young expanded leaves in the upper crown (Fig. 1C). While WT, co-
18 suppressed (D4) and CDSP32-overexpressing (D10) plants displayed contents between 36 and 38 $\mu\text{g. cm}^{-2}$,
19 a significantly lower content (less than 33 $\mu\text{g. cm}^{-2}$) was measured in plants overexpressing the
20 mutated TRX. No difference in the chlorophyll *a/b* ratio was observed, indicating that both chlorophyll
21 types were similarly affected in this line (Fig. S1E).

22 **Growth characteristics upon high salt treatments**

23 *CDSP32* gene expression is upregulated in WT potato plants upon severe osmotic stress conditions such
24 as drought or exposure to high NaCl concentration (0.3 M) for eight days (Pruvot et al. 1996). Here, we
25 subjected transgenic potato lines to a less deleterious concentration (0.125 M) in watering solution for
26 three weeks. Upon NaCl exposure, the stem height was strongly reduced in all lines (12 cm *vs* 21 cm in
27 control conditions (Figs. 1B, S1A, C). The treatment also led to a dramatic decrease in the weight of
28 aerial parts to *ca.* 17 g (against 30 g in control conditions) in the four potato lines, (Fig. S1D). We noticed
29 that NaCl-treated plants were greener than control ones (Fig. 1B). Consistently, we measured a
30 chlorophyll content higher than 45 $\mu\text{g.cm}^{-2}$ in the same range in all lines (Fig. 1D). This increased level
31 is likely due to the leaf size reduction and thickness increase induced by the NaCl treatment (Fig. 1B),
32 resulting in higher chloroplast density per leaf surface area (Munns and Tester, 2008). Altogether, these
33 data show that the phenotype of potato plants exposed to osmotic stress *via* NaCl treatment at 0.125 M
34 is unaffected by modifications of *CDSP32* expression.
35
36

1 Since some CDSP32 targets like MSRBs are involved in responses to saline-alkaline conditions (Sun et
2 al. 2016) and *CDSP32* expression is down-regulated in such stress conditions (Huihui et al. 2020), we
3 exposed plants to 0.1 M NaHCO₃ for three weeks. Similarly to NaCl treatment, watering with NaHCO₃
4 resulted in a strong decrease in plant size for all lines (Fig. 1B and S1B) with a stem height in the range
5 of 12 cm (Fig. S1C). The weight of aerial parts was also strongly reduced upon salt treatment.
6 Interestingly, WT and CDSP32-overexpressing plants exhibited weights of around 15 and 18 g,
7 respectively, while substantially lower values were recorded for the two other lines, particularly for the
8 one overexpressing the mutated form (less than 11 g) where the difference was significant compared to
9 WT (Fig. S1D). Exposure to NaHCO₃, in contrast to NaCl, did not affect leaf expansion (Fig 1B). The
10 four potato lines watered with NaHCO₃ displayed pale green to yellow leaves, particularly the youngest
11 ones (Fig. S1B). Chlorophyll content measurements revealed that the pigment level decreased in all
12 lines including the WT (15.5 µg.cm⁻²), but it was significantly lower in plants overexpressing mutated
13 CDSP32 (ca. 9 µg.cm⁻²) (Fig. 1E). In this line, as well as in the one overexpressing the non-mutated
14 CDSP32, the chl *a/b* ratio was also significantly lower than in WT (Fig. S1E). Taken together, these
15 data show that plants overexpressing inactive CDSP32 are more sensitive than WT to the saline-alkaline
16 stress induced by NaHCO₃ exposure.

17

18 **Abundance of CDSP32 partners and of proteins involved in maintenance of redox homeostasis**

19 We performed Western blot analyses to investigate the abundance of several thiol reductases, most being
20 known to interact with CDSP32. First, we reassessed the abundance of the CDSP32 TRX in the four
21 potato lines grown in control conditions (Fig. 2A). While the protein was not detected in the D4 co-
22 suppressed line, much higher abundances were observed compared to WT in over-expressing lines, with
23 5- and 8-fold more intense bands in plants expressing WT or mutated forms, D10 and DM19,
24 respectively, as previously reported (Rey et al., 2005). In agreement with previous reports (Pruvot et al.
25 1996), NaCl treatment led to a noticeable increase in the abundance of the TRX in WT plants. Upon
26 watering with NaHCO₃ (Fig. 2A), no change in protein abundance was noticed in WT plants, and
27 decreased amounts were observed in overexpressing lines compared to control conditions.

28 We then analysed the abundance of known partners of CDSP32, namely PRXs and MSRs. No change
29 in the total abundance of 2-Cys PRX was noticed in control conditions and upon salt treatments
30 depending on *CDSP32* expression level. However, variations were observed regarding the over-oxidized
31 form of the 2-Cys PRX, which is involved in redox signalling (Rey et al. 2007) and detected using
32 specific antibodies (Table S1). In control conditions and compared to WT, this form was less abundant
33 in co-suppressed lines and more abundant in lines overexpressing WT CDSP32, as previously reported
34 (Cerveau et al. 2016b). Interestingly, over-oxidized 2-Cys PRX was also significantly less abundant in
35 plants over-expressing inactive TRX (Fig. 2A, lane 4; Fig. S2A). Almost no difference was noticed in
36 the abundance of over-oxidized PRX among the four genotypes upon NaCl treatment. Upon NaHCO₃
37 exposure, significantly higher amounts of this redox form were revealed in co-suppressed plants and in

1 plants over-expressing the mutated TRX compared to WT (Fig. 2A, B) in sharp contrast with control
2 conditions. With regard to PRXQ, no noticeable variation was observed in the four lines either in control
3 conditions or upon salt treatments (Fig. 2A). Plastidial NTRC is a main reducer of 2-Cys PRX (Pulido
4 et al. 2010), and involved in response to high salt (Serrato et al. 2004). In control conditions, a band at
5 *ca.* 54 kDa was revealed by the serum raised against Arabidopsis NTRC, with another upper band at *ca.*
6 56 kDa. The latter could correspond to an oxidized form, clearly detected in non-reducing conditions
7 (Chae et al. 2013). Both forms could be observed in our experiments due to incomplete reduction, as
8 observed for various types of thiol reductases in reducing SDS-PAGE (Cerveau et al., 2016b). Higher
9 amounts of the upper form were noticed upon NaCl treatment in all lines. No substantial change in the
10 abundance of MSRB1, which is efficiently reduced by CDSP32 (Tarrago et al. 2010), was observed
11 depending on potato genotype in control or NaCl conditions (Fig. 2A). In plants watered with NaHCO₃,
12 a significantly higher MSRB1 abundance was observed in both co-suppressed (D4) and the
13 overexpression (D10) lines compared to WT (Fig. 2A, B). Finally, we did not observe any substantial
14 variation in MSRA4 amount, except in co-suppressed plants treated with NaHCO₃, which showed a
15 significantly higher level compared to other lines (Fig. 2A, B). These data indicate that modifying
16 *CDSP32* expression level substantially affects the redox status of plastidial PRXs and the abundance of
17 MSRs, notably in NaHCO₃ salt-stress conditions.

18 We investigated the abundance of several ROS-scavenging enzymes, since CDSP32 interacts with 2-
19 Cys PRX, a main player in plastidial ROS homeostasis. Stromal, peroxisomal and cytosolic ascorbate
20 peroxidases (APXs) were revealed in potato extracts (Fig. S2B). APXs levels were similar in all
21 genotypes in control conditions, except for somewhat higher levels of peroxisomal and stromal isoforms
22 in D10 plants, and did not change significantly upon NaCl treatment. Of note, watering with NaHCO₃
23 resulted in a strong decrease in the level of cytosolic APX in all lines (Fig. S2B). No significant variation
24 in catalase (CAT) abundance was observed depending on genotype in control and NaCl conditions. In
25 plants watered with NaHCO₃, a lower protein level was noticed in the three transgenic lines compared
26 to WT, with the difference being significant in the lines overexpressing the WT and mutated CDSP32
27 (Fig. S2B, C). In control conditions and upon NaCl treatment, the abundance of plastidial Cu-Zn
28 superoxide dismutase (SOD) was similar in all lines. In plants watered with NaHCO₃, we noticed a
29 significantly higher Cu-Zn SOD amount (by more than 40%) in plants over-expressing WT CDSP32
30 compared to WT (Fig. S2B, C).

31

32 **Abundance of photosynthetic components and photoprotective proteins**

33 We analysed the abundance of various photosynthetic components, starting with enzymes participating
34 in the Calvin-Benson cycle (CBC), whose activity is redox regulated by TRXs. No genotype-dependent
35 variation in large RuBisCO subunit abundance was observed in plants grown in control conditions or
36 treated with NaHCO₃, while somewhat higher levels were observed upon NaCl treatment (Fig. 3A).
37 Phosphoribulokinase (PRK) allows the regeneration of ribulose 1,5-bisphosphate, the CO₂-acceptor

1 substrate in CBC. No substantial variation was noticed in PRK abundance as a function of growth
2 condition or of genotype (Fig. 3A).

3 We then investigated the level of several thylakoid proteins involved in light collection, electron transfer,
4 or protective mechanisms. The abundance of major proteins of light-harvesting complexes, LHCB1 and
5 LHCB2, was very similar in the four potato lines in control and salt conditions. In contrast, in
6 comparison with control conditions, the LHCA1 amount was increased upon NaCl watering, and
7 noticeably decreased in plants treated with NaHCO₃, particularly those overexpressing mutated CDSP32
8 (Fig. 3A and S3). Regarding PSII components, we noticed in control conditions a strong decrease (more
9 than 50%) in the abundance of PsbO, one the three subunits of the PSII oxygen-evolving complex
10 located in lumen, in CDSP32 over-expressing plants compared to WT (Fig. 3A lanes 1, 3 and 3B).
11 Further, the protein level was also found to be substantially lower in this line and the one expressing
12 mutated CDSP32 upon NaHCO₃ treatment. Most interestingly, similar changes were observed for the
13 D1 protein that together with D2 forms the PSI reaction centre. Indeed, the abundance of D1 was
14 significantly lower in CDSP32 over-expressing plants in control conditions and in plants expressing the
15 mutated TRX upon NaHCO₃ exposure (Fig. 3A lanes 1, 3 and 3B).

16 Zhang et al. (2021) proposed that CDSP32 could regulate photosynthetic cyclic electron transport.
17 Therefore, we investigated the abundance of components of the two cyclic pathways operating around
18 PSI. The protein levels of NDH-H, a subunit of NAD(P)H-like dehydrogenase complex (Rumeau et al.
19 2007), were strongly reduced protein level in all lines watered with NaCl compared to control and
20 alkaline-saline conditions and no substantial genotype-dependent variation was noticed (Fig. 3).
21 Conversely, the abundance of PGRL1, which has been proposed to be involved in the
22 ferredoxin:plastoquinone reductase cyclic pathway (Hertle et al. 2013), was much higher in plants
23 watered with NaCl, the protein being barely detected in plants grown in control conditions or treated
24 with NaHCO₃.

25 We previously reported that the ATP-synthase γ subunit interacts with CDSP32 in plant extracts
26 overexpressing the mutant TRX form (Rey et al. 2005). In control conditions, we observed a lower
27 amount of this subunit in plants overexpressing the non-mutated TRX than in WT (Fig. 3A lanes 1, 3
28 and 7B). Salt treatments resulted in an increased abundance of the γ subunit, with a lower level in plants
29 overexpressing CDSP32 compared to WT upon exposure to NaHCO₃ (Fig. 3B).

30 We analysed the abundance of PsbS, a photoprotective protein involved in dissipation of excess energy
31 in conditions of high illumination (Li et al. 2000). We did not observe any significant change in the
32 protein level in relation with CDSP32 expression level, but noticed a slightly higher protein abundance
33 in all lines exposed to NaCl (Fig. 3A). Finally, we examined the abundance of fibrillins (FBN), a family
34 of proteins possessing a lipid-binding motif and presumed among other functions to reduce
35 photooxidative damage to PSII (Yang et al. 2006; Kim and Kim, 2022). We used the serum that we
36 generated against FBN clade1-type in potato, initially named CDSP34 for Chloroplastic-Drought
37 induced Stress Protein of 34 kDa (Pruvot et al. 1996). In control conditions, two FBNs were revealed at

1 around 34 and 37 kDa, the latter form being more abundant in plants lacking CDSP32 or over-expressing
2 the mutated TRX. Upon NaCl treatment, the FBN1 isoform was much more abundant in the four lines,
3 consistently with our previous results (Pruvot et al. 1996). In contrast, watering with NaHCO₃ resulted
4 in a strong decrease of the abundance of the two isoforms in all lines.

