Drought stress -induced expression of cyclic electron transfer components

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Drought stress -induced upregulation of components involved in ferredoxin-dependent cyclic electron transfer

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Summary

Linear photosynthetic electron transfer, consisting of both Photosystem (PS) II and PSI, converts light energy into chemical form of ATP and NADPH, whereas PSI cyclic electron transfer (CET) is exclusively involved in ATP synthesis. In the chloroplasts of higher plants, there are two partially redundant CET routes. The ferredoxin (FD) or ferredoxin-plastoquinone reductase (FQR) -dependent route cycles electrons from PSI to plastoquinone via ferredoxin (FD), while in the NDH-dependent route NADPH donates electrons to the NDH-complex for reduction of the plastoquinone pool. In the present study, we show that drought stress induces transcriptional and translational upregulation of the *PGR5* and *PGRL1* genes, which so far are the only characterized components of the FQR-dependent CET. In contrast, the expression of the *NDH-H* gene, a representative of the NDH-complex, did not differ between the drought-stressed and the control plants. The overall expression level of the ferredoxin-$NADP^+$-oxidoreductase (FNR) genes increased upon drought stress, with concomitant release of FNR from the thylakoid membrane. Moreover, drought stress accelerated the rate of $P700^+$ re-reduction, which may indicate induction of CET. Responses of the *PSAE*, *FD* and *PSAD* gene families upon drought stress are also described.

Key words: cyclic electron transfer; gene expression; photosynthesis; PGR5; PGRL1

Abbreviations: CET, cyclic electron transfer; Cyt b_6f, cytochrome b_6f complex; FD, ferredoxin; FNR, ferredoxin-$NADP^+$ oxidoreductase; FQR, ferredoxin-plastoquinone reductase; NDH-complex, NADPH dehydrogenase complex; PS, photosystem; S, soluble fraction; T, thylakoid membrane fraction

Introduction

Linear photosynthetic electron transfer results in production of energy-rich compounds NADPH and ATP, which are further used in fixation of CO_2 into carbohydrates. Electron transfer is fuelled by solar energy, which is trapped by protein-bound chlorophyll molecules. Physically these 'light reactions' are located in the thylakoid membranes of chloroplasts, where several large pigment-protein complexes Photosystem (PS) II, Cytochrome b_6f complex (Cyt b_6f), PSI and ATP synthase participate in trapping and conversion of light energy into chemical form.

In addition to linear electron transfer, electrons may be cycled around PSI (Fig. 1). At least two distinct pathways have been introduced: (1) the ferredoxin or FQR-dependent route, and (2) the NDH-dependent route (reviewed by Johnson, 2005; Shikanai, 2007). The FQR-dependent route cycles electrons from PSI via ferredoxin back to the plastoquinone pool. Only two components involved in these reactions have been identified this far, namely PGR5 and PGRL1 (Munekage et al., 2004; DalCorso et al., 2008). The FQR enzyme has remained hypothetical, but some studies have suggested the involvement of ferredoxin-$NADP^+$-oxidoreductase (FNR) protein in FQR-dependent cyclic route, due to the fact that FNR has been found attached to the Cyt b_6f complex (Zhang et al., 2001). Additionally, FNR has been shown to physically interact with the PGRL1 protein (DalCorso et al., 2008). In NDH-dependent pathway, electrons are cycled from PSI to $NADP^+$ via ferredoxin and FNR, and thereafter the thylakoid-embedded NDH complex abstracts electrons from NADPH to reduce plastoquinone (Burrows et al., 1998).

In the present study, we show that drought stress results in transcriptional and translational upregulation of *PGR5* and *PGRL1*, and discuss the possibility of specific induction of FQR-dependent CET pathway upon water deficit.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana ecotype Columbia was grown under standard conditions in phytotron (100 μmol photons $m^{-2}sec^{-1}$, 8-h light/16-h dark cycles, +23°C). Plants were grown on soil:vermiculite (1:1) for four weeks. Thereafter, for the induction of drought stress normal watering of the plants was ceased and the plants were doused with 250 ml of water 7 and 10 days after onset of stress, while control plants were doused as normally. The experiments were performed on day 12 after the onset of stress.

