Effects of Exogenous Salicylic Acid on Photosynthesis and Nitrogen Metabolism of Tobacco under Drought Stress and Transcriptome Analysis

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Introduction

Background

- Water is a major ecological factor for the growth, physiological metabolism and quality formation of tobacco.

- Drought causes not only the decrease of tobacco yield and quality, but also the lowering of nitrogen utilization rate, leading to a large amount of nitrate accumulation and increased levels of tobacco specific nitrosamine in cured and stored leaves.

- Enhancing plant drought tolerance has been an important topic for many researchers around the world. And many studies found adding exogenous regulatory substances is an efficient mean of improving resistance to abiotic stresses.

- Previous studies revealed that Salicylic Acid (SA), a phenolic compound and signal substance, was able to alleviate drought stress by an increase in antioxidant enzyme activities and photosynthesis. However, there is currently no in-depth coverage about the drought tolerance mechanisms at the genome-wide transcriptional level in SA-regulated tobacco seedlings.

In this study:

Two experiments were designed to investigate the physiological responses of tobacco to salicylic acid under PEG drought stress and natural drought stress conditions. Transcriptome sequencing and GO/KEGG analysis were also performed to explain the regulatory mechanism.

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Material and Method

Materials and method

Materials

1. • **Varieties:** TN90 and K326
2. • **PEG Drought treatment:** CK (water + Hoagland nutrient solution), D (water +15 % PEG6000-treated nutrient solution), D+SA (0.3 mmol/L SA + 15 % PEG6000)
3. • **Natural Drought treatment:** CK (70 % soil moisture content), D (50 % soil moisture content), D+SA (0.3 mmol/L SA + 50 % soil moisture content)

Methods

1. • Photosynthesis parameters: Pn, Tr, Gs and Ci
2. • Antioxidant parameters: SOD, POD, CAT, MDA, PRO and protein content
3. • Key nitrogen metabolism enzyme activities (NR and GS) and nitrate content
4. • Transcriptome sequencing