6 **PSII function and activity of the electron transport chain**

7 Our biochemical analyses revealed decreased abundance of the PsbO, D1 and ATP-synthase γ subunit
8 in the line overexpressing CDSP32 in the absence of stress (Fig. 3), so we performed several
9 measurements of the photosynthetic activity to investigate the involvement of the TRX in its regulation.
10 We performed fluorescence measurements to assess the effects of altered CDSP32 expression on the
11 maximal PSII quantum yield (Fv/Fm) in dark-adapted leaves and the yield during the transition from
12 darkness to moderate-low light (80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, Fig. 4A). Fv/Fm values were similar in all
13 genotypes (~0.79, Fig. 4C and Table S2), indicating a normal PSII function. The transient fluorescence
14 increase in the first seconds of moderate-low illumination was instead significantly higher in the
15 CDSP32 co-suppressed line (D4, Fig. 4B), although it then decreased to similar stationary levels as in
16 the other genotypes during the following 4 minutes of illumination. This resulted in a lower PSII yield
17 (Φ_{PSII}) measured in D4 plants 4 seconds after the light onset (Fig. 4D and Table S2), but a normal Φ_{PSII}
18 *ca.* 200 seconds after the light onset (Fig. 4E and Table S2). We observed an increased transient
19 fluorescence in D4 also during the transition from darkness to moderate-high light (170 $\mu\text{mol photons}$
20 $\text{m}^{-2} \text{s}^{-1}$), although the difference was less pronounced because the increase was generally higher in all
21 genotypes (Fig. S4A, B). In high light (750 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the initial fluorescence increase
22 reached values close to Fm in all plants (Fig. S4C, D). The Non Photochemical Quenching (NPQ) of
23 Fm' induced by the medium-high and high lights was similar in all lines (Fig. S4A, C). At all light
24 intensities, the fluorescence rise kinetics in the first hundreds of milliseconds of illumination were
25 similar in all genotypes, suggesting a similar PSII antenna size.

26 In agreement with the fluorescence results, the PSII+PSI antenna size and total steady-state electron
27 transport rates measured by ElectroChromic Shift (ECS) as a function of light intensity were also similar
28 in all genotypes (Fig. S5).

30 **Light-dependent activation of the ATP-synthase activity**

31 Zimmer et al. (2021) have shown that, during the transition from darkness to low light, the reduction of
32 CDSP32 follows kinetics similar to those of TRX f and of ATP-synthase γ subunit, and here we revealed
33 a lower abundance of this subunit in lines overexpressing CDSP32 in control conditions (Fig. 3). Based
34 on these results and on the interaction between CDSP32 and the γ subunit (Rey et al. 2005), we
35 investigated the redox-dependent activation of ATP-synthase in dark-adapted potato leaves.

36 The ElectroChromic Shift (ECS) measures the transthylakoid membrane potential ($\Delta\psi$) generated by
37 the photosynthetic electron and proton transport activity, whose dissipation mostly depends on the ATP-

1 synthase activity. We thus measured the activity of the ATP-synthase based on the kinetics of ECS decay
2 after a single-turnover flash, inducing charge separation in PSII and PSI, as previously done by Kramer
3 and Crofts (1989). Since high $\Delta\psi$ levels can also induce an increase in ATP-synthase activity (Kramer
4 and Crofts, 1989), we used sub-saturating flashes inducing charge separation in ~55-59% of
5 photosystems in all genotypes (Table S3).

6 We measured the flash-dependent ECS decay kinetics 2 minutes after different numbers of pre-flashes.
7 After 2 minutes, the $\Delta\psi$ generated by the pre-flashes is dissipated, while the ATP-synthase reduced by
8 the pre-flashes is not yet re-oxidised. In these conditions, an acceleration of the flash-dependent ECS
9 decay thus reflects an increase in the ATP-synthase activity induced by its reduction during the pre-
10 flashes. The ECS decay kinetics were similarly slow in dark-adapted leaves in all genotype, indicating
11 an oxidised and thus inactive ATP-synthase, and fully accelerated after a train of 300 pre-flashes, that
12 induced the reduction-dependent activation of the ATP-synthase. Although the acceleration after 300
13 pre-flashes was comparable in all genotypes, that for intermediate numbers of pre-flashes was delayed
14 in the co-suppressed D4 line (Fig. 5A). Consequently, the number of charge separations required to
15 achieve a 50% acceleration of the slow ECS decay phase was significantly higher in D4 than in the other
16 genotypes (Fig. 5B, Fig. S6A, B and Table S4). In the DM19 line, expressing a mutated inactive form
17 of CDSP32 in addition to the WT form, we observed a higher degree of variability in the acceleration
18 of the ECS decay kinetics than in the other genotypes. For this reason, we present the results obtained
19 in 5 DM19 biological replicates, instead of 3. Overall, the average number of charge separations required
20 to achieve a 50% acceleration of the slow ECS decay phase in DM19 plants was still significantly lower
21 than in D4 plants (Fig. 5B, Fig. S6B and Table S4).

22 We then investigated the ATP-synthase de-activation in darkness by measuring the flash-dependent ECS
23 decay kinetics at different times after a train of 300 pre-flashes. While the ECS decay kinetics were
24 equally fast in all genotypes 2 minutes after the pre-flashes, their subsequent deceleration in darkness
25 was faster in the D4 line (Fig. 5C). Consequently, the dark time required to achieve 50% recovery of the
26 slow ECS decay phase was significantly shorter in the D4 line (Fig. 5D, Fig. S6C, D and Table S5).

27 Together, these results show that the kinetics of ATP-synthase activation and de-activation during
28 dark/light and light/dark transitions are modified in the D4 line, where CDSP32 is absent. The amounts
29 of ATP-synthase γ subunit are close in WT and D4, but significantly lower in the D10 overexpression
30 line (Fig. 3B), where we did not observe changes in kinetics with respect to WT. Therefore, the modified
31 kinetics observed in D4 likely reflect altered redox regulation of the ATP-synthase, that could involve
32 CDSP32 in WT and another TRX with different redox properties in its absence. This hypothesis is in
33 line with the previously identified interaction between CDSP32 and the ATP-synthase γ subunit (Rey et
34 al. 2005), where the regulatory cysteines are present. Both CDSP32 and TRX f have been shown to be
35 reduced with similar kinetics during dark/light transitions (Zimmer et al. 2021), so we made the
36 hypothesis that TRX f could replace CDSP32 in the redox regulation of the ATP-synthase in the D4 line.
37 If this were the case, and assuming that CDSP32 and TRX f are in thermodynamic equilibrium (as

1 suggested by Zimmer et al. 2021), the data in Fig. 5B and D could be used to calculate an hypothetical
2 equilibrium constant (K) between the two TRXs, as detailed in the Supplementary Information. Based
3 on the calculated equilibrium constants (*ca.* 0.3 for the flash-dependent activation data and of *ca.* 0.5 for
4 the dark-dependent de-activation data, Fig. S7), the E_m of CDSP32 should be between 16 and 9 mV
5 higher than the E_m of the putative substitute TRX, TRX f. This would be in accordance with the
6 published E_m values of the two proteins (*ca.* -290 mV for TRX f and -280 mV for CDSP32, as reported
7 in Zimmer et al. 2021).

8 Thanks to its slightly higher E_m , CDSP32 could ensure a fast redox-dependent activation of the ATP-
9 synthase when plants are exposed to low light, and modulate the kinetics of its de-activation in
10 darkness. This could explain the delayed ATP-synthase activation/deactivation phenotype we observed
11 in the D4 co-suppressed line. The transient nature of such phenotype and the poor reactivity of the
12 available antibody with the *S. tuberosum* γ subunit (Fig. S8) have so far prevented us to obtain molecular
13 evidence of a direct redox regulation of the ATP-synthase γ subunit by CDSP32. We therefore used
14 sequence analysis and structure modelling to assess whether CDSP32 could directly modulate activity of
15 the ATP-synthase via its γ subunit.

17 **CDSP32 sequence and structure features**

18 We first investigated whether the CDSP32 sequence features confer specific structural and electrostatic
19 characteristics to the TRX notably compared to canonical plastidial ones such as TRX f. The analysis
20 of CDSP32 sequences in Dicotyledon and Monocotyledon species revealed that the TRX is highly
21 conserved, except the transit peptide, as shown by multiple sequence alignment (Fig. S9). We
22 particularly noticed the presence of a conserved SXXS motif in the N-ter domain at the active site
23 position in the C-ter domain (Rey et al. 1998), and very high identity in the sequence surrounding the
24 active site. This highly conserved sequence constitutes a typical CDSP32 19-residue motif,
25 VLDVGLKHCGPCVKVYPTV, not found in plant canonical TRXs.

26 The 3D structure of *S. tuberosum* CDSP32 predicted by AlphaFold2 with high confidence shows two
27 TRX-fold domains as expected (TRX_D1 and TRX_D2), each with four α helices surrounding four
28 β strands, separated by a 10-residue flexible region (Fig. 6A, Fig. S10). The AlphaFold2 structure of *S.*
29 *tuberosum* TRX f shows a single TRX-fold domain that perfectly superimposes on the TRX_D2 domain
30 of CDSP32 including the same position of the redox site motif (H/WCGPC) (Fig. 6A, Fig. S11).
31 Although these TRXs show the same folding, they present different electrostatic properties (Fig. 6B)
32 that can play a crucial role in distinct substrate specificity via electrostatic complementarity with their
33 partners as shown for *Arabidopsis thaliana* TRXs isoforms (Bodnar et al. 2023). Of note, the two TRX-
34 fold domains of CDSP32 (that share 26.8 % identity) also perfectly superimpose but show different
35 electrostatic properties (Fig. 6B, Fig. S10).

37 **Modelling of the CDSP32-ATP-synthase complex**

1 The 3D structure of the complex formed between CDSP32 and the γ subunit of *S. tuberosum* ATP-
2 synthase was modelled using AlphaFold2 to see how these two proteins could interact. Chloroplast ATP-
3 synthase is a macromolecular machine made of 26 protein subunits, 17 of them being embedded in the
4 membrane and the regulatory γ subunit being part of the central stalk as shown in the cryo-EM structure
5 of *Spinacia oleracea* ATP-synthase (Hahn et al. 2018). The AlphaFold2 3D structure of *S. tuberosum*
6 ATP-synthase γ subunit alone shows the same folding as the spinach γ subunit (PDB 6FKF) (83.4 %
7 identity between these two γ -subunits) with two long α -helices in a tight coiled coil that forms the rotor
8 shaft and a L-shaped structure containing the redox loop and a β -hairpin (Fig. 7A, Fig. S12A-B). In the
9 modelled ATP-synthase γ subunit-CDSP32 complex, the γ subunit redox loop and the redox active motif
10 of CDSP32 TRX_D2 are in close proximity with a distance of 4Å between the S of the catalytic Cys of
11 each partner, showing that CDSP32 could reduce the disulphide bridge of the γ subunit redox loop
12 (Fig. 7B, Fig. S13 for all AlphaFold2 confidence metrics). The superposition of the *S. tuberosum* γ
13 subunit-CDSP32 complex on the 3D structure of *S. oleracea* ATP-synthase shows that CDSP32 TRX
14 would have sufficient space to interact with the γ subunit without steric hindrance (Fig 7B). The spinach
15 ATP-synthase γ subunit-CDSP32 complex was also modelled using AlphaFold2 and shows the same
16 result (data not shown). It has to be noticed that the L-shaped structure present in the γ subunit alone is
17 no longer structured in the modelled ATP-synthase γ subunit-CDSP32 complex (Fig. 7). These changes
18 are in agreement with the recent proposed model of redox regulation of the ATP-synthase by cooperative
19 function of the redox loop and the β -hairpin (Akiyama et al. 2023).

20 Modelisation of *S. tuberosum* TRX f in complex with the ATP-synthase γ subunit was also generated
21 using AlphaFold2. In this complex, the redox loop of the γ subunit and the redox active motif of TRX f
22 are not close, with a distance of 30Å between the S of the catalytic Cys of each partner (Fig. S14, all
23 AlphaFold2 confidence metrics in legend). This model suggests that it could be more difficult for TRX f
24 to interact with the γ subunit. Therefore, both redox and structural differences could explain the modified
25 kinetics of ATP-synthase regulation observed in absence of CDSP32, if they depended on TRX f activity.

26

27 **CDSP32 presence and ATP-synthase redox features in photosynthetic organisms**

28 We then performed an extensive search of databases to investigate the presence of CDSP32 in
29 photosynthetic organisms. We retained proteins displaying only the two very specific features of the
30 TRX, *i.e.* two TRX-fold domains and an atypical active site motif, HCGPC. When extending the search
31 outside Dicotyledons and Monocotyledons (Fig. 8, Figs. S15, S16), we observed that the protein,
32 particularly the typical 19-residue motif, is well-conserved in primitive Angiosperms, one class of
33 Gymnosperms, Pinales, and several classes of Pteridophytes. In Bryophytes, related proteins display
34 sequence divergence in the active site (NCGPC and SCGPC in *P. patens* and *M. polymorpha*,
35 respectively) and in the surrounding sequence (Fig. 8, Fig. S15). The CDSP32 homologous proteins
36 identified in Charophytes, such as *C. braunii*, harbour active sites containing two Cys residues, but no

1 His preceding the catalytic Cys. In Chlorophytes, very poor conservation of the typical CDSP32
2 sequence motif was observed, since the potential active site carries only one Cys, for example SAGPC
3 in *C. reinhardtii* (Fig. 8, Fig. S16). Finally, no homologous proteins were identified in Cyanobacteria,
4 indicating a non-prokaryotic origin of the gene, as well as in red Algae, Diatoms and Coccolithophores.
5 Interestingly, the region of the ATP-synthase γ subunit containing the two cysteines that are the targets
6 of redox regulation is also present in plants and green algae, but absent in non-green algae and
7 cyanobacteria (Fig. S17; Akiyama et al. 2023). In conclusion, the CDSP32 TRX is particularly well
8 conserved in vascular plants and the presence of related proteins in Bryophytes and green Algae
9 indicates that this TRX type is specific of the green lineage, like the presence of the redox loop in ATP-
10 synthase γ subunit.

