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Comparative Transcriptome-Based Analysis of the Regulation of Leaf Senescence in the Upper and Middle Canopy of Different Soybean Cultivars

Nan Wang, Zhenghao Zhang, Jiayi Li, Ruoning Li, Xuejing Zhang, Xingdong Yao and Futi Xie *

Soybean Research Institute, Shenyang Agricultural University, Shenyang 110866, China

* Correspondence: xft299@syau.edu.cn

Abstract: Premature leaf failure is one of the major factors contributing to crop yield reduction. High-yielding soybean cultivars exhibit a longer leaf lifespan during the reproductive period, promoting higher yields. In this experiment, physiological indexes and transcriptomics analysis were carried out on leaves of different canopy parts of two soybean cultivars with different senescence sensitivities of LD32 and SND28 as test materials. The results showed that the leaf senescence rates of the two soybean cultivars, tested at the grain-filling stage, were significantly different, and the senescence rates of the upper and middle canopy leaves of LD32 were significantly lower than those of SND28. In comparison to SND28, LD32 exhibited slower decreases in chlorophyll, net photosynthetic rate, and SPAD values in the upper and middle canopy leaves. The differentially expressed genes for senescence of upper and middle canopy leaves of both cultivars were enriched in four pathways: the photosynthesis pathway, the photosynthesis–antenna protein pathway, the MAPK signaling pathway–plant hormone signal transduction pathway, and the plant hormone signal transduction pathway. The differential expression of 20 genes (*Ribose-5-phosphate isomerase*, *fructose-1,6-bisphosphatase*, etc.) in the “carbon fixation in photosynthetic organisms” pathway of LD32 may be involved in the regulation of reducing the rate of leaf senescence in the middle of the canopy at the grain-filling stage of LD32. *Ribose-5-phosphate isomerase* and *fructose-1,6-bisphosphatase* in LD32 may reduce the rate of leaf senescence in the middle of the canopy during seed filling.

Keywords: soybean; canopy; leaf senescence; photosynthesis pathway; photosynthesis–antenna proteins; carbon fixation in photosynthetic organisms pathway



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1. Introduction

Leaf senescence is a necessary stage of crop growth and development, and senescence is an orderly degradation process of the programmed death of leaf cells, which is jointly regulated by internal and external factors, such as the development of the leaf itself, light, hormones, and genetic inheritance [1,2]. Soybean (*Glycine max* (L.) Merr.) is an important food crop and economic crop [3]. In the process of soybean growth and development, the upper leaves of the plant will have a shade impact on the leaves below the middle [4] but also will often—subject to the external environment, including sunlight, high temperature, drought, and other adverse factors—trigger premature leaf senescence [5]. Early leaf failure in different canopy parts will lead to weakened photosynthesis and reduced availability of photosynthetic assimilates, thus affecting the yield and quality of the soybean [6,7]. The most critical growth period for high soybean yield is from pod to seed formation: during this critical period, the leaves in different canopy parts of soybean plants are subjected to different degrees of photosynthesis and may exhibit different rates of leaf senescence [8]. Understanding the mechanisms governing leaf senescence in various canopy regions of soybean plants can aid in mitigating premature senescence, thereby enhancing soybean yield.

Leaf senescence triggers a complex signaling network [9], but the specific mechanisms controlling the whole process are not fully understood. Different hormones have specific functions in activating/inhibiting and controlling leaf senescence, and photosynthesis [10], hormone transduction [11], MAPK signaling [12], and carbon fixation by photosynthetic organisms all play important regulatory roles in leaf growth and development [13,14]. Different canopy leaves of crops are subjected to different light intensities, resulting in changes in leaf photosynthesis [15] that have a great impact on the subsequent physiological characteristics of leaves in terms of photochemical energy, photosynthesis, and carbon assimilation [16]. One of the most striking features of leaf senescence is the decline in photosynthetic capacity. This is mainly due to a decrease in the number of chloroplasts, which ultimately leads to chloroplast decomposition [17,18]. The decomposition of chloroplasts leads to the visualization of leaf senescence, i.e., the color of the leaf changes from green to yellow [19]. The whole process of chloroplast catabolism is strictly regulated by metabolic pathways, such as the gene expression control pathway, the hormone signaling pathway, and the MAPK signaling pathway [20,21]. It has been demonstrated that delaying leaf senescence in soybean plants at the grain-filling stage can effectively improve soybean yield and quality [22], but the molecular mechanisms that regulate leaf senescence in different canopies are still unclear, and there is a lack of theoretical basis for improving soybean leaf senescence traits by using modern breeding techniques.

Transcriptome sequencing is an effective method to study the plant leaf senescence process [23]. Studies have shown that leaf senescence induces changes in the transcript levels of genes through specific pathways' internal and environmental senescence signals. Arabidopsis genes such as *ATAF1/2*, *ANAC092*, and *AtNAC2* were identified as regulators of senescence-induced cell death in Arabidopsis leaves [24–26]. Differential expression of *OsNAP* in rice leads to delayed leaf senescence and a prolonged seed-filling period, resulting in a 6.3% and 10.3% higher seed yield, respectively [27]. *GmNAC81* and *GmNAC30* in soybean have been shown to be associated with the regulation of leaf senescence [28,29]. Using transcriptome sequencing technology to study the leaf senescence process during the critical period of soybean grain yield formation, discovering new genes related to leaf senescence, and exploring the specific mechanisms involved in the regulation of senescence have important theoretical value and practical significance for high-yield soybean cultivation and breeding.

In this study, two soybean cultivars with significantly different canopy leaf senescence phenotypes, namely, the high-yielding soybean cultivars CV. LD32 and conventional CV. SND28, were used as research materials. Physiological indexes and transcriptome sequencing technology were used to compare and analyze the physiological characteristics and gene expression of different canopy leaf senescence characteristics of the two soybean varieties at the grain-filling stage. The results of the study help us understand the regulatory mechanism of leaf senescence in different canopy parts of soybean and provide new ideas for further delaying leaf senescence and achieving high crop yield.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The soybean cultivars Liaodou32 (LD32; maturity at 128 days and slow phenotypic senescence rate of canopy leaves) and Shennongdou28 (SND28; maturity at 130 days and a faster phenotypic senescence rate of canopy leaves) were used as the research materials. The two soybean varieties are local soybean varieties in Liaoning Province, and the maturity of the two soybean varieties is close. Flowering period is the same, all about 60 days after sowing until the beginning of flowering. The experiment was located at the Agronomy Experimental Base of Shenyang Agricultural University in Shenyang, Liaoning Province (41°48'11.75" N, 123°25'31.18" E). The experiment was conducted using a randomized group design with three replicates, and each plot was 20 m². The fields were managed according to local soybean management practices (the soil in the area contained

42.3 g/kg organic matter, 0.201 g/kg total nitrogen, 39.5 mg/kg fast-acting phosphorus, and 226 mg/kg fast-acting potassium, and it had a pH value of 6.30).

2.2. Determination of Samples, Location and Period of Sampling

Samples were obtained at the three key stages (R5, R6, and R7) of soybean grain-filling growth and development. In this experiment, these stages were recorded as T1, T2, and T3, respectively. The upper and middle leaves of the canopy of the soybean plant were used as the leaf determination and sampling locations.

2.3. Determination of Chlorophyll and Leaf SPAD

Chlorophyll was extracted from fresh leaves by the ethanol method [30]. The absorbance values were measured by using a spectrophotometer (665 nm and 649 nm) under two light wavelengths and, finally, calculated by the formula (chlorophyll a (Chla) = $(13.95 * \text{od}_{665} - 6.88 * \text{od}_{649}) * v / 1000 * s$, chlorophyll b (Chlb) = $(24.96 * \text{od}_{649} - 7.32 * \text{od}_{665}) * v / 1000 * s$ where v is the volume of the test liquid). A handheld SPAD meter (SPAD-502PLUS, Konica Minolta, Tokyo, Japan) was clamped to determine the position of the leaves; readings were obtained, values were recorded, and three biological replicates were performed for each treatment.

2.4. Determination of Antioxidant Reductase

From each sample, 0.5 g of leaf material was cut and placed into a pre-cooling bowl with a small amount of quartz sand and 10 mL of pre-cooled phosphate buffer (pH = 7.8, 0.05 mmol/L) and ground in an ice bath. Subsequently, 10 mL of the pulp was centrifuged at 10,000 rpm for 20 min at 25 °C and stored at 0–4 °C. Activity levels of superoxide dismutase (SOD) and peroxidase (POD) were determined by nitroblue tetrazolium assay and guaiacol assay, respectively. These methods were performed in accordance with the experimental method of Wang [31].

2.5. Leaf gas Exchange and Chlorophyll Fluorescence Analysis

Gas exchange parameters of the test material were measured using a portable photosynthesis assay instrument (LI-6800Inc., Lincoln, NE, USA). Sample leaves were placed in the instrument's measuring folder, and measurements were made under normal soybean plant parameters ($1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, ambient CO_2 concentration $380\text{--}400 \mu\text{mol mol}^{-1}$ air CO_2) and during clear daytime hours from 10:00 a.m. to 11:00 a.m. The instrument directly recorded the net photosynthetic rate of the sample leaves. The chlorophyll fluorescence parameters of sample leaves were recorded using a portable chlorophyll recorder (PAM2500, Walz, Nuremberg, Germany). Measured parameters included F_o , F_m , F_s , F_o' , and F_m' , and calculated parameters, including F_v/F_m , qP , NPQ , and ETR , were obtained directly from the instrument. Calculation formulas that may be used in measuring instruments are as follows: $F_v/F_m = (F_m - F_o)/F_m$, $Q_p = (F_m' - F_s)/(F_m' - F_o')$, and $NPQ = F_m/F_m' - 1$ [32,33]. The same samples are used for the above measurements to avoid errors.

