Altered gene expression by sedaxane increases PSII efficiency, photosynthesis and growth and improves tolerance to drought in wheat seedlings

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KEYWORDS

Sedaxane, Photosystem II, Gene expression, Drought, Wheat

ABSTRACT

Succinate dehydrogenase inhibitor (SDHI) fungicides have been shown to increase PSII efficiency and photosynthesis under drought stress in the absence of disease to enhance the biomass and yield of winter wheat. However, the molecular mechanism of improved photosynthetic efficiency observed in SDHI-treated wheat has not been previously elucidated. Here we used a combination of chlorophyll fluorescence, gas exchange and gene expression analysis, to aid our understanding of the basis of the physiological responses of wheat seedlings under drought conditions to sedaxane, a novel SDHI seed treatment. We show that sedaxane increased the efficiency of PSII photochemistry, reduced non-photochemical quenching and improved the photosynthesis and biomass in wheat correlating with systemic changes in the expression of genes involved in defense, chlorophyll synthesis and cell wall modification. We applied a coexpression network-based approach using differentially expressed genes of leaves, roots and pregerminated seeds from our wheat array datasets to identify the most important hub genes, with top ranked correlation (higher gene association

value and z-score) involved in cell wall expansion and strengthening, wax and pigment biosynthesis and defense. The results indicate that sedaxane confers tolerant responses of wheat plants grown under drought conditions by redirecting metabolites from defense/stress responses towards growth and adaptive development.

1. INTRODUCTION

Drought is considered the most important environmental factor limiting growth, plant metabolism and crop productivity worldwide [1]. Photosystem II (PSII) is the most important protein-pigment complex in the chloroplast that is also most vulnerable to drought stress [2]. Under severe drought, often associated with elevated leaf temperatures and light levels, the limitation in CO_2 uptake coupled with an increased excitation energy in PSII and absorption of light energy in excess of that required for photosynthesis causes an imbalance between PSII activity and the Calvin cycle. This can result in photodamage to the PSII oxygen-evolving complex [3],[4], disruption of D1 protein involved in PSII repair, and subsequent inactivation of PSII reaction centers [5]. To protect the chloroplast, plants have evolved photoprotective responses to rapidly dissipate excess excitation energy as heat. Thermal dissipation of light energy by the light-harvesting antenna complex of PSII, measured as non-photochemical quenching (NPQ), is one of the most important rapidly activated regulatory mechanisms in plants to avoid irreversible photodamage [6]. NPQ is triggered by the light-driven build-up of a transthylakoid proton gradient (ΔpH). The acidification of the thylakoid lumen results in the protonation of PSII LHC antenna regulatory proteins such as PsbS [7] and the de-epoxidation of xanthophyll cycle pigment violaxanthin into zeaxanthin [6],[8]. Whilst reducing the likelihood of photoinhibitory damage, NPQ momentarily reduces the quantum yield of CO_2 assimilation. Although this is a highly regulated process that reduces the likelihood of oxidative stress, photoprotection can also be considered to compete with photochemistry for absorbed energy [9]. Plant under drought stress typically show rapid increase in NPQ with increasing illumination coupled with decreased capacity for photosynthesis [10]

Fungicides of the class of succinate dehydrogenase complex II inhibitors (SDHIs) however have been recently shown to significantly increase the efficiency of PSII photochemistry (Fv′/Fm′) of wheat grown under drought stress, in the absence of disease, resulting in improved photosynthesis and yield under controlled and field conditions [11],[12]. Changes in Fv′/Fm′ were detected in plants grown in field and under controlled environments within 4 h of

fungicide application. Fv′/Fm′ is indicative of changes in PSII operating efficiency attributed to thermal dissipation, which correlates in a non-linear fashion with decreasing thermal dissipation of excitation energy in the light harvesting complexes of PSII, estimated as non-photochemical quenching or NPQ [2]. Thus it is likely that increased PSII efficiency (indicated as Fv′/Fm′) and improved photosynthesis in SDHIs treated plants may be accompanied by reductions in NPQ. It is currently unclear how this effect on PSII and photosynthesis occurs: the succinate dehydrogenase (SDH; succinate: ubiquinone oxidoreductase) complex plays a central role in mitochondrial metabolism, catalyzing the oxidation of succinate to fumarate and the reduction of ubiquinone to ubiquinol, thereby linking the tricarboxylic acid (TCA) cycle and the electron transport system. In fungi, SDHIs specifically block the ubiquinone-binding sites in the mitochondrial complex to disrupt cellular respiration and energy generation [13]. Although the mode of action of these compounds on fungal metabolism is well understood, the effects on plant metabolism and the molecular basis of the observed physiological responses to drought stress in SDHI treated plants remain unknown.

In this work, we investigated the effects of sedaxane, a novel SDHI fungicide, belonging to the chemical class of pyrazole-carboxamides, formulated to use on crops as seed treatment to provide local and systemic protection of the seed, seedling and roots against soil-borne plant pathogenic fungi [14]. The active ingredient is typically absorbed from the soil matrix by the developing plant roots and translocated within the seedling with systemic activity of 4-6 weeks following seed germination. We combined chlorophyll fluorescence with gas exchange measurements to measure PSII efficiency and photosynthesis of plants grown from sedaxane treated seeds. Our aim was to better understand the molecular mechanism for improved photosynthetic efficiency, growth and biomass of SDHI-treated wheat grown under drought stress in the absence of disease using transcriptomics approach. The objective of this paper was to address the following questions. (1) Can sedaxane improve photosynthesis and PSII efficiency and is this characterized by low NPQ under drought? (2) Are these phenotypic effects associated with transcriptomic changes? (3) Do these changes lead to modifications

in physiological processes with sedaxane applied as seed treatment? We integrated whole plant physiological responses with changes in global gene expression in leaf tissues to obtain more comprehensive understanding of the regulatory genetic mechanisms underlying the physiological responses of SDHI treated plants under drought stress.

The focus of our investigation was the leaf as the main photosynthetic organ maximizing carbon assimilation [15] and a major target for improving photosynthetic efficiency. However, the root and pregerminated seed tissues were included in the gene co-expression and gene network analysis to aid our understanding of the interactions regulating plant responses to sedaxane under drought conditions because of translocation and systemic activity of sedaxane into developing tissues following seed germination.

2. MATERIALS AND METHODS

2.1. Plant Growth Conditions and Experimental Design

Two experiments were carried out. Experiment 1 was used to measure sedaxane treated plants for photosystem II efficiency and photosynthesis using detailed chlorophyll fluorescence and gas exchange analysis. Experiment 2 was designed for transcriptomics analysis. Plant tissues were collected for RNA isolation as soon as changes in PSII efficiency were confirmed on indicator plants using portable fluorometer (Fluorpen FP100, Photon System Instruments, Czech. Republic).

Winter wheat seed (cv. Gallant) were treated with Sedaxane at 10g a.i/100kg seed (Syngenta Crop Protection UK, Cambridge) or left untreated. Untreated and treated seeds were initially tested on potato dextrose agar medium (PDA) for any fungal or bacterial infection to ensure that only healthy seeds were used in all experiments [16]. Plants were grown in a walk-in growth chamber at the University of Nottingham with controlled temperature and light intensity of 15^{o}C and 300 µmolm^{-2}s^{-1}, respectively. Photoperiod was maintained at 8 h light/16 h dark throughout the course of the experiment. Seeds were pre - germinated on water moistened filter paper for 2 d prior to planting into 9cm, 0.36L pots filled with either compost (John Innes 2, experiment 1) or γ-radiated loamy sand soil (experiment 2) prepared as described by Sturrock et al. [17]. The amount of water in soil available to the plants at field capacity was determined as described by Ajigboye et al. [11]. Water was initially supplied to 60% of available water at full field capacity (AW_{FC}) and maintained at either 10% or 90% AW_{FC}.

Experiment 1 was designed as randomized block with two factors, fungicide treatment (sedaxane treated or untreated) and soil moisture (90% or 10% available water at full field capacity). There were seven replications of each treatment. Plants were divided into two groups; “drought-stressed” and “non-stressed”, each group with equal number of treated and untreated seedlings. Water was withheld from the drought-stressed plants to attain 10% available water at full field capacity (AW_{FC}) by 8 days after germination (DAG) while non-

stressed plants were supplied with sufficient water to attain 90% AW_{FC} at 3 DAG and maintained at the same available water until the end of the experiment. Experiment 2 was designed as randomized block with two treatments, sedaxane or untreated and consisted of 22 replicates, seven of which were considered as indicator plants while samples for RNA isolation were collected from the remaining 15 replicates. All plants were maintained at 10% AW_{FC} from 5 DAG.

2.2. Experiment 1: Photosynthetic Efficiency and Growth Analysis

The polyphasic rise in chlorophyll a fluorescence (OJIP) transient was measured using portable fluorometer (Fluorpen FP100, Photon System Instruments, Czech Republic) between 12-2pm daily from 9 to 11 DAG. Leaves were not dark adapted prior to obtaining measurements. Therefore, we describe minimal and maximal fluorescence as Fo' and Fm', respectively. OJIP transient was induced by strong light pulse of 3000 µmol $m^{-2}s^{-1}$. Data extracted along the recorded transient include fluorescence intensity at 50 µs, considered to be minimal fluorescence Fo', fluorescence intensity at J-step (2 ms), i-step (60 ms) and at the peak of the transient P (=Fm'). Fv'/Fm' was computed as [(Fm'-Fo')/Fm']. Biophysical parameters involving energy fluxes per reaction centers were automatically computed from the transient curve using the JIP test as defined by Strasser et al [18]. Photosystem II quantum yield was measured independently of the OJIP transient. Measuring light of 900 µmol $m^{-2}s^{-1}$, was applied to acquire minimal fluorescence *Fo'* followed by a saturating light pulse of 3000 µmol $m^{-2}s^{-1}$ to measure *Fm'*. QY is considered equivalent to [Fv'/Fm'] in light adapted plants. All measurements were made on the youngest fully expanded leaf on each plant.

At 12 DAG, light response of gas exchange and chlorophyll fluorescence were quantified simultaneously using an infra-red gas analyzer, LI6400XT, equipped with leaf chamber pulse-amplitude modulated fluorometer LI6400-40 (LI-COR, Lincoln, NE, USA). Leaves were dark adapted in the growth chamber for 60 min prior to measurement by wrapping sections

of the leaf in low-weight silver aluminium foil. The dark-adapted leaves were placed in the chamber, they were left for 5 min in the dark before F_0 was measured and then a saturating pulse applied to measure F_m. At this point the actinic light was applied. In the light-adapted state F_m' was measured by applying a saturating pulse of 7000 µmol m^{-2} s^{-1} (for 0.8 s). F_0' was measured by switching off the actinic for 2 s after the saturating pulse and applying far-red (FR) light. A series of illumination at PAR values was started at 0 and shifting to 2000, waiting for 3 min at each light intensity before measurement. Fluorescence and gas exchange parameters were calculated directly from the Licor software (https://www.licor.com/env/products/photosynthesis/LI-6400XT/software_downloads.html). Measurements were made under constant leaf temperature of 18^oC, CO_2 concentration of 400µl L^{-1}, relative humidity 50- 55%, gas flow rate 500µmol air s^{-1} and photosynthetic photon flux density (PPFD) of 1000 µmol$m^{-2}s^{-1}$.