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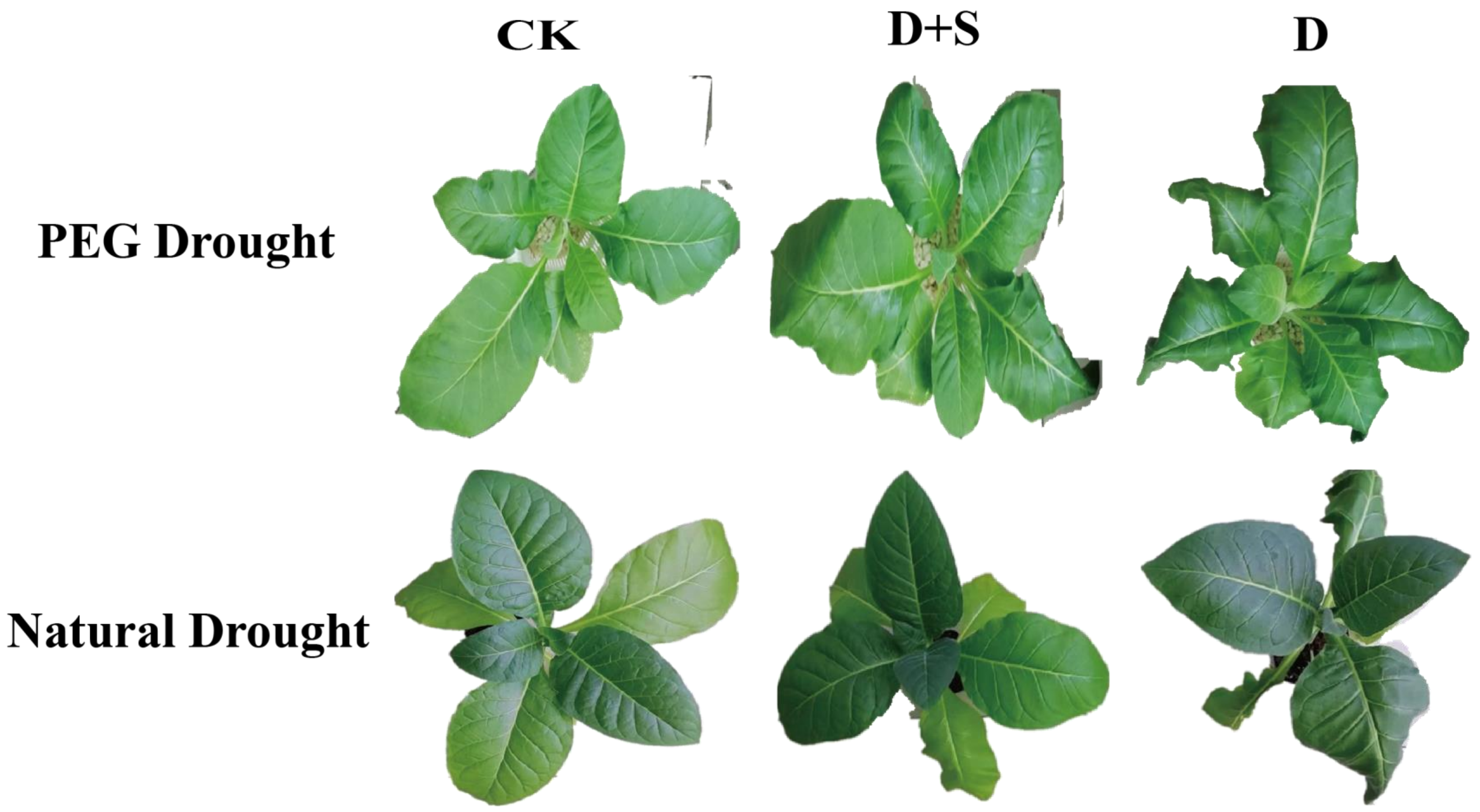