2.6. RNA-Seq Analysis

The samples were snap-frozen in liquid nitrogen for 30 min, removed, and then placed in a refrigerator at -80°C . After all samples were collected, they were sent to Lianchuan Biotech (Hangzhou, China) for sequencing and analysis. Genetic differences in the leaves of different canopy parts of different soybean varieties were investigated using RNA-Seq technology (LD32; slow phenotypic senescence rate of canopy leaves and SND28; a faster phenotypic senescence rate of canopy leaves). The experiment was conducted on eight sample groups, numbered 1–12; the representative meaning of each number is shown in Table 1. Total RNA was extracted using TRIzol reagent (Thermo Fisher, Santa Clara, CA, USA) following the manufacturer's instructions. High-quality RNA samples with RIN numbers greater than 7.0 were used to construct sequencing libraries. After total RNA

extraction, two rounds of purification of total RNA (5 µg) were performed using Dynabeads Oligo (dT) (Thermo Fisher, Santa Clara, CA, USA). The cDNA libraries constructed from the pooled RNA of the samples were sequenced using the Illumina Novaseq 6000 sequence platform. The transcriptome was sequenced using the Illumina paired-end RNA-Seq method. Reads from all samples were mapped to the soybean reference genome using the HISAT2 (version: hisat2-2.0.4) software package. A portion of the reads were initially removed (based on the quality information accompanying each read), and the remaining reads were mapped to the reference genome. The transcriptomes of all samples were then merged using software (version: gffcompare-0.9.8) to reconstruct a comprehensive transcriptome. After the final transcriptome was generated, StringTie and BallGown were used to estimate the expression levels of all transcripts and the expression abundance of mRNAs by calculating the fragments per kilobase of exon model per million mapped fragments (FPKM) values. The false discovery rate (FDR) parameter was set to <0.05, and a fold change ≥ 2 ($|\log_2\text{FC}| \geq 1$) and $q < 0.05$ (q value is the corrected p value) were used as the criteria for screening differentially expressed genes (DEGs) [34]. DEGs were then analyzed for GO function and enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [35].

Table 1. Number of transcriptome samples and corresponding physiological significance.

Sample Code	Representative Meaning
LD32-T1-UP (S1)	LD32 upper canopy leaves sampled for the first time during the seed filling
LD32-T1-MID (S2)	LD32 middle canopy leaves sampled for the first time during the seed filling
LD32-T2-UP (S3)	LD32 upper canopy leaves sampled for the second time during the grain filling
LD32-T2-MID (S4)	LD32 middle canopy leaves sampled for the second time during the grain filling
LD32-T3-UP (S5)	LD32 upper canopy leaves sampled for the third time during the grain filling
LD32-T3-MID (S6)	LD32 middle canopy leaves sampled for the third time during the grain filling
SND28-T1-UP (S7)	SND28 upper canopy leaves sampled for the first time during the seed filling
SND28-T1-MID (S8)	SND28 middle canopy leaves sampled for the first time during the seed filling
SND28-T2-UP (S9)	SND28 upper canopy leaves sampled for the second time during the grain filling
SND28-T2-MID (S10)	SND28 middle canopy leaves sampled for the second time during the grain filling
SND28-T3-UP (S11)	SND28 upper canopy leaves sampled for the third time during the grain filling
SND28-T3-MID (S12)	SND28 middle canopy leaves sampled for the third time during the grain filling

2.7. Quantitative Real-Time PCR (qRT-PCR) Analysis

The relative gene expression of the DEGs was verified using qRT-PCR. The fluorescent quantification primer sequences of the differential genes were designed, β -actin was selected as the internal reference gene control, and RNA was extracted from the samples by reagents provided by OMEGA Bio-tek. The All-in-One First-Strand cDNA Synthesis Kit was used to reverse transcribe RNA into cDNA, which was used as a template for qRT-PCR validation using an Agilent Mx3000p real-time fluorescence quantitative analyzer (Agilent, Santa Clara, California, USA). The target gene results were quantified using the $2^{-\Delta\Delta C_t}$ method (the $2^{-\Delta\Delta C_t}$ method is a simple way to analyze relative changes in gene expression in real-time quantitative PCR experiments, i.e., a simple method for relative quantification).

2.8. Data Analysis

All experiments in this study were independently repeated three times. The data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test ($p < 0.05$) using SPSS 22.0 Statistics (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Leaf Phenotypes and Physiological Indices of Soybean

The color of different canopy leaves of the two soybeans was determined, and it was found (Figure 1) that the color of the leaves in the middle part of the canopy of SND28 changed from green to yellow from the T2 period and wilted rapidly in the T3 period

compared to LD32. The different canopy leaves of LD32 showed longer green holding (slower senescence) compared to SND28.

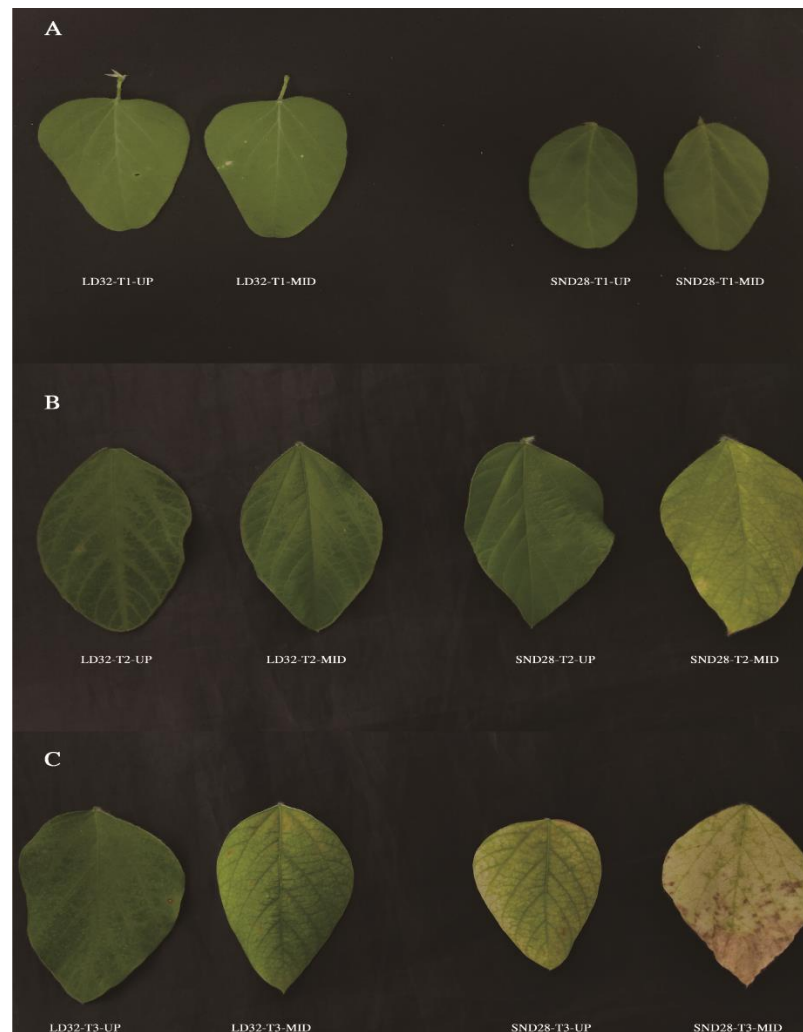


Figure 1. Phenotypes of two soybean cultivars. (Phenotypes of LD32 and SND28 in upper and middle leaves at soybean grain-filling stage). (A) Phenotypes of two soybean cultivars during T1. (B) Phenotypes of two soybean cultivars during T2. (C) Phenotypes of two soybean cultivars during T3.

Chl a content, Chl b content, and SPAD values in different canopy leaves of LD32 and SND28 decreased as the sampling period advanced. The SPAD values of the upper canopy leaves of both soybean varieties were larger than those of the middle canopy leaves, and the SPAD values of the upper and middle canopy leaves showed a decreasing trend. At the same sampling period, the SPAD values of the upper and middle canopy leaves of LD32 were higher than those of the upper and middle canopy leaves of SND28 (Figure 2A). The results of Chl a and Chl b contents showed that chlorophyll degradation was slower in the different canopy leaves of LD32 compared to SND28. In the T2 period, the rate of decline of Chl a content and Chl b content in the middle canopy leaves of SND28 was higher than that of LD32 (Figure 2C,D). During the T1 to T3 periods, the net photosynthetic rates of leaves in the middle of the canopy were higher in LD32 than in SND28 (Figure 2B). In summary, compared with SND28, the leaves of LD32 were characterized by a slower senescence rate at the grain-filling stage of the soybean.

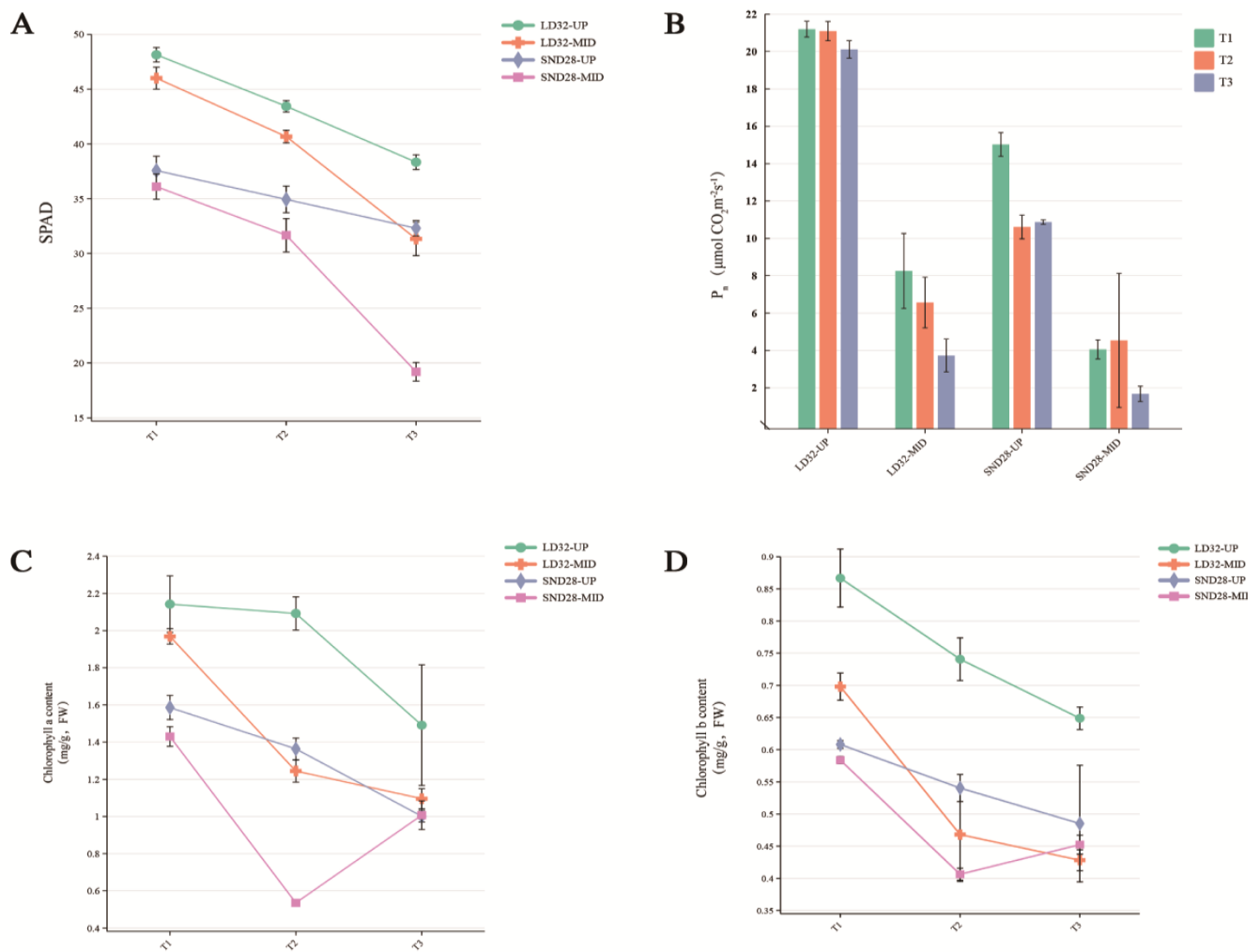


Figure 2. (A) SPAD values of LD32 and SND28 in the upper and middle leaves at soybean grain-filling stage. (B) Net photosynthetic rates of LD32 and SND28 in upper and middle leaves at soybean grain-filling stage. (C) Chlorophyll a content of upper and middle leaves of LD32 and SND28 at soybean grain-filling stage. (D) Chlorophyll b content of upper and middle leaves of LD32 and SND28 at soybean grain-filling stage.