Plant height was measured from the base of the plant to the tip of the longest leaf. Plants were harvested 32 days after transplanting, fresh weights were measured before plants were oven-dried at 80^oC for 72 h to a constant weight. Dry weight was defined as dry weight/fresh weight. Percentage water content was defined as (fresh weight – dry weight)/fresh weight.

2.3. Experiment 2: Gene Expression Analysis

2.3.1. Sampling

Chlorophyll (Chl) a fluorescence transient (OJIP) induced as described in experiment 1, was measured daily on the fully expanded leaf on the main shoot of plants considered as indicator plants from 5 DAG. OJIP was induced as described in experiment 1. As soon as significant differences ($P< 0.05$) in PSII efficiency (Fv'/Fm') between treatments were detected in the indicator plants at 9 DAG, leaf and root samples were collected individually from the remaining 15 replicates for RNA extraction.

2.3.2. RNA Extraction

Harvested tissues were immediately frozen in liquid nitrogen and stored at −80°C prior to processing. Total RNA from leaf and roots of sampled plants as well as pre-germinated seeds (2 d) was extracted from 100mg tissue. Frozen tissues were homogenized in TRIzol using a FastPrep-24 (MP BIO) and lysing matrix D. Extracted RNA was then purified (RNeasy Mini Kit, Qiagen). The extracted RNA was quantified with a NanoDrop ND-2000 UN-VIS spectrophotometer (Thermo Scientific) and the integrity checked by fragment length on 2% agarose gel electrophoresis.

2.3.3. Microarray Experiments

RNA from 3-5 individual plants was combined into one sample per treatment and replicate. Eighteen arrays were used in total, representing two treatments, three tissue types and three replicates. Hybridization of biotin-labelled RNA to Affymetrix Wheat GeneChip arrays and array scanning were carried out at the University of Nottingham Affymetrix Microarray service according to the manufacturer's instructions (www.affymetrix.com/support/technical/manual/expression.manual.affx). Normalization and analysis of differential expression was carried out using GeneSpring GX13 (Agilent Technologies). Baseline preprocessing and normalization were carried out using the Robust Multiarray Average summarization algorithm (RMA), as described by Irizarry et al. [19]. Tissues from the leaf root and pre-germinated seed were examined separately. A one-way ANOVA with Benjamini Hochberg FDR multiple test correction was applied in order to select genes that reveal significant changes ($P<0.05$) in their expression. All treatments for the tissues were compared with the control experiment of corresponding tissue. A cutoff value of 1.5-fold change was adopted to discriminate expression of genes that were differentially expressed in response to sedaxane treatment.

2.3.4. Gene Ontology Enrichment and Functional Pathway Analysis

To categorize differentially expressed genes based on their biological functions, list of genes identified by microarray analysis (≥ 1.5-fold change) were submitted to MapMan for analysis

[20]. Transcripts were assigned into functional categories (or bins) of metabolism and cell function. The Wilcoxon Rank Sum test corrected with Benjamini Hochberg FDR multiple test was used to identify differentially regulated bins. Gene ontology enrichment of the gene lists was O carried out using the Parametric Analysis of Gene Set Enrichment (PAGE) in the agriGO toolkit (http://bioinfo.cau.edu.cn/agriGO/analysis.php) [21]. Benjamini-Hochberg multi-test adjustment method for the P-value was selected. P-value of 0.05 and false discovery rate (FDR) <0.05 was used as a cutoff to select significantly enriched GO terms.

2.3.5. Genome-scale gene network analysis

A web-based Genome-scale gene network method was used. RMA normalized microarray data were uploaded to the DeGNServer http://plantgrn.noble.org/DeGNServer/Analysis.jsp. Networks with reduced edge densities were generated on the basis of co-expression (cut-off >0.8) and Context Likelihood or Relatedness (CLR, at a cut-off of >3.6). The constructed network and sub network were uploaded into Cytoscape [22] for visualization. The ranked genes and common subgraphs of key differentially expressed genes were produced in both the DEGNserver and Cytoscape. All the differential expressed genes of each set were selected to build a sub-expression profile unit, and were implemented for correlation analysis by value-based co-expression network method (gene association value). Considering the computative speed and empirical accuracy comparison, z-scores value based co-expression method and Spearman's rank correlation estimation method were applied in our analysis.

2.3.6. qRT-PCR

To validate the microarray experiment, RNA from microarray as well as from an independent experiment was used for qRT-PCR. DNase treated RNA were from the microarray experiment and from plants of a different seed lot grown under the same controlled environmental conditions described earlier. qRT-PCR was performed for six genes from the gene network analysis (Table S1). CFX96 (Bio-Rad, UK) was used for qPCR with iTaq™ Universal SYBR® Green one-step kit (Bio-Rad). Reactions consisted of 2 µL of 20 ng of total

RNA, 0.012 µL of 300nM forward and reverse primers, 5 µL of iTaq™ Universal SYBR® Green reaction mix (2x), 0.125 µL of iScript reverse transcriptase and 2.8 µL of nuclease-free water for a final reaction volume of 10 µL. Reactions were under the following conditions: 50°C for 10 min, 95°C for 1 min, then 40 cycles of 10 s at 95°C for denaturation and 15 s at 60°C for annealing, extension and plate read. At the end of each reaction, dissociation curve was performed from 65°C–95°C in 0.5°C increments for 0.05 s, which confirmed a single peak for each set of primers. No-template controls were included for each primer set per run to confirm the absence of contamination and primer dimer. No-template control consistently recorded no signal or were significantly supressed, with signals recorded after 10 or more cycle threshold above the target signal. No-reverse transcription controls were run for each RNA sample to confirm the absence of genomic DNA contamination. The PCR reactions were performed in triplicates for each gene being validated. The quantification cycle (Cq) for each type of PCR product were determined for all samples using Bio-Rad CFX Manager 3.1 (Bio-Rad, UK). All Cq values were normalized to two reference genes, Ubiquitin-conjugating enzyme and Cell division control protein, AAA-superfamily of ATPases [23].

2.4. Statistical analysis

Analysis of variance (ANOVA) of chlorophyll fluorescence, and gas exchange parameters, and biomass were performed with Genstat 16th Edition (VSN International). Treatments were considered significantly different at Least Significant Difference (LSD) of 5% ($P \leq 0.05$).

3. RESULTS

3.1. Sedaxane Improves Wheat Photosynthetic Efficiency under Drought Stress Conditions

We first used fast induction chlorophyll fluorescence (OJIP) transient to rapidly quantify changes in chlorophyll fluorescence parameters measured on plants grown at 10% (drought-stressed) and 90% (non-stressed) available water at field capacity (AW_{FC}). This technique allowed us to monitor the efficiency of photosystem (PS) II of the youngest fully expanded leaf of the primary plant tiller over a 3 d period. Across both treatments, there were no interactions between sedaxane and water regime. The main effect of sedaxane treatment from 9 to 11 DAG on efficiency of PSII photochemistry (Fv'Fm'), quantum yield (QY) and dissipated energy flux (DIo/RC) are shown in Fig. 1. The efficiency of PSII photochemistry (Fv′/Fm′) increased with sedaxane treatment from 9 DAG compared with the untreated control, with the highest increase ($P<0.05$) observed at 11 DAG (Fig. 1A). A similar trend was observed for QY (Fig. 1B). Dissipated energy flux (DIo/RC) per PSII active reaction center was lower in plants grown from sedaxane treated seeds from 9 DAG and remained lower (8% $P<0.05$) than the untreated plants 11 DAG (Fig. 1C). The above results showed that sedaxane had a significant impact on PSII photochemistry. Therefore, we quantified the effect of sedaxane on the photosynthetic performance of drought-stressed and non-stressed plants through simultaneous measurement of leaf chlorophyll fluorescence (CF) and gas exchange parameters 12 DAG under a range of incident light fluxes. Drought stress (10% AW_{FC}) at 12 DAG resulted in a significant decrease in qP compared to non-stressed plants (90% AW_{FC}) at higher light intensities above 750 µmol $m^{-2}s^{-1}$ (Fig. 2). The rate of stomatal conductance under drought stress declined by almost 17% ($P<0.05$) at each light intensity compared to the non-stressed control (Fig. S1A). A similar trend was observed for the rate of leaf transpiration although the effect was significant ($P<0.05$) under higher light intensities between 1200 to 2000 µmol m^{-2} s^{-1} (Fig. S1B). Sedaxane-treated plants under both water availability regimes had 8% lower NPQ compared to untreated ($P<0.05$) at high light intensities above 1000 µmol m^{-2} s^{-1} (Fig.

278 3A). Generally, under the range of incident light fluxes, the rate of photosynthesis was 10%
279 lower in drought-stressed plants than in non-stressed control. However, interactions between
280 fungicide and AW_{FC} treatment showed that photosynthesis was 8% higher in sedaxane-treated
281 plants grown under drought stress compared to untreated control ($P< 0.05$; Fig. 3B).

282 At 12 DAG no further interactions were detected between treatments and the main effects of
283 sedaxane or water availability on ΦPSII, Fv/Fm and Fv'/Fm' were not significant at LSD of 5%
284 (data not shown).

285 Drought stress (10% AW_{FC}) 36 DAG significantly reduced ($P<0.001$) tiller number by 78%
286 (Table 1). There were significant interactions between seed treatment and AW_{FC} for plant
287 height, total percentage water content and dry weight. Under drought stress, sedaxane
288 increased plant height by 7% ($P=0.044$), reduced percentage water content ($P=0.027$) and
289 increased dry weight by 37%, ($P=0.027$) compared to untreated control.

