Flavodoxin and ferredoxin-NADP\(^+\) reductase confers increased tolerance to oxidative stress in plants

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1. Introduction

The photosynthetic electron transport chain (PETC) of plant chloroplasts provides reduced ferredoxin (Fd), NADPH and ATP for oxygenic photosynthesis. Electrons abstracted by light-dependent reactions at the photosystems promote water splitting at the oxidative end of the PETC, and are conveyed through soluble and thylakoid-bound electron carriers to Fd at the reducing side [1]. Ferredoxins are small, soluble [2Fe–2S] proteins that play a key role in electron distribution in all types of plastids [2]. In chloroplasts, photoreduced Fd can interact with Fd–NADP\(^+\) reductase (FNR) to generate the NADPH required for carbon assimilation and other biosynthetic and protective pathways. It can also divert reducing equivalents back to the PETC via cyclic electron flow, or to a plethora of soluble electron acceptor enzymes involved in various metabolic, regulatory and dissipative routes of the stroma [3]. In cyanobacteria and some marine algae, Fd can be replaced by flavodoxin (Fld), an iso-functional electron shuttle not present in plants which contains flavin mononucleotide as the prosthetic group [4–6]. When present, Fd usually acts as a backup for Fd when the levels of the iron–sulfur protein decline due to environmental hardships such as iron deficiency [4]. Under these conditions, Fd expression is induced and the flavoprotein takes over Fd functions in photosynthesis and other metabolic pathways [4].

Dioxygen may act as an adventitious electron acceptor of the PETC, yielding excited or partially reduced forms such as singlet oxygen and the superoxide radical, collectively known as reactive oxygen species (ROS). They react with different biomolecules, inactive them and causing severe damage [7]. Under normal growth conditions, electron leakage to oxygen is minimal (<10%), and the small amounts of ROS generated are readily scavenged by plastidic antioxidants [8]. However, adverse environmental situations increase ROS production several-fold. Two non-excluding mechanisms have been invoked to explain this phenomenon. First, stress conditions inhibit CO\(_2\) assimilation and hence, NADPH oxidation in the regenerative stages of the Calvin cycle [7]. Second, many environmental insults cause down-regulation of Fd expression in plants and cyanobacteria [9–11]. Both effects contribute to decrease the levels of the major physiological PETC acceptors (oxidized Fd and NADP\(^+\)). Under these conditions, the PETC should become over-reduced and the ability of O\(_2\) to subtract electrons from the chain is expected to increase, leading to runaway ROS propagation [7].
Therefore, depletion of the electron sink at the reducing side of the PETC is proposed to be a major determinant for the establishment of oxidative stress in plants [12], although the evidence for such a mechanism is circumstantial. For instance, knocked-down plants in which the levels of either FNR or Fd had been decreased by RNA antisense or RNA interference approaches display enhanced susceptibility to several sources of stress, in addition to growth and reproductive penalties [13–17]. This line of reasoning predicts that replenishment of the electron acceptor pool and reconstruction of electron delivery to productive routes might alleviate this situation. However, transformation of tobacco plants with a plastid-targeted Fd failed to improve stress tolerance, due to down-regulation of the transgene under oxidative conditions [18]. Instead, expression of a cyanobacterial Fld in tobacco chloroplasts led to transgenic lines with enhanced tolerance to multiple sources of stress, including drought, chilling and redox-cycling herbicides [11]. Consistent with the previous arguments, the effect of Fld was dose-dependent, required plastid targeting and resulted in higher degrees of tolerance could be achieved by improving the capacity for Fld reduction in planta under stress conditions, and by relieving electron acceptor limitation of the PETC. Since NADPH build-up is another unwanted consequence of stress episodes, overexpression of FNR seems to be the logical choice in order to use the excess of NADPH as electron source for Fld reduction. Soluble FNR catalyzes Fld reduction by NADPH with high efficiency [19], but FNR normally binds to the thylakoid membrane, and when membrane-bound the preferred direction of the reaction is still under debate [20]. In this report we describe the generation of a soluble cyanobacterial FNR (sFNR) obtained by removing a membrane-binding N-terminal domain, and the design of an in vivo system for Fld reduction and NADPH oxidation, involving co-expression of Fld and sFNR in tobacco chloroplasts. The resulting transgenic lines displayed higher tolerance to oxidative stress and lower ROS build-up as compared to wild-type (wt) plants and single transformants.