In order to further understand the relevant regulators of leaf senescence in different canopies of soybean at the grain-filling stage, chlorophyll fluorescence values and antioxidant enzyme activities of leaves were determined. Maximum photosynthetic quantum yield (F_v/F_m) reflects the maximum light energy conversion efficiency of the PS II reaction center. PS II potential vigor (F_v/F_o) and photochemical quenching coefficient (qP) reflect the high or low photosynthetic activity of the plant. A higher PS II electron transfer rate (ETR) indicates a stronger photosynthetic capacity. As the sampling period progressed, the photosynthetic capacity of both SND28 and LD32 gradually weakened; the photosynthetic capacity of leaves in the upper part of the canopy was greater than that of leaves in the center. Heat dissipation NPQ (nonphotochemical quenching) reflects the ability of plants to dissipate excess light energy into heat (photoprotective capacity), and the results indicated that LD32 was more photoprotective than SND28 (Figure 3A). The antioxidant enzyme activity assay showed that the antioxidant enzyme activities in the middle leaves of the LD32 canopy were higher than those in the middle leaves of the SND28 canopy in the T2 period (Figure 3B,C).

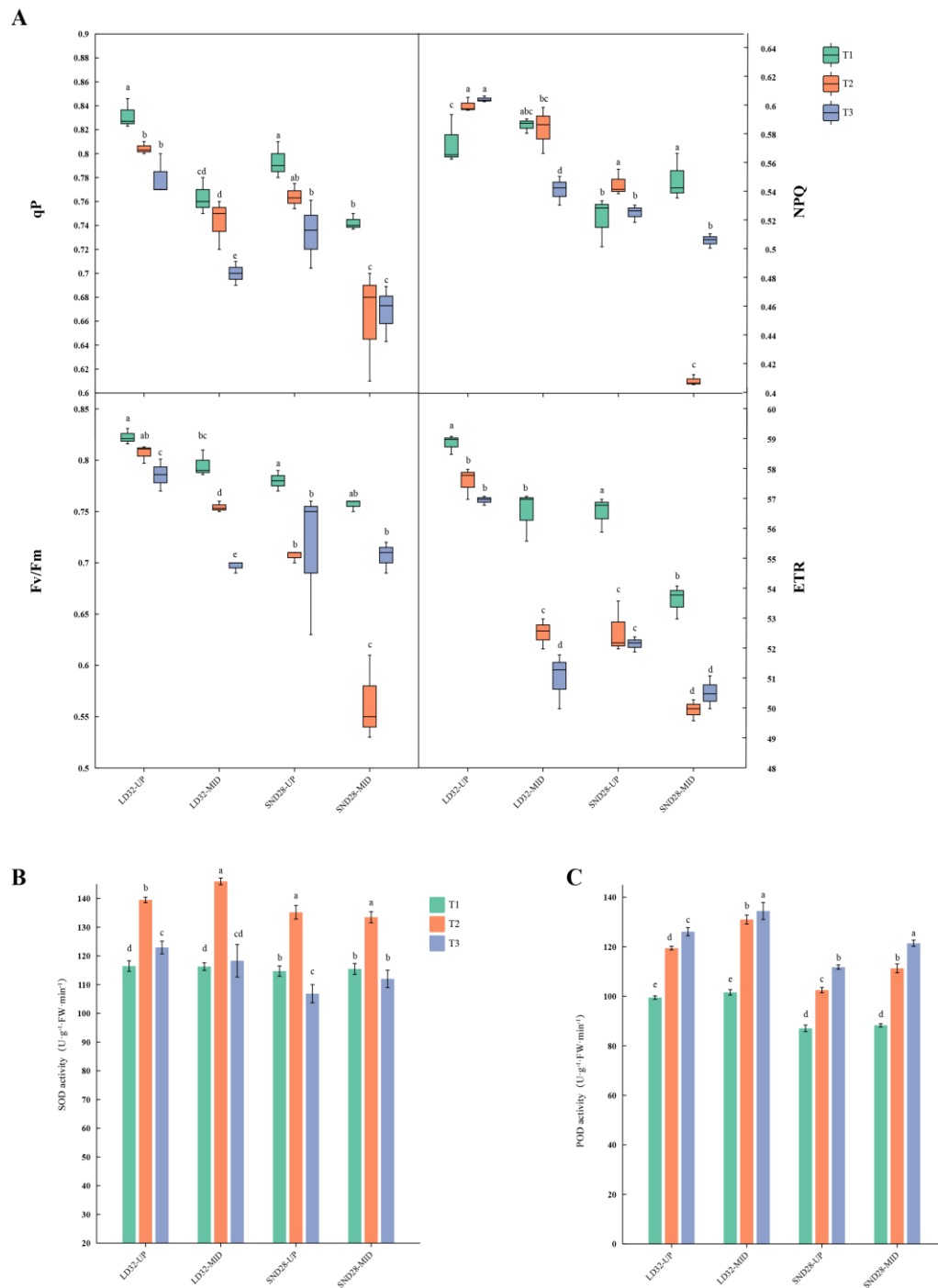


Figure 3. (A) Chlorophyll fluorescence parameters of LD32 and SND28 in upper and middle leaves at soybean grain-filling stage. (B) SOD enzyme activities of LD32 and SND28 in upper and middle leaves at soybean grain-filling stage. (C) POD enzyme activities of LD32 and SND28 in upper and middle leaves at soybean grain-filling stage.

3.2. Transcriptome Differences and Enrichment Analysis

In order to understand the transcriptional landscape of different canopy leaves of soybean during senescence, RNA-Seq analyses were performed on the upper and middle leaves of SND28 and LD32 canopies. The transcriptome sequencing data of the 12 comparative groups were analyzed for quality control, and the results are shown in Table S1. The effective data volume of all samples was in the range of 5.31–8.39 G, the number of effective reads was 86.93–97.22% of the total reads, the bases with quality value $Q \geq 20$ accounted

for 99.91–99.97% of the total reads, the number of bases with quality value $Q \geq 30$ accounted for 97.82–98.89% of the total reads, and the GC content was 43–44% of the total GC content (accounting for 43–44.5% of the total reads). The comprehensive evaluation results showed that the transcriptome sequencing results were qualified and could be analyzed downstream.

Further analysis of the number of different differential genes in the 12 comparison groups showed that in the T1 period there were 7312 differential genes in the upper and middle canopy leaves of SND28 (SND28-T1-UP vs. SND28-T1-MID) and 12,883 differential genes in the upper and middle canopy leaves of LD32 (LD32-T1-UP vs. LD32-T1-MID). In the T2 period, there were 6084 differential genes in the upper and middle canopy leaves of SND28 (SND28-T2-UP vs. SND28-T2-MID), and LD32 had a total of 13,770 differential genes in the upper and middle canopy leaves (LD32-T2-UP vs. LD32-T2-MID). In the T3 period, there were a total of 13,557 differential genes in the upper and middle canopy leaves of SND28 (SND28-T3-UP vs. SND28-T3-MID), and LD32 had a total of 11,766 differential genes in the upper and middle canopy leaves (LD32-T3-UP vs. LD32-T3-MID). Therefore, enrichment analysis can be performed for differential genes in the upper and middle leaves of the canopy of different soybean varieties (LD32 vs. SND28) during seed filling.

3.3. Transcriptional Analysis Reveals Enrichment Pathways Involved in Upper and Middle Leaf Senescence in the Soybean Canopy at Soybean Grain-Filling Stage

In the three periods (T1, T2, and T3), there were 3057 differentially expressed genes (DEGs) detected between the upper and middle leaves of the LD32 canopy (LD32-T1-UP vs. LD32-T1-MID), (LD32-T2-UP vs. LD32-T2-MID), and (LD32-T3-UP vs. LD32-T3-MID) (Figure 4A). The common DEGs were subjected to GO enrichment analysis, and 168 pathways were found to be enriched, including the photosynthesis pathway, the light-harvesting in photosystem I pathway, the photosystem II pathway, and other GO pathways (Figure 4B). These DEGs were subjected to KEGG analysis, and 19 pathways were found to be enriched in these genes. They included metabolic pathways such as the photosynthesis–antenna protein pathway, the photosynthesis pathway, the MAPK signaling pathway–plants pathway, and other metabolic pathways (Figure 4C). There were 1314 differentially expressed genes (DEGs) detected between the upper and middle leaves of the SND28 canopy (SND28-T1-UP vs. SND28-T1-MID), (SND28-T2-UP vs. SND28-T2-MID), and (SND28-T3-UP vs. SND28-T3-MID) (Figure 4D). The common DEGs were subjected to GO enrichment analysis, and 216 pathways were found to be enriched, including the photosynthesis pathway, the light-harvesting in photosystem I pathway, and other GO pathways (Figure 4E). These DEGs were subjected to KEGG analysis, and 16 pathways were found to be enriched in these genes. They included metabolic pathways such as the photosynthesis–antenna protein pathway, the photosynthesis pathway, the MAPK signaling pathway–plants pathway, and other metabolic pathways (Figure 4F).