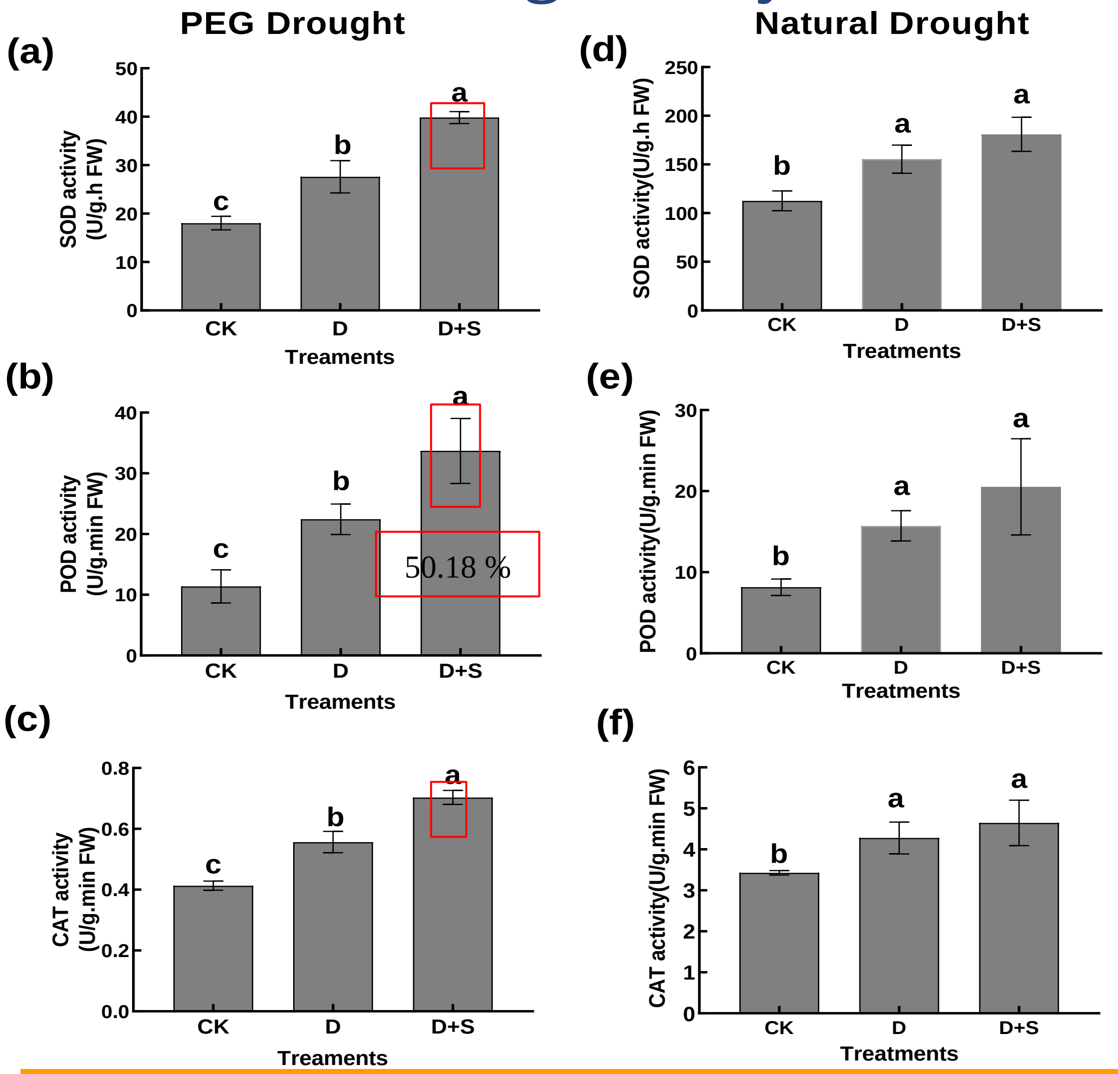
Results and Discussion