11

12 **Discussion**

13 **Participation of the CDSP32 TRX in salt stress responses**

14 The CDSP32 TRX exhibits very unique features with the presence of two TRX-fold domains in tandem
15 and one atypical HCGPC redox active site. The remarkable conservation of CDSP32 in vascular plants
16 suggests that this atypical TRX fulfils specific physiological functions that cannot be performed as
17 efficiently by other plastidial TRXs, despite their remarkable diversity in higher plants (Meyer et al.
18 2012). The first data on transgenic potato plants highlighted the participation of CDSP32 in responses
19 to oxidative stress (Broin et al. 2002). We proposed that the TRX fulfils an antioxidant role allowing the
20 maintenance of plastidial redox homeostasis upon environmental constraints. TRXs are recognized as
21 essential components in redox and ROS-related transduction pathways allowing integration of multiple
22 signals generated by climatic constraints (Vieira dos Santos and Rey, 2006; Mittler et al. 2022). This is
23 illustrated by the participation of apoplasmic TRX h, mitochondrial TRX o, and plastidial TRXs m in
24 responses to salt or light constraints in rice and *Arabidopsis* (Zhang et al. 2011; Calderon et al. 2018 ;
25 Serrato et al. 2021). However, while we observed an increased CDSP32 abundance in WT potato leaves
26 upon various NaCl treatments (Fig. 2A; Pruvot et al. 1996), no phenotype was noticed in plants modified
27 in *CDSP32* expression under such a salt exposure (Fig. S1). Potato is classified as a moderately salt-
28 sensitive crop (Hütsch et al. 2018; Chourasia et al. 2021), so the NaCl concentration used here might
29 not be high enough to reveal a CDSP32-dependent response, that we cannot exclude, might occur at
30 higher salt concentrations. Consistently, we observed upon high salinity a decreased CDSP32 level in
31 *Arabidopsis* while its abundance was unaffected in a halophyte related-species (Mrah et al. 2007).

32 When subjected to 0.1 M NaHCO_3 , potato plants were much more affected compared to plants watered
33 with 0.125 M NaCl. By comparing the two conditions, we can exclude that the stronger stress phenotype
34 observed upon NaHCO_3 treatment is due to sodium toxicity, and attribute it to the fact that NaHCO_3 ,
35 compared to neutral salts, generates also alkaline stress in addition to osmotic stress. Plants
36 overexpressing a mutated form were more susceptible to this treatment as evidenced by growth and
37 chlorophyll content measurements (Fig. 1E, Fig. S1B, D, E). Plant adaptation to saline-alkaline stress

1 has been only recently investigated (Cao et al. 2022; Rao et al. 2023), and involves common responses
2 with neutral salt stress like accumulation of osmolytes and regulation of ion homeostasis, but also more
3 specific responses to limit pH modification within cells (Cao et al. 2022). Saline-alkaline conditions
4 strongly impair ROS production levels and cell redox homeostasis (Rao et al. 2023). Consistently, we
5 observed that exposure to NaHCO₃, unlike to NaCl, results in strongly reduced abundance of cytosolic
6 APX in potato (Fig. S2B). CDSP32 could be involved in a pathway regulating the ROS-scavenging
7 capacity upon alkaline stress since the three lines modified for its expression displayed a reduced CAT
8 abundance. In addition, we noticed a significantly higher abundance of Cu/Zn SOD in the line
9 overexpressing the TRX (Fig. S2C). In other respects, we observed substantial changes in the abundance
10 and/or redox status of CDSP32 partners in saline-alkaline stress conditions. For instance, compared to
11 WT, potato plants lacking the TRX or overexpressing its non-functional form displayed a much higher
12 level of over-oxidized 2-Cys PRX, although overall levels of this protein were unaffected, and the three
13 modified lines exhibited higher abundances of plastidial MSRB1 upon NaHCO₃ exposure (Fig. 2).
14 Interestingly, overexpression of 2-Cys PRX was recently reported to confer tolerance to NaHCO₃, that
15 was associated to maintained photosynthetic activity and increased ROS scavenging capacity (Wang et
16 al. 2023). In other respects, Sun et al. (2016) revealed interaction of one cytosolic methionine sulfoxide
17 reductase B with a Ca²⁺-kinase in soybean, leading to activation of ROS-signalling under alkaline stress.
18 Altogether, these findings prompt us to propose that CDSP32 and its PRX and MSR partners, which are
19 involved in signalling pathways (Liebthal et al.2018; Rey and Tarrago 2018), participate in plant
20 responses to saline-alkaline stress, and could help protecting from damage the photosynthetic machinery.

21

22 **Participation of CDSP32 in photosynthetic metabolism and modulation of ATP-synthase activity**

23 Abundance of components of the photosynthetic apparatus

24 In comparison with typical TRXs f or m, CDSP32 has been considered to be mainly involved in the
25 maintenance of plastidial redox homeostasis upon stress conditions through interaction with other thiol-
26 reductases such as PRXs and MSRs (Rey et al. 2005). In this work, we provide several lines of evidence
27 showing that the TRX is also involved in the regulation of photosynthetic metabolism at several levels
28 in the absence of stress. Indeed, the DM19 line over-expressing mutated CDSP32 exhibits a reduced
29 chlorophyll content (Fig. 1C), revealing that the presence of a high proportion of inactive CDSP32
30 affects chlorophyll metabolism and/or biosynthesis. The activity of several enzymes involved in
31 chlorophyll biosynthesis is redox-regulated by various plastidial TRX types including NTRC in relation
32 with 2-Cys PRX (Stenbaek et al. 2008; Da et al. 2017; Richter et al. 2018). The phenotype trait of DM19
33 could result from irreversible trapping by the mutated TRX of redox-sensitive enzymes involved in
34 chlorophyll metabolism or from the modified redox status of 2-Cys PRX observed in this line (Fig. 2A).
35 In control conditions, the D1 and PsbO subunits of PSII accumulated to significantly lower levels in
36 plants overexpressing CDSP32 than in WT (Fig. 3). Of note, Wang et al. (2013) reported substantially
37 reduced levels of these two PSII subunits in *Arabidopsis* plants lacking three TRX m isoforms, and also

1 showed redox-based interaction between the D1 subunit and TRXs m. PsbO also interacts with TRX
2 (Montrichard et al. 2009) and thus could be possibly redox-regulated through sensitive cysteines, which
3 would be reduced via a NTRC-dependent pathway providing electrons to this PSII subunit located in
4 the lumen (Ameztoy et al. 2019). A lower abundance of D1 and PsbO subunits could result from
5 impaired expression or increased degradation due to redox modifications, and might be associated with
6 subsequent impairment of PSII function. Nonetheless, the PSII maximum quantum yield (F_v/F_m , Fig.
7 4C) is normal in the overexpression line, indicating that assembled PSII complexes properly function.
8 From the protein analyses in Fig. 3, we did not observe any difference regarding PSI and PSII light
9 harvesting antennas (LHCA1 and LHCB1/2, respectively) in any of the genotypes grown upon standard
10 conditions. This agrees with the fact that the functional antenna size of PSII measured by fluorescence
11 (Figs. 4B and S4B, D) and of PSII+PSI measured by ECS (Fig. S5B) are similar in all lines. Taken
12 together, these data indicate that PSII function and antenna size are independent on CDSP32 abundance.
13 On the other hand, the fluorescence induction curves show that electron transport downstream of PSII
14 is transiently impaired in the line lacking CDSP32 during dark/light transitions (Fig. 4B, D). This
15 suggests a delay in the early stages of photosynthesis activation. Once the activation is completed, the
16 activity of the electron transport chain is independent of *CDSP32* expression, as indicated by the similar
17 levels of stationary Φ_{PSII} and NPQ (which depends on the lumen acidification induced by electron
18 transport) reached in all genotypes (Fig. 4 and S4). This is further confirmed by the similar steady-state
19 electron transport rates measured by ECS as a function of light intensity (Fig. S5).

20

21 Regulation of ATP-synthase activity during dark/light and light/dark transitions

22 Zimmer et al. (2021) have shown that, during the transition from darkness to low light ($40 \mu\text{mol photons}$
23 $\text{m}^{-2} \text{s}^{-1}$), the reduction of the ATP-synthase γ subunit is completed within the first 30 seconds of
24 illumination, while that of the Calvin-Benson cycle enzymes requires a couple of minutes, in accordance
25 with the kinetics of their light-dependent activation reported in previous works (Laing et al. 1981, Wirtz
26 et al. 1982, Kramer et al. 1990). The reduction of CDSP32 and TRX f also peaks at 30 seconds of
27 illumination. This agrees with the proposal made by Kramer and Crofts (1989) that the ATP-synthase
28 activation, which only requires very low light intensities, depends on the redox equilibrium between the
29 γ subunit and TRXs, and should not be limiting for photosynthesis in normal conditions. In our induction
30 curves in medium-low light (Fig. 4), we can attribute the initial transient increase of fluorescence to an
31 over-reduction of the electron transport chain caused by an inactive CBC. This fluorescence transient is
32 higher in the absence of CDSP32 at few seconds after the light onset, but then decreases with similar
33 kinetics in all genotypes during the first minute of illumination. This suggests a similar activation of
34 Calvin-Benson cycle enzymes in all genotypes, with the fluorescence phenotype in the D4 line being
35 due to an impairment in a process that occurs over a shorter time-scale. Based on the previously detected
36 *ex planta* interaction between CDSP32 and the γ subunit (Rey et al. 2005), we hypothesize this process
37 to be the activation of the ATP-synthase. Our ECS measurements show that the light-dependent

1 activation of the ATP-synthase still occurs in the absence of CDSP32 (D4 plants), but requires more
2 charge separations in PSII and PSI than in the WT, and therefore a higher reduction level of the stroma
3 (Fig. 5B). Concomitantly, the dark reoxidation of the ATP-synthase after illumination is faster in the
4 absence of CDSP32 (Fig. 5D). When the mutated CDSP32 is expressed in addition to the active form
5 (DM19), the kinetics of ATP-synthase activation remain on average faster than in absence of the TRX,
6 but present a higher degree of variability between biological replicates (Fig. 5B and Fig. S6B), possibly
7 reflecting a variability in the relative accumulation levels of the two CDSP32 forms. Plants
8 overexpressing the WT TRX form did not show any significant difference in the kinetics of ATP-
9 synthase activation and de-activation, although a reduced abundance of γ subunit was observed in this
10 line (Fig. 5 and Fig. 3B). Based on these results, we propose that CDSP32 could be responsible for the
11 early stages of redox activation of the ATP-synthase (via the reduction of its γ subunit) during dark/light
12 transition. Additionally, CDSP32 might delay ATP-synthase reoxidation during light/dark transition,
13 possibly acting as a storage of reducing power due to its high abundance in the chloroplast. In D4 plants
14 lacking CDSP32, this redox regulation could be performed by another thioredoxin, but with delayed
15 kinetics. Zimmer et al. (2021) have shown that TRX f is reduced with very similar kinetics to both
16 CDSP32 and ATP-synthase γ subunit during the initial phases of illumination, so this canonical TRX
17 could fulfil the CDSP32 function in co-suppressed plants. In DM19 plants, the mutated CDSP32 could
18 prevent a fraction of the ATP-synthase to be activated during the dark/light transition, in competition
19 with the WT form or TRX f, by irreversible trapping of the γ subunit.

20 The ATP-synthase has been identified as one of the first photosynthetic components regulated by the
21 TRX system (Mc Kinney et al. 1978) via the control of the redox status of two Cys residues situated in
22 a 9-residue loop domain of the γ subunit (called the redox loop) (Akiyama et al. 2023). Various plastidial
23 TRX systems depending on NTRC or FTR (via TRXs f and m) have been reported to modulate the
24 activity of the ATP-synthase complex (Yoshida et al. 2022; Diaz et al. 2020; Sekiguchi et al. 2020).
25 Rapid reduction of ATP-synthase γ subunit has been noticed under low-light conditions that do not lead
26 to reduction of CBC enzymes, revealing differential dependency on linear electron transfer between this
27 subunit and enzymes like SBPase or FBPase (Hisabori et al. 2013; Yoshida et al. 2014). Of note, the
28 NTRC system has been proposed to be a key activator of the ATP-synthase under low irradiance
29 conditions (Nikkanen et al. 2016). Our data reveal that plants lacking CDSP32 display delayed activation
30 of ATP-synthase during transition from dark to low light, indicating that, if CDSP32 is indeed
31 responsible for the fast activation step, other plastidial TRXs cannot compensate as efficiently the
32 absence of the TRX. This could arise from specific intrinsic properties of this atypical TRX such as a
33 slightly higher E_m , or the more negative electrostatic surface charge compared to TRX f (Fig. 6B).