290 **3.2. Microarray Analysis of Sedaxane-Responsive Genes**

291 We showed that photosynthetic efficiency, photosynthesis and biomass increased in wheat
292 plants from sedaxane treated seeds under drought conditions. To further understand the
293 molecular basis of the observed physiological effects, we performed microarray analysis
294 (Wheat GeneChips; Affymetrix) to determine gene expression. Genes were considered
295 differentially regulated if their expression was significantly different from the untreated control
296 ($P<0.05$). A total number of 4369 differentially regulated genes (adjusted P value of <0.05)
297 were identified and we used a minimum cut-off of 1.5-fold change to identify genes that were
298 robustly regulated by sedaxane (Table S2). The number of genes differentially regulated in
299 response to sedaxane was 2200 in leaves (898 up-regulated and 1302 down-regulated), 514
300 in roots (237 up-regulated and 271 down-regulated) and 2066 in pre-germinated seeds (615
301 up-regulated and 1452 down-regulated). Comparison of the microarray data from all of the
302 three tissues did not show any up-or down-regulated (Fig. 4), however less than 3% of the
303 differentially expressed genes overlapped between any two tissues Thus, most of the

regulated genes were tissue specific, indicating that pre-germinated seeds, leaves or root tissues respond to sedaxane by activating distinct sets of genes.

3.3. Functional Classification of Sedaxane Responsive Genes with Altered Expression

MAPMAN software [20] was used to gain insight into the biological processes affected by sedaxane in each of the three tissues considered (Table 2 & 3; $P < 0.05$, Wilcoxon rank sum test in the MapMan tool). In the overview of cell function, analysis of differential gene expression in pregerminated seeds revealed a down-regulation of genes assigned to the categories DNA synthesis/chromatin structure encoding core histone H2A/H2B/H3/H4 domain containing protein and biotic stress generally encoding genes associated with disease resistance proteins, HEVEIN FAMILY PROTEIN, PATHOGENESIS-RELATED (PR) PROTEINS although majority of genes in the seeds were unassigned (Tables 2 & Table S3A). In roots, genes involved in biotic stress such as those encoding the PATHOGENESIS-RELATED PROTEINS and DEFENSIN-LIKE PROTEINS were down regulated. (Tables 2 & Table S3B). In the leaf, an overview of the transcriptional responses affecting genes coupled to cell function showed that genes connected to protein synthesis were up-regulated (Table 2 & Table S3C). These up-regulated genes encode the various sub units (30S, 40S, 50S and 60S) of ribosomal protein from the chloroplasts. In contrast, genes involved in hormone metabolism, signaling and biotic stress were generally down regulated (Table 2 & Table S3C). For example, in pathways involved in hormone metabolism, genes encoding jasmonate biosynthetic precursors, ethylene, auxin and abscisic acids were down-regulated. Similarly, most of the genes involved in signaling were down-regulated, including genes associated with CALCIUM SIGNALING, MITOGEN-ACTIVATED PROTEIN KINASES, LEUCINE RICH REPEAT PROTEIN KINASES FAMILY PROTEIN, although two genes associated with light signaling, encoding the EARLY LIGHT INDUCIBLE PROTEIN HV58, known to function against chlorophyll induced oxidative damage [24] were activated. In the stress category,

genes encoding defense related proteins and PR-proteins were generally down regulated except a dirigent-like protein which was upregulated (Table S3C).

In the overview of metabolism, enriched functional categories were detected in the leaf tissue only (Table 3). It is likely that several important transcripts which can exert significant changes in downstream gene expression to lead to a substantial biological effect were eliminated [25] in pregerminated seeds and roots due to our stringent criteria (fold change ≥1.5). A closer look at the categories showed that genes involved in cell wall modification and tetrapyrrole synthesis categories were up-regulated (Table 3; Table S4). Upregulated genes related to cell wall modifications include the cell wall loosening EXPANSINS and cell wall-strengthening enzymes, XYLOGLUCAN ENDOTRANSGLYCOSYLASES (XTHs, Table S4). The set of genes involved in tetrapyrrole synthesis include those encoding chlorophyll precursors corresponding with the various steps in chlorophyll biosynthesis including DELTA-AMINOLEVULINIC ACID DEHYDRATASE, UROPORPHYRINOGEN DECARBOXYLASE, PROTOPORPHYRINOGEN IX OXIDASE, MG-PROTOPORPHYRIN IX and PROTOCHLOROPHYLLIDE REDUCTASE (Table S4).

We also explored changes in the abundance of transcripts from genes that mediate known biological processes and molecular function in the tissues using the Parametric Analysis of Gene Enrichment Analysis (PAGE) tool of agriGO [21]. Results showing the most enriched GO terms from these analyses are shown in Table 4 and 5. In pregerminated seeds, GO analysis identified molecular functions that were significantly enriched in up-regulated genes with the terms glutathione transferase activity and cofactor binding while down-regulated genes were enriched in DNA binding (Table 4). For biological processes, the most significantly enriched biological process was glutathione metabolic processes and nucleosome assembly for up-regulated and down regulated genes respectively (Table 4). There were no significantly enriched GO terms in the root. In the leaf, molecular functions with highly enriched GO terms for up-regulated genes were ribosomal RNA binding, GTP binding, structural constituent of ribosome and transferase activity while down-regulated genes enriched GO terms were

related to protein serine/threonine kinase activity and co-enzyme binding (Table 5). Enriched GO terms involved in biological processes for up-regulated genes were translation, ribosome biogenesis and chlorophyll metabolic process and significantly enriched categories for down-regulated genes were jasmonic acid biosynthetic process, defense response and response to other organisms (Table 5).

3.4. Co-expression and Gene Regulatory Network Analysis

We analyzed co-expressed genes to identify the functional associations between sedaxane responsive genes that are part of the same biological process and may be under similar transcriptional control in all three different tissues. To identify genes associated with stress, we submitted the top 10 up/down-regulated genes as seed genes to exact sub-networks, the sub-networks were visualized with the DEGNServer and Cytoscape. The centrality to co-expression networks of hubs tend to be associated with essential roles in biological processes [26],[27]. Forty genes with the highest stress centrality followed by degree centrality (Fig. 5 and Table S5) were annotated using the PLEXdb annotation portal [28] and HarvEST (version 1.59). About 75% of the top genes were upregulated in the leaf, while only about 50% and 35% were upregulated in the root and pregerminated seeds respectively (Table S5). Among differentially induced genes associated with drought tolerance in the leaf and roots but down regulated in the pre-germinated seeds, were AQUAPORIN, CHOLINE DEHYDROGENASE, HESSIAN FLY RESPONSE GENE 1 PROTEIN, DIRIGENT LIKE PROTEIN (DIR), ZINC FINGER PROTEIN, 2-OXOGLUTARATE DEPENDENT OXYGENASE and DEHYDRIN. An exception to the group is the TYPE 1 NON SPECIFIC LIPID TRANSFER PROTEIN (nsLTPs), XYLOGLUCAN ENDOTRANSGLYCOSYLASES (XTHs) and WAX2 protein which were upregulated only in the leaf tissues. The GLYCINE-RICH PROTEIN (GRPs), MALTO-OLIGOSYLTREHALOSE TREHALOHYDROLASE, TRANSCRIPTIONAL REGULATOR LYSR, FRUCTAN EXOHYDROLASE and CYSTEINE SYNTHASE were among the down regulated genes in the leaf.

Six of the top genes with high stress and degree centrality (HFR1 and DIR, nsLTPs GRP – like, XTHs and an unknown gene *TaAffx.30098.1.S1_at*) were selected for qRT-PCR analysis in the leaf tissue (Table S4 and Table S5). Excess RNA produced during microarray target preparation and from an independent experiment was used separately to provide template for qRT-PCR. The expression ratios produced by qRT-PCR and the microarray experiments were similar (Fig. 6), and except for the gene encoding GRPs, all the genes were confirmed as preferentially upregulated in leaf by both the microarray and qRT-PCR.

4 DISCUSSION

Sedaxane applied as seed treatment induced significant increase in the efficiency of excitation energy capture by open PSII reaction centers (Fv'/Fm') in drought stressed plants. This is in agreement with previous studies showing similar effect exerted by another SDHI, isopyrazam, shown to enhance the photosynthetic efficiency of disease-free wheat plants under drought conditions [11]. Changes in Fv'/Fm' and DIo/RC were used in this study as early indicators of modifications in PSII operating efficiencies attributed to thermal dissipation of excessive excitation energy in the light harvesting complexes of PSII, estimated as non-photochemical quenching or NPQ [29]. Under stress conditions, NPQ acts as a photoprotective mechanism by which PSII activity is down-regulated to prevent damage to PSII reaction centers. Consequently, decrease in NPQ accompanied with an increased rate of leaf photosynthesis in sedaxane treated plants under drought conditions suggests that sedaxane treatment led to preferential allocation of excitation energy into photochemical processes [30].

Sedaxane inhibits the succinate dehydrogenase (SDH) complex II in the fungal mitochondria and there is a possibility that similar effect may be exerted on the plant mitochondrial complex II although this hypothesis was not tested in the present study. Inhibition of SDH by partial reduction of SDH subunits has been reported previously to improve leaf photosynthesis and biomass by increasing stomatal conductance in tomato and Arabidopsis [31],[32]. Acevedo et al [33] recently reported that an SDH flavoprotein subunit (*SDH1-like*) transcript was upregulated in *Ilex paraguariensis* plants exposed to drought. This authors showed that increase in *SDH1-like* transcripts correlated with elevated ABA concentration. ABA accumulates in the guard cells of drought stressed plants to induce stomatal closure and conserve water. In the present study, genes encoding ABA were downregulated in sedaxane treated plants under drought. In addition, we detected interactions between sedaxane and AW_{FC} on stomatal conductance which were significant at 10% LSD (results not shown), suggesting that, treatment with sedaxane may have contributed to the

maintenance of stomata function under drought consistent with our observations of improved photosynthesis, increased biomass and reduced water content of sedaxane-treated wheat seedlings.

Transcriptome Response to Sedaxane in Plant Tissues

In total, 4369 genes, around 7% of the genes present on the chip were found to be differentially expressed ($P<0.05$) in response to sedaxane seed treatment in all three tissues considered under drought conditions. About 50 % (≥ 1.5-fold change) of the differentially expressed genes (DEGs) were found in leaves and pregerminated seeds, while only 12% were found in the roots. When comparing DEGs in the three tissues collectively, no common DEGs were identified. However, about 3% DEGs overlapped in the leaf and pregerminated seed, and less than 1% in the leaf and root or the pregerminated seed and root tissues. Hence, distinct sets of genes were generally activated in individual tissues of drought stressed wheat seedlings in response to sedaxane. Our work thus offers the first comprehensive picture of transcriptional changes triggered by an SDHI, sedaxane, in distinct tissues of drought-stressed wheat plants associated with increased PSII efficiency and photosynthesis.