Quantitative RT-PCR

Total RNA extraction, DNase treatment using TURBO DNA-free™ Kit (Ambion, Applied Biosystems, TX, USA), cDNA synthesis with iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), RealTime-PCR reactions using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as well as determinantion of efficiency values were performed as described in (Lintala et al., 2009). Primer sequences are presented in Table 1.

Content and detection of proteins

Thylakoid membrane and soluble proteins were extracted, and SDS-PAGE and immunoblotting performed as in (Lintala et al., 2007). Samples were loaded on protein basis in the linear range for each antibody.

Far-red light induced oxidation and dark re-reduction of P700

PAM-Fluorometer PAM-101/102/103 (Walz) equipped with an ED-P700DW-E emitter-detector unit was used to monitor the redox state of P700 from the dark adapted leaves by absorbance changes at 810 nm using 860 nm as a reference. Leaves were kept in darkness for 10 min prior to the measurement. P700 was oxidized by far-red light from a photodiode (FR-102, Walz) for 30 s, and the subsequent re-reduction of $P700^+$ in darkness was monitored. Kinetic curves of $P700^+$ re-

reduction in the dark was fitted by a single exponential term and the half-times calculated as $t_{½} = \ln 2\ \tau$.

Results

PGR5 and PGRL1 genes are upregulated upon drought stress

Exposure of Arabidopsis plants to drought stress resulted in the distinct phenotype of plants, as the stressed plants were smaller and contained higher quantity of anthocyanins than the control plants (Fig. 2). The plants exposed to drought stress were submitted to gene expression studies by quantitative RT-PCR to elucidate whether the expression of genes encoding proteins potentially participating in CET was changed. *PGR5*, *PGRL1a* and *PGRL1b* expression as the representatives of FQR-dependent CET and the expression of *NDH-H* as a representative of the NDH complex were analyzed. Moreover, distinct gene families coding for different chloroplast isoenzymes of FNR, FD, PSAD and PSAE were monitored. These proteins function on the stromal side of Photosystem I (PSI), and are possibly co-regulated with CET-specific proteins.

Water deficit induced marked upregulation in the expression of *PGR5* and *PGRL1b* (Fig. 3). The expression of *PGRL1a* was likewise consistently enhanced, although the level of upregulation was not so significant. Importantly, the transcriptional upregulation was also reflected at the level of translation. More PGR5 and PGRL1 proteins accumulated in the thylakoid membrane of drought-stressed plants than in those of the control plants (Fig 3B). In contrast, drought stress did not affect the expression of the *NDH-H* gene at transcriptional level, and neither did the accumulation of the NDH-H protein differ between the stressed and control plants (Fig. 3). Next, the expression of the *PSAE*, *PSAD*, *FD* and *FNR* gene families upon drought stress was investigated. Both *FNR* genes as well as *FD1* and *PSAE2* were slightly upregulated at transcriptional level, whereas *FD2*, *PSAD1*, *PSAD2* and *PSAE1* were downregulated (Fig. 3A). In chloroplasts, both FNR isoenzymes are present as membrane-bound and soluble pools (Lintala et al., 2007). Drought stress led to a marked release of FNR from the thylakoid membrane, whereas no

changes in soluble FNR content could be detected (Fig 3B). Downregulation of the *PSAD* genes was evident both on transcriptional and translational levels (Fig. 3). Interestingly, the members of *FD* and *PSAE* gene families had unique expression patterns at transcription level, *FD1* and *PSAE2* being somewhat upregulated upon drought stress. Upregulation of the PSAE protein(s) could not be detected in the stressed plants, whereas markedly more FD accumulated into the leaves of drought stressed plants as compared to the control plants (Fig. 3). However, the method used did not allow a distinction between the different PSAE and FD isoforms.

Relative expression of genes within a given gene family

Under standard conditions, 99% of *PGRL1* mRNA pool originated from *PGRL1A*, whereas *PGRL1B* transcripts comprised only 1% of the total leaf *PGRL1* mRNA pool. The total *PGRL1* transcript pool showed a marked increase upon drought stress, and especially the expression of *PGRL1B* gene was enhanced (Fig. 4). Similarly, the expression of both *FNR* genes increased ca. 50% upon drought stress, but the ratio of *FNR1* to *FNR2* transcript (36% *FNR2* mRNA, 64% *FNR1*mRNA) did not change due to the stress treatment (Fig 4; Lintala et al., 2009). In contrast, drought stress downregulated the total amount of transcripts in all other studied gene families. Nevertheless, the relative expression of genes within a given family showed modified expression under water deficit, including enhanced expression of *FD1* at the expense of *FD2*, and *PSAE2* at the expense of *PSAE1*, whereas the relative expression of *PSAD* genes remained unchanged (Fig. 4).