3.4. Analysis of Senescence Regulatory Pathways in the Upper and Middle Leaves of Soybean Canopy at Soybean Grain-Filling Stage

Different canopy leaves of two soybean varieties possessed common enrichment of metabolic pathways and differential genes at T1–T3. In six comparison groups (LD32-T1-UP vs. LD32-T1-MID, LD32-T2-UP vs. LD32-T2-MID, LD32-T3-UP vs. LD32-T3-MID, SND28-T1-UP vs. SND28-T1-MID, SND28-T2-UP vs. SND28-T2-MID, and SND28-T3-UP vs. SND28-T3-MID), 140 common differential genes were obtained (Figure 5A), which were enriched in four pathways: the photosynthesis pathway, the photosynthesis–antenna protein pathway, the phytohormone signaling pathway, and the MAPK signaling pathway–plant pathway (Figure 5B). Among them, there were two photosynthesis-related genes (Figure 5C), three photosynthesis–antenna proteins-related genes (Figure 5D), twelve plant hormone signaling pathway-related genes (Figure 5E), and fourteen MAPK signaling pathway-related genes (Figure 5F). The results (Table S3) revealed that at the T3 period, in the photosynthesis pathway, *psbA* was upregulated for expression in both LD32 (LD32-T3-UP vs. LD32-T3-MID) and SND28 (SND28-T3-UP vs. SND28-T3-MID), and

SoyZH13_12G084100 was upregulated for expression in LD32 (LD32-T3-UP vs. LD32-T3-MID) but downregulated for expression in SND28 (SND28-T3-UP vs. SND28-T3-MID); in the photosynthesis–antenna protein pathway, all differential genes enriched were downregulated for expression in LD32 (LD32-T3-UP vs. LD32-T3-MID), but all were upregulated for expression in SND28 (SND28-T3-UP vs. SND28-T3-MID). In the phytohormone signaling pathway and MAPK signaling pathway—plant pathway, the differentially expressed genes enriched to SND28 (SND28-T3-UP vs. SND28-T3-MID) and LD32 (LD32-T3-UP vs. LD32-T3-MID) showed opposite expression trends (the same DEGs were upregulated for expression in LD32 but downregulated for expression in SND28, and vice versa), except for SoyZH13_19G058000, SoyZH13_19G210900, and SoyZH13_15G047900. In conclusion, the above enrichment pathway is involved in the regulation of leaf senescence in different canopies of two soybean varieties at the grain-filling stage, and the differential expression of genes in the enrichment pathway may be the main reason for the difference in leaf senescence of the canopy between the two varieties. However, the specific process of the differential genes in regulating leaf senescence needs to be confirmed by further functional experiments.

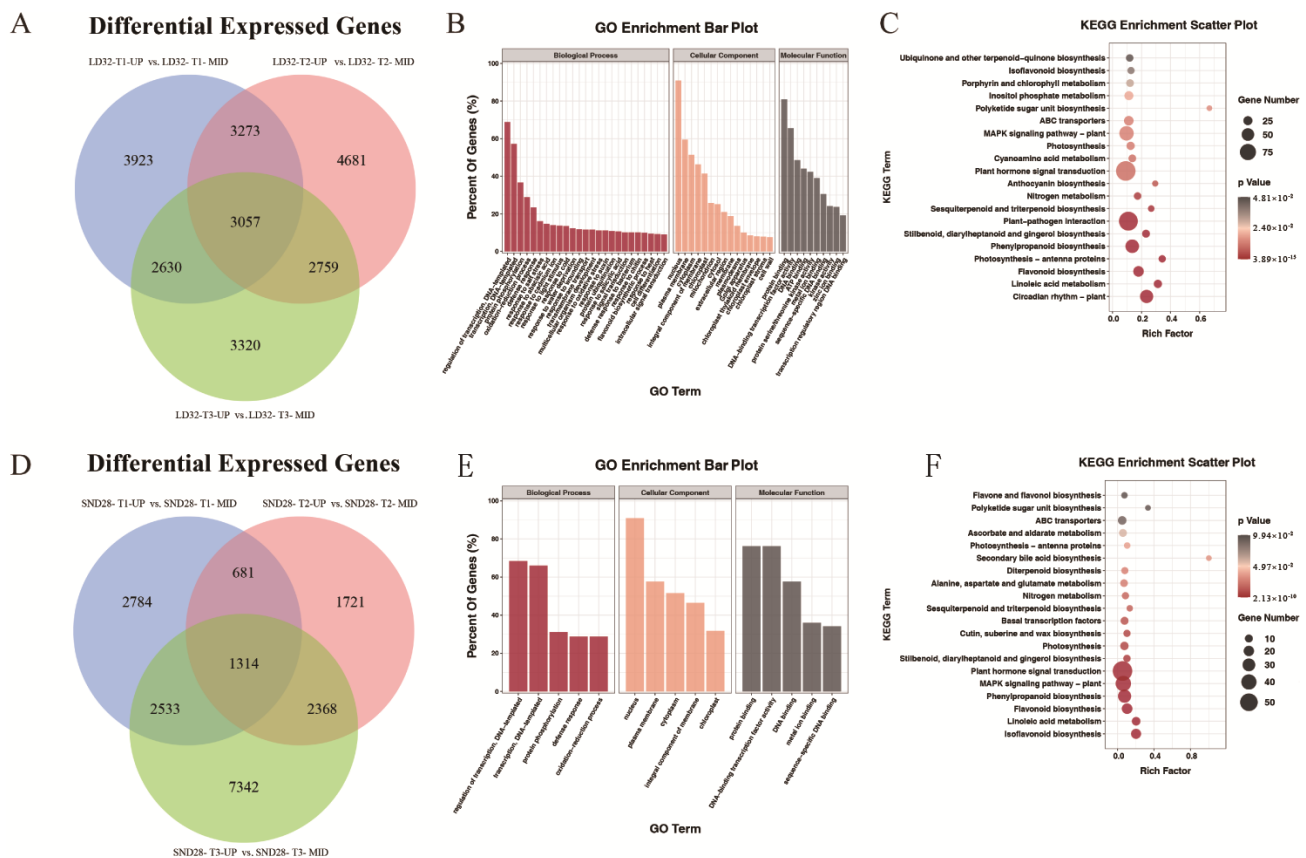


Figure 4. Analysis of differentially expressed genes in the upper and middle leaves of LD32 and SND28 canopies. (A) Wayne plots of the differentially expressed genes of LD32 in the upper and middle leaves at soybean grain-filling stage. (B) GO functional analysis of the differential genes of LD32 in the upper and middle leaves at soybean grain-filling stage. (C) KEGG enrichment analysis of the differential genes of LD32 in the upper and middle leaves at soybean grain-filling stage. (D) Wayne plots of the differential genes of SND28 in the upper and middle leaves during the soybean seed-filling period. (E) GO function analysis of the differential gene of SND28 in the upper and middle leaves at soybean grain-filling stage. (F) KEGG enrichment analysis of the differential gene of SND28 in the upper and middle leaves at soybean grain-filling stage.

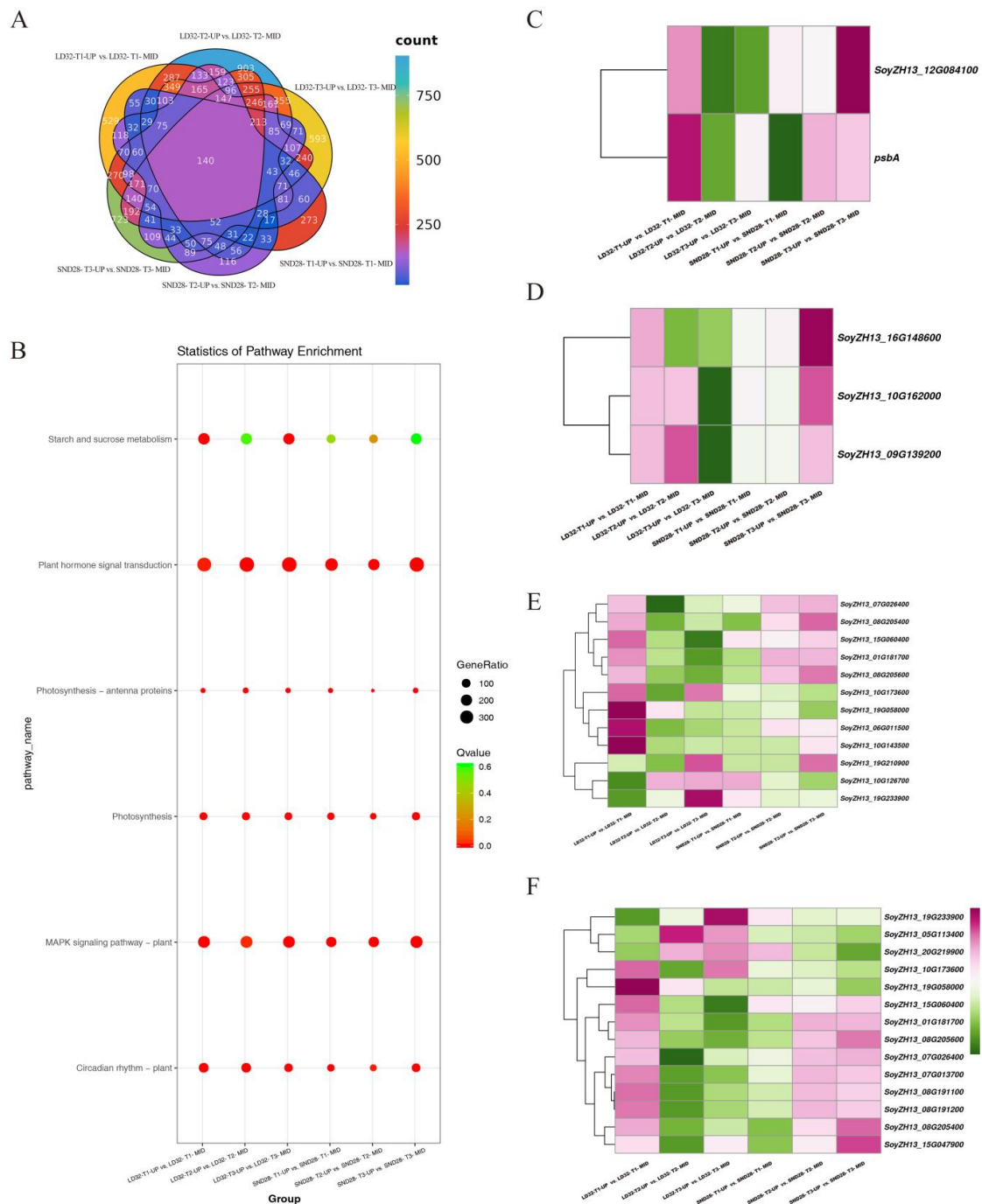


Figure 5. (A) Wayne plots of genes co-differentially expressed by LD32 and SND28 in the upper and middle leaves of the canopy at soybean grain-filling stage. (B) KEGG enriched iso-pathway plots of genes co-differentially expressed by LD32 and SND28 in the upper and middle leaves of the canopy at soybean grain-filling stage. (C) Heat map of LD32 and SND28 enriched for common differentially expressed genes in the photosynthesis pathway in the upper and middle leaves of the canopy at soybean grain-filling stage. (D) Heat map of LD32 and SND28 enriched for common di-fifferential genes in the photosynthesis-hapten pathway in the upper and middle leaves of the canopy at soybean grain-filling stage. (E) Heat map of LD32 and SND28 enriched for common differential genes in the phytohormone signaling pathway in upper and middle leaves of the canopy at soybean grain-filling stage. (F) Heat map of LD32 and SND28 enriched for common differential genes in the MAPK signaling pathway-plant in upper and middle leaves of the canopy at soybean grain-filling stage.