2. Materials and methods

2.1. Design and characterization of transgenic tobacco plants expressing Fld and sFNR

A DNA fragment encoding a C-terminal region of FNR (sFNR) from *Anabaena* PCC7119 (without the phycobilisome binding domain) was obtained by PCR amplification of the whole gene cloned into plasmid pTrc99a [21], using primers 5’-CGAGCTCATCATGACCTAGCGA-3’ and 5’-ACGTCAACACCTATGTTTCTAC-3’. A PCR product of the predicted length (940 bp) was digested with ScaI and SalI and cloned into compatible sites of a pUC9-derived recombinant plasmid harboring the entire pea FNR precursor gene between BamHI and SalI restriction sites, and from which the DNA fragment encoding the mature region of pea FNR had been removed by digestion with ScaI and SalI. This generated an in-frame fusion of the chloroplast transit peptide (TP) derived from pea FNR with the mature region of *Anabaena* FNR. The chimeric gene was excised from the corresponding plasmid by digestion with BamHI and SalI, cloned between the CaMV 35S promoter and polyadenylation regions of pDH51 [22]. The entire cassette was further isolated as an EcoRI fragment and inserted into the corresponding EcoRI site of the binary vector pCAMBIA2300 [23]. The resulting plasmid was introduced into the genome of tobacco (*Nicotiana tabacum* cv. Petit Havana) through *Agrobacterium tumefaciens*-mediated leaf disc transformation [24].

Primary transformants expressing high levels of sFNR, as evaluated by SDS–PAGE and immunoblotting, were self-pollinated and all subsequent experiments were carried out with the homozygous progeny.

The preparation of double expressing plants was performed by cross-pollination. Transgenic plants expressing sFNR from *Anabaena* (psfnr) and a stable homozygous line expressing high levels of *Anabaena* Fld in chloroplasts (pfld) [11] were used as parents. Primary double heterozygous transgenic plants expressing sFNR and Fld (pfld/psfnr) were self-pollinated and double homozygous plants selected by SDS–PAGE and immunoblotting.

Seeds were germinated on Murashige–Skoog agar supplemented with 3% (w/v) sucrose and, in the case of transformants, 100 µg mL⁻¹ kanamycin. Three-week-old seedlings were transferred to soil or grown hydroponically in nutrient medium [25], and illuminated at 200 µmol quanta m⁻² s⁻¹ and 25 °C to provide a 16-h photoperiod. Intact chloroplasts were isolated and osmotically shocked as described [26]. Levels of sFNR and Fld in cleared leaf and chloroplast extracts were analyzed by 12% SDS–PAGE, followed by immunoblot analysis with antisera raised against *Anabaena* FNR and Fld.

2.2. Determination of photosynthetic parameters

Chlorophyll fluorescence measurements were performed at 25 °C on dark-adapted leaves using a Qubit Systems pulse-modulated fluorometer. The F₀ and Fm parameters were determined after 30 min in the dark, and the light-adapted values (Fₚ and Fₚm) were measured after 30 min of illumination at 200 µmol quanta m⁻² s⁻¹. Photosynthetic parameters (Fₚ/Fₚm, ΦSIS, NPQ, 1-qP) were calculated as described [27]. CO₂ assimilation rates were measured with an infra-red gas analyzer LI-6200 (LI-COR) at 500 µmol quanta m⁻² s⁻¹. Induction/relaxation NPQ curves were determined using the program of the MINI-PAM 2000 fluorometer. Light induction measurements were performed on dark-adapted leaves by application