4.2. Cellular and Metabolic Responses to Sedaxane

4.2.1 Pregerminated seeds and Roots

No drought stress was introduced to the pregerminated seeds in this study; therefore, we consider gene differential expression in this tissue a direct effect of sedaxane seed treatment under non-stress conditions. Upregulated genes in response to sedaxane were significantly enriched in glutathione-s-transferase (GST) activity. GSTs are important proteins involved in efficient scavenging of plant toxins such as ROS, which accumulate as a consequence of increased oxidative stress [34] and thus maintain redox homeostasis in plant tissues [35]. Our results showed that the transcripts of these ROS scavenging proteins, GSTs accumulated during germination, to suggest a close association between sedaxane seed treatment protection of the plant (leaf) from oxidative stress under drought conditions.

Pathway analysis of all differentially expressed genes in the root showed that biotic stress was the only enriched pathway (Table 2). These genes encode pathogenesis-related proteins and defensin-like proteins, generally upregulated in response to pathogen attack which ultimately impede further pathogen invasion and enhance the capacity of the host to limit subsequent pathogen infection [36],[37]. Interestingly, many pathogenesis-related genes are also induced upon exposure of a plant to abiotic stress ensuring disease resistance [38]. In our study, all the genes in this category were downregulated suggesting treated roots were not exhibiting biotic stress related responses under drought possibly due to the protective properties of sedaxane.

4.2.2 Leaves

4.2.2.1 Jasmonate Biosynthesis and Signaling

Early plant responses to drought involve the adjustment of the levels of endogenous hormones to activate physiological pathways for adaptation, thereby modulating the expression of genes involved in processes relating to PSII, photosynthesis, cell modification, growth and development under abiotic stress conditions [39]-[41]. Hormone metabolism was one of the enriched pathways involved in key cellular functions in our study in particular genes involved in the jasmonate synthesis. This is substantiated by GO analysis showing enrichment for genes involved in jasmonic acid biosynthetic and metabolic processes (Table 5). JAs have been shown to play critical role in the early priming (preconditioning stage) to moderate drought in Arabidopsis (*Arabidopsis thaliana*), stimulating preparatory response for drought acclimation (for example stomatal closure and cell wall modification) [42]. Our datasets show that genes encoding the various derivatives of jasmonates among which are jasmonic acid and genes encoding enzymes involved in the biosynthetic pathway including allene oxide synthase, allene oxide cyclase; lipoxygenase and 12-oxophytodienoic reductase were downregulated. Under drought stress conditions in sedaxane treated plants, the JA-signaling genes involved in calcium signaling and mitogen-activated protein kinases were also downregulated. Calcium ion influx (Ca^{2+}) and mitogen-activated protein kinases are key

components of JA signal transduction, accumulating in response to abiotic and biotic stress [43],[44]. Treatment with JA has been shown to induce cytosolic free-Ca^{2+} concentration ($[Ca^{2+}]$ cyt) in *Arabidopsis thaliana* leaves [45]. However, high concentrations of JA inhibit cell expansion and cell wall modification and reduce plant growth [46],[47]. Thus downregulation of JA biosynthesis and signaling under drought stress in sedaxane treated plants is likely to act to establish new homeostasis through altered signaling and redirection of metabolism from defense/stress responses towards modification of plant growth and development.

4.2.2.2 Cell Wall Modifications

Physical properties of the cell wall play a crucial role in the response of plants to drought [48]. Expansins mediate cell wall -loosening factors that directly induce turgor-driven cell wall extension [49]. Secondary wall-loosening enzymes such as xyloglucan endoglycosylase/hydrolases (XTHs) modify the structures of the cell wall, aiding cell wall loosening [50],[51]. Our microarray and qRT-PCR analyses showed upregulation of genes encoding cell-wall-loosening expansins and XTHs thus indicating that sedaxane is likely to confer adaptive responses to drought stress facilitating cellular expansion and modification of shoot growth and development. Upregulation of expansins genes have been implicated in increased drought tolerance in plants [42],[52].

4.2.2.3 Tetrapyrrole biosynthesis

The tetrapyrrole biosynthetic pathway is responsible for the synthesis of different types of porphyrins in higher plants including chlorophyll and heme essential for several primary metabolic processes [53]. The major site of tetrapyrrole biosynthesis in plants occurs in plastids except the last steps of heme biosynthesis, which are possibly localized in both mitochondria and plastids [53]. In this study, expression of genes encoding various intermediates of the tetrapyrrole biosynthesis pathway was strongly upregulated in sedaxane treated plants suggesting that these plants were able to maintain a flux through the tetrapyrrole pathway under drought conditions. High level of tetrapyrrole intermediates has been

previously associated with improved drought tolerance in transgenic rice expressing *Myxococcus xanthus* protoporphyrinogen oxidase [54],[55]. Insertion of Mg2+ into Protoporphyrin- IX by the enzyme Mg-chelatase was shown to favor the chlorophyll branch of the pathway [56]. In our data, genes encoding enzymes Magnesium-chelatase subunit and Mg-protoporphyrin IX, precursors for chlorophyll biosynthesis were upregulated. In plants, the protochlorophyllide reductase oxidoreductase (POR) step in tetrapyrrole pathway is strictly light-dependent, as it requires protochlorophyllide to be activated by light [57],[58]. In illuminated plants, protons are translocated from the stroma into the intra thylakoid lumen [59]. This movement is coupled with the release of Mg2+, into the stroma. These ion fluxes are known to contribute to an increase of the pH of the stroma from 7 to 8, an optimum pH of most enzymes involved in the Benson- Calvin cycle [60],[61], including rubisco, fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, and phosphoribulokinase. Hence, the light-mediated increase of Mg2+ and H+ enhances the activity of key enzymes of the Calvin-Benson cycle. This coupled with the observed increased rate of photosynthesis would indicate a maintained balance between PSII activity and the Calvin cycle, thus protecting the PSII from photo damage.

Based on our results, the tetrapyrrole biosynthetic pathway is likely to be the target of sedaxane in wheat metabolism, driven by the production of glutamate in the mitochondria. Glutamate, the precursor for the synthesis of tetrapyrroles in plants is formed from 2-oxoglutarate and glutamine. 2-Oxoglutarate mainly produced in the mitochondria and transported to the chloroplast is an obligatory substrate for 2-oxoglutarate-dependent dioxygenases [62] and a key metabolite required for ammonia assimilation [63]. In this study, one of the upregulated top genes encode 2-oxoglutarate- dependent oxygenase. In addition, the tetrapyrrole intermediate Mg-protoporphyrin IX has been postulated to act as a signal molecule in signaling pathways between the chloroplast, nucleus and the mitochondria, and the accumulation of this metabolite is required to regulate the expression of genes encoding proteins associated with photosynthesis [64],[65].

4.3. Central Players in the Sedaxane Regulated Network

We aimed to identify a hub subnetwork to provide more insight on the physiological impact of sedaxane under drought conditions and ultimately to identify transcription factors that can potentially be used as candidate genes to improve photosynthetic performance of wheat. We used datasets from the leaves, roots and pregerminated seeds to identify 40 genes using inferred network stress and degree centralities, computed for each of the coexpressed regulatory genes in the network.

Our data suggest that dirigent proteins play a central role in protecting wheat plants against the effects of severe drought through their impact on mechanical strength and flexibility of cell wall. DIR-like family proteins have been implicated in cell wall lignin biosynthesis, which are structural cell wall components of vascular tissues [66]. The hub with the highest stress centrality in our network encoded the Hessian fly responsive protein 1 (HFR1), highly upregulated in the leaf and root, also considered a dirigent-like protein involved in modulating plant response to biotic stress [67] and previously implicated in cell wall strengthening via deposition of phenolics [68] and secretion of protective surface waxes [69].

Increased dry weight has been associated with accumulation of cell wall expansin [47]. One of the top ranked genes in the hub of the network encoded xyloglucan endotransglycosylase (XTH), involved in strengthening and cell wall plasticity leading to water uptake in leaves under drought conditions [51],[70]. The enrichment of pathways involved in cell wall modification, and upregulation of genes encoding XTHs and expansins in the leaf is an indication that, the selective loosening and strengthening of the cell wall in growing plant tissue under drought conditions is likely to stimulate water uptake to increase growth and development in the plant [71] which is consistent with increased dry weight in sedaxane treated plants under drought stress. A proline-rich protein precursor was also upregulated. Recent discoveries point out that proline is a key determinant of many cell wall proteins that plays important roles in plant growth and development. Interestingly, a gene encoding aquaporin, involved in regulating

water movement across cell membranes [72] was also upregulated in both leaf and root tissues. This suggests that water movement and use under drought conditions was enhanced with sedaxane treatment consistent with the physiological phenotype of increased photosynthesis and growth as well as reduction in NPQ of fungicide treated plants.

A gene hub encoding the calmodulin binding protein (CaM) was upregulated in both the leaf and pregerminated seeds. CaM is small Ca^{2+}- sensing protein that acts as signal transducer in a wide array of physiological processes including drought stress in plants [73],[74]. Using knockout mutants of the CaM transcription factors (CAMTAs), Pandey et al [75] showed that CaM was positively involved in drought stress tolerance in Arabidopsis. A new family of CaM-binding proteins, the type 1 non-specific lipid transfer proteins (nsLTPs) was identified in Arabidopsis [76]. In this study, we identified three hub genes encoding the nsLTPs, all upregulated in the leaf tissue. nsLTPs also act as wax transporters, able to transfer lipids and fatty acids across different membranes and are induced under drought stress [77]. nsLTPs have been shown to be involved in epicuticular wax or cuticle biosynthesis [78]. Kottapalli et al. [79] showed that epicuticular wax content increased in drought tolerant genotype of peanut (*Arachis hypogaea*). Loss of epicuticular wax has been associated with increased water loss in plants. In our study, one of the identified hub gene, the wax biosynthesis annotated as Ecriferum 1 (CER1) and WAX2-like protein (WAX2) was also upregulated.

Another interesting gene in the network which plays a regulatory role in signaling and abiotic stress tolerance is a transcriptional factor for glycine rich proteins (GRPs) [80]. The expression of GRP genes is modulated by plant hormones, which in turn regulate plant growth, development and stress responses [81]-[83]. In our study, GRP hub of genes was downregulated whereas GRPs have been shown to accumulate under drought [84].

4.4. CONCLUSION

This study showed that the SDHI sedaxane, applied as seed treatment, improved PSII efficiency, photosynthesis and biomass production of wheat under drought. These effects

were accompanied by low NPQ, as a result of a homeostasis between PSII activity and the Calvin cycle.

Transcriptomic analysis suggests that sedaxane enhances wheat seedling tolerance/resistance to drought stress by altering the expression of key genes/transcriptional factors from seed germination. We propose a schematic of the effects of sedaxane on plant physiology (Fig. 7) associated with differential patterns of nsLTPs, XTHs, CaM, HFR1, Zinc finger protein 1 known to regulate the expression of drought tolerance/resistance traits in crops. Initial responses were first observed in pregerminated seeds, where ROS scavenging genes were upregulated involved in the reduction of oxidative stress. In the root, defense-related genes were downregulated most likely to allow metabolites to be redirected towards adaptive development. The most differentially expressed genes were observed in leaves characterized by downregulation of jasmonate biosynthesis and signaling and increased chlorophyll biosynthesis allowing for the remobilization of assimilates from stress-related responses towards modified growth and development.