3.5. Differentially Expressed Genes in Photosynthesis-Related Pathways Involved in the Regulation of Senescence of Upper and Middle Leaves in the Soybean Canopy at Soybean Grain-Filling Stage

In the photosynthesis-related enrichment pathway, LD32 was enriched with more differential genes compared with SND28 in T1–T3. A total of twenty differential genes in the photosynthesis pathway were co-enriched by LD32, of which fourteen were upregulated and six were downregulated in the T1 period (Figure 6A). A total of twelve differential genes in the photosynthesis pathway were identified by SND28 co-enrichment, of which only two were upregulated and the remaining ten were downregulated in the T1 period (Figure 6B). A total of 13 differential genes co-enriched for LD32 in the photosynthesis–antenna protein pathway were upregulated and expressed in all of them at T1 (Figure 6C). A total of four differential genes co-enriched for SND28 in the photosynthesis–antenna protein pathway were upregulated and expressed in all of them at T1 (Figure 6D). In the photosynthetic system I in the photosynthetic pathway, relative to SND28, all genes in LD32 were upregulated for expression at T1 (i.e., photosystem I subunit Psd-SoyZH13_10G229700, photosystem I reaction center subunit VI-SoyZH13_13G329300) and downregulated for expression at T3. Meanwhile, among the genes in photosystem II, photosystem II 22 kDa protein (SoyZH13_04G224800 and SoyZH13_06G108400) and photosystem II reaction center PSB28 protein (SoyZH13_10G039100 and SoyZH13_13G109300) were relatively downregulated in LD32 at both T1 and T3, but all other genes were upregulated for expression at T1. These results indicated that LD32 showed more upregulated genes in both the photosynthesis pathway and the photosynthesis–antenna protein pathway, and SND28 showed more downregulated genes in T1. LD32 was in a period of strong gene expression in T1, and gene expression in SND28 was weakened (Figure 6A,C). It is conjectured that this may be the reason that the central leaves in the canopy of SND28 showed, from the T1 period onward, a premature senescence phenotype.

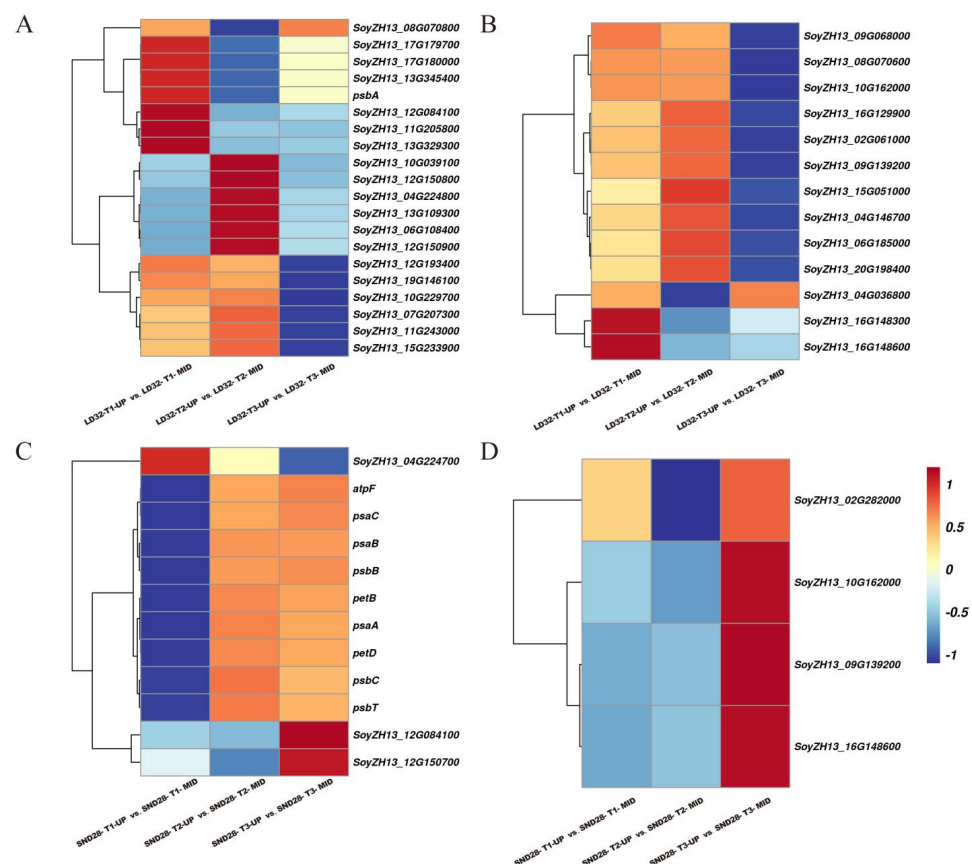


Figure 6. Heat map analysis of differentially expressed genes in the upper and middle leaves of LD32 and SND28 canopies. (A) Heatmap of common differential genes of LD32 in the photosynthesis pathway

in the upper and middle leaves at soybean grain-filling stage. **(B)** Heatmap of LD32 enrichment of common differential genes in the photosynthesis-antenna proteins pathway in the upper and middle leaves at soybean grain-filling stage. **(C)** Heat map of common differential genes in the photosynthesis pathway enriched by SND28 in the upper and middle leaves at soybean grain-filling stage. **(D)** Heat map of SND28 enrichment of common differential genes in the photosynthesis-antenna proteins pathway in the upper and middle leaves at soybean grain-filling stage.

3.6. Transcriptional Analysis Reveals Differentially Expressed Genes Involved in Leaf Senescence in the Middle of the Soybean Canopy at Soybean Grain-Filling Stage

In order to gain a clearer understanding of the mechanisms and differential genes that regulate the senescence rate of the mid-canopy leaves of different senescence phenotypes, the mid-canopy leaves of LD32 and the mid-canopy leaves of SND28 from T1 to T3 were analyzed. The carbon fixation in the photosynthetic organisms pathway was found to be enriched in both varieties. A total of 108 identical differential genes in this pathway were found in the mid-canopy leaves of both varieties (Figure 7A). Compared with SND28, LD32 mid-canopy leaves had 20 differential genes in the carbon fixation in the photosynthetic organisms pathway (ribose-5-phosphate isomerase, fructose-1,6-bisphosphatase, and other putative proteins) that were specifically expressed individually (Figure 7C). The differential expression of the 20 aforementioned differential genes is likely to play a key role in the regulation of leaf senescence in the mid-canopy of LD32 at T1–T3 (Figure 7B).

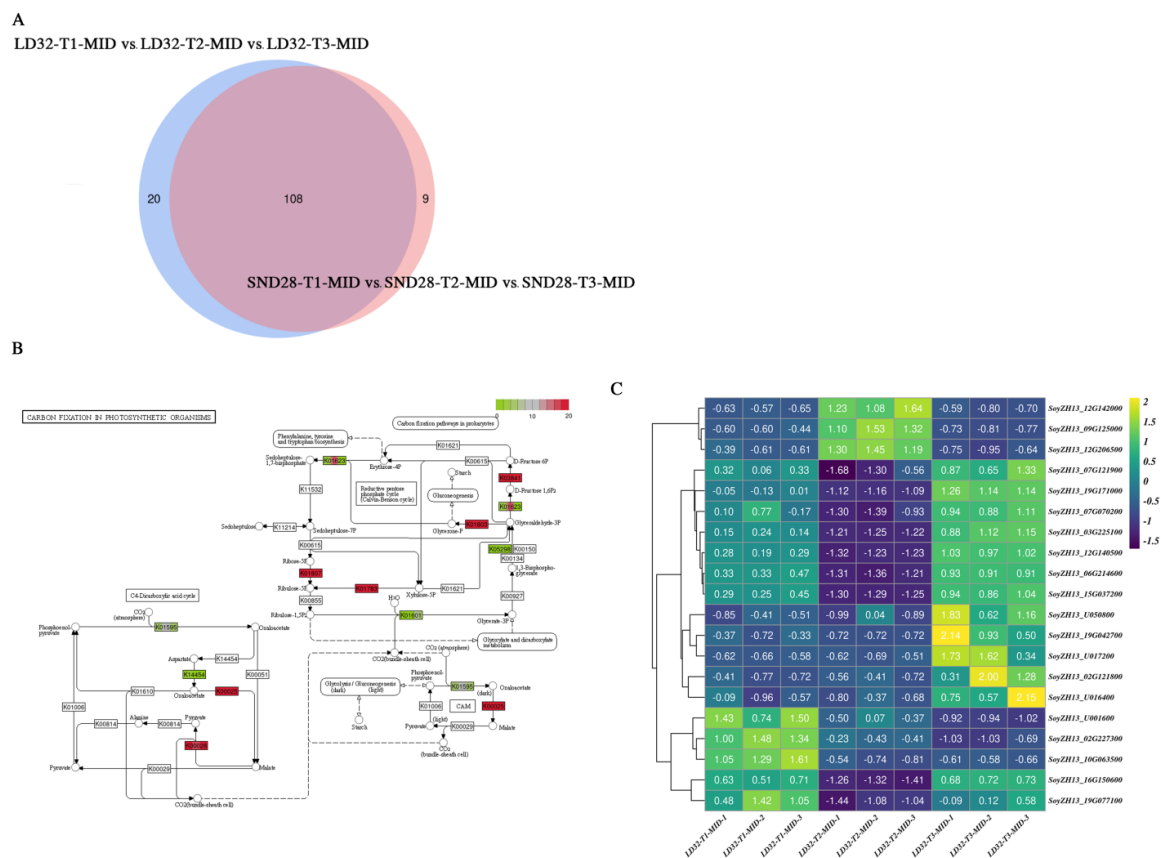


Figure 7. (A) Plots of genes co-differentially expressed by LD32 and SND28 in mid-canopy leaves at soybean grain-filling stage. (B) LD32 and SND28 enrich differentially expressed genes in the carbon fixation in photosynthetic organisms pathway in mid-canopy leaves at soybean grain-filling stage. Red, green, and gray indicate the expression of the differentially expressed genes, respectively. (C) Heatmap analysis of common differentially expressed genes in the carbon fixation in photosynthetic organisms pathway.