Drought stress enhances the rate of $P700^+$ re-reduction

To examine physiological status of PSI upon drought stress, the redox state of P700 was studied in the darkness following far-red illumination. Exposing of plants to drought resulted in acceleration of $P700^+$ re-reduction ($t_{½}$ = 0.82 s ± 0.007), as compared to the control plants ($t_{½}$ = 1.13 s ± 0.010). These results are in line with previous findings suggesting that cyclic electron flow around PSI is activated under drought stress (Golding et al., 2004).

Discussion

The biological relevance and the routes of CET around PSI have been under extensive study and intense discussion during the past decades. Recent discoveries of new molecular components of the thylakoid membrane, such as plastidial NDH complex composed of several nuclear-encoded and eleven plastid-encoded subunits (Rumeau et al., 2005; Suorsa et al. 2009), the PGR5 (Munekage et al., 2004) and PGRL1 (DalCorso et al., 2008) proteins as well as the plastid terminal oxidase (Wu et al., 1999) have provided evidence for the existence of these alternative electron transfer pathways also in C_3 plants (Fig. 1). In the present study, we tested whether the components of distinct pathways are induced upon drought stress.

The genes encoding the only known components of FQR-dependent CET, PGR5, PGRL1a and PGRL1b, were clearly and consistently upregulated upon drought stress, both at transcriptional and translational level (Fig. 3), suggesting that the FQR-dependent pathway may be specifically induced upon drought stress. In contrast, no such increase could be detected in the expression of the *NDH-H* gene, implying that the NDH-dependent pathway may not be the dominating route of CET upon water deficit.

The two genes encoding leaf FNR isoforms were somewhat upregulated at transcriptional level (Fig. 3), whereas the total amount of the FNR protein in the water-stressed leaves decreased. FNR seems to be released from the thylakoid membrane upon drought stress, and is probably degraded, since no net increase in the soluble FNR pool was detected (Fig. 3). Similar release of FNR from the thylakoid membrane occurs when the plants are exposed to high light (Benz et al., 2009). In case the attachment of FNR to the Cyt b_6f complex is a prerequisite for CET, as suggested in (Zhang et al., 2001), the release of FNR from the thylakoid membrane upon drought stress points to the possibility that FNR is not a crucial component of FQR-dependent CET. The *PSAE1* gene product has been suggested to bind FNR to PSI (Andersen et al., 1992), and this gene showed transcriptional downregulation upon drought stress. However, *PSAE2* was slightly

upregulated and no difference in the total level of the PSAE protein was detected between the stressed and the control plants (Fig. 3). Thus, it remains to be elucidated whether PSAE2 also is important in membrane tethering of FNR.

The genes encoding the two isoforms of PSAD, which is docking ferredoxin to PSI, were downregulated both at the transcriptional and translational level. Although the expression of the FD gene family was decreased at transcriptional level, the relative expression of *FD1* gene was upregulated (Fig. 4), and the level of FD protein in the drought-stressed plants was increased as compared to control. Thus, FD1 might be dedicated to CET, while linear electron flow may mainly rely on FD2, which upon normal growth conditions is the dominant form of FD in Arabidopsis (Hanke and Hase 2008; Voss et al., 2008).

Moreover, drought stress enhanced the rate of $P700^+$ re-reduction. $P700^+$ re-reduction in darkness has been used as an indication of CET rate in some studies (Bukhov et al., 2004; Golding et al., 2004; Fan et al., 2007), although there is no consensus whether this method actually represents CET in general, nor a distinct CET pathway. Nevertheless, it is well known that in response to drought, higher plants close the stomata to restrict transpiration, which leads to lowered internal CO_2 concentration and enhanced CET (Golding et al., 2004; Johnson, 2005; Rumeau et al., 2007).