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**Fig. 1.** Expression of soluble cyanobacterial FNR in chloroplasts. (A) Schematic representation of the chimeric gene used to generate transgenic plants expressing sFNR in chloroplasts. Phyc-D, fad-D and nadp-D indicate the phycobilisome-, FAD- and NADP(H)-binding domains, respectively. The coding sequence of FNR was fused in-frame to the pea FNR transit peptide (TP) and cloned in pCAMBIA2200. (B) sFNR accumulation in chloroplasts. Stomatal and membrane fractions from osmotically shocked chloroplasts (corresponding to 4 µg of chlorophyll) from wt and two independent transgenic lines (psfnr18 and psfnr22) were fractionated by SDS–PAGE and blotted onto nitrocellulose membranes for immunodetection with antisera directed against *Anabaena* FNR. (C) NADP(H)-diaphorase activity in stromal fractions of wt and transgenic plants. Extracts containing 30 µg of stromal proteins were resolved by native electrophoresis and stained as indicated in Section 2.
of a saturating pulse to obtain the $F_m$ values, and a second pulse 5 s later. After 30 s, NPQ induction was followed over 6 min at 200, 600, 1000 and 2500 μmol quanta m$^{-2}$s$^{-1}$ of actinic light, and saturating pulses were applied every 30 s to obtain $F_m$. ETR values were obtained at 200 and 600 μmol quanta m$^{-2}$s$^{-1}$ of actinic light using the equation: $\Phi_{\text{PSII}} \times$ actinic light intensity $\times$ 0.5 $\times$ 0.84. The actinic light was subsequently turned off, and the dark relaxation process was monitored at various times (30 s and 1, 2, 5 and 10 min) using saturating pulses [17].

### 2.3. In-gel diaphorase activity

For the identification of enzymes displaying NADPH-dependent diaphorase activity (largely FNR), stromal extracts corresponding to 30 μg of soluble protein were resolved by non-denaturing PAGE on 12% polyacrylamide gels. After electrophoresis, the gel was stained by incubation in 50 mM Tris–HCl, pH 8.5, 0.3 mM NADP$^+$, 3 mM Glc-6-P, 1 unit mL$^{-1}$ Glc-6-P dehydrogenase, and 1 mg mL$^{-1}$ nitroblue tetrazolium until the appearance of the purple formazan bands [26].

### 2.4. Methyl viologen treatments

Leaf discs (12-mm diameter) were floated topside up in 1 mL water or 40 μM MV, and illuminated at 1000 μmol quanta m$^{-2}$s$^{-1}$ for 7 h. Electrolyte leakage was measured as described previously [11]. For plants cultured in hydroponics, 100 μM MV was added to the nutrient solution.
2.5. Analytical procedures

Chlorophylls and carotenoids were determined spectrophotometrically after extraction with 96% (v/v) ethanol [28]. NADP(H) levels were estimated by a redox cycling assay, essentially as described by Slater and Sawyer [29], after extraction of the pyridine nucleotides from leaf tissue. Hydroperoxides were measured using a modified ferrous oxidation–xylenol orange assay [30].

3. Results and discussion

The preparation and phenotypic characterization of tobacco line pfld5–8, expressing Fld from Anabaena PCC7119, have been described elsewhere [11]. While plant FNRs are made up of two structural domains and bind extrinsically to membranes, Anabaena FNR is a 3-domain flavoenzyme, with the two C-terminal domains being homologous to their plant counterparts [19]. The N-terminal region in the cyanobacterial reductase is responsible for membrane attachment through phycobilisome binding [31]. This domain can be excised to abolish thylakoid interaction, without affecting enzyme activity [32]. Then, for the generation of sFNR-expressing plants, a truncated form of the FNR from Anabaena PCC7119 lacking the membrane-binding domain was fused in-frame to the transit peptide of pea FNR (Fig. 1A), and introduced into tobacco plants using Agrobacterium-mediated leaf disc transformation, to yield psfnr lines. The presence of sFNR was determined in cleared leaf extracts by SDS–PAGE and immunoblotting. Lines containing the highest amounts of the foreign flavoenzyme were made homozygous by self-pollination. Homozygosity of the transgene was confirmed in all cases by back-crosses to the wild type and by quantification of sFNR amounts (data not shown, but see below). Segregation analysis confirmed a single insertion locus per genome in selected lines. The sFNR product was targeted to chloroplasts and accumulated in the stroma, as revealed by immunoblots performed on different fractions of intact chloroplasts isolated from two different homozygous lines (Fig. 1B, lanes psfnr18 and psfnr22). It is worth noting that antisera raised against Anabaena FNR did not cross-react with endogenous