34 Modelisation data suggest that it could be more difficult for TRX f, compared to CDSP32, to interact
35 with the γ subunit. Indeed, in the modelled γ subunit-CDSP32 complex, the interaction zone of CDSP32
36 is located almost exclusively in TRX_D2 (Fig. S13D) in a region showing different electrostatic
37 properties with those of TRX f (Fig. 6B). Of note, the complex generated by AlphaFold2 between the

1 γ subunit and CDSP32 TRX-D2 only shows the same position for TRX-D2 as in the complex involving
2 the whole CDSP32 indicating that CDSP32 TRX_D1 might not be crucial for the interaction with the
3 ATP-synthase γ subunit (data not shown). These data raise the question of the role of this N-terminal
4 domain, which is unique to CDSP32. Other large TRXs carry domains with known functions such as
5 protein disulphide isomerase activity or interaction with heat shock proteins (Meyer et al. 2012). Taking
6 into account the beneficial stabilizing properties of the TRX-fold, which are widely used for producing
7 recombinant proteins, TRX-D1 might help stabilizing and/or positioning the CDSP32 TRX within the
8 ATP-synthase complex.

9 Taken collectively, our previous data revealing that the ATP-synthase γ subunit is an *ex planta* target of
10 CDSP32 (Rey et al. 2005), the impaired light-dependent activation of ATP-synthase activity in potato
11 lines lacking the TRX and the modelisation data of the CDSP32-ATP-synthase complex argue for a
12 physiological function for CDSP32 in redox modulation of ATP-synthase activity in relation with
13 environmental conditions and plastidial redox status. However, molecular evidence of a direct redox
14 regulation of the ATP-synthase γ by CDSP32 is still missing, and further work will be required to fully
15 validate this hypothesis.

16 Nonetheless, it is interesting to note that both CDSP32 and the region of the ATP-synthase γ subunit
17 containing the two cysteines that are the targets of redox regulation are present in plants and green algae,
18 but absent in Cyanobacteria (Figs. 8, S15; Akiyama et al. 2023). When extending the search for CDSP32
19 homologues to other eukaryotic lineages, we could not find any in red Algae and in organisms whose
20 chloroplast derives from them, such as diatoms and coccolithophores. Interestingly, the ATP-synthase
21 γ subunit of these organisms also lacks the two cysteines that are the targets of redox regulation in plants
22 and green algae (Fig. 8, Fig. S17). It is tempting to speculate that CDSP32 and the γ subunit redox-
23 active cysteines could have evolved together after the endosymbiotic event, from which the chloroplast
24 originated. Indeed, a redox fine-tuning of the ATP-synthase activity during light transitions makes sense
25 in the chloroplast, where the stroma is relatively isolated from the surrounding cellular environment,
26 and its redox poise closely reflects the photosynthetic electron transport activity. This supports the idea
27 of a possible evolutionary correlation between the presence of CDSP32 and the redox fine-tuning of the
28 ATP-synthase activity, that seems to be specific to the green eukaryotic lineage. In Cyanobacteria,
29 instead, the thylakoid membranes are embedded in the cytoplasm, whose redox poise does not change
30 rapidly in function of the sole photosynthetic electron transport activity. Sugar catabolism reduces the
31 cytosolic NADPH pool also in darkness to sustain the activity of the respiratory electron transport chain
32 (Mi et al. 1994) that is also located in the thylakoids (Mullineaux, 2013). The reducing conditions
33 maintain the ATP-synthase active in darkness (Viola et al. 2019), to produce ATP using the proton
34 motive force generated by respiration. The absence of this fine-tuning in red Algae and derivatives might
35 reflect a closer metabolic interaction between chloroplasts and mitochondria, as characterised in diatoms
36 (Bailleul et al. 2015), that could partially uncouple the redox poise of the stroma from photosynthetic
37 electron transport.

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Conclusions

CDSP32 is one of the most highly expressed TRX in photosynthetic cells, and its synthesis is strongly triggered upon stress conditions (Rey et al. 1998; Broin et al. 2000; Belin et al. 2015). This argues for an essential function of CDSP32 in the maintenance of chloroplast redox homeostasis and regulation of photosynthetic activity in relation to environmental conditions. CDSP32 interacts with the 2-Cys PRX, a major plastidial thiol peroxidase acting as a redox sensor of H₂O₂, the concentration of which is increased upon stress conditions (Vogelsang and Dietz, 2020). H₂O₂ reacts with 2-Cys PRXs (Dietz, 2008), which in turn can oxidize TRXs and deactivate photosynthetic enzymes (Yoshida et al. 2018). H₂O₂ would thus fulfil, in addition to an electron sink role, a regulatory function via thiol reductases under stress conditions (Driever and Baker, 2011). In addition to its possible involvement in the regulation of ATP-synthase activity during light/dark and dark/light transitions, CDSP32 could thus modulate the activity of the photosynthetic machinery under stress conditions leading to ROS production. Salt-induced osmotic stress conditions results in increased ATP-synthase abundance particularly upon NaCl treatment (Fig. 3A, B) Interestingly, Kohzuma et al. (2009) reported a substantially reduced abundance of this subunit in watermelon plants subjected to drought. Thus, such stress conditions are likely to result in impaired activity of this complex. Kohzuma et al. (2009) proposed that ATP-synthase represents a key point allowing proper photosynthetic proton circuit during acclimation of plant to long-term environmental constraints. It is tempting to speculate that the higher CDSP32 abundance observed in drought-stressed potato plants (Rey et al. 1998) could help maintaining ATP-synthase activation despite a reduced amount of this complex. In other respects, all potato lines exhibited strongly reduced growth and decreased chlorophyll content when exposed to NaHCO₃ (Fig. S1). This could originate from pH alteration within plastidial sub-compartments due to saline-alkaline stress, and subsequent impaired functioning of ATP-synthase. Of note, the line expressing the CDSP32 mutant form was found to be more sensitive than WT to NaHCO₃, possibly due to more severe disturbance of the ATP-synthase machinery resulting from irreversible trapping of the γ subunit (Rey et al. 2005).

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8

9 **References**

10 Akiyama K, Ozawa SI, Takahashi Y, Yoshida K, Suzuki T, Kondo K, Wakabayashi KI, Hisabori T.
11 Two specific domains of the γ subunit of chloroplast FoF1 provide redox regulation of the ATP synthesis
12 through conformational changes. *Proc Natl Acad Sci USA*. 2023;120(6):e2218187120.
13 <https://doi.org/10.1073/pnas.2218187120>

14 Amezttoy K, Baslam M, Sánchez-López ÁM, Muñoz FJ, Bahaji A, Almagro G, García-Gómez P,
15 Baroja-Fernández E, De Diego N, Humplík JF, Ugena L, Spíchal L, Doležal K, Kaneko K, Mitsui T,
16 Cejudo FJ, Pozueta-Romero J. Plant responses to fungal volatiles involve global posttranslational thiol
17 redox proteome changes that affect photosynthesis. *Plant Cell Environ*. 2019;42(9):2627-2644.
18 <https://doi.org/10.1111/pce.13601>

19 Bailleul B, Berne N, Murik O, Petroustos D, Prihoda J, Tanaka A, Villanova V, Bligny R, Flori S,
20 Falconet D, Krieger-Liszkay A, Santabarbara S, Rappaport F, Joliot P, Tirichine L, Falkowski PG,
21 Cardol P, Bowler C, Finazzi G. Energetic coupling between plastids and mitochondria drives CO₂
22 assimilation in diatoms. *Nature* 2015;524:366–369.
23 <https://doi.org/10.1038/nature14599>.

24 Belin C, Bashandy T, Cela J, Delorme-Hinoux V, Riondet C, Reichheld JP. A comprehensive study of
25 thiol reduction gene expression under stress conditions in *Arabidopsis thaliana*. *Plant Cell Environ*.
26 2015;38(2):299-314.
27 <https://doi.org/10.1111/pce.12276>

28 Bodnar Y, Gellert M, Hossain FM, Lillig CH. Breakdown of Arabidopsis thaliana thioredoxins and
29 glutaredoxins based on electrostatic similarity-Leads to common and unique interaction partners and
30 functions. *PLoS One*. 2023;18(9):e0291272.
31 <https://doi.org/10.1371/journal.pone.0291272>

32 Broin M, Cuiné S, Eymery F, Rey P. The plastidic 2-cysteine peroxiredoxin is a target for a thioredoxin
33 involved in the protection of the photosynthetic apparatus against oxidative damage. *Plant Cell*.
34 2002;14(6):1417-1432. <https://doi.org/10.1105/tpc.001644>

35 Broin M, Cuiné S, Peltier G, Rey P. Involvement of CDSP 32, a drought-induced thioredoxin, in the
36 response to oxidative stress in potato plants. *FEBS Lett*. 2000;467(2-3):245-248.
37 [https://doi.org/10.1016/s0014-5793\(00\)01165-0](https://doi.org/10.1016/s0014-5793(00)01165-0)

38 Broin M, Rey P. Potato plants lacking the CDSP32 plastidic thioredoxin exhibit overoxidation of the
39 BAS1 2-cysteine peroxiredoxin and increased lipid peroxidation in thylakoids under photooxidative
40 stress. *Plant Physiol*. 2003;132(3):1335-1343.
41 <https://doi.org/10.1104/pp.103.021626>

42 Calderón A., Sánchez-Guerrero A., Ortiz-Espín A., Martínez-Alcalá I., Camejo D., Jiménez A. &
43 Sevilla F. Lack of mitochondrial thioredoxin o1 is compensated by antioxidant components under
44 salinity in *Arabidopsis thaliana* plants. *Physiol Plant*. 2018;164(3): 251-267.
45 <https://doi.org/10.1111/ppl.12708>

1 Cao Y, Song H, Zhang L. New insight into plant saline-alkali tolerance mechanisms and application to
2 breeding. *Int J Mol Sci.* 2022;23(24):16048.
3 <https://doi.org/10.3390/ijms232416048>
4
5 Cerveau D, Kraut A, Stotz HU, Mueller MJ, Couté Y, Rey P. Characterization of the *Arabidopsis*
6 *thaliana* 2-Cys peroxiredoxin interactome. *Plant Sci.* 2016a;252:30-41.
7 <https://doi.org/10.1016/j.plantsci.2016.07.003>
8
9 Cerveau D, Ouahrani D, Marok MA, Blanchard L, Rey P. Physiological relevance of plant 2-Cys
10 peroxiredoxin overoxidation level and oligomerization status. *Plant Cell Environ.* 2016b;39(1):103-19.
11 <https://doi.org/10.1111/pce.12596>
12
13 Chae HB, Moon JC, Shin MR, Chi YH, Jung YJ, Lee SY, Nawkar GM, Jung HS, Hyun JK, Kim WY,
14 Kang CH, Yun DJ, Lee KO, Lee SY. Thioredoxin reductase type C (NTRC) orchestrates enhanced
15 thermotolerance to *Arabidopsis* by its redox-dependent holdase chaperone function. *Mol Plant.*
16 2013;6(2):323-336.
17 <https://doi.org/10.1093/mp/sss105>
18
19 Chourasia KN, Lal MK, Tiwari RK, Dev D, Kardile HB, Patil VU, Kumar A, Vanishree G, Kumar D,
20 Bhardwaj V, Meena JK, Mangal V, Shelake RM, Kim JY, Pramanik D. Salinity stress in potato:
21 Understanding physiological, biochemical and molecular responses. *Life (Basel).* 2021 :11(6):545.
22 <https://doi.org/10.3390/life11060545>
23
24 Coic Y, Lesaint C. Comment assurer une bonne nutrition en eau et ions minéraux en horticulture.
25 *Horticulture Française.* 1971 :8 :11-14.
26
27 Courteille A, Vesa S, Sanz-Barrio R, Cazalé AC, Becuwe-Linka N, Farran I, Havaux M, Rey P,
28 Rumeau D. Thioredoxin m4 controls photosynthetic alternative electron pathways in *Arabidopsis*.
29 *Plant Physiol.* 2013 :161(1):508-20.
30 <https://doi.org/10.1104/pp.112.207019>
31
32 Da Q, Wang P, Wang M, Sun T, Jin H, Liu B, Wang J, Grimm B, Wang HB. Thioredoxin and NADPH-
33 dependent thioredoxin reductase C regulation of tetrapyrrole biosynthesis. *Plant Physiol.*
34 2017;175(2):652-666.
35 <https://doi.org/10.1104/pp.16.01500>
36
37 Davies MJ. The oxidative environment and protein damage. *Biochim Biophys Acta.* 2005;1703(2):93-
38 109.
39 <https://doi.org/10.1016/j.bbapap.2004.08.007>
40
41 Delaunay A, Pflieger D, Barrault MB, Vinh J, Toledano MB. A thiol peroxidase is an H₂O₂ receptor and
42 redox-transducer in gene activation. *Cell.* 2002;111(4):471-‘81.
43 [https://doi.org/10.1016/s0092-8674\(02\)01048-6](https://doi.org/10.1016/s0092-8674(02)01048-6)
44
45 Dietz KJ. Redox signal integration: from stimulus to networks and genes. *Physiol Plant.*
46 2008;133(3):459-‘68.
47 <https://doi.org/10.1111/j.1399-3054.2008.01120.x>
48
49 Driever SM, Baker NR. The water-water cycle in leaves is not a major alternative electron sink for
50 dissipation of excess excitation energy when CO₂ assimilation is restricted. *Plant Cell Environ.*
51 2011;34:837-846.
52 <https://doi.org/10.1111/j.1365-3040.2011.02288.x>
53