ACKNOWLEDGEMENTS

This research was funded by Syngenta grant RK3537. We thank Dr Guillermina Mendiondo for assistance with qRT-PCR.

Competing interests

The authors declare that they have no competing interests.

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TABLES

Table 1. Biomass of wheat plants grown from sedaxane treated and untreated seeds 36 days after germination. Each value is a mean (n=7) followed by standard error. SDX, sedaxane. UNT, untreated. AW_{FC}, available water at field capacity

Table 2. Mapman functional categories (BINs) in the cell function pathway for significantly up-and down-regulated genes (≥ 1.5-fold change; P<0.05) in (A) seeds after 48 h pregermination, and in (B) roots and (C) leaves of wheat plants grown from sedaxane treated and untreated seeds under drought stress (10% AW_{FC}) 9 days after germination.

Table 3. Mapman functional categories in the metabolic pathways for significantly up-and down-regulated genes (≥ 1.5) in leaves of wheat plants grown from sedaxane treated and untreated seeds under drought stress (10% AW_{FC}) 9 days after germination.

Table 4. GO enrichment analysis for significantly up-and down-regulated genes (≥ 1.5-fold change; P<0.05) in seeds after 48 h pregermination. Analysis was performed using parametric analysis of gene set enrichment in AgriGO with Bonferroni multitest adjustment method. FC, fold change; FDR, false discovery rate; P, biological process; F, molecular function; C, cellular component. Red color system indicates upregulated and blue indicate downregulated terms.

Table 5. GO enrichment analysis for significantly up-and down-regulated genes (≥ 1.5-fold change; P<0.05) in leaves of wheat plants grown from sedaxane treated and untreated seeds under drought stress (10% AWFC) 9 days after germination. Analysis was performed using analysis of gene set enrichment in AgriGO with Bonferroni multitest adjustment method. FC, fold change; FDR, false discovery rate; P, biological process; F, molecular function; C, cellular component. Red color system indicates upregulated and blue indicate downregulated terms.

FIGURES

Fig. 1. A, Efficiency of photosystem II (PSII) photochemistry (Fv'/Fm') in light adapted samples; B, Quantum yield (QY); C, Dissipated energy flux per active reaction center (DIo/RC), of leaves of wheat seedlings grown from sedaxane treated and untreated seeds. Error bars indicate mean ± SE, n = 7. Asterisks indicate significant difference (P<0.05) from the untreated control. SDX, Sedaxane, UNT, Untreated.

Fig. 2. Light response of photochemical quenching (qP) of drought-stressed (10% AW_{FC}) and non-stressed (90% AW_{FC}) plants 12 days after germination. Error bars indicate mean ± SE, n = 7. Asterisks show a significant difference (P<0.05) from the untreated control. AW_{FC} - available water at field capacity.

Fig. 3. Light response of A, dissipated excess excitation energy measured as non-photochemical quenching (NPQ) and B, rate of CO_2 assimilation (*A*) in leaves of wheat plants grown from sedaxane treated and untreated seeds 12 DAG. Error bars indicate mean ± SE, n = 7. Asterisks in A, indicate differences (P<0.05) from the untreated control. In B, asterisks indicate significant interaction (P<0.05) between fungicide sedaxane and available water at field capacity (AW_{FC}). SDX, sedaxane. UNT, Untreated.

Fig. 4. Venn diagram comparing up-regulated genes (adjusted P<0.05; Fold change ≥ 1.5) in leaf and root tissues of plants grown from sedaxane treated seeds and treated seed after 48hr pregermination.

Fig. 5. Coexpression and regulatory interaction network of common top differentially expressed genes across the tissues (leaf, root and pregerminated seeds). The subnetwork was implemented and visualized in Cytoscape. Nodes were coloured based on stress degree, red, brown and yellow represented highest, high and middle stress respectively. The edge colour and thickness represent the degree of co-expressed connections from strong (thick and brown) to weak (thin and green).

Fig. 6. Expression levels of candidate genes by microarray and qRT-PCR. Genes were selected from gene network analysis across leaf, root and pregerminated tissues. The array and qRT-PCR data are averages of 3 biological replicates of minimum of 3 plants each. Error bars indicate mean ± SE. Asterisks show significant differences in candidate gene expression levels compared to the corresponding control (*P<0.05). NE: new experiment.

Fig. 7. Molecular responses to sedaxane in individual plant tissues and across tissues and their effect on plant physiology. Sedaxane induced transcriptional regulation of genes and transcriptional factors resulting in protection against oxidative stress in pregerminated seeds, downregulation of pathogenesis related genes at 9 days after germination under drought conditions in the root tissues; coupled with altered hormone signaling and metabolism in the leaves to mobilize metabolites towards growth and adaptive development leading to increased drought tolerance with improved photosynthesis and growth.

Appendix A. Supplementary data

Fig. S1. Light response of plants drought-stressed (10% AW_{FC}) and non-stressed (90% AW_{FC}) plants 12 days after germination. A, Stomatal conductance. B, Transpiration rates. Error bars indicate mean ± SE, n = 7. Asterisks show a significant difference (P<0.05) from the untreated control. AW_{FC} - available water at field capacity.

Table S1. List of targeted genes for qRT-PCR.

Table S4. Functional categories in the MapMan 'metabolism overview' of differentially regulated genes (adjusted P<0.05) in the leaf of drought stressed wheat plants grown from sedaxane treated seeds.

Table S5. Centralities based analysis and the values of the top 40 ranked genes

Appendix B. Supplementary data

Table S2.

A. Differentially regulated transcripts (adjusted P<0.05) in sedaxane treated- pregerminated seeds

B. Differentially regulated transcripts (adjusted P<0.05) in the root of drought stressed wheat plants grown from sedaxane treated seeds

C. Differentially regulated transcripts (adjusted P<0.05) in the leaf of drought stressed wheat plants grown from sedaxane treated seeds

Appendix C. Supplementary data

Table S3.

A. Functional categories in the Mapman 'cell function overview' of differentially regulated genes (adjusted P<0.05) in sedaxane treated pregerminated seeds

B. Functional categories in the MapMan 'cell function overview' of differentially regulated genes (adjusted P<0.05) in the root of drought stressed wheat plants grown from sedaxane treated seeds.

C. Functional categories in the MapMan 'cell function overview' of differentially regulated genes (adjusted P<0.05) in the leaf of drought stressed wheat plants grown from sedaxane treated seeds.

TABLE 1.

Biomass of wheat plants grown from sedaxane treated and untreated seeds 36 days after germination.

	Tiller no.		Height (cm)		Water content (%)		Dry weight (g)	
Fungicide	10% AW_{FC}	90% AW_{FC}	10% AW_{FC}	90% AW_{FC}	10% AW_{FC}	90% AW_{FC}	10% AW_{FC}	90% AW_{FC}
SDX	8 ± 1	35 ± 2	18.31 ± 0.23	36.05 ± 0.48	73.51 ± 2.77	86.68 ± 0.51	0.27 ± 0.03	0.13 ± 0.01
UNT	7 ± 1	33 ± 2	17.03 ± 0.51	36.71 ± 0.52	83.12 ± 1.1	88.4 ± 0.95	0.17 ± 0.01	0.12 ± 0.01
Effects	P	LSD	P	LSD	P	LSD	P	LSD
Fungicide	0.432	3.176	0.478	0.955	0.003	3.438	0.003	0.035
AW_{FC}	<0.001	3.176	<0.001	0.955	<0.001	3.438	<0.001	0.035
Fungicide x AW_{FC}	0.547	4.491	0.044	1.351	0.027	4.862	0.027	0.049

Each value is a mean (n=7) followed by standard error. SDX, sedaxane. UNT, untreated. AW_{FC}, available water at field capacity. Dry weight is expressed relative to fresh weight

TABLE 2.

Mapman functional categories (BINs) in the cell function pathway for significantly up-and down-regulated genes (≥ 1.5 fold change; P<0.05) in (A) seeds after 48 hrs pregermination, and in (B) roots and (C) leaves of wheat plants grown from sedaxane treated and untreated seeds under drought stress (10% AW_{FC}) 9 days after germination.

	Bin	Name	Up	Down	P Value
A. Pregerminated seeds	28.1	DNA.synthesis/Chromatin structure.histone	2	84	3.67E-12
	20.1	Stress.biotic	3	18	2.30E-02
	35.2	Not assigned/unknown	355	750	1.10E-02
B. Roots	20.1	Stress. biotic	0	10	2.00E-02
C. Leaves	29.2	Protein synthesis	58	16	6.56E-13
	17	Hormone metabolism	5	47	2.20E-07
	20.1	Stress.biotic	4	29	3.84E-05
	29.4	Protein.postranslational	7	35	5.40E-02
	30	Signalling	13	53	5.40E-02
	26	Misc	65	54	5.40E-02

AW_{FC}, available water at field capacity

TABLE 3.

Mapman functional categories in the metabolic pathway for significantly up-and down-regulated genes (≥ 1.5) in leaves of wheat plants grown from sedaxane treated and untreated seeds under drought stress (10% AW_{FC}) 9 days after germination.

Bin	Name	Up	Down	*P* value
10.7	Cell wall.modification	12	1	2.64E-06
19	Tetrapyrrole synthesis	8	0	4.00E-02

AW_{FC}, available water at field capacity

TABLE 4.

GO enrichment analysis of pathways for significantly up-and down-regulated genes (≥ 1.5 fold change; P<0.05) in seeds after 48 h pregermination.