Taken together, we conclude that drought stress i) induces the expression of genes encoding components specifically involved in FQR-dependent CET route, and ii) results in an increase of the overall expression level of the *FNR* gene family at the transcriptional level, as well as iii) in a release of FNR from the thylakoid membrane. Furthermore, iv) drought stress accelerates the rate of $P700^+$ re-reduction in darkness.

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Tables

Table 1.

Gene	Primers
PGR5	for 5'-ACC AAA CCA TGC TCT CCA AG-3'
At2g05620	rev 5'-CAA TGG CTT TTC CTC TGA GC-3'
PGRL1a	for 5'- CAC ATC TTC AAC CAC AGG TTC -3'
At4g22890	rev5'- GAA GAG GAA GGT TTG CGA GA -3'
PGRL1b	for5'- CAA CCA CAC AAA TCC AAA GC-3'
At4g11960	rev5'- TTT GCG AGA AAT TGC AGA AA -3'
NDH-H	for 5'-ATG GGA AAT TCA ATG GCA AA-3'
AtCg01110	rev 5'-TCA AAG CCC CTG CTT TCT AA-3'
FNR1	for 5'-CTG CAG TCT CTT TAC CTT CCT CC-3'
At5g66190	rev 5'-GAC AAC AAT CCC TTC TTC CTG TTT C-3'
FNR2	for 5'-GGC GAC TAC CAT GAA TGC TGC-3'
At1g20020	rev 5'-GTC TGT ACC TGT TAA CAA TCA CAC -3'
FD1	for 5'-AAT TTC ATC AAA AGA GAA ATT ACT TGA-3'
At1g10960	rev 5'-TTG ATT GAT CTT ATA AAA GGA TGA GC-3'
FD2	for 5'-GAA GAA GAC ATT GTT TAA GCC TCA-3'
At1g60950	rev 5'-GAT TGA TGG TGA GCC AAA CC-3'
PSAD1	for 5'-CCA AAA ACT ATG TGC ATG TGG-3'
At4g02770	rev 5'-TTT AGG CCC ATA AAA GAT CCA-3'
PSAD2	for 5'-CAT GTA AAA TCT TGC GGA TGT-3'
At1g03130	rev 5'-ACC CTG TCC CAA GTA ATG GA-3'
PSAE1	for 5'-CCG CAA AAG TTT ACC AAT TAT TTC-3'
At4g28750	rev 5'-GAA AGA GAC TTT TAA CTG AAT TTT CCA-3'
PSAE2	for 5'-CCG CTA AGG CTA AAC CTC CT-3'
At2g20260	rev 5'-ATT CGC GTA ATT CAC CTT GG-3'
PSBO	for 5'-TGC TCA CAG CTT TGG ATC AC-3'
At5g66570	rev 5'-ACT GGA AGG AGC AAG TGA GG-3'

Legends of figures

Figure 1. Putative routes of cyclic electron transfer. Linear electron transfer from water through Photosystem II (PSII), plastoquinone (PQ) pool, Cyt b_6f complex (Cyt b_6f), Photosystem I (PSI), ferredoxin (Fd) and ferredoxin-$NADP^+$-oxidoreductase (FNR) to $NADP^+$ is presented as solid arrows. FNR is present both as soluble and thylakoid-bound forms. Routes for cyclic electron transfer are shown as dash/dotted arrows. In Fd-dependent pathway (1), electrons are transferred from P700 of PSI to the plastoquinone pool, via ferredoxin, hypothetical ferredoxin-plastoquinone reductase (FQR), PGR5 and PGRL1 proteins, and possibly FNR. NDH-dependent pathway functions in two steps. In reaction 2a electrons are transferred from NADPH to NDH-1 complex and in reaction 2b electrons are transferred from NDH-1 to Cyt b_6f via plastoquinone pool.

Figure 2. Phenotype of the Arabidopsis control and drought-stressed plants after 12 days treatment.