1 Elasad M, Ahmad A, Wang H, Ma L, Yu S, Wei H. Overexpression of CDSP32 (GhTRX134) cotton
2 gene enhances drought, salt, and oxidative stress tolerance in Arabidopsis. *Plants (Basel)*.
3 2020;9(10):1388.
4 <https://doi.org/10.3390/plants9101388>
5
6 Geigenberger P, Thormählen I, Daloso DM, Fernie AR. The unprecedented versatility of the plant
7 thioredoxin system. *Trends Plant Sci*. 2017;22(3):249-262.
8 <https://doi.org/10.1016/j.tplants.2016.12.008>
9
10 Gelhaye E, Rouhier N, Navrot N, Jacquot JP. The plant thioredoxin system. *Cell Mol Life Sci*.
11 2005;62(1):24-35.
12 <https://doi.org/10.1007/s00018-004-4296-4>
13
14 Guinea Diaz M, Nikkanen L, Himanen K, Toivola J, Rintamäki E. Two chloroplast thioredoxin systems
15 differentially modulate photosynthesis in Arabidopsis depending on light intensity and leaf age. *Plant*
16 *J*. 2020;104(3):718-734.
17 <https://doi.org/10.1111/tpj.14959>
18
19 Hägglund P, Finnie C, Yano H, Shahpiri A, Buchanan BB, Henriksen A, Svensson B. Seed thioredoxin
20 h. *Biochim Biophys Acta*. 2016;1864(8):974-882.
21 <https://doi.org/10.1016/j.bbapap.2016.02.014>
22
23 Hahn A, Vonck J, Mills DJ, Meier T, Kuhlbrandt W. Structure, mechanism, and regulation of the
24 chloroplast ATP synthase. *Science*. 2018;360(6389):eaat4318.
25 <https://doi.org/10.1126/science.aat4318>.
26
27 Hertle AP, Blunder T, Wunder T, Pesaresi P, Pribil M, Armbruster U, Leister D. PGRL1 is the elusive
28 ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. *Mol Cell*. 2013;49(3):511-
29 23.
30 <https://doi.org/10.1016/j.molcel.2012.11.030>.
31
32 Hisabori T, Sunamura E, Kim Y, Konno H. The chloroplast ATP synthase features the characteristic
33 redox regulation machinery. *Antioxid Redox Signal*. 2013;19(15):1846-1854.
34 <https://doi.org/10.1089/ars.2012.5044>
35
36 Huihui Z, Xin L, Yupeng G, Mabo L, Yue W, Meijun A, Yuehui Z, Guanjun L, Nan X, Guangyu S.
37 Physiological and proteomic responses of reactive oxygen species metabolism and antioxidant
38 machinery in mulberry (*Morus alba* L.) seedling leaves to NaCl and NaHCO₃ stress. *Ecotoxicol Environ*
39 *Saf*. 2020;193:110259.
40 <https://doi.org/10.1016/j.ecoenv.2020.110259>
41
42 Hütsch BW, Keipp K, Glaser AK, Schubert S. Potato plants (*Solanum tuberosum* L.) are chloride-
43 sensitive: Is this dogma valid? *J Sci Food Agric*. 2018;98(8):3161-3168.
44 <https://doi.org/10.1002/jsfa.8819>
45
46 Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R,
47 Židek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B,
48 Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M,
49 Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli
50 P, Hassabis D. Highly accurate protein structure prediction with AlphaFold. *Nature*.
51 2021;596(7873):583-589.
52 <https://doi.org/10.1038/s41586-021-03819-2>.
53
54 Kim I, Kim HU. The mysterious role of fibrillin in plastid metabolism: current advances in
55 understanding. *J Exp Bot*. 2022;73(9):2751-2764.

1 <https://doi.org/10.1093/jxb/erac087>
2
3 Kohzuma K, Cruz JA, Akashi K, Hoshiyasu S, Munekage YN, Yokota A, Kramer DM. The long-term
4 responses of the photosynthetic proton circuit to drought. *Plant Cell Environ.* 2009;32(3):209-19.
5 <https://doi.org/10.1111/j.1365-3040.2008.01912.x>.
6
7 Kramer D.M., Crofts A.R. Activation of the chloroplast ATPase measured by the electrochromic change
8 in leaves of intact plants. *Biochim Biophys Acta Bioenerg.* 1989;976, 28–41.
9 [https://doi.org/10.1016/S0005-2728\(89\)80186-0](https://doi.org/10.1016/S0005-2728(89)80186-0)
10
11 Kramer D.M., Wise R.R., Frederick J.R., Alm D.M., Hesketh J.D., Ort D.R., Crofts A.R., Regulation of
12 coupling factor in field-grown sunflower: A Redox model relating coupling factor activity to the
13 activities of other thioredoxin-dependent chloroplast enzymes, *Photosynth Res.* 1990;26, 213–222.
14 <https://doi.org/10.1007/BF00033134>.
15
16 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4.
17 *Nature.* 1970;227(5259):680-685.
18 <https://doi.org/10.1038/227680a0>
19
20 Laing W.A., Stitt M., Heldt H.W., Control of CO₂ fixation. Changes in the activity of ribulosephosphate
21 kinase and fructose- and sedoheptulose-bisphosphatase in chloroplasts, *Biochim Biophys Acta Bioenerg.*
22 1981;637,348–359.
23 [https://doi.org/10.1016/0005-2728\(81\)90174-2](https://doi.org/10.1016/0005-2728(81)90174-2).
24
25 Lampl N, Lev R, Nissan I, Gilad G, Hipsch M, Rosenwasser S. Systematic monitoring of 2-Cys
26 peroxiredoxin-derived redox signals unveiled its role in attenuating carbon assimilation rate. *Proc Natl*
27 *Acad Sci USA.* 2022;119(23):e2119719119.
28 <https://doi.org/10.1073/pnas.2119719119>
29
30 Laugier E, Tarrago L, Vieira Dos Santos C, Eymery F, Havaux M, Rey P. *Arabidopsis thaliana* plastidial
31 methionine sulfoxide reductases B, MSRBs, account for most leaf peptide MSR activity and are essential
32 for growth under environmental constraints through a role in the preservation of photosystem antennae.
33 *Plant J.* 2010;61(2):271-282.
34 <https://doi.org/10.1111/j.1365-313X.2009.04053.x>
35
36 Li XP, Björkman O, Shih C, Grossman AR, Rosenquist M, Jansson S, Niyogi KK. A pigment-binding
37 protein essential for regulation of photosynthetic light harvesting. *Nature* 2000;403:391–395.
38 <https://doi.org/10.1038/35000131>
39
40 Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Meth*
41 *Enzymol* 1987;148,350-382.
42 [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
43
44 Liebthal M, Maynard D, Dietz KJ. Peroxiredoxins and redox signaling in plants. *Antioxid Redox Signal.*
45 2018;28(7):609-624.
46 <https://doi.org/10.1089/ars.2017.7164>
47
48 Lu, J., Holmgren, A. The Thioredoxin Antioxidant System. *Free Radic Biol Med* 2014;66, 75–87.
49 <https://doi.org/10.1016/j.freeradbiomed.2013.07.036>.
50
51 Mathiot C, Alric J. Standard units for ElectroChromic Shift measurements in plant biology. *J Exp Bot.*
52 2021;72(18):6467-6473.
53 <https://doi.org/10.1093/jxb/erab261>.
54

1 Mckinney DW, Buchanan BB, Wolosiuk RA. Activation of chloroplast ATPase by reduced thioredoxin.
2 *Phytochemistry* 1978;17, 794–795.
3 [https://doi.org/10.1016/S0031-9422\(00\)94230-4](https://doi.org/10.1016/S0031-9422(00)94230-4)
4

5 Meyer Y, Belin C, Delorme-Hinoux V, Reichheld JP, Riondet C. Thioredoxin and glutaredoxin systems
6 in plants: molecular mechanisms, crosstalks, and functional significance. *Antioxid Redox Signal*.
7 2012;17(8):1124-1160.
8 <https://doi.org/10.1089/ars.2011.4327>
9

10 Mi H, Endo T, Schreiber U, Ogawa T, Asada K. NAD(P)H dehydrogenase-dependent cyclic electron
11 flow around photosystem I in the cyanobacterium *Synechocystis* PCC 6803: a study of dark-starved
12 cells and spheroplasts. *Plant Cell Phys*. 1994;35(2):163–173.
13 <https://doi.org/10.1093/oxfordjournals.pcp.a078580>.
14

15 Mirdita M, Schütze K, Moriwaki Y, Heo L, Ovchinnikov S, Steinegger M. ColabFold: making protein
16 folding accessible to all. *Nat Methods*. 2022; 19(6): 679–682.
17 <https://doi.org/10.1038/s41592-022-01488-1>.
18

19 Mittler R, Zandalinas SI, Fichman Y, Van Breusegem F. Reactive oxygen species signalling in plant
20 stress responses. *Nat Rev Mol Cell Biol*. 2022;23(10):663-679.
21 <https://doi.org/10.1038/s41580-022-00499-2>
22

23 M'rah S, Ouerghi Z, Eymery F, Rey P, Hajji M, Grignon C, Lachaâl M. Efficiency of biochemical
24 protection against toxic effects of accumulated salt differentiates *Thellungiella halophila* from
25 *Arabidopsis thaliana*. *J Plant Physiol*. 2007;164(4):375-384.
26 <https://doi.org/10.1016/j.jplph.2006.07.013>
27

28 Montillet JL, Rondet D, Brugière S, Henri P, Rumeau D, Reichheld JP, Couté Y, Leonhardt N, Rey P.
29 Plastidial and cytosolic thiol reductases participate in the control of stomatal functioning. *Plant Cell*
30 *Environ*. 2021;44(5):1417-1435.
31 <https://doi.org/10.1111/pce.14013>
32

33 Montrichard F, Alkhalfioui F, Yano H, Vensel WH, Hurkman WJ, Buchanan BB. Thioredoxin targets
34 in plants: the first 30 years. *J Proteomics*. 2009;72(3):452-474.
35 <https://doi.org/10.1016/j.jprot.2008.12.002>
36

37 Mullineaux CW. Co-existence of photosynthetic and respiratory activities in cyanobacterial thylakoid
38 membranes. *Biochim Biophys Acta*. 2014;1837(4):503-11.
39 <https://doi.org/10.1016/j.bbabi.2013.11.017>
40

41 Munns R, Tester M. Mechanisms of salinity tolerance. *Annu Rev Plant Biol*. 2008;59:651-681.
42 <https://doi.org/10.1146/annurev.arplant.59.032607.092911>
43

44 Nikkanen L, Toivola J, Rintamäki E. Crosstalk between chloroplast thioredoxin systems in regulation
45 of photosynthesis. *Plant Cell Environ*. 2016;39(8):1691-1705.
46 <https://doi.org/10.1111/pce.12718>
47

48 Pruvot G, Massimino J, Peltier G, Rey P (1996) Effects of low temperature, high salinity and exogenous
49 ABA on the synthesis of two chloroplastic drought-induced proteins in *Solanum tuberosum*. *Physiol*
50 *Plant* 1996;97(1):123-131.
51 <https://doi.org/10.1111/j.1399-3054.1996.tb00488.x>
52

53 Pulido P., Spinola M.C., Kirchsteiger K., Guinea M., Pascual M.B., Sahrawy M., Sandalio L.M., Dietz
54 K.J., Gonzalez M. & Cejudo F.J. (2010) Functional analysis of the pathways for 2-Cys peroxiredoxin
55 reduction in *Arabidopsis thaliana* chloroplasts. *J Exp Bot*. 2010;61(14):4043-54.