Fig. 3. Increased NADP+/NADPH ratio in pfld/psfnr line (47). NADP(H) contents were measured in leaf extracts of 3-week-old plants as described in Section 2. Measurements were done in triplicate, and values are the mean ratios ± propagation of SE.

Fig. 4. Expression of Fld and sFNR in chloroplasts increase tolerance to MV toxicity. Membrane damage (A), bleaching (B), and pigment degradation (C), were analyzed in five leaf discs from 6-week-old wt and transgenic plants exposed to 40 μM MV at 1000 μmol quanta m⁻² s⁻¹ for 7 h. Two pfld/psfnr lines were assayed. Values are expressed as the percentages ± SD of ion leakage relative to zero time of discs incubated under the same conditions (A), or as means ± SD of the percentages calculated against control discs incubated in water (C). (D) Leaf discs were challenged with 10 μM MV at 700 μmol quanta m⁻² s⁻¹ for 3 h, and hydroperoxides (LOOH) were determined by the xylenol orange method [30]. Bars are means of four independent determinations ± SD. (E) Four-week-old plants grown in soil were transferred to hydroponics solution for 3 days, and subsequently supplemented with new nutrient solution containing 100 μM MV. Pictures were taken 24 h after treatment.
tobacco FNR (Fig. 1B). Diaphorase activity staining of native gels revealed several reactive bands in the stroma of transformed plants (Fig. 1C, lanes psfnr18 and psfnr22), migrating ahead of those corresponding to the tobacco flavoenzymes (Fig. 1C, lane wt). Micro-heterogeneity at the N-terminus during transit peptide processing might account for the various molecular species observed, as already reported [33]. Although in-gel determinations do not provide quantitative values, it is clear from Fig. 1C that the cyanobacterial reductase accounts for most FNR activity in the chloroplast stroma of the transgenic lines. Growth rates, biomass accumulation and photosynthetic activities of psfnr plants did not differ significantly from those displayed by wt siblings (Table S1).

Plants co-expressing Fld and sFNR were prepared by cross-fertilization of the respective individual transformants, and double homozygous lines were selected by a combination of segregation analysis and protein level determinations (Fig. S1). The general phenotypes of the double-transgenic lines (pfld/psfnr) were similar to those of wt plants (Table S1). Steady-state photosynthetic parameters, including photosystem (PS) II operating efficiency, maximum quantum yield of PSII and CO2 assimilation activities, also failed to show significant differences (Table S1). The results indicate that the presence of the two interacting proteins remained largely unnoticed, in phenotypic terms, in unstressed plants. However, some subtle differences could be detected. First, the time course of NPQ build-up at high light intensities was faster and more extensive in the double transgenics, without significant changes in electron transfer rates through PSII (Fig. 2). NPQ is a dissipative response deployed by plants to relieve excess excitation energy on the PETC, and largely relies on Fd-dependent cyclic electron flow through PSI. Second, the NADP+/NADPH ratio was higher in the pfld/psfnr lines relative to wt and single transformants (Fig. 3).

Methyl viologen (MV) is a redox-cycling herbicide which propa- gates ROS by accepting electrons at PSI, one at a time, and transferring them to oxygen to yield superoxide radicals [34]. Previous