3.7. Validation of DEGs by qRT-PCR Analysis

Based on the results of pre-transcriptional enrichment, we have enriched and focused on eight, and the photosynthesis pathways (*SoyZH13_12G084100*), the photosynthesis–antenna protein pathway (*SoyZH13_10G162000*, *SoyZH13_09G139200*, and *SoyZH13_16G148600*), the MAPK signaling pathway (*SoyZH13_08G205600* and *SoyZH13_08G191200*), and the phytohormone pathway (*SoyZH13_01G181700* and *SoyZH13_10G126700*)-related differential genes were detected by fluorescence quantification. Based on the fluorescence quantification results, we found that *SoyZH13_09G139200* had the highest expression in LD32-T1-up. *SoyZH13_01G181700*, *SoyZH13_08G191200*, and *SoyZH13_08G205600* had the highest expression in SND28-T1-MID. *SoyZH13_16G148600* had higher expression in LD32-T1-up and SND28-T3-up, and the rest of the expression was lower (Figure S1). Finally, we found that the transcriptome data showed the same trend as the fluorescence quantification results by correlation analysis ($R^2 = 0.8629$), proving the accuracy of the RNA-Seq results (Figure 8).

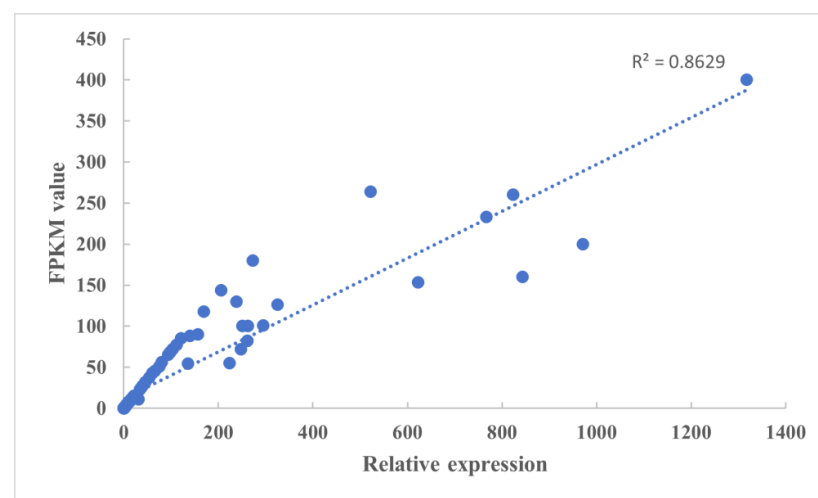


Figure 8. Correlation analysis of RNA-Seq and qRT-PCR.

4. Discussion

For various soybean canopy parts, leaves, and the entire crop, senescence is related to seed yield. During the bulge period, soybean's photosynthetic capacity maintenance and yield increase are closely related. In previous studies, senescence retardation in different crops was mainly reflected in leaves, and delaying leaf senescence in crop plants is one of the most important ways to increase crop yields [36]. Sustained photosynthesis in different canopy leaves of soybean is crucial for seed bulging during crop senescence. In this study, by measuring the phenotypic and physiological indexes of leaves in different canopy parts of various soybean varieties, we found that compared with SND28, leaves in different canopy parts of LD32 had greater SPAD values, slower leaf senescence (more green holding), a lower rate of chlorophyll content degradation, a higher rate of net photosynthetic rate in the later period of reproduction, and higher final grain yield (Table S2). This suggests that LD32 has more senescence-resistant characteristics and high yield, but the regulatory mechanisms of leaf senescence in different canopy layers need to be further investigated.

Transcriptome sequencing of upper and lower canopy leaves of two soybean varieties collected from the T1 to T3 periods showed that differentially expressed genes were mainly enriched in the photosynthesis–antenna protein pathway and the photosynthesis pathway. The higher plant chlorophyll a-b binding protein CP26 belongs to the Lhc family of proteins and can simultaneously play a central role in the balance between effective photoprotection and energy dissipation. The higher plant photosystem II antenna protein CP26 is an important component of photosystem II, which plays a key role in light energy absorption, transmission, and regulation of excitation energy distribution in the two photosystems [37,38]. In plant photosynthesis, the CP26 protein realizes efficient light energy

capture and conversion through its unique structure and function. When light is irradiated onto plant leaves, the CP26 protein is able to absorb light energy and transfer it to the light reaction center, thus driving photosynthesis [39]. In addition, it is able to regulate the distribution of excitation energy between the two photosystems according to changes in light conditions to ensure the smooth progress of photosynthesis [40]. The chlorophyll a-b binding protein CP26 is not only involved in light harvesting but also in the coordination of the light-harvesting antenna LHCII; furthermore, it influences NPQ in an indirect way. The chlorophyll a-b binding protein CP26 may remove excess excitation energy from the light-trapping antenna LHCII that reaches the PSII reaction centers, and it may consume a certain amount of energy without damaging the reaction centers under photoinhibitory conditions. As a member of the Lhc family of proteins, changes in gene expression of the chlorophyll a-b binding protein CP26 affect light harvesting in photosystem I and photosystem II, thereby influencing the growth, development, and senescence of upper and middle leaves in the canopy of soybean plants at the bulging stage. However, further studies and explorations are needed regarding the specific mechanism of action of the CP26 protein and the interaction relationship with other photosynthesis-related proteins. In the results of this experiment, the chlorophyll a-b binding protein CP26 was significantly enriched in the upper and middle leaves of both LD32 and SND28 canopies. There were three chlorophyll a-b binding protein CP26-related genes significantly enriched in LD32, namely, *SoyZH13_10G162000*, *SoyZH13_09G139200*, and *SoyZH13_15G051000*. In the three DEGs mentioned above, at the T1 and T2 periods, the expression of the chlorophyll a-b binding protein CP26 gene was lower in the middle leaves of the LD32 canopy than in the upper leaves of the LD32 canopy; at the T3 period, the expression of the chlorophyll a-b binding protein CP26 gene in the middle leaves of the LD32 canopy was higher than the content in the upper leaves of the LD32 canopy. Two chlorophyll a-b binding protein-related genes were significantly enriched in SND28: *SoyZH13_10G162000* and *SoyZH13_09G139200*. The expression of the chlorophyll a-b binding protein CP26 gene of the above two DEGs in the middle leaves of the SND28 canopy was lower than the content in the upper leaves of the SND28 canopy at the T1, T2, and T3 periods (SND28-T1-UP vs. SND28-T1-MID, SND28-T2-UP vs. SND28-T2-MID, and SND28-T3UP vs. SND28-T3-MID). Based on these results, it is hypothesized that differences in the expression patterns of the chlorophyll a-b binding protein CP26 are the influencing factors for the differences in leaf senescence in the upper and middle parts of the SND28 and LD32 canopies.

Chloroplast photosystem II (PSII) plays a central role in plant photosynthesis. It is responsible for absorbing light energy and converting it into chemical energy to drive the process of photosynthesis. The PSII reaction center is a key site in this process, and its protein composition and functional status have a direct impact on the efficiency of photosynthesis. Leaf senescence is a complex physiological process involving the action of a variety of internal and external factors, among which the decline in photosynthetic capacity is one of the important signs of leaf senescence. As leaves senesce, the function of PSII gradually declines, which may cause changes in the structure or function of proteins within the system, including PSB28, PsbY, and PsbQ [41]. Among them, changes in PsbQ protein directly lead to changes in the exothermic activity of PSII, which, in turn, affects the photosynthetic efficiency and physiological state of leaves, and the above changes might lead to changes in the absorption and conversion efficiency of light energy by PSII [42]. This, in turn, affects the entire photosynthetic process and directly affects the senescence process of leaves. In the results of this experiment, only PSB28 (*SoyZH13_10G039100*, *SoyZH13_13G109300*), PsbY (*SoyZH13_11G243000*), and PsbQ (*SoyZH13_11G205800*) were significantly enriched in the upper and middle leaves of the LD32 canopy. In order to gain a deeper understanding of the direct relationship between PSB28, PsbY, and PsbQ proteins and leaf senescence, more experimental studies may be needed, such as observing the expression, structural, and functional changes in PSB28, PsbY, and PsbQ proteins in leaves at different stages of senescence, as well as further investigating how the above changes affect the overall function of PSII and the senescence process of leaves.

Ribulose-5-phosphate isomerase (RPI) is a naturally occurring widespread and highly conserved protease that plays a key enzymatic role in the pentose phosphate oxidation cycle [43,44]; it is also an important player in the carbon fixation in the photosynthetic organisms pathway, participating in the prokaryotic and eukaryotic pentose phosphate pathway (PPP) and the Calvin cycle for CO₂ fixation in plants. *Ribulose-5-phosphate isomerase* plays a crucial role in photosynthesis [45]. *Fructose-1,6-bisphosphatase* is present in soybean chloroplasts, and its reductive activation process also affects chloroplast photosynthesis [46,47]. Studies have shown that the joint involvement of multiple genes for photosynthetic carbon assimilation positively affects photosynthesis and biomass yield [48,49]. In this study, *ribulose-5-phosphate isomerase* (*SoyZH13_03G225100*) and *fructose-1,6-bisphosphatase* (*SoyZH13_16G150600*) were detected to be differentially expressed in the mid-canopy leaves at T1–T3 of LD32, but no expression of the above differential genes was detected in SND28. It is hypothesized that the longer green-holding and slower senescence rate of the mid-canopy leaves of LD32 are related to the specific expression of the above DEGs in the carbon fixation in the photosynthetic organisms pathway.