1 <https://doi.org/10.1093/jxb/erq218.4043-4054>
2
3 Rao Y, Peng T, Xue S. Mechanisms of plant saline-alkaline tolerance. *J Plant Physiol.* 2023;281:153916.
4 <https://doi.org/10.1016/j.jplph.2023.153916>
5
6 Rey P, Bécuwe N, Barrault MB, Rumeau D, Havaux M, Biteau B, Toledano MB. The *Arabidopsis*
7 *thaliana* sulfiredoxin is a plastidic cysteine-sulfinic acid reductase involved in the photooxidative stress
8 response. *Plant J.* 2007;49(3):505-514.
9 <https://doi.org/10.1111/j.1365-313X.2006.02969.x>
10
11 Rey P, Cuiné S, Eymery F, Garin J, Court M, Jacquot JP, Rouhier N, Broin M. Analysis of the proteins
12 targeted by CDSP32, a plastidic thioredoxin participating in oxidative stress responses. *Plant J.*
13 2005;41(1):31-42.
14 <https://doi.org/10.1111/j.1365-313X.2004.02271.x>
15
16 Rey P, Pruvot G, Becuwe N, Eymery F, Rumeau D, Peltier G. A novel thioredoxin-like protein located
17 in the chloroplast is induced by water deficit in *Solanum tuberosum* L. plants. *Plant J.* 1998;13(1):97-
18 107.
19 <https://doi.org/10.1046/j.1365-313x.1998.00015.x>
20
21 Rey P, Tarrago L. Physiological roles of plant methionine sulfoxide reductases in redox homeostasis
22 and signaling. *Antioxidants (Basel).* 2018;7(9):114.
23 <https://doi.org/10.3390/antiox7090114>
24
25 Rhee SG, Woo HA. Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the
26 intracellular messenger H₂O₂ and protein chaperones. *Antioxid Redox Signal.* 2011;15(3):781-794.
27 <https://doi.org/10.1089/ars.2010.3393>
28
29 Richter AS, Pérez-Ruiz JM, Cejudo FJ, Grimm B. Redox-control of chlorophyll biosynthesis mainly
30 depends on thioredoxins. *FEBS Lett.* 2018;592(18):3111-3115.
31 <https://doi.org/10.1002/1873-3468.13216>
32
33 Rouhier N, Lemaire SD, Jacquot JP. The role of glutathione in photosynthetic organisms: emerging
34 functions for glutaredoxins and glutathionylation. *Annu Rev Plant Biol.* 2008;59:143-66.
35 <https://doi.org/10.1146/annurev.arplant.59.032607.092811>
36
37 Rumeau D, Peltier G, Cournac L. Chlororespiration and cyclic electron flow around PSI during
38 photosynthesis and plant stress response. *Plant Cell Environ.* 2007;30(9):1041-1051.
39 <https://doi.org/10.1111/j.1365-3040.2007.01675.x>
40
41 Schürmann P, Buchanan BB. The ferredoxin/thioredoxin system of oxygenic photosynthesis. *Antioxid*
42 *Redox Signal.* 2008;10(7):1235-74.
43 <https://doi.org/10.1089/ars.2007.1931>
44
45 Sekiguchi T, Yoshida K, Okegawa Y, Motohashi K, Wakabayashi KI, Hisabori T. Chloroplast ATP
46 synthase is reduced by both f-type and m-type thioredoxins. *Biochim Biophys Acta Bioenerg.*
47 2020;1861(11):148261.
48 <https://doi.org/10.1016/j.bbabi.2020.148261>
49
50 Serrato AJ, Pérez-Ruiz JM, Spínola MC, Cejudo FJ. A novel NADPH thioredoxin reductase, localized
51 in the chloroplast, which deficiency causes hypersensitivity to abiotic stress in *Arabidopsis thaliana*.
52 *J Biol Chem.* 2004;279(42):43821-43827.
53 <https://doi.org/10.1074/jbc.M404696200>
54

1 Serrato AJ, Rojas-González JA, Torres-Romero D, Vargas P, Mérida Á, Sahrawy M. Thioredoxins m
2 are major players in the multifaceted light-adaptive response in *Arabidopsis thaliana*. *Plant J*.
3 2021;108(1):120-133.
4 <https://doi.org/10.1111/tpj.15429>
5
6 Stenbaek A, Hansson A, Wulff RP, Hansson M, Dietz KJ, Jensen PE. NADPH-dependent thioredoxin
7 reductase and 2-Cys peroxiredoxins are needed for the protection of Mg-protoporphyrin monomethyl
8 ester cyclase. *FEBS Lett*. 2008;582(18):2773-8.
9 <https://doi.org/10.1016/j.febslet.2008.07.006>
10
11
12 Sun X, Sun M, Jia B, Qin Z, Yang K, Chen C, Yu Q, Zhu Y. A *Glycine soja* methionine sulfoxide
13 reductase B5a interacts with the Ca²⁺ /CAM-binding kinase GsCBRLK and activates ROS signaling
14 under carbonate alkaline stress. *Plant J*. 2016;86(6):514-29.
15 <https://doi.org/10.1111/tpj.13187>
16
17 Sun H, Zhao W, Liu H, Su C, Qian Y, Jiao F. MaCDSP32 from mulberry enhances resilience post-
18 drought by regulating antioxidant activity and the osmotic content in transgenic tobacco. *Front Plant*
19 *Sci*. 2020;11:419.
20 <https://doi.org/10.3389/fpls.2020.00419>
21
22 Sweat TA, Wolpert TJ. Thioredoxin h5 is required for victorin sensitivity mediated by a CC-NBS-LRR
23 gene in *Arabidopsis*. *Plant Cell*. 2007;19(2):673-687.
24 <https://doi.org/10.1105/tpc.106.047563>
25
26 Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X. Plant immunity
27 requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. *Science*.
28 2008;321(5891):952-956.
29 <https://doi.org/10.1126/science.1156970>
30
31 Tarrago L, Laugier E, Rey P. Protein-repairing methionine sulfoxide reductases in photosynthetic
32 organisms: gene organization, reduction mechanisms, and physiological roles. *Mol Plant*.
33 2009;2(2):202-217.
34 <https://doi.org/10.1093/mp/ssn06>
35
36 Tarrago L, Laugier E, Zaffagnini M, Marchand CH, Le Maréchal P, Lemaire SD, Rey P. Plant
37 thioredoxin CDSP32 regenerates 1-cys methionine sulfoxide reductase B activity through the direct
38 reduction of sulfenic acid. *J Biol Chem*. 2010;285(20):14964-14972.
39 <https://doi.org/10.1074/jbc.M110.108373>
40
41 Vieira Dos Santos C, Cuiné S, Rouhier N, Rey P. The *Arabidopsis* plastidic methionine sulfoxide
42 reductase B proteins. Sequence and activity characteristics, comparison of the expression with plastidic
43 methionine sulfoxide reductase A, and induction by photooxidative stress. *Plant Physiol*.
44 2005;138(2):909-922.
45 <https://doi.org/10.1104/pp.105.062430>
46
47 Vieira Dos Santos C, Laugier E, Tarrago L, Massot V, Issakidis-Bourguet E, Rouhier N, Rey P.
48 Specificity of thioredoxins and glutaredoxins as electron donors to two distinct classes of *Arabidopsis*
49 plastidial methionine sulfoxide reductases B. *FEBS Lett*. 2007;581(23):4371-6.
50 <https://doi.org/10.1016/j.febslet.2007.07.081>
51
52 Vieira Dos Santos C, Rey P. Plant thioredoxins are key actors in the oxidative stress response.
53 *Trends Plant Sci*. 2006;11(7):329-334.
54 <https://doi.org/10.1016/j.tplants.2006.05.005>
55

1 Viola S, Bailleul B, Yu J, Nixon P, Sellés J, Joliot P, Wollman FA. Probing the electric field across
2 thylakoid membranes in cyanobacteria. *Proc Natl Acad Sci USA*. 2019;116(43):21900-21906.
3 <https://doi.org/10.1073/pnas.1913099116>
4
5 Vogelsang L, Dietz KJ. Regulatory thiol oxidation in chloroplast metabolism, oxidative stress response
6 and environmental signaling in plants. *Biochem J*. 2020;477(10):1865-1878.
7 <https://doi.org/10.1042/BCJ20190124>
8
9 Wang P, Liu J, Liu B, Feng D, Da Q, Wang P, Shu S, Su J, Zhang Y, Wang J, Wang HB. Evidence for
10 a role of chloroplastic m-type thioredoxins in the biogenesis of photosystem II in Arabidopsis. *Plant*
11 *Physiol*. 2013;163(4):1710-28. <https://doi.org/10.1104/pp.113.228353>
12
13 Wang J, Song J, Qi H, Zhang H, Wang L, Zhang H, Cui C, Ji G, Muhammad S, Sun G, Xu Z, Zhang H.
14 Overexpression of 2-Cys Peroxiredoxin alleviates the NaHCO₃ stress-induced photoinhibition and
15 reactive oxygen species damage of tobacco. *Plant Physiol Biochem*. 2023;201:107876.
16 <https://doi.org/10.1016/j.plaphy.2023.107876>
17
18 Wirtz W, Stitt M, Heldt HW, Light activation of calvin cycle enzymes as measured in pea leaves, *FEBS*
19 *Letters* 1982;142, 223–226.
20 [https://doi.org/10.1016/0014-5793\(82\)80139-7](https://doi.org/10.1016/0014-5793(82)80139-7).
21
22 Yang Y, Sulpice R, Himmelbach A, Meinhard M, Christmann A, Grill E. 2006. Fibrillin expression is
23 regulated by abscisic acid response regulators and is involved in abscisic acid-mediated photoprotection.
24 *Proc Natl Acad Sci USA*. 2006;103(15):6061-6066.
25 <https://doi.org/10.1073/pnas.0501720103>
26
27 Yoshida K, Hara A, Sugiura K, Fukaya Y, Hisabori T. Thioredoxin-like2/2-Cys peroxiredoxin redox
28 cascade supports oxidative thiol modulation in chloroplasts. *Proc Natl Acad Sci USA*.
29 2018;115(35):E8296-E8304.
30 <https://doi.org/10.1073/pnas.1808284115>
31
32 Yoshida K, Matsuoka Y, Hara S, Konno H, Hisabori T. Distinct redox behaviors of chloroplast thiol
33 enzymes and their relationships with photosynthetic electron transport in *Arabidopsis thaliana*. *Plant*
34 *Cell Physiol*. 2014;55(8):1415-25.
35 <https://doi.org/10.1093/pcp/pcu066>
36
37 Yoshida K, Yokochi Y, Tanaka K, Hisabori T. The ferredoxin/thioredoxin pathway constitutes an
38 indispensable redox-signaling cascade for light-dependent reduction of chloroplast stromal proteins. *J*
39 *Biol Chem*. 2022;298(12):102650.
40 <https://doi.org/10.1016/j.jbc.2022.102650>
41
42 Zhang H, Liu X, Zhang H, Wang Y, Li T, Che Y, Wang J, Guo D, Sun G, Li X. Thioredoxin-like protein
43 CDSP32 alleviates Cd-induced photosynthetic inhibition in tobacco leaves by regulating cyclic electron
44 flow and excess energy dissipation. *Plant Physiol Biochem*. 2021;167:831-839.
45 <https://doi.org/10.1016/j.plaphy.2021.09.016>
46
47 Zhang H, Xu Z, Huo Y, Guo K, Wang Y, He G, Sun H, Li M, Li X, Xu N, Sun G. Overexpression of
48 Trx CDSP32 gene promotes chlorophyll synthesis and photosynthetic electron transfer and alleviates
49 cadmium-induced photoinhibition of PSII and PSI in tobacco leaves. *J Hazard Mater*. 2020 :398:122899.
50 <https://doi.org/10.1016/j.jhazmat.2020.122899>
51
52 Zhang CJ, Zhao BC, Ge WN, Zhang YF, Song Y, Sun DY, Guo Y. An apoplasmic h-type thioredoxin
53 is involved in the stress response through regulation of the apoplasmic reactive oxygen species in rice.
54 *Plant Physiol*. 2011;157(4):1884-1899.
55 <https://doi.org/10.1104/pp.111.182808>

1
2 Zimmer D, Swart C, Graf A, Arrivault S, Tillich M, Proost S, Nikoloski Z, Stitt M, Bock R, Mühlhaus
3 T, Boulouis A. Topology of the redox network during induction of photosynthesis as revealed by time-
4 resolved proteomics in tobacco. *Sci Adv.* 2021;7(51):eabi8307.
5 <https://doi.org/10.1126/sciadv.abi8307>
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8 9 **Figure legends**

10 11 **Figure 1. Growth of potato plants modified in *CDSP32* expression upon standard or high salinity**

12 **conditions. A.** Three-week old plants grown on soil in a phytotron under standard watering conditions.
13 **B.** Three-week old WT plants watered with standard nutritive solution or solutions containing 0.125 M
14 NaCl or 0.1 M NaHCO₃. Arrows indicate leaves at similar developmental stages, smaller in plants
15 treated with NaCl than in those treated with NaHCO₃. Histogram representations of the chlorophyll
16 content of young expanded leaves from the upper crown of 24-day old plants grown in standard
17 conditions (C), watered with solutions containing 0.125 M NaCl (D) or 0.1 M NaHCO₃ (E). WT, wild
18 type; D4, line co-suppressed for *CDSP32*; D10, line overexpressing *CDSP32*; DM19, line
19 overexpressing *CDSP32* active site mutant. Chlorophyll data are means ± SD from 22 (NaCl) to 35
20 (standard and NaHCO₃) independent measurements. ** and ***, significantly different from the WT
21 value with P < 0.01 and P < 0.001, respectively (t-test).

22 **Figure 2. Abundance of *CDSP32* and various *CDSP32*-related plastidial thiol reductases in potato**

23 **plants modified in *CDSP32* expression grown in standard conditions or exposed to high salinity.**
24 **A.** Western blot analysis of the abundance of *CDSP32*, 2-Cys PRX, overoxidized 2-CysPRX (2-Cys
25 PRX-Ox), PRXQ, NTRC, MSRB1 and MSRA4 in plants grown in standard conditions or exposed to
26 high salinity treatments, 0.125 M NaCl or 0.1 M NaHCO₃, n.s., non specific. **B.** Histogram
27 representation showing the abundance of 2-Cys PRX-Ox, MSRB1 and MSRA4 in plants exposed to
28 NaHCO₃. Data are means ± S.D. of four values originating from independent experiments. *, * and ***,
29 significantly different from the WT value with P < 0.05, P < 0.01 and P < 0.001, respectively (t-test).
30 WT, wild type; D4, line co-suppressed for *CDSP32*; D10, line overexpressing *CDSP32*; DM19, line
31 overexpressing *CDSP32* active site mutant.