GO Term	Ontology Source	Description	No. Input List	Mean Log_2 FC	Z-score	FDR
GO:0006575	P	cellular amino acid derivative metabolic process	20	0.28	3.3	8.70E-03
GO:0006790	P	sulfur metabolic process	16	0.37	3.3	8.70E-03
GO:0006803	P	glutathione conjugation reaction	10	0.59	3.3	8.70E-03
GO:0006749	P	glutathione metabolic process	10	0.59	3.3	8.70E-03
GO:0006518	P	peptide metabolic process	10	0.59	3.3	8.70E-03
GO:0051186	P	cofactor metabolic process	18	0.26	3.1	1.40E-02
GO:0009057	P	macromolecule catabolic process	13	0.36	3	2.00E-02
GO:0006732	P	coenzyme metabolic process	15	0.27	2.8	2.80E-02
GO:0006091	P	generation of precursor metabolites and energy	19	0.17	2.8	3.30E-02
GO:0009056	P	catabolic process	22	0.09	2.6	4.70E-02
GO:0051707	P	response to other organism	18	-1.2	-2.6	4.70E-02
GO:0009607	P	response to biotic stimulus	23	-1.1	-2.7	4.60E-02
GO:0044085	P	cellular component biogenesis	40	-1	-3.1	1.40E-02
GO:0065003	P	macromolecular complex assembly	35	-1.1	-3.3	8.70E-03
GO:0043933	P	macromolecular complex subunit organization	35	-1.1	-3.3	8.70E-03
GO:0034622	P	cellular macromolecular complex assembly	35	-1.1	-3.3	8.70E-03
GO:0034621	P	cellular macromolecular complex subunit organization	35	-1.1	-3.3	8.70E-03
GO:0022607	P	cellular component assembly	35	-1.1	-3.3	8.70E-03
GO:0016043	P	cellular component organization	50	-1	-3.5	6.90E-03
GO:0051276	P	chromosome organization	36	-1.2	-3.7	3.90E-03
GO:0006996	P	organelle organization	42	-1.1	-3.7	3.90E-03
GO:0065004	P	protein-DNA complex assembly	29	-1.3	-3.8	2.90E-03
GO:0034728	P	nucleosome organization	29	-1.3	-3.8	2.90E-03
GO:0031497	P	chromatin assembly	29	-1.3	-3.8	2.90E-03
GO:0006334	P	nucleosome assembly	29	-1.3	-3.8	2.90E-03
GO:0006323	P	DNA packaging	30	-1.2	-3.8	2.90E-03
GO:0006333	P	chromatin assembly or disassembly	31	-1.3	-4	2.90E-03
GO:0006325	P	chromatin organization	33	-1.2	-4	2.90E-03
GO:0071103	P	DNA conformation change	31	-1.3	-4	2.90E-03
GO:0004364	F	glutathione transferase activity	12	0.74	4.1	4.20E-03
GO:0048037	F	cofactor binding	32	0.15	3.5	1.30E-02
GO:0016765	F	transferase activity, transferring alkyl or aryl (other than methyl) groups	14	0.46	3.4	1.40E-02
GO:0003676	F	nucleic acid binding	96	-0.85	-3.2	2.50E-02
GO:0003677	F	DNA binding	79	-0.93	-3.5	1.30E-02

Analysis was performed using parametric analysis of gene set enrichment in AgriGO with Bonferroni multitest adjustment method. FC, fold change; FDR, false discovery rate; P, biological process; F, molecular function; C, cellular component. Red color system indicates upregulated and blue indicate downregulated terms

TABLE 5.

GO enrichment analysis of pathways for significantly up-and down-regulated genes (≥ 1.5 fold change; P<0.05) in leaves of wheat plants grown from sedaxane treated and untreated seeds under drought stress (10% AW_{FC}) 9 days after germination.

GO Term	Ontology Source	Description	No. Input List	Mean log_2FC	Z-score	FDR
GO:0006412	P	translation	56	0.56	6.3	5.10E-08
GO:0042254	P	ribosome biogenesis	41	0.59	5.6	2.00E-06
GO:0022613	P	ribonucleoprotein complex biogenesis	42	0.56	5.5	3.10E-06
GO:0044085	P	cellular component biogenesis	57	0.34	4.5	2.00E-04
GO:0009059	P	macromolecule biosynthetic process	123	0.15	4.1	9.30E-04
GO:0034645	P	cellular macromolecule biosynthetic process	109	0.12	3.5	8.50E-03
GO:0044249	P	cellular biosynthetic process	208	0.04	3.4	8.50E-03
GO:0033013	P	tetrapyrrole metabolic process	11	0.72	3.4	8.50E-03
GO:0015994	P	chlorophyll metabolic process	11	0.72	3.4	8.50E-03
GO:0006778	P	porphyrin metabolic process	11	0.72	3.4	8.50E-03
GO:0009058	P	biosynthetic process	220	0.03	3.4	9.60E-03
GO:0010467	P	gene expression	108	0.09	3.1	2.50E-02
GO:0009309	P	amine biosynthetic process	11	0.58	2.9	3.90E-02
GO:0008652	P	cellular amino acid biosynthetic process	11	0.58	2.9	3.90E-02
GO:0044267	P	cellular protein metabolic process	146	0.03	2.8	4.60E-02
GO:0009607	P	response to biotic stimulus	26	-0.64	-2.8	4.60E-02
GO:0051707	P	response to other organism	24	-0.69	-3	3.30E-02
GO:0051704	P	multi-organism process	24	-0.69	-3	3.30E-02
GO:0006952	P	defense response	31	-0.74	-3.7	4.50E-03
GO:0009695	P	jasmonic acid biosynthetic process	11	-1.3	-4.5	2.00E-04
GO:0009694	P	jasmonic acid metabolic process	11	-1.3	-4.5	2.00E-04
GO:0031408	P	oxylipin biosynthetic process	20	-1	-4.5	2.00E-04
GO:0031407	P	oxylipin metabolic process	20	-1	-4.5	2.00E-04
GO:0003735	F	structural constituent of ribosome	61	0.57	6.7	1.40E-09
GO:0005198	F	structural molecule activity	66	0.54	6.7	1.40E-09
GO:0019843	F	rRNA binding	17	0.97	5.4	1.60E-06
GO:0003723	F	RNA binding	42	0.50	5	6.30E-06
GO:0016757	F	transferase activity, transferring glycosyl groups	32	0.32	3.2	1.20E-02
GO:0016758	F	transferase activity, transferring hexosyl groups	24	0.35	2.9	2.90E-02
GO:0032561	F	guanyl ribonucleotide binding	11	0.55	2.8	3.60E-02
GO:0019001	F	guanyl nucleotide binding	11	0.55	2.8	3.60E-02
GO:0005525	F	GTP binding	11	0.55	2.8	3.60E-02
GO:0048037	F	cofactor binding	32	-0.58	-2.7	4.40E-02
GO:0050662	F	coenzyme binding	25	-0.65	-2.8	3.60E-02
GO:0004674	F	protein serine/threonine kinase activity	44	-0.78	-4.7	2.40E-05
GO:0004672	F	protein kinase activity	58	-0.74	-5.1	5.70E-06
GO:0016772	F	transferase activity, transferring phosphorus-containing groups	97	-0.61	-5.1	5.70E-06
GO:0016773	F	phosphotransferase activity, alcohol group as acceptor	64	-0.74	-5.3	2.70E-06
GO:0016301	F	kinase activity	83	-0.67	-5.3	2.50E-06

Analysis was performed using analysis of gene set enrichment in AgriGO with Bonferroni multitest adjustment method. FC, fold change; FDR, false discovery rate; P, biological process; F, molecular function; C, cellular component. Red color system indicates upregulated and blue indicate downregulated terms. AW_{FC}, available water at field capacity

Fig. 1

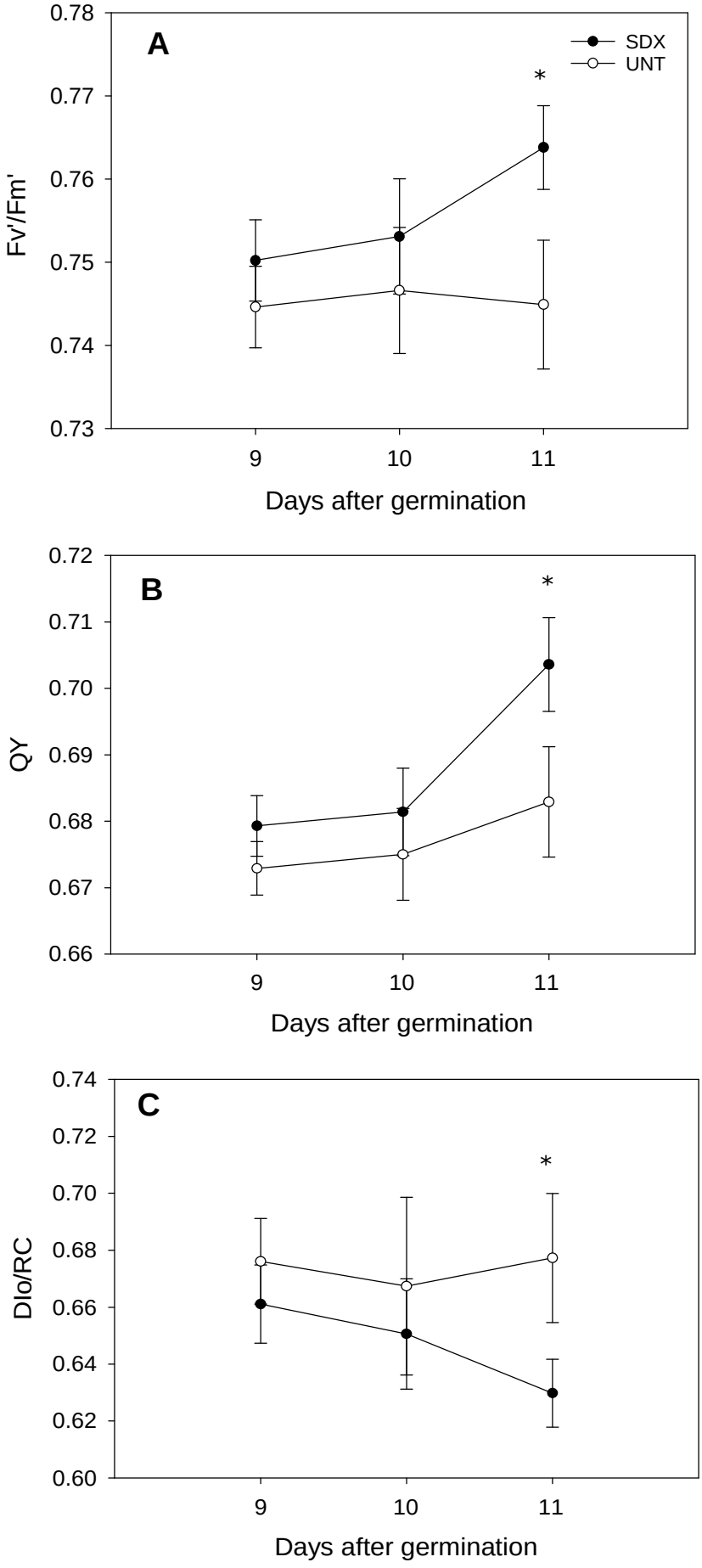
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Fig. 2.