Figure 3. Relative amount of transcripts in the drought-stressed plants as compared to the control.
A) Expression of the *PGR5, PGRL1a, PGRL1b, NDH-H, PSAE1, PSAE2, PSAD1, PSAD2, FD1, FD2, FNR1* and *FNR2* genes that potentially participate in CET. The columns denote the relative amount of transcripts of the studied genes upon drought stress as compared to the control, normalized to the expression of a reference gene *PSBO*, and studied by quantitative RT-PCR. 1 denotes for no change in transcript levels, <1 denotes for decreased level of transcripts, and >1 denotes for increased level of transcripts upon drought stress as compared to the control.
B) Protein content of the control and drought stress treated plants. After SDS-PAGE, proteins were electroblotted on a PVDF membrane and probed with protein-specific antisera.
Total RNA and proteins were extracted from control plants and the plants grown under drought stress. RT-PCR reactions and protein detection were performed as described in Material and methods. Results are representatives of three biological replicates with similar results.

Figure 4. Relative expression of genes within a given gene family under control growth conditions and under drought stress.

A) Relative expression of the members of *PGRL1* gene family in control and drought stressed plants.

B) Relative expression of the members of *PSAE* gene family in control and drought stressed plants.

C) Relative expression of the members of *PSAD* gene family in control and drought stressed plants.

D) Relative expression of the members of *FD* gene family in control and drought stressed plants.

E) Relative expression of the members of *FNR* gene family in control and drought stressed plants.

Total RNA was extracted from plants grown under control conditions and drought stress, and RT-PCR reactions were performed as described in Material and methods. The bars indicate the relative amount of transcripts in percentages per total amount of the control (100%). The numbers within (or above) the bars indicate the fraction (%) of each transcript in the same gene family. Results are means from three biological replicates.

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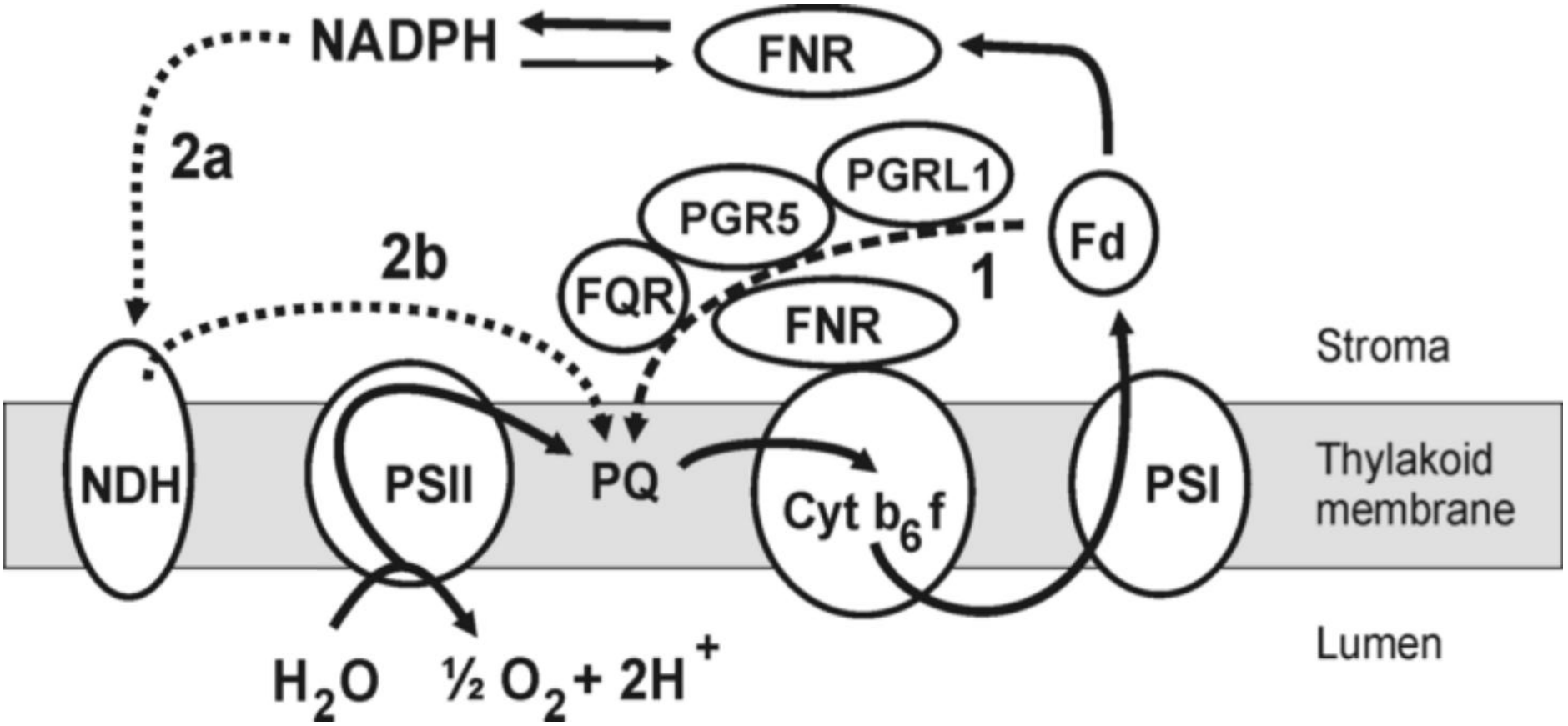