32 **Figure 3. Abundance of photosynthetic and photoprotective components in potato plants modified**

33 **in *CDSP32* expression grown in standard conditions or exposed to high salinity. A.** Western blot
34 analysis of the abundance of RubisCO large subunit, PRK, LHCB1, LHCB2, LHCA1, PsbO,
35 PsbA, NDH-H, PGR-L1, ATP-synthase γ subunit, PSBS and FBN/*CDSP34* in plants grown in
36 standard conditions or exposed to high salinity treatments, 0.125 M NaCl or 0.1 M NaHCO₃.
37 **B.** Histogram representation showing the abundance of PsbO, PsbA and ATP-synthase γ subunit

1 in plants grown in standard conditions or exposed to high salinity treatments. Data are means \pm
2 S.D. of three or four values originating from independent experiments. * and **, significantly
3 different from the WT value with $P < 0.05$ and $P < 0.01$, respectively (t-test). WT, wild type; D4, line
4 co-suppressed for CDSP32; D10, line overexpressing CDSP32; DM19, line overexpressing CDSP32
5 active site mutant.

6 **Figure 4. Chlorophyll fluorescence parameters in medium-low light. A.** Fluorescence induction
7 curves recorded by applying to dark-adapted leaf fragments a multi-turnover saturating pulse followed
8 by 4 minutes of actinic illumination at $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with superimposed saturating pulses. All
9 curves are normalised on $F_m - F_o$. The red and black arrows indicate the onset (time 0) and offset of the
10 actinic illumination. The means \pm S.D. of 3 biological replicates per genotype are shown. The first 4.5
11 seconds from actinic light onset of the curves are shown with an expanded time scale in panel **B**, to
12 better display the increased transient fluorescence in D4. **C, D** and **E.** Maximal PSII quantum yield
13 (F_v/F_m), and PSII yield (Φ_{PSII}) after 4 s and 216 s of actinic illumination, respectively, calculated from
14 the curves in (A). All values represent the means \pm S.D. of 3 biological replicates and are reported in
15 Table S2. The $\Phi_{\text{PSII}} 4 \text{ s}$ in D4 is significantly lower than in the other genotypes (t-test, ** $P \leq 0.01$, ***
16 $P \leq 0.001$).

17 **Figure 5. Redox-dependent regulation of the chloroplast ATP-synthase activity measured by ECS.**
18 **A.** Representative flash-dependent ECS curves recorded in dark-adapted leaves (closed symbol, solid
19 line), or 2 minutes after a train of 100 (open symbol, solid line) or 300 (closed symbol, dashed line) pre-
20 flashes. **B.** Loss in amplitude of the slow ECS decay phase induced by a variable number of pre-flashes
21 applied to dark-adapted leaves ($-A_n/A_{\text{Dark}}$), plotted in function of the number of charge separations
22 $\text{PSII} + \text{PSI}^{-1}$ generated by the pre-flashes (means \pm S.D. of 3 biological replicates for WT, D4 and D10,
23 5 replicates for DM19). The black and red solid lines represent the Hill fit of the WT and D4 datasets,
24 respectively, and the vertical dashed lines indicate the number of charge separations required to achieve
25 a 50% acceleration of the slow ECS decay phase in the two samples. This number is significantly higher
26 in D4 (t-test, ** $P \leq 0.01$). The fits of the D10 and DM19 datasets are shown in Fig. S6A and B, all fit
27 results are reported in Table S4. **C.** Representative flash-dependent ECS curves recorded in dark-adapted
28 leaves (closed symbol, solid line), or 2 minutes (closed symbol, dashed line) or 8 minutes (open symbol,
29 solid line) after a train of 300 pre-flashes. **D.** Recovery in amplitude of the slow ECS decay phase during
30 a variable dark incubation time after a train of 300 pre-flashes applied to dark-adapted leaves (A_v/A_{Dark}),
31 plotted in function of the dark incubation time (means \pm S.D. of 4 biological replicates per genotype).
32 The black and red solid lines represent the Hill fit of the WT and D4 datasets, respectively, and the
33 vertical dashed lines indicate the time of dark incubation required to achieve a 50% recovery of the slow
34 ECS decay phase in the two samples. This time is significantly shorter in D4 (t-test, **** $P \leq 0.0001$).

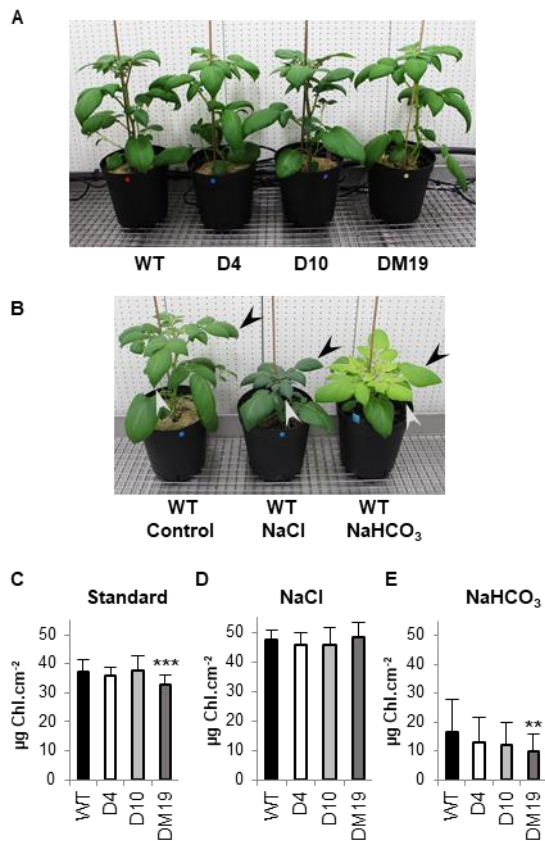
1 The fits of the D10 and DM19 datasets are shown in Fig. S6C and D, all fit results are reported in Table
2 S5.

3 **Figure 6. AlphaFold2 structure of *S. tuberosum* CDSP32 (top) and TRX f (bottom).** **A.** The 3D
4 structures are colored according to secondary structure elements (α -helices in pink and β -strands in teal
5 blue). CDSP32 presents two TRX-fold domains (D1 and D2) in tandem, and TRX f a single TRX-fold
6 domain. The Cys residues of the active motif and the Ser residues of the corresponding motif in CDSP32
7 TRX_D1 are shown in blue. **B.** Surface representation with the same orientation as in **A** displaying
8 atoms colored-coded according to the surface electrostatic potential from red (negative) to blue
9 (positive). Different electrostatic properties are observed between CDSP32 and TRX f in the region
10 surrounding the two redox-active Cys indicated by the yellow circle.

11 **Figure 7. *S. tuberosum* CDSP32 in complex with ATP synthase γ subunit.** **A.** AlphaFold2 3D
12 structure of *S. tuberosum* ATP-synthase γ subunit alone showing the L-shaped structure (yellow)
13 containing the redox loop and the β -hairpin. **B.** Complex between CDSP32 and ATP synthase γ subunit
14 generated by AlphaFold2 showing a close proximity between the γ subunit redox loop and the CDSP32
15 redox active motif, with a close proximity (4Å) of the two catalytic Cys residues of each partner.
16 Superposition of the *S. tuberosum* CDSP32-ATP synthase γ subunit complex onto the spinach ATP-
17 synthase (PDB 6FKF) is shown aside.

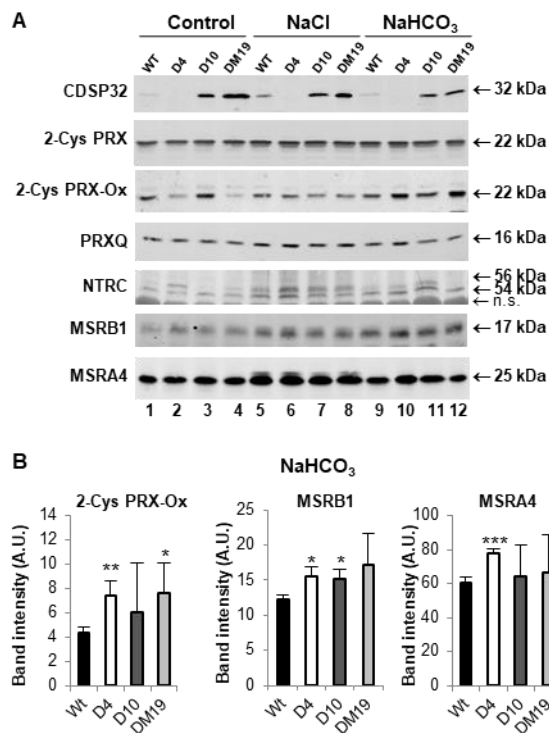
18 **Figure 8. Presence of the CDSP32 TRX and of the redox loop in the ATP-synthase γ subunit among**
19 **photosynthetic organisms.** CDSP32 and ATP-synthase γ subunit sequences were analyzed from
20 several species of each main representative group of photosynthetic organisms. The typical CDSP32 19-
21 residue motif surrounding the active site and the redox loop in ATP-synthase γ subunit are shown and
22 Cys residues are underlined. Species: St, *Solanum tuberosum*; Ta, *Triticum aestivum*; Am, *Amborella*
23 *trichopoda*; Ps, *Picea sitchensis*; Sm, *Selaginella moellendorffii*; Mp, *Marchantia polymorpha*; Cb,
24 *Chara braunii*; Cr, *Chlamydomonas reinhardtii*; Cc, *Chondrus crispus*; Pt, *Phaeodactylum tricoratum*;
25 Eh, *Emiliana huxleyi*; Nc, *Nostoc commune*. Species and accession numbers for CDSP32 : St,
26 NP_001305492.1; Ta, XP_044421501.1; Am, XP_006828837.1; Ps, ABK25354.1; Sm,
27 XP_002989497.2; Mp, PTQ43510.1; Cb, CBR_g26413; Cr, CHLRE_14g624201v5. Species and
28 accession numbers for ATP-synthase γ subunit : St, XP_006348117.1; Ta, XP_044323458.1; Am,
29 XP_006855900.1; Ps, ABK26845.1; Sm, XP_002960916.2; Mp, PTQ27329.1; Cb, GBG67951.1; Cr,
30 XP_001696335.1; Cc, XP_005710884.1; Pt, AAO43198.1; Eh, XP_005761783.1; Nc,
31 WP_196527105.1. ---, absence of CDSP32 gene or of redox loop in γ subunit.

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1
2 **Figure 1. Growth of potato plants modified in *CDSP32* expression upon standard or high salinity**
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4 **B.** Three-week old WT plants watered with standard nutritive solution or solutions containing 0.125 M
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7 content of young expanded leaves from the upper crown of 24-day old plants grown in standard
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9 type; D4, line co-suppressed for *CDSP32*; D10, line overexpressing *CDSP32*; DM19, line
10 overexpressing *CDSP32* active site mutant. Chlorophyll data are means ± SD from 22 (NaCl) to 35
11 (standard and NaHCO₃) independent measurements. ** and ***, significantly different from the WT
12 value with P < 0.01 and P < 0.001, respectively (t-test).