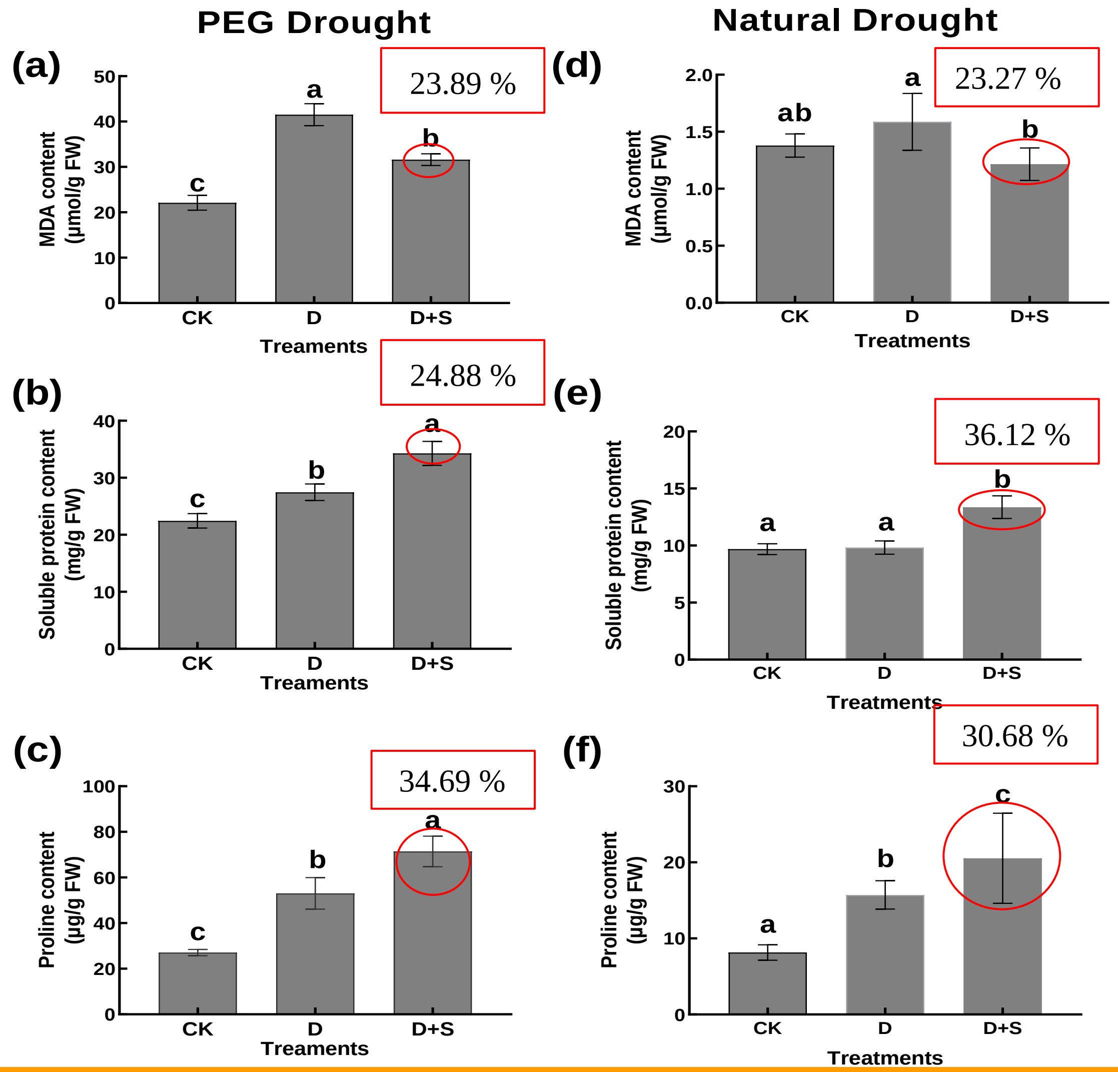
Phenotypic observation under PEG drought stress and natural drought stress