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**Fig. 5.** Proposed model for the protective mechanism of Fld and sFNR in chloroplasts. Under normal growth conditions Fd/Fld transfers electrons to different productive routes in chloroplasts. Under stress situations, Fd levels decline. In pfld/psfnr plants, sFNR oxidizes NADPH, regenerating NADP+ and contributing to Fld reduction. Blue arrows indicate cyclic electron transport. Cytb6/f, cytochrome b6/f; PC, plastocyanin; PQ, plastoquinone; ox, oxidized; red, reduced.
results have shown that plants expressing Fld in chloroplasts displayed enhanced tolerance to several sources of stress including MV [11]. To probe the tolerance of the single and double transgen-

ants, membrane damage in the presence of MV was evaluated by measuring ion release from leaf discs. Initial rates of electrolyte leakage were ∼2-fold lower in pfdl foliar tissue as compared to wt discs (Fig. 4A), whereas plants transformed with the gene encoding sFNR displayed wt levels of tolerance (Fig. S2). The combination of both flavoproteins in pfdl/pfsnr lines led to a further 10-fold decrease (relative to pfdl siblings) in the rates of ion release, a dramatic improvement that was also evident at the end of the treatment (Fig. 4A). During that 7–8 h period of MV exposure, both wt (Fig. 4B) and pfsnr discs (data not shown) were completely bleached, reflecting nearly quantitative destruction of leaf pigments (Fig. 4C). Plants expressing Fld alone retained about 50% and 25% of chlorophylls and carotenoids, respectively, in good agreement with previous reports [11]. Once again, the double transgen-

ants displayed the highest levels of pigment preservation: 70–80% for chlorophylls and 55–70% for carotenoids (Fig. 4C). In line with the stress protection conferred, ROS build-up was almost entirely prevented in lines co-expressing Fld and sFNR, about 5- and 10-fold below the levels accumulated in pfdl and wt lines, respectively (Fig. 4D). When whole plants were exposed to MV toxicity in a hydroponic system, the extent of damage undergone by the leaves followed the same trend observed in disc experiments, with pfdl/pfsnr lines displaying the highest degree of tolerance (Fig. 4E).

The collected results indicate that replenishment of the acceptor sink at the PETC, and the presence of an additional electron source for Fld reduction in the double-transgenic plants significantly increased tolerance to oxidative stress. We propose that this system functions by recycling NADP(H) through the sFNR/Fld couple, thus relieving the electron pressure on the PETC and prevent-

ing excessive reduction of the NADP(H) pool under adverse situations. At the same time, proper delivery of reducing equiva-

lents generated at the PETC to productive oxido-reductive path-

ways of the chloroplast will be favored by a continuous stream of reduced Fld. A model describing these findings and interpreta-

tion is provided in Fig. 5.

According to this rationale, under non-stressed conditions, Fd in wt plants will collect reducing equivalents from the PETC and dis-

tribute them to the Calvin cycle (via NADP* photoreduction), to other stomatal acceptors, and back to the PETC via cyclic electron flow (Fig. 5A). In the double transgenics (Fig. 5B), Fd and Fld can be reduced by both the PETC and NADPH (via sFNR). Fld can act in the same chloroplast oxido-reductive pathways as Fd, but the iron-sulfur protein will likely be the preferred electron shuttle [11]. The activity of sFNR has only limited effect on redox homeo-

stasis, reflected by a moderate increase in the NADP+/NADPH ratio (Fig. 3) and faster NPQ build-up (Fig. 2). Under stress conditions, however, Fd levels decline and NADPH accumulates in wt plants, leading to over-reduction of the PETC and ROS build-up (Fig. 5C). Fld can replace declining Fd, restoring delivery of reducing equiva-

lents to productive electron accepting routes [3,11]. The activity of sFNR will contribute to these Fld functions, and at the same time will consume the NADPH surplus, preventing over-reduction of the PETC and ROS propagation (Fig. 5D). Although the proposed mechanism is consistent with the observations reported here, some of its steps are speculative and require further experimenta-

tion to substantiate the contentions made. Accordingly, the model of Fig. 5 should be regarded as a working hypothesis.

Then, the combined action of Fld and sFNR can play a transient but crucial role in maintaining proper electron distribution during oxidative stress episodes. The results emphasize the paramount importance of chloroplast redox homeostasis in plants exposed to adverse conditions. Many environmental insults of agronomic relevance, such as drought, chilling and salinity, lead to the establishment of an oxidative stress situation that significantly contributes to the damage suffered by the plant [35]. Observations reported here indicate that the Fld/sFNR system could provide a promising biotechnological tool to improve crop tolerance towards these environmental hardships. Research is currently underway to evaluate this possibility.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.fob.2011.10.004.