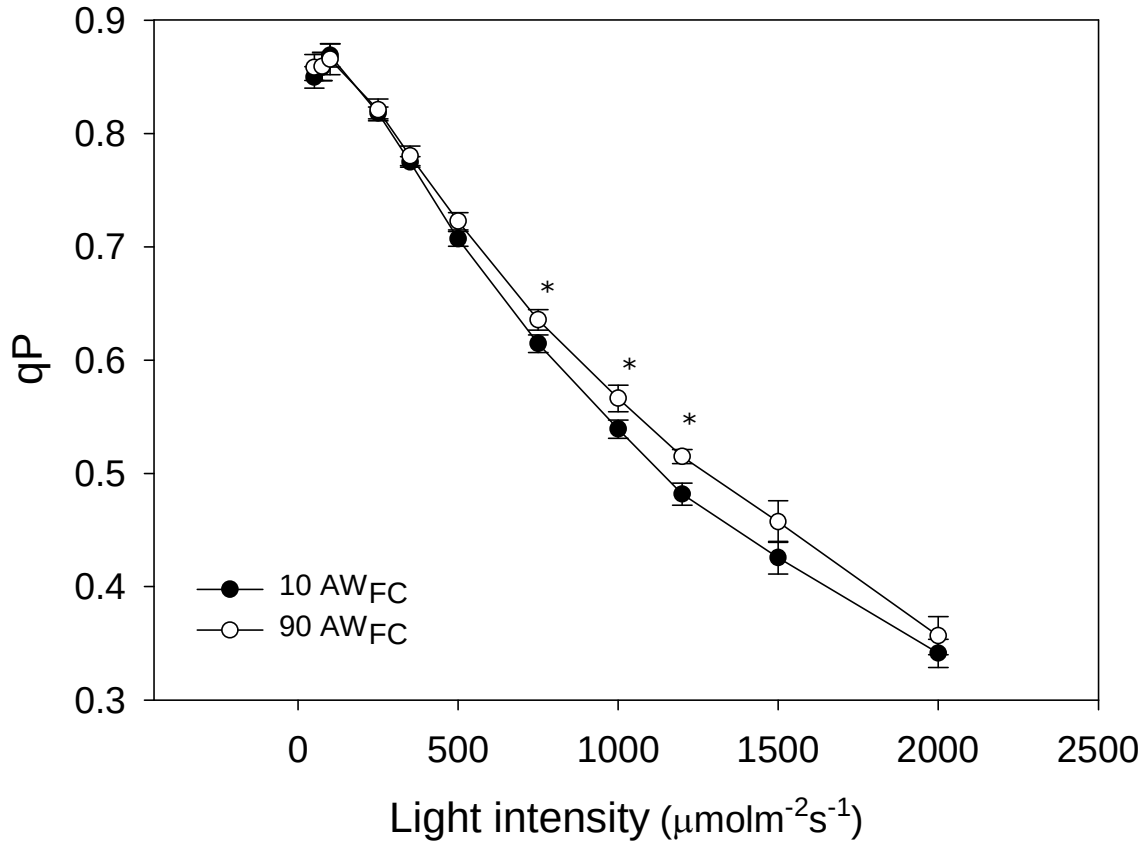
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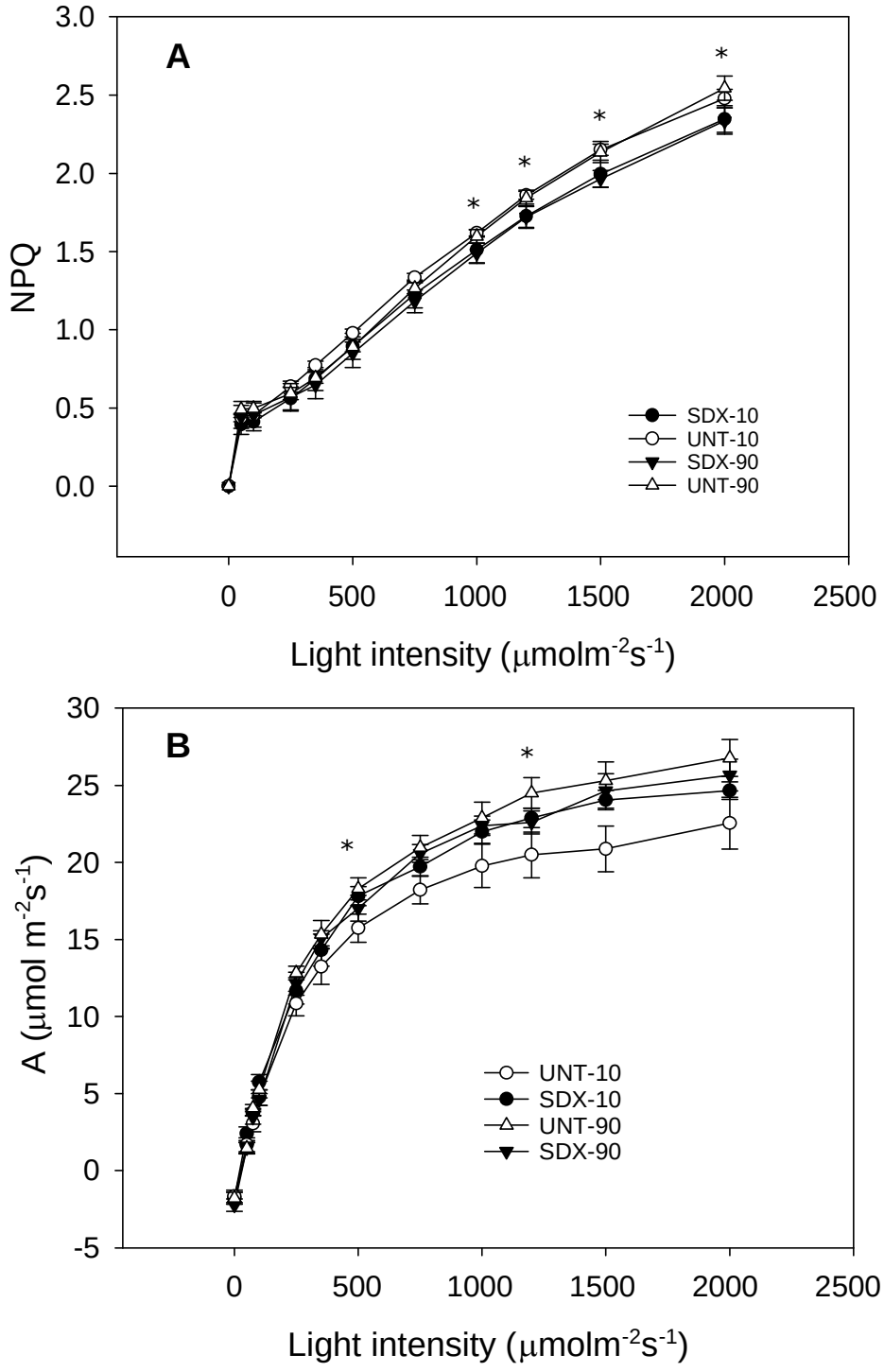
Fig. 3.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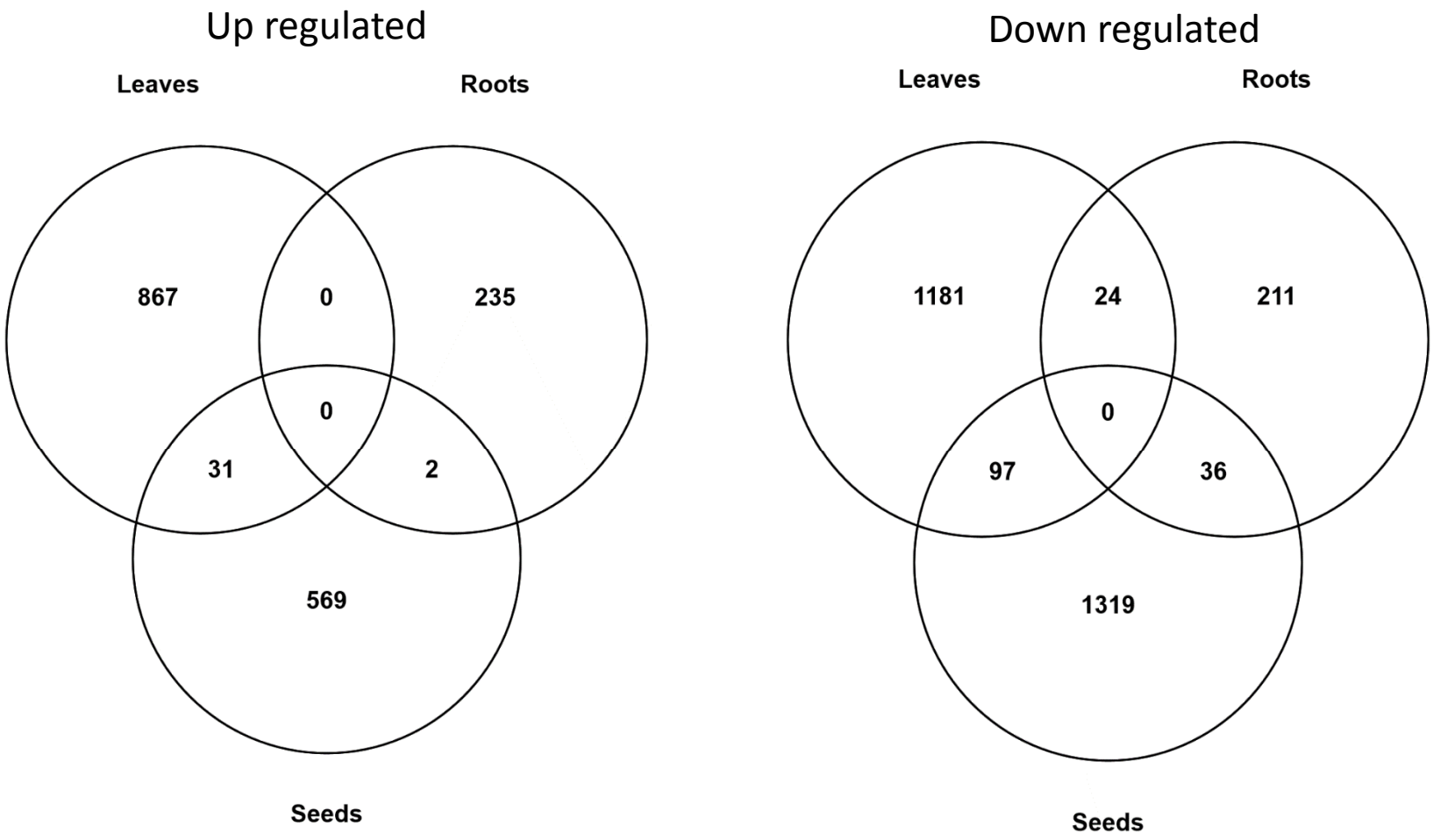
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Fig. 5.