It was worth noting that under drought stress (D), all the leaves began wilting. In contrast, the leaves of flue-cured tobacco only had slighter symptoms of wilting under SA treatment (D+S).

Reduction of MDA and increase of antioxidant enzyme activities and osmotic regulatory substance by SA

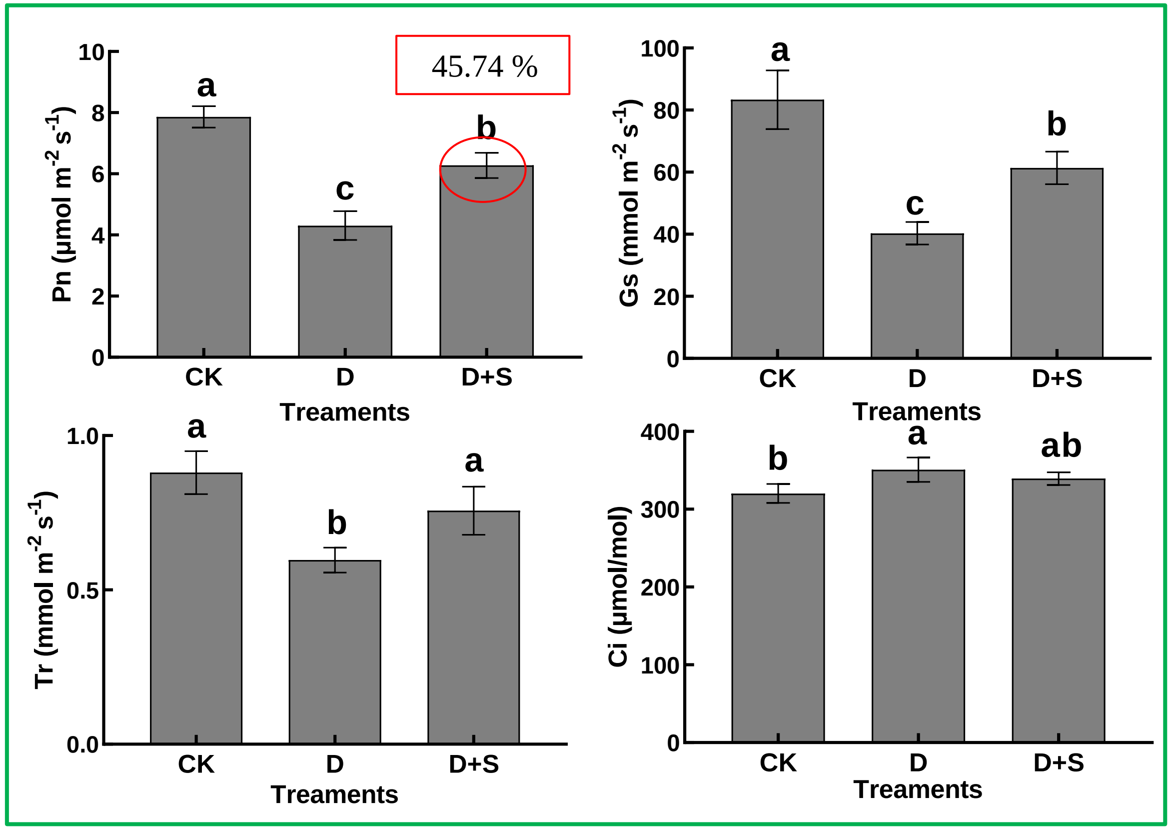




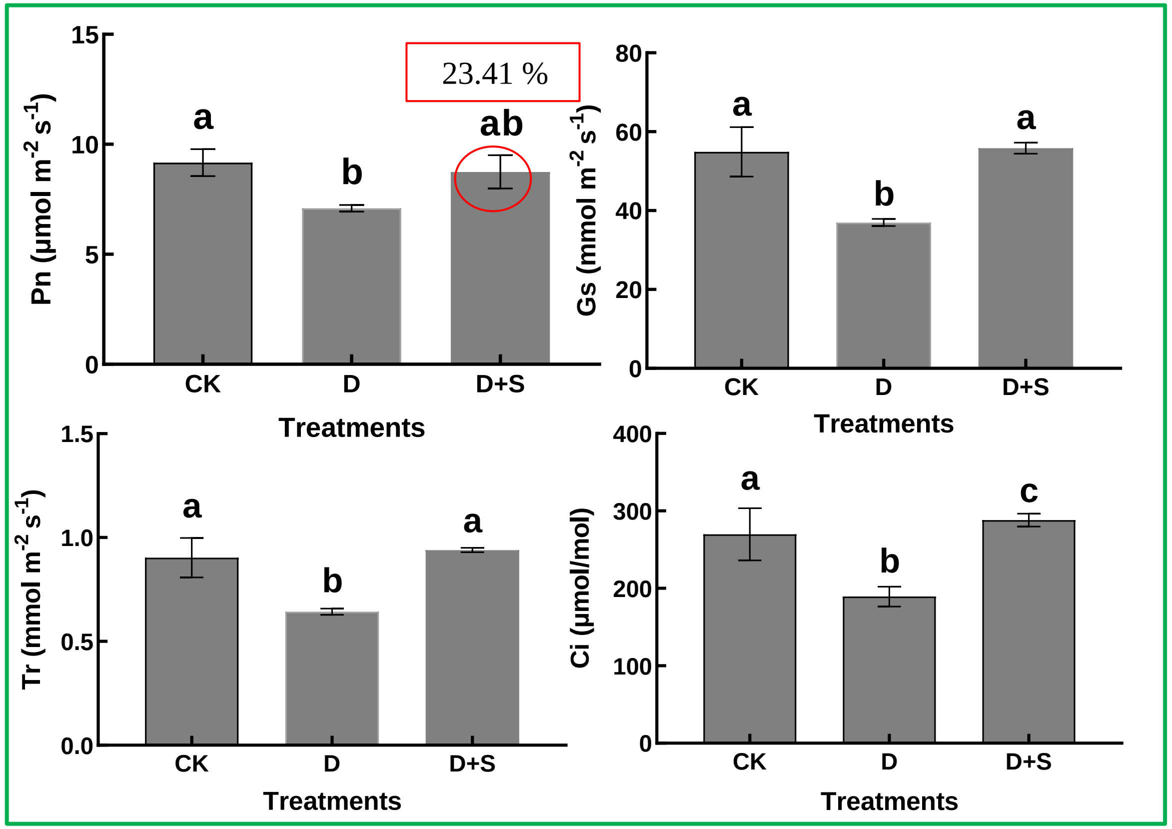
The pictures showed that compared with PEG drought stress, SA spraying significantly increased the antioxidant enzyme activities, soluble protein content and proline content while decreased the MDA.