References

[1] Rochaix, J.D. (2011) Regulation of photosynthetic electron transport. Biochim. Biophys. Acta 1807, 375–383.
[2] Hase, T., Schürmann, P. and Knaff, D.B. (2006) The Light-Driven Plastocyanin–

ferredoxin Oxidoreductase in Photosystem I (Golbeck, J.H., Ed.), pp. 477–498, Springer, Dordrecht, Netherlands.
[3] Zurbriggen, M.D., Tognetti, V.B., Millat, M.F., Hajirezaei, M.R., Valle, E.M. and Carrillo, N. (2008) Combating stress with flavodoxin: a promising route for crop improvement. Trends Biotechnol. 26, 531–537.
[4] Erdier, D.L., Price, N.M., Doucette, G.J., Peleato, M.L. and Anderson, D.M. (1999) Characterization of ferredoxin and flavodoxin as markers of iron limitation in marine phytoplankton. Mar. Ecol. Prog. 184, 43–53.
[5] Sancho, J. (2006) Flavodoxins: sequence, folding, binding, function and beyond. Cell. Mol. Life Sci. 63, 855–864.
[6] Gofi, G., Herguedas, B., Hervás, M., Perajerina, J.R., De la Rosa, M.A., Gómez Moreno, C., Navarro, J.A., Hermoso, J.A., Martínez-Júlvez, M. and Medina, M. (2009) Flavodoxin: a compromise between efficiency and versatility in the electron transfer from photosystem I to ferredoxin–NADP (+) reductase. Biochim. Biophys. Acta 1778, 144–154.
[7] Apel, K. and Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–395.
[8] Foyer, C.H. and Noctor, G. (2000) Oxidation processing in photosynthesis: regulation and signalling. New Phytol. 146, 359–388.
[9] Mazouni, K., Domain, F., Chauvat, F. and Casart-Chauvat, C. (2003) Expression and regulation of the crucial plant-like ferredoxin of cyanobacteria. Mol. Microbiol. 49, 1019–1029.
[10] Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L. and Gruissem, W. (2004) GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol. 136, 2621–2629.
[11] Tognetti, V.B., Palatnik, J.F., Fillat, M.F., Melzer, M., Hajirezaei, M.R., Valle, E.M. and Carrillo, N. (2006) Functional replacement of ferredoxin by a cyanobacterial flavodoxin in tobacco confers broad-range stress tolerance. Plant Cell 18, 2035–2050.
[12] Scheibe, R., Backhausen, J.E., Emmerlich, V. and Holtgreve, S. (2005) Strategies to maintain redox homeostasis during photosynthesis under changing conditions. J. Exp. Bot. 56, 1481–1489.
[13] Hajirezaei, M.R., Peisker, M., Tschiersch, H., Palatnik, J.F., Valle, E.M., Carrillo, N. and Sonnewald, U. (2002) Small changes in the activity of chloroplastic NADP (+)-dependent ferredoxin oxidoreductase lead to impaired plant growth and restrict photosynthetic activity of transgenic tobacco plants. Plant J. 29, 281–293.
[14] Palatnik, J.F., Tognetti, V.B., Poli, H.O., Rodríguez, R.E., Blanco, N., Gattuso, M., Hajirezaei, M.R., Sonnewald, U., Valle, E.M. and Carrillo, N. (2003) Transgenic tobacco plants expressing antisense ferredoxin-NADP(H) reductase transcripts display increased susceptibility to photo-oxidative damage. Plant J. 35, 332–341.
[15] Holtgreve, S., Bader, K.P., Horton, P., Scheibe, R., Schwaenew, A. and Backhausen, J.E. (2003) Decreased content of leaf ferredoxin changes electron distribution and limits photosynthesis in transgenic potato plants. Plant Physiol. 133, 1768–1778.
[16] Hald, S., Sandba, B., Gallois, P. and Johnson, G.N. (2008) Feedback regulation of photosynthetic electron transport by NADP(H) redox poise. Biochim. Biophys. Acta 1777, 433–440.
[17] Almonacid, F.F., Scheibe, R., Hajirezaei, M.R. and Carrillo, N. (2011) Photosynthesis and regulation of the crucial plant-like ferredoxin of cyanobacteria. Mol. Microbiol. 49, 1019–1029.
[18] Ceccoli, R.D., Blanco, N.E., Medina, M. and Carrillo, N. (2011) Stress response of transgenic tobacco plants expressing a cyanobacterial ferredoxin in chloroplasts. Plant Mol. Biol. 76, 535–544.
Carrillo, N. and Ceccarelli, E.A. (2003) Open questions in ferredoxin–NADP+ reductase catalytic mechanism. Eur. J. Biochem. 270, 1900–1915.