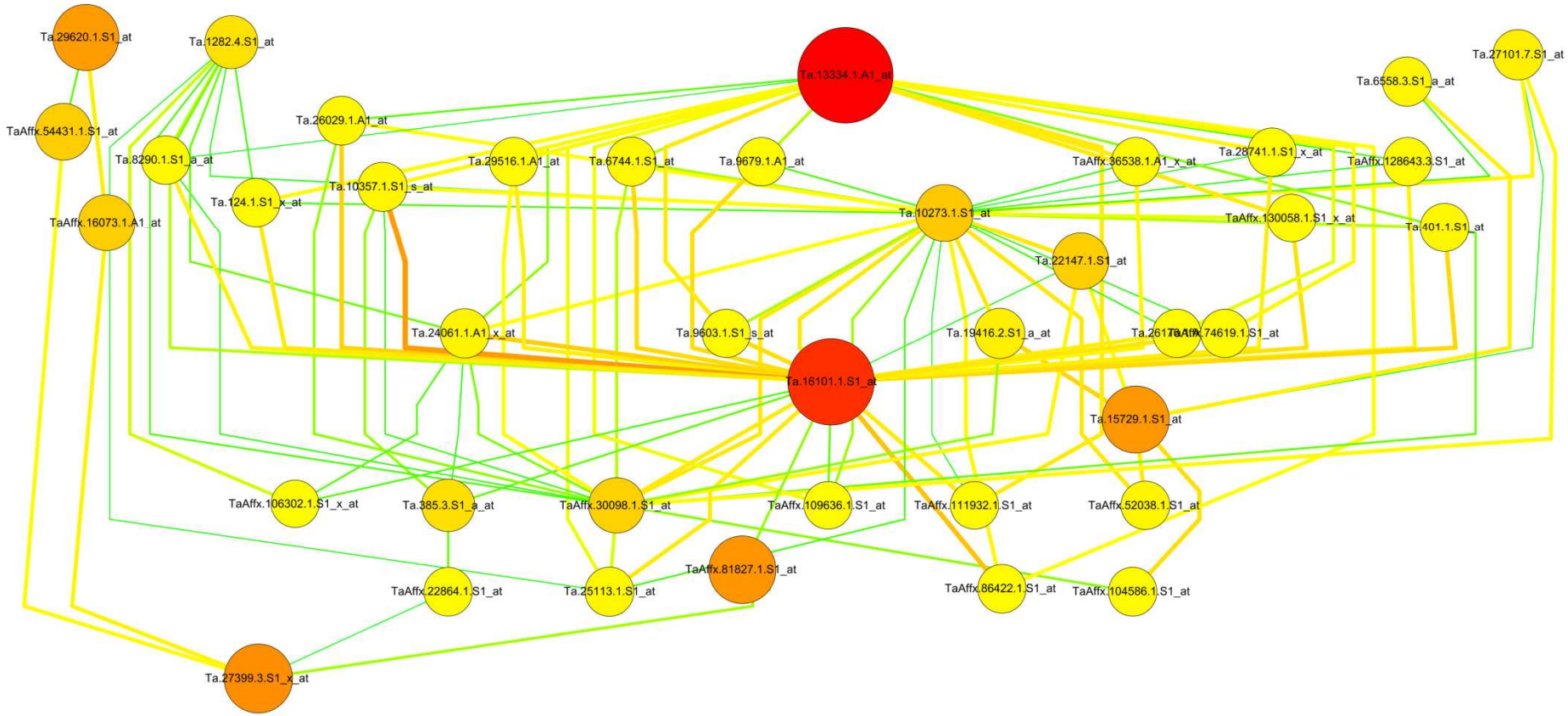
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Fig. 6.

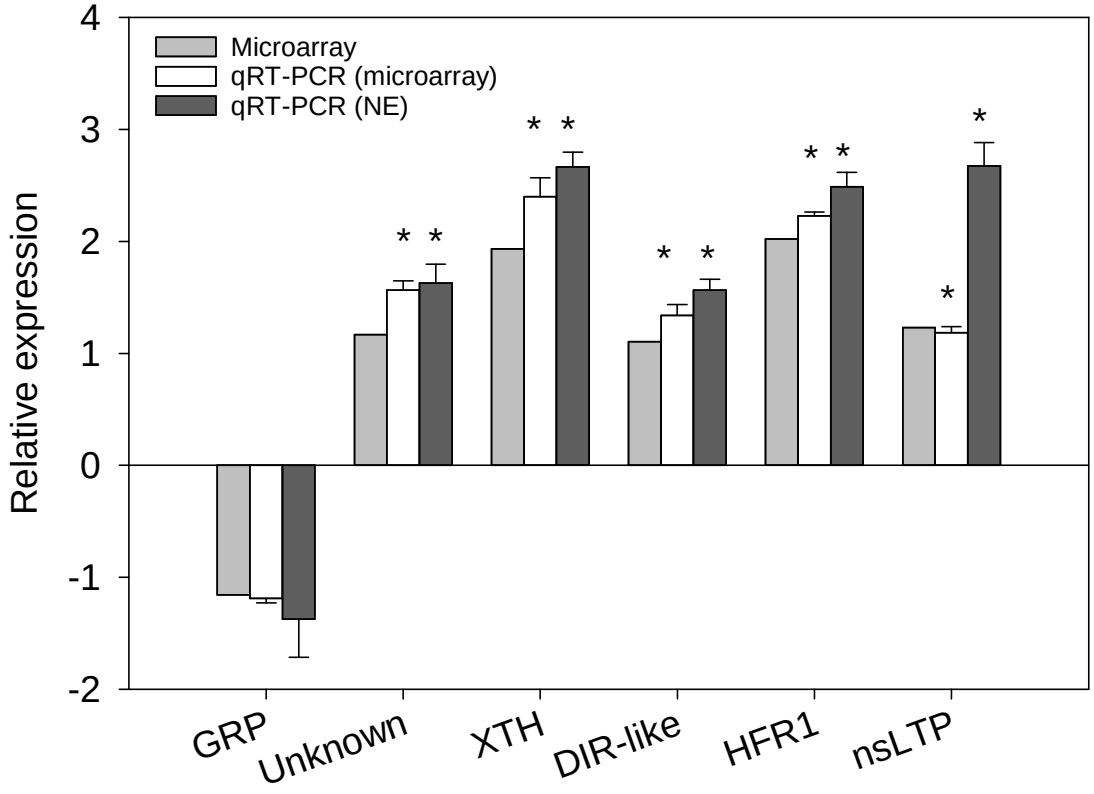
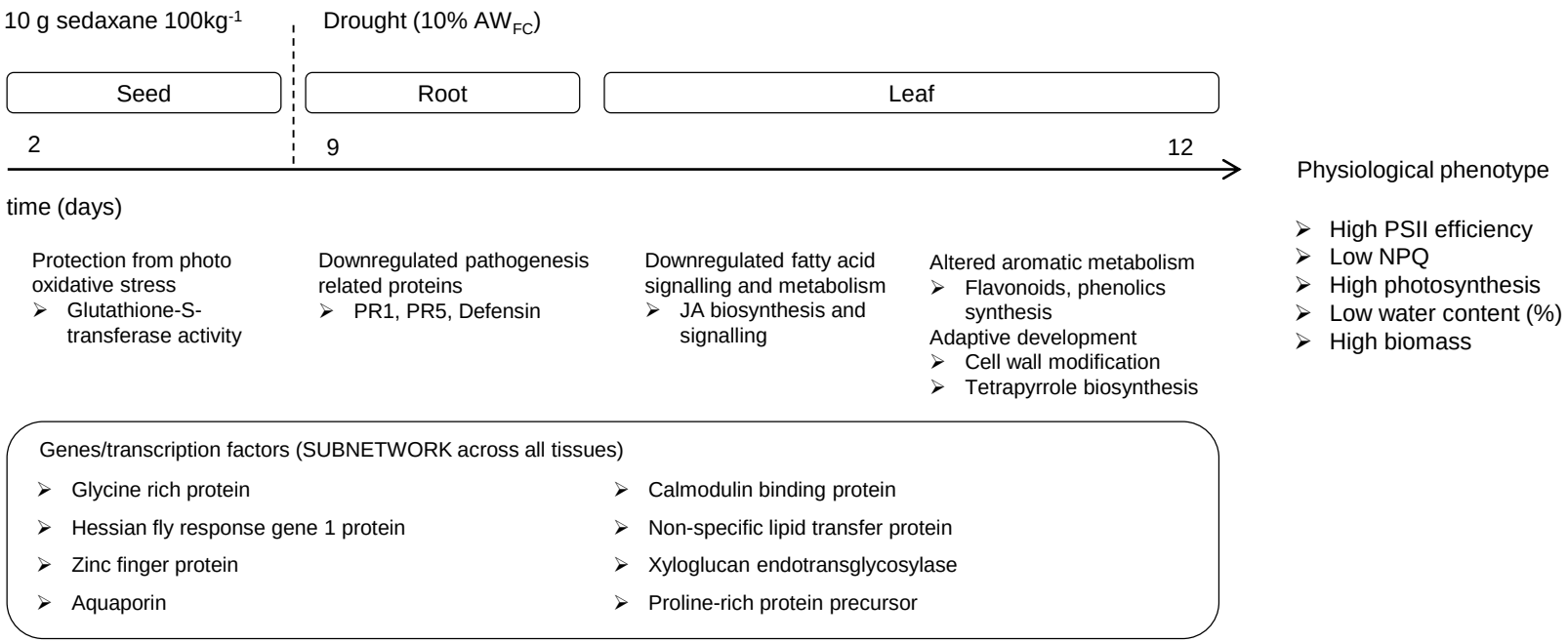

Microarray
qRT-PCR (microarray)
qRT-PCR (NE)
Relative expression
4
3
2
1
0
-1
-2
GRP
Unknown
XTH
DIR-like
HFR1
nsLTP

Fig. 7.


10 g sedaxane 100kg-1
Drought (10% AWFC)
Seed
Root
Leaf
2
9
12
time (days)
Protection from photo oxidative stress
Glutathione-S-transferase activity
Downregulated pathogenesis related proteins
PR1, PR5, Defensin
Downregulated fatty acid signalling and metabolism
JA biosynthesis and signalling
Altered aromatic metabolism
Flavonoids, phenolics synthesis
Adaptive development
Cell wall modification
Tetrapyrrole biosynthesis
Physiological phenotype
High PSII efficiency
Low NPQ
High photosynthesis
Low water content (%)
High biomass
Genes/transcription factors (SUBNETWORK across all tissues)
Glycine rich protein
Hessian fly response gene 1 protein
Zinc finger protein
Aquaporin
Calmodulin binding protein
Non-specific lipid transfer protein
Xyloglucan endotransglycosylase
Proline-rich protein precursor