Effect of SA on photosynthesis parameters

PEG Drought

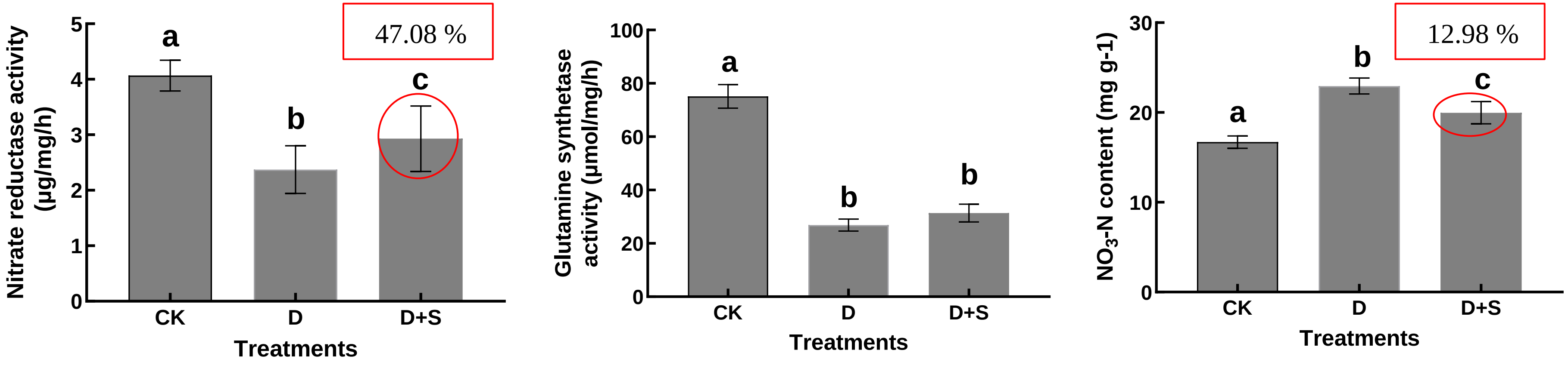


Natural Drought



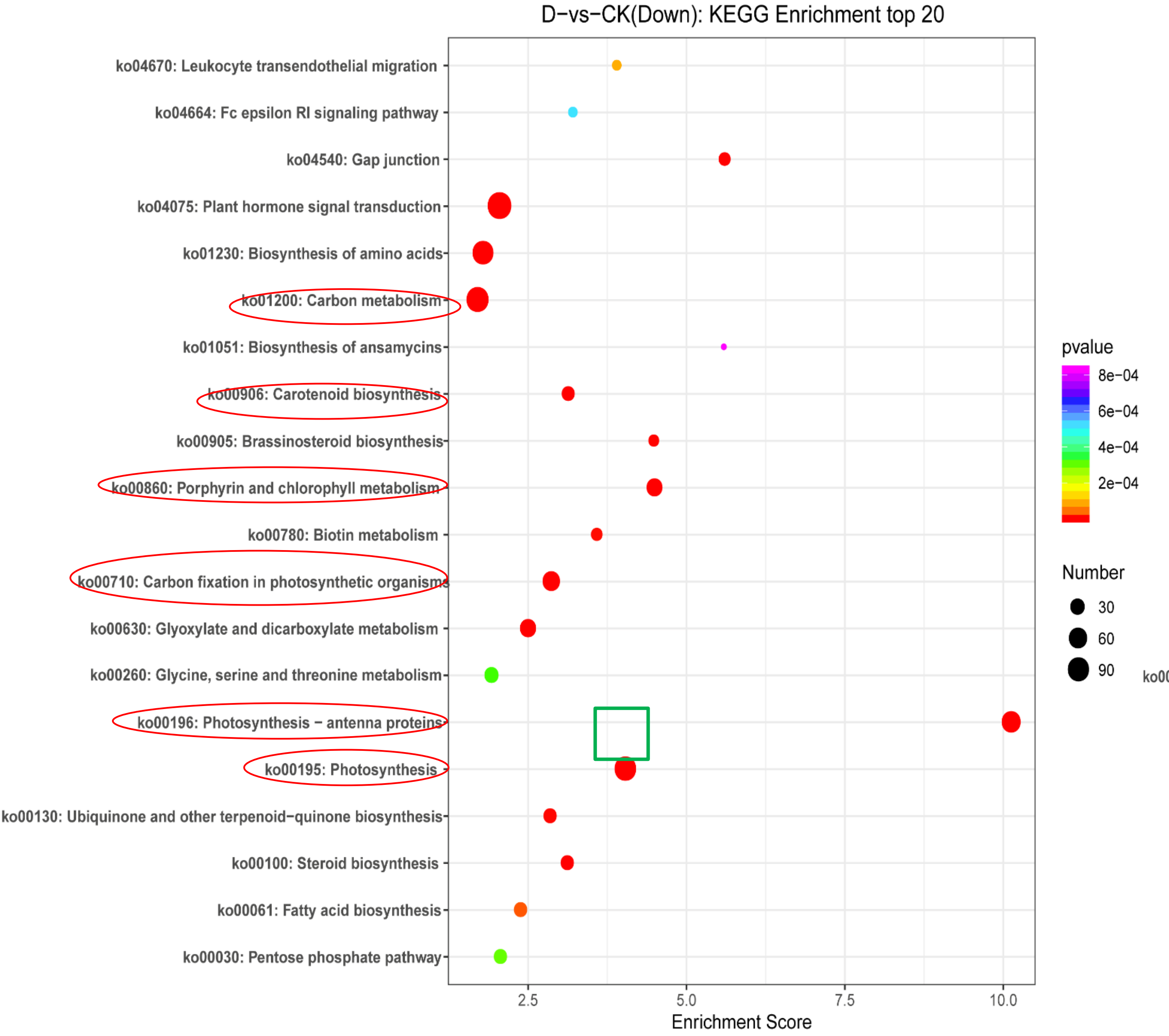
The results showed that SA spraying significantly improved all the photosynthesis parameters. Compared with PEG drought stress(D), SA treated leaves had higher Pn of 23.41 % and 45.74 % under PEG drought stress and natural drought stress, respectively.