Benz, J.P., Lintala, M., Soili, J., Mulo, P. and Bolter, B. (2010) A new concept for ferredoxin–NADP(H) oxidoreductase binding to plant thylakoids. Trends Plant Sci. 15, 608–613.

Fillat, M.F., Bakker, H.A. and Weisbeek, P.J. (1990) Sequence of the ferredoxin–NADP(H) + reductase gene from *Anabaena* PCC 7119. Nucleic Acids Res. 18, 7161.

Pietrzk, M., Shillito, K.M., Hohn, T. and Potrikus, I. (1986) Expression in plants of two bacterial antibiotic resistance genes after protoplast transformation with a new plant expression vector. Plant J. 5857–5868.

Fillat, M.F., Flores, E. and Gómez-Moreno, C. (1993) Homology of the N-terminal domain of the petH gene product from *Anabaena* sp. PCC 7119 to the CpcD phycobilisome linker polypeptide. Plant Mol. Biol. 22, 725–729.

Gallosi, P. and Marinho, P. (1995) Leaf disk transformation using *Agrobacterium tumefaciens*-expression of heterologous genes in tobacco. Methods Mol. Biol. 49, 39–48.

Hoagland, D.R. (1920) Optimum nutrient solutions for plants. Science 52, 562–564.

Rodríguez, R.E., Lodeyro, A., Poli, H.O., Zurbriggen, M., Peisker, M., Palatnik, J.F., Tognetti, V.B., Tschierech, H., Hajirezaei, M.R., Valle, E.M. and Carrillo, N. (2007) Transgenic tobacco plants overexpressing chloroplastic ferredoxin–NADP(H) reductase display normal rates of photosynthesis and increased tolerance to oxidative stress. Plant Physiol. 143, 639–649.

Baker, N.R. (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59, 89–113.

Lichtenthaler, H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–383.

Slater, T.F. and Sawyer, B. (1962) A colorimetric method for estimating the pyridine nucleotide content of small amounts of animal tissue. Nature 193, 454–456.

DeLong, J.M., Prange, R.K., Hodges, D.M., Forney, C.F., Bishop, M.C. and Quilliam, M. (2002) Using a modified ferrous oxidation–xylene orange (FOX) assay for detection of lipid hydroperoxides in plant tissue. J. Agric. Food Chem. 50, 248–254.

Martínez-Júlvez, M., Hurley, J.K., Tollin, C., Gómez-Moreno, C. and Fillat, M.F. (1996) Overexpression in *E. coli* of the complete petH gene product from *Anabaena*: purification and properties of a 49 kDa ferredoxin–NADP+ reductase. Biochim. Biophys. Acta 1297, 200–206.

Martínez-Júlvez, M. (1985) Biosynthesis of ferredoxin–NADP+ oxidoreductase. Evidence for the formation of a functional preholoenzyme in the cytoplasmic compartment. Eur. J. Biochem. 150, 469–474.

Babbs, C.F., Pham, J.A. and Coolbaugh, R.C. (1989) Lethal hydroxyl radical production in paraquat-treated plants. Plant Physiol. 90, 1267–1270.

Vinocur, B. and Altman, A. (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr. Opin. Biotechnol. 16, 123–132.