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1

2 **Figure 2. Abundance of CDSP32 and various CDSP32-related plastidial thiol reductases in potato**

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4 **A.** Western blot analysis of the abundance of CDSP32, 2-Cys PRX, overoxidized 2-CysPRX (2-Cys

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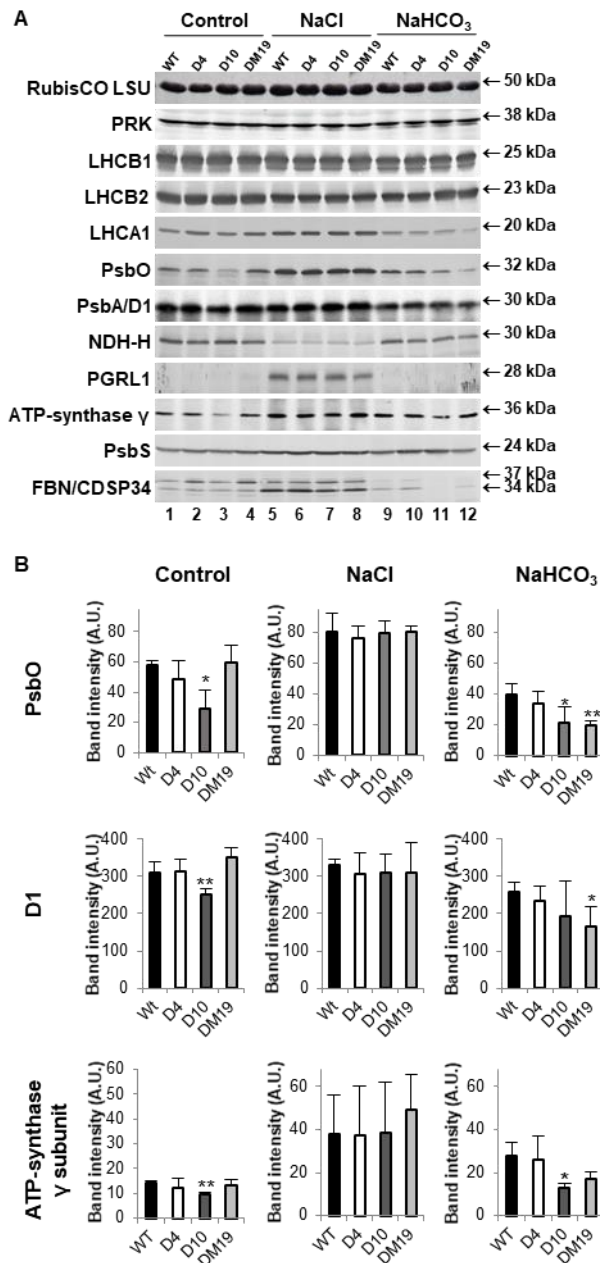
7 representation showing the abundance of 2-Cys PRX-Ox, MSRB1 and MSRA4 in plants exposed to

8 NaHCO₃. Data are means ± S.D. of four values originating from independent experiments. *, * and ***,

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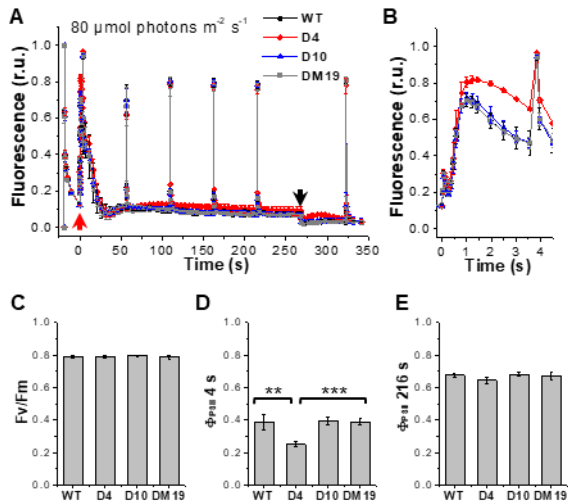


1
2 **Figure 3. Abundance of photosynthetic and photoprotective components in potato plants modified**
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6 standard conditions or exposed to high salinity treatments, 0.125 M NaCl or 0.1 M NaHCO₃.
7 **B.** Histogram representation showing the abundance of PsbO, PsbA and ATP-synthase γ subunit
8 in plants grown in standard conditions or exposed to high salinity treatments. Data are means \pm
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10 actinic illumination. The means \pm S.D. of 3 biological replicates per genotype are shown. The first 4.5
11 seconds from actinic light onset of the curves are shown with an expanded time scale in panel B, to
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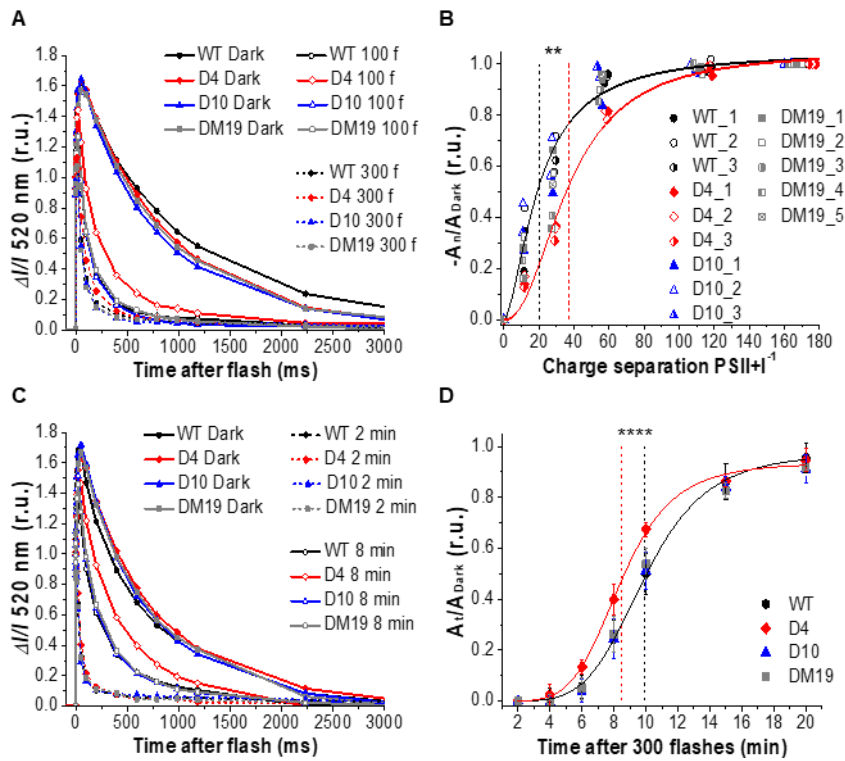
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16 plotted in function of the dark incubation time (means \pm S.D. of 4 biological replicates per genotype).

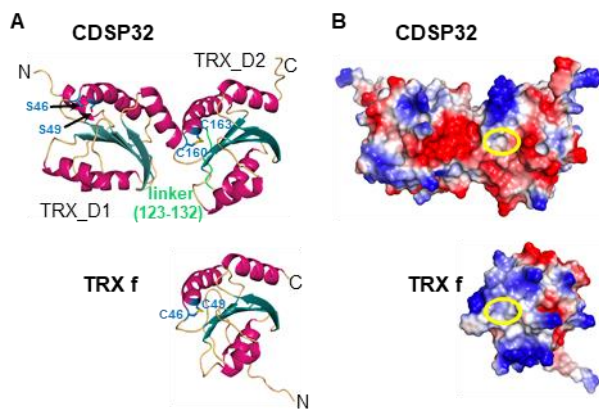
17 The black and red solid lines represent the Hill fit of the WT and D4 datasets, respectively, and the

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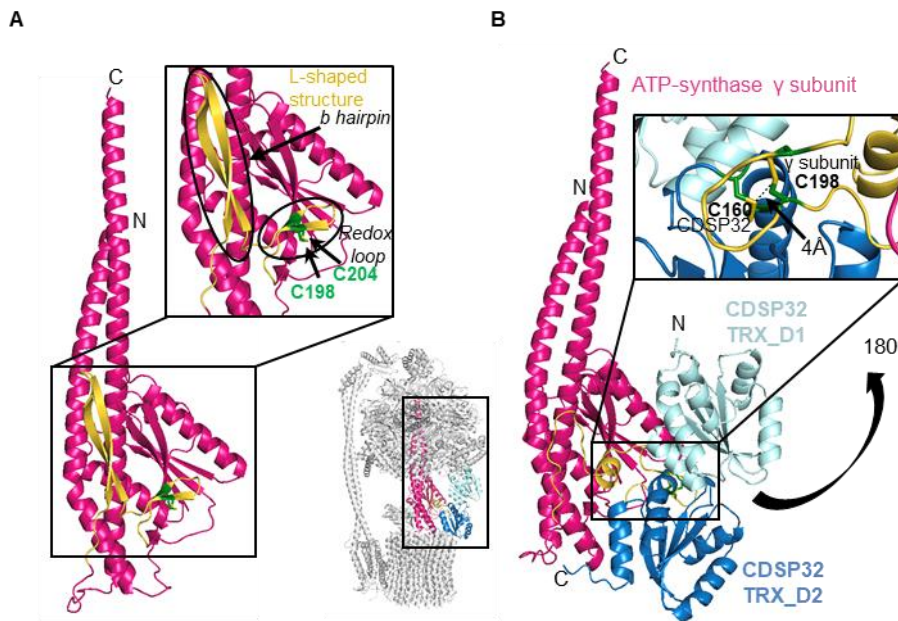
21 S5.



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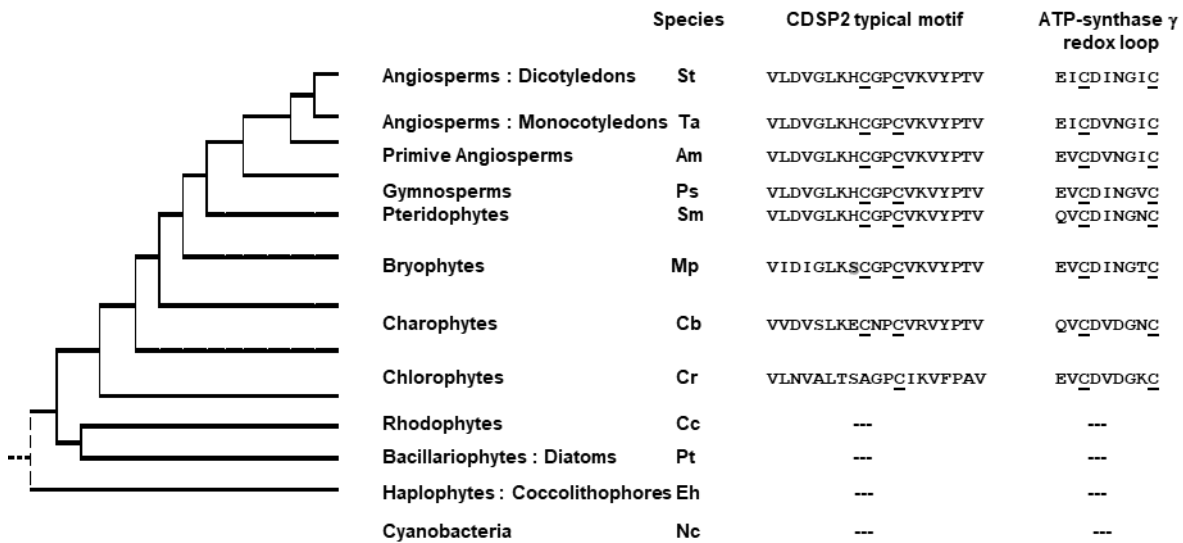
2 **Figure 6. AlphaFold2 structure of *S. tuberosum* CDSP32 (top) and TRX f (bottom).** **A.** The 3D
 3 structures are colored according to secondary structure elements (α -helices in pink and β -strands in teal
 4 blue). CDSP32 presents two TRX-fold domains (D1 and D2) in tandem, and TRX f a single TRX-fold
 5 domain. The Cys residues of the active motif and the Ser residues of the corresponding motif in CDSP32
 6 TRX_D1 are shown in blue. **B.** Surface representation with the same orientation as in **A** displaying
 7 atoms colored-coded according to the surface electrostatic potential from red (negative) to blue
 8 (positive). Different electrostatic properties are observed between CDSP32 and TRX f in the region
 9 surrounding the two redox-active Cys indicated by the yellow circle.

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Figure 7. *S. tuberosum* CDSP32 in complex with ATP synthase γ subunit. **A.** AlphaFold2 3D structure of *S. tuberosum* ATP-synthase γ subunit alone showing the L-shaped structure (yellow) containing the redox loop and the β -hairpin. **B.** Complex between CDSP32 and ATP synthase γ subunit generated by AlphaFold2 showing a close proximity between the γ subunit redox loop and the CDSP32 redox active motif, with a close proximity (4Å) of the two catalytic Cys residues of each partner. Superposition of the *S. tuberosum* CDSP32-ATP synthase γ subunit complex onto the spinach ATP-synthase (PDB 6FKF) is shown aside.



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2 **Figure 8. Presence of the CDSP32 TRX and of the redox loop in the ATP-synthase γ subunit among**
3 **photosynthetic organisms.** CDSP32 and ATP-synthase γ subunit sequences were analyzed from
4 several species of each main representative group of photosynthetic organisms. The typical CDSP32 19-
5 residue motif surrounding the active site and the redox loop in ATP-synthase γ subunit are shown and
6 Cys residues are underlined. Species: St, *Solanum tuberosum*; Ta, *Triticum aestivum*; Am, *Amborella*
7 *trichopoda*; Ps, *Picea sitchensis*; Sm, *Selaginella moellendorffii*; Mp, *Marchantia polymorpha*; Cb,
8 *Chara braunii*; Cr, *Chlamydomonas reinhardtii*; Cc, *Chondrus crispus*; Pt, *Phaeodactylum tricorutum*;
9 Eh, *Emiliana huxleyi*; Nc, *Nostoc commune*. Species and accession numbers for CDSP32 : St,
10 NP_001305492.1; Ta, XP_044421501.1; Am, XP_006828837.1; Ps, ABK25354.1; Sm,
11 XP_002989497.2; Mp, PTQ43510.1; Cb, CBR_g26413; Cr, CHLRE_14g624201v5. Species and
12 accession numbers for ATP-synthase γ subunit : St, XP_006348117.1; Ta, XP_044323458.1; Am,
13 XP_006855900.1; Ps, ABK26845.1; Sm, XP_002960916.2; Mp, PTQ27329.1; Cb, GBG67951.1; Cr,
14 XP_001696335.1; Cc, XP_005710884.1; Pt, AAO43198.1; Eh, XP_005761783.1; Nc,
15 WP_196527105.1. ---, absence of CDSP32 gene or of redox loop in γ subunit.

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