Compared with SND28, LD32 leaves in the upper and middle parts of the canopy possessed fourteen upregulated genes in the photosynthesis pathway at the T1 period, including six upregulated genes in photosystem II (*SoyZH13_11G243000*, *SoyZH13_13G345400*, *SoyZH13_15G233900*, *SoyZH13_17G179700*, *SoyZH13_17G180000*, and *psbA*), three upregulated genes in photosystem I (*SoyZH13_10G229700*, *SoyZH13_11G205800*, and *SoyZH13_13G329300*), and five upregulated genes acts on the remaining functional parts of the photosynthesis pathway (*SoyZH13_12G084100*, *SoyZH13_07G207300*, *SoyZH13_08G070800*, *SoyZH13_12G193400*, and *SoyZH13_19G146100*). LD32 leaves in the upper and middle parts of the canopy possessed thirteen upregulated genes in the photosynthesis–antenna protein pathway at the T1 stage, including eight upregulated genes in Lhc I and five upregulated genes in Lhc II. It is speculated that the upregulated expression of the above differential genes is related to the higher Chl a content, Chl b content, SPAD, and net photosynthetic rate in the upper and middle leaves of the LD32 canopy at T1, indicating that the upregulated expression of the above genes is likely to play a key role in the regulation and control of leaf aging in the upper and middle leaves canopy at the grain-filling stage of LD32 (Figure 9).

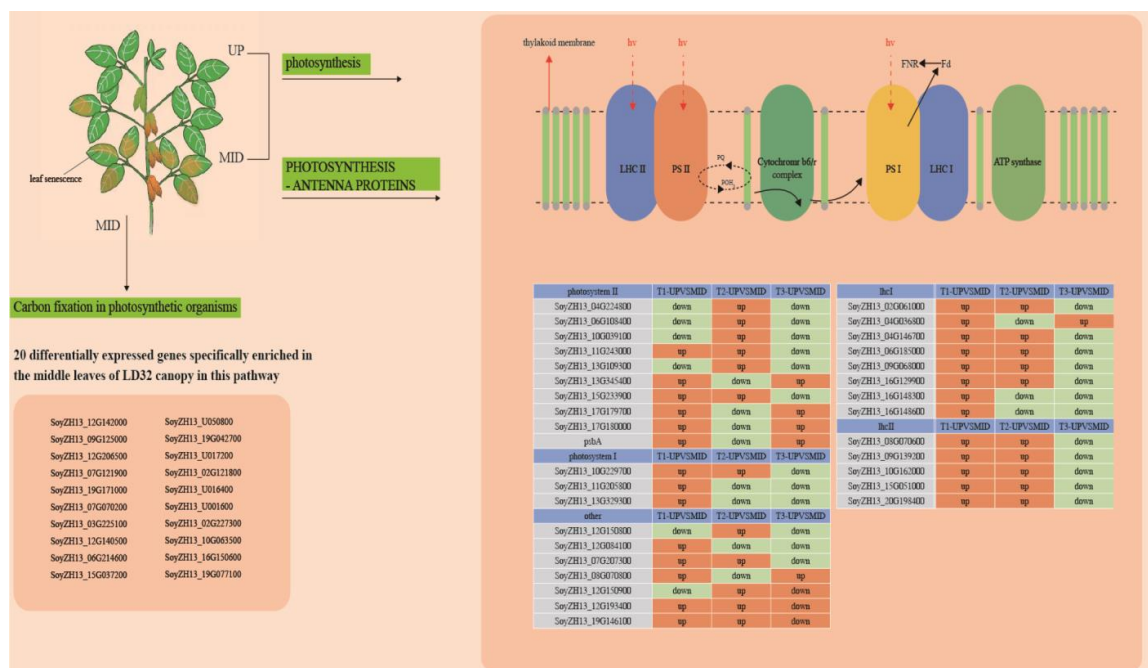


Figure 9. The proposed model represents the impact of aging on different crown and middle leaf segments of LD32. The orange box indicates an increase, the green box indicates a decrease, and the black arrow indicates direction guidance.

5. Conclusions

In this study, two soybean varieties, LD32 and SND28, with different rates of leaf senescence in different parts of the canopy, were subjected to physiological indexes and transcriptome analyses. The results showed that the photosynthesis pathway, the photosynthesis–antenna protein pathway, the MAPK signaling pathway–plant pathway (mitogen-activated protein kinase (MAPK) cascades, the evolutionarily conserved signaling modules in all eukaryotes that adapt to various stresses and play pivotal roles in processes such as hormonal and developmental signaling), the phytohormone signaling pathway, and differentially expressed genes were involved in the regulation of leaf senescence in the upper and middle parts of the canopy at the grain-filling stage. Compared with SND28, LD32 showed resistance to early senescence during seed filling, which may be influenced by the expression of differentially expressed genes and their specific genes in the above pathways.

Based on these results, we proposed a hypothetical model to illustrate the regulatory mechanisms affecting upper and middle leaf senescence in the canopy site during seed filling in LD32 (LD32-T1-UP vs. LD32-T1-MID, LD32-T2-UP vs. LD32-T2-MID, and LD32-T3-UP vs. LD32-T3-MID). Differential expression of the same DEGs in the photosynthesis pathway and the photosynthesis–antenna protein pathway in different sampling periods are involved in the regulation of leaf senescence in the upper and middle leaves of the canopy at the grain-filling stage of LD32, and 20 genes specifically expressed in the metabolic pathway of the carbon fixation in the photosynthetic organisms pathway in the leaves of the middle part of the canopy at the grain-filling stage of LD32 could have an effect on chloroplast photosynthesis through the reduction activation process. The effect on chloroplast photosynthesis through the process of reductive activation may delay leaf senescence. However, precisely how the differential expression of genes in the metabolic pathway of the carbon fixation in the photosynthetic organisms pathway affects chloroplast photosynthesis and the photosynthesis–antenna protein pathway through the reduction activation process remains to be investigated further (Figure 9).

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy14061250/s1>, Table S1: Sequencing sequence statistics and accusations of soybean leaves, Table S2: Yield statistics for two soybean varieties, Table S3: Up- and down-regulated expression of genes, Figure S1: Validation of differential genes in different comparison groups by qRT-PCR.

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References

1. Leng, Y.; Ye, G.; Zeng, D. Genetic Dissection of Leaf Senescence in Rice. *Int. J. Mol. Sci.* **2017**, *18*, 2686. [CrossRef] [PubMed]
2. Li, Z.; Lyu, X.; Li, H.; Tu, Q.; Zhao, T.; Liu, J.; Liu, B. Author Correction: The mechanism of low blue light-induced leaf senescence mediated by GmCRY1s in soybean. *Nat. Commun.* **2024**, *15*, 798. [CrossRef] [PubMed]
3. Board, J.E.; Harville, B.G. Soybean Yield Component Responses to a Light Interception Gradient during the Reproductive Period. *Crop Sci.* **1993**, *33*, 772–777. [CrossRef]
4. Tranbarger, T.J.; Tucker, M.L.; Roberts, J.A.; Meir, S. Editorial: Plant Organ Abscission: From Models to Crops. *Front. Plant Sci.* **2017**, *8*, 196. [CrossRef] [PubMed]