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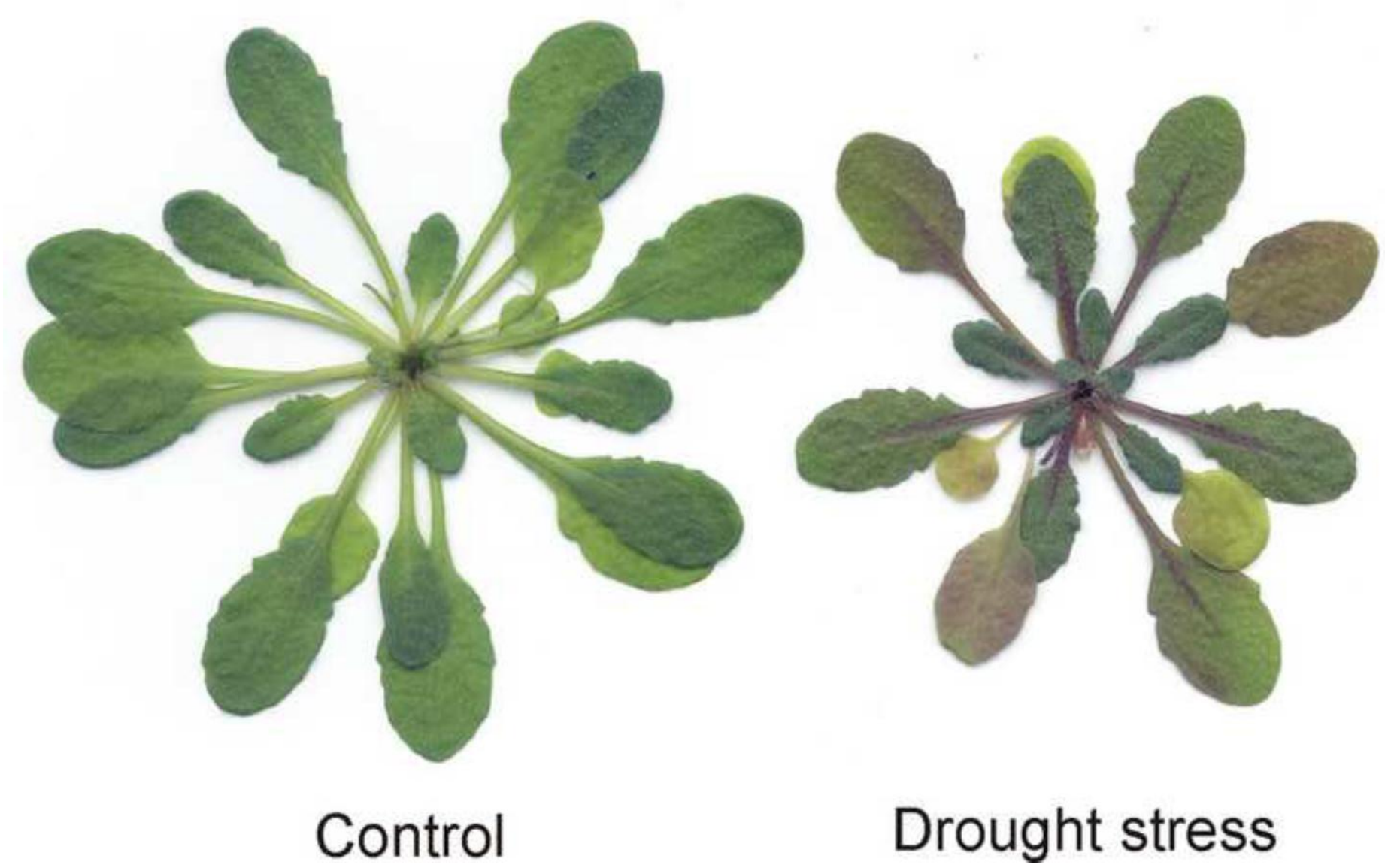

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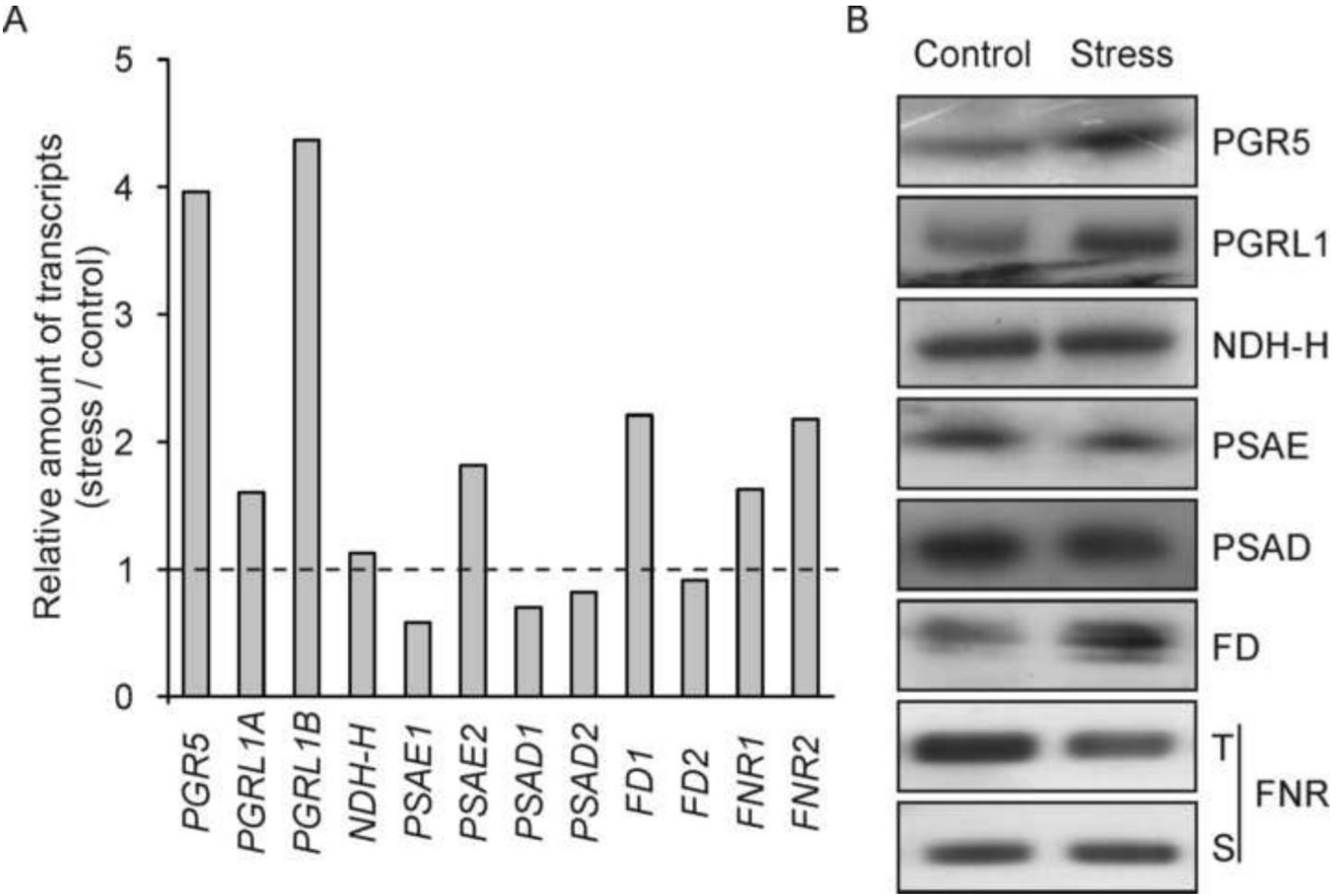

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