5. Ouyang, X.; Zhong, X.; Chang, S.; Qian, Q.; Zhang, Y.; Zhu, X. Partially functional NARROW LEAF1 balances leaf photosynthesis and plant architecture for greater rice yield. *Plant Physiol.* **2022**, *189*, 772–789. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Burkey, K.O.; Wells, R. Response of soybean photosynthesis and chloroplast membrane function to canopy development and mutual shading. *Plant Physiol.* **1991**, *97*, 245–252. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Jumrani, K.; Bhatia, V.S.; Pandey, G.P. Impact of elevated temperatures on specific leaf weight, stomatal density, photosynthesis and chlorophyll fluorescence in soybean. *Photosynth. Res.* **2017**, *131*, 333–350. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Kim, J.; Yang, J.; Yang, R.; Sicher, R.C.; Chang, C.; Tucker, M.L. Transcriptome Analysis of Soybean Leaf Abscission Identifies Transcriptional Regulators of Organ Polarity and Cell Fate. *Front. Plant Sci.* **2016**, *7*, 125. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Li, S.; Zhao, Y.; Tan, S.; Li, Z. Non-coding RNAs and leaf senescence: Small molecules with important roles. *Plant Physiol. Bioch* **2024**, *207*, 108399. [\[CrossRef\]](#)
10. Gordon, A.J.; Hesketh, J.D.; Peters, D.B. Soybean leaf photosynthesis in relation to maturity classification and stage of growth. *Photosynth. Res.* **1982**, *3*, 81–93. [\[CrossRef\]](#)
11. Chen, C.; Favero, B.T.; Nardy, R.; He, J.; de Godoy Maia, I.; Liu, F.; Lütken, H. *Rhizobium rhizogenes* rolC gene promotes leaf senescence and enhances osmotic stress resistance in Arabidopsis: The positive role of abscisic acid. *Physiol. Plant.* **2024**, *176*, e14142. [\[CrossRef\]](#)
12. Yadav, N.; Nagar, P.; Rawat, A.; Mustafiz, A. Phytosulfokine receptor 1 (AtPSKR1) acts as a positive regulator of leaf senescence by mediating ROS signaling in Arabidopsis thaliana. *Env. Exp. Bot.* **2024**, *220*, 105674. [\[CrossRef\]](#)
13. Zhang, Q.; Chen, C.; Guo, R.; Zhu, X.; Tao, X.; He, M.; Li, Z.; Shen, L.; Li, Q.; Ren, D.; et al. Plasma membrane-localized hexose transporter OsSWEET1b, affects sugar metabolism and leaf senescence. *Plant Cell Rep.* **2024**, *43*, 29. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Tice, M.M.; Lowe, D.R. Hydrogen-based carbon fixation in the earliest known photosynthetic organisms. *Geology* **2006**, *34*, 37–40. [\[CrossRef\]](#)
15. Fan, Y.; Chen, J.; Wang, Z.; Tan, T.; Li, S.; Li, J.; Wang, B.; Zhang, J.; Cheng, Y.; Wu, X.; et al. Soybean (*Glycine max* L. Merr.) seedlings response to shading: Leaf structure, photosynthesis and proteomic analysis. *Bmc Plant Biol.* **2019**, *19*, 34. [\[CrossRef\]](#)
16. Wang, J.; Chen, G.; Li, X.; Fu, X.; Li, S.; Tao, X.; Chen, Z.; Xu, S. Transcriptome and Metabolome Analysis of a Late-Senescent Vegetable Soybean during Seed Development Provides New Insights into Degradation of Chlorophyll. *Antioxidants* **2022**, *11*, 2480. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Tanaka, R.; Tanaka, A. Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes. *Acta (BBA) Bioenerg.* **2011**, *1807*, 968–976. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Tian, Y.N.; Zhong, R.H.; Wei, J.B.; Luo, H.H.; Eyal, Y.; Jin, H.L.; Wu, L.; Liang, K.; Li, Y.; Chen, S.; et al. Arabidopsis CHLORO-PHYLLASE 1 protects young leaves from long-term photodamage by facilitating FtsH-mediated D1 degradation in photosystem II repair. *Mol. Plant* **2021**, *14*, 1149–1167. [\[CrossRef\]](#)
19. Hörtensteiner, S.; Kräutler, B. Chlorophyll breakdown in higher plants. *Acta (BBA) Bioenerg.* **2010**, *1807*, 977–988. [\[CrossRef\]](#)
20. Martyna, Z.; Zbigniew, T. Biochemical and physiological aspects of chlorophyll breakdown. *Postep. Biochem.* **2019**, *65*, 128–134.
21. Ulrich, E.; Bernhard, G.; Stefan, H. Recent advances in chlorophyll biosynthesis and breakdown in higher plants. *Plant Mol. Biol.* **2004**, *56*, 1–14.
22. Ferreira, D.O.; Fraga, O.T.; Pimenta, M.R.; Caetano, H.D.N.; Machado, J.P.B.; Carpinetti, P.A.; Brustolini, O.J.B.; Quadros, I.P.S.; Reis, P.A.B.; Fontes, E.P.B. GmNAC81 Inversely Modulates Leaf Senescence and Drought Tolerance. *Front. Genet.* **2020**, *11*, 601876. [\[CrossRef\]](#)
23. Di, H.; Zhang, C.; Zhou, A.; Huang, H.; Tang, Y.; Li, H.; Huang, Z.; Zhang, F.; Sun, B. Transcriptome Analysis Reveals the Mechanism by Which Exogenous Melatonin Treatment Delays Leaf Senescence of Postharvest Chinese Kale (*Brassica oleracea* var. *alboglabra*). *Int. J. Mol. Sci.* **2024**, *25*, 2250. [\[CrossRef\]](#)
24. Wu, A.; Allu, A.D.; Garapati, P.; Siddiqui, H.; Dortay, H.; Zanol, M.I.; Asensi-Fabado, M.A.; Munné-Bosch, S.; Antonio, C.; Tohge, T.; et al. JUNGBRUNNEN1, a reactive oxygen species-responsive NAC transcription factor, regulates longevity in Arabidopsis. *Plant Cell* **2012**, *24*, 482–506. [\[CrossRef\]](#)
25. Kim, J.H.; Woo, H.R.; Kim, J.; Lim, P.O.; Lee, I.C.; Choi, S.H.; Hwang, D.; Gil Nam, H. Trifurcate Feed-Forward Regulation of Age-Dependent Cell Death Involving miR164 in Arabidopsis. *Science* **2009**, *323*, 1053–1057. [\[CrossRef\]](#)
26. Kim, Y.S.; Sakuraba, Y.; Han, S.H.; Yoo, S.C.; Paek, N.C. Mutation of the Arabidopsis NAC016 transcription factor delays leaf senescence. *Plant Cell Physiol.* **2013**, *54*, 1660–1672. [\[CrossRef\]](#)
27. Liang, C.; Wang, Y.; Zhu, Y.; Tang, J.; Hu, B.; Liu, L.; Ou, S.; Wu, H.; Sun, X.; Chu, J.; et al. OsNAP connects abscisic acid and leaf senescence by fine-tuning abscisic acid biosynthesis and directly targeting senescence-associated genes in rice. *Prac. Natl. Acad. Sci. USA* **2014**, *111*, 10013–10018. [\[CrossRef\]](#)
28. Mendes, G.C.; Reis, P.A.; Calil, I.P.; Carvalho, H.H.; Aragão, F.J.; Fontes, E.P. GmNAC30 and GmNAC81 integrate the endoplasmic reticulum stress- and osmotic stress-induced cell death responses through a vacuolar processing enzyme. *Prac. Natl. Acad. Sci. USA* **2013**, *110*, 19627–19632. [\[CrossRef\]](#)
29. Teixeira, F.O.; de Melo, B.P.; Silva, Q.I.P.; Reis, P.A.B.; Fontes, E.P.B. Senescence-Associated Glycine max (Gm)NAC Genes: Integration of Natural and Stress-Induced Leaf Senescence. *Int. J. Mol. Sci.* **2021**, *22*, 8287.
30. Sartory, D.P.; Grobbelaar, J.U. Extraction of Chlorophyll a from Freshwater Phytoplankton for Spectrophotometric Analysis. *Hydrobiologia* **1984**, *114*, 177–187. [\[CrossRef\]](#)

31. Wang, X.; Wu, Z.; Zhou, Q.; Wang, X.; Song, S.; Dong, S. Physiological Response of Soybean Plants to Water Deficit. *Front. Plant Sci.* **2022**, *12*, 809692. [\[CrossRef\]](#)
32. van Kooten, O.; Snel, J.F. The Use of Chlorophyll Fluorescence Nomenclature in Plant Stress Physiology. *Photosynth. Res.* **1990**, *25*, 147–150. [\[CrossRef\]](#)
33. Krause, G.H.; Weis, E. Chlorophyll Fluorescence and Photosynthesis: The Basics. *Annu. Rev. Plant Biol.* **1991**, *42*, 313–349. [\[CrossRef\]](#)
34. Ebrahim, S.S.M.; Marghoob, M.; Robert, S.; Tilgner, H.; Afshar, P.T.; Au, K.F.; Bani Asadi, N.; Gerstein, M.B.; Wong, W.H.; Snyder, M.P.; et al. Gaining comprehensive biological insight into the transcriptome by performing a broad-spectrum RNA-seq analysis. *Nat. Commun.* **2017**, *8*, 59.
35. Yi, L.; Pimentel, H.; Bray, N.L.; Pachter, L. Gene-level differential analysis at transcript-level resolution. *Genome Biol.* **2018**, *19*, 53. [\[CrossRef\]](#)
36. Xue, J.; Lu, D.; Wang, S.; Lu, Z.; Liu, W.; Wang, X.; Fang, Z.; He, X. Integrated transcriptomic and metabolomic analysis provides insight into the regulation of leaf senescence in rice. *Sci. Rep.* **2021**, *11*, 1–12.
37. de Bianchi, S.; Dall'Osto, L.; Tognon, G.; Morosinotto, T.; Bassi, R. Minor antenna proteins CP24 and CP26 affect the interactions between photosystem II subunits and the electron transport rate in grana membranes of Arabidopsis. *Plant Cell Online* **2008**, *20*, 1012–1028. [\[CrossRef\]](#)
38. Mullet, J.E.; Burke, J.J.; Arntzen, C.J. A Developmental Study of Photosystem I Peripheral Chlorophyll Proteins. *Plant Physiol.* **1980**, *65*, 823–827. [\[CrossRef\]](#)
39. Andersson, J.; Walters, R.G.; Jansson, H.S. Antisense Inhibition of the Photosynthetic Antenna Proteins CP29 and CP26: Implications for the Mechanism of Protective Energy Dissipation. *Plant Cell Online* **2001**, *13*, 1193–1204. [\[CrossRef\]](#)
40. Zienkiewicz, M.; Ferenc, A.; Wasilewska, W.; Romanowska, E. High light stimulates Deg1-dependent cleavage of the minor LHClI antenna proteins CP26 and CP29 and the PsbS protein in Arabidopsis thaliana. *Planta* **2012**, *235*, 279–288. [\[CrossRef\]](#)
41. Yu, M.; Huang, L.; Feng, N.; Zheng, D.; Zhao, J. Exogenous Uniconazole Enhances Tolerance to Chilling Stress in Mung Beans (*Vigna Radiata* L.) through Cross Talk among Photosynthesis, Antioxidant System, Sucrose Metabolism, and Hormones. *J. Plant Physiol.* **2022**, *276*, 153772. [\[CrossRef\]](#)
42. Yuan, L.; Zheng, Y.; Nie, L.; Zhang, L.; Wu, Y.; Zhu, S.; Hou, J.; Shan, G.L.; Liu, T.K.; Chen, G.; et al. Transcriptional Profiling Reveals Changes in Gene Regulation and Signaling Transduction Pathways during Temperature Stress in Wucui (*Brassica Campestris* L.). *BMC Genom.* **2021**, *22*, 687. [\[CrossRef\]](#)
43. Chen, J.; Wu, H.; Zhang, W.; Mu, W. Ribose-5-phosphate isomerases: Characteristics, structural features, and applications. *Appl. Microbiol. Biot.* **2020**, *104*, 6429–6441. [\[CrossRef\]](#)
44. Han, E.; Wang, H.; Han, L.; Li, J.; Li, S.; Wang, B. Cloning and Functional Analysis of GmABCG1 Gene in Glycine max. *Mol. Plant Breed.* **2018**, *24*, 7925–7932.
45. Hamada, K.; Ago, H.; Sugahara, M.; Nodake, Y.; Kuramitsu, S.; Miyano, M. Oxyanion hole-stabilized stereospecific isomerization in ribose-5-phosphate isomerase (Rpi). *J. Biol. Chem.* **2003**, *278*, 49183–49190. [\[CrossRef\]](#)
46. Ruth, A.H. Chloroplast Alkaline Fructose 1,6-Bisphosphatase Exists in a Membrane-Bound Form. *Plant Physiol.* **1982**, *3*, 728–734.
47. Slovacek, R.E.; Monahan, B.C. Reductive activation of fructose-1,6-bisphosphatase and the peroxide effect on chloroplast photosynthesis. *Arch. Biochem. Biophys.* **1983**, *224*, 310–318. [\[CrossRef\]](#)
48. Chen, Z.-F.; Wang, T.-H.; Feng, C.-Y.; Guo, H.-F.; Guan, X.-X.; Zhang, T.-L.; Li, W.-Z.; Xing, G.-M.; Sun, S.; Tan, G.-F. Multigene manipulation of photosynthetic carbon metabolism enhances the photosynthetic capacity and biomass yield of cucumber under low-CO₂ environment. *Front. Plant Sci.* **2022**, *13*, 1005261. [\[CrossRef\]](#)
49. Simkin, A.J.; McAusland, L.; Headland, L.R.; Lawson, T.; Raines, C.A. Multigene manipulation of photosynthetic carbon assimilation increases CO₂ fixation and biomass yield in tobacco. *J. Exp. Bot.* **2015**, *66*, 4075. [\[CrossRef\]](#)

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