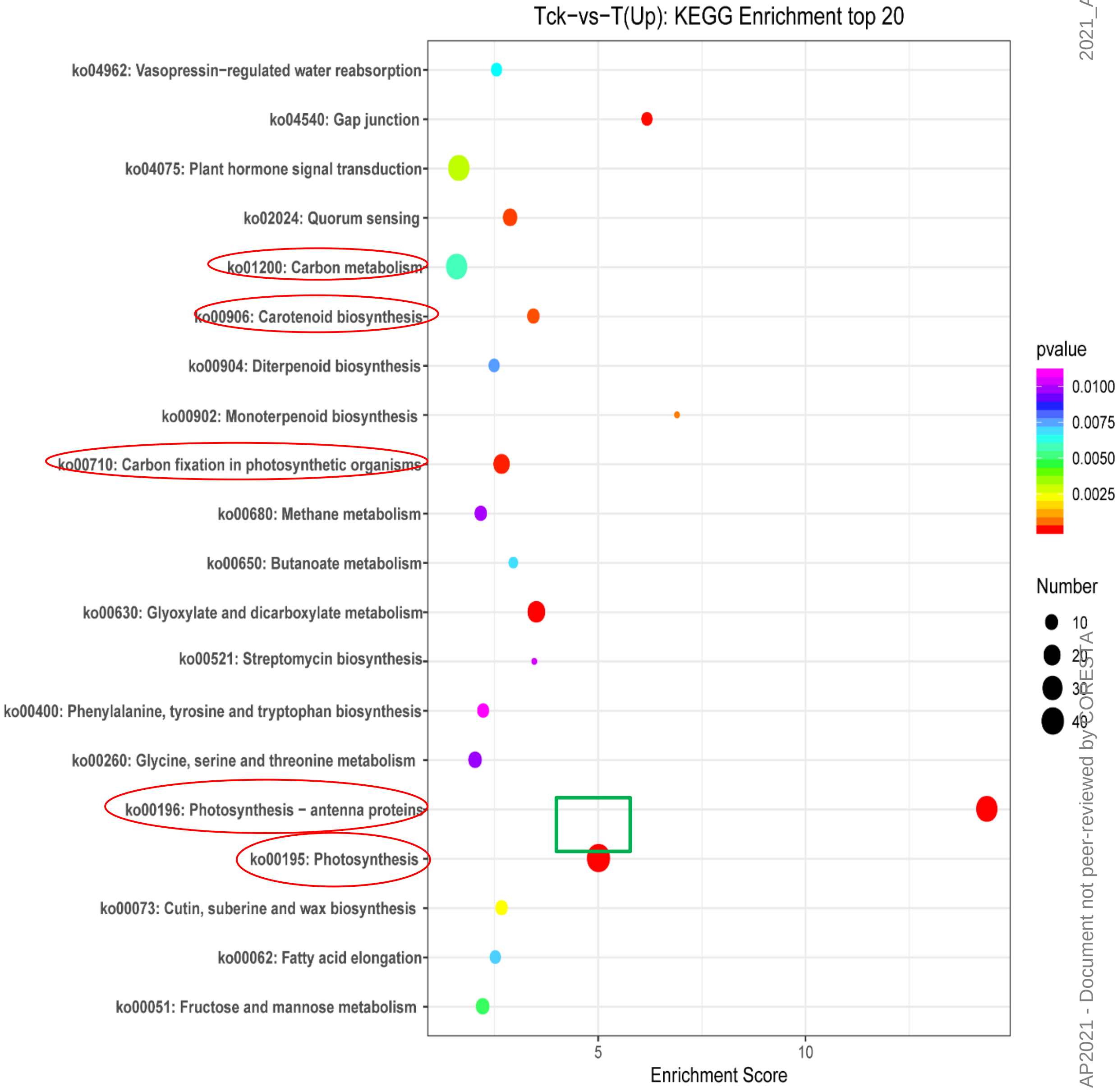
Effect of SA on key nitrogen metabolism enzyme activities and nitrate content under natural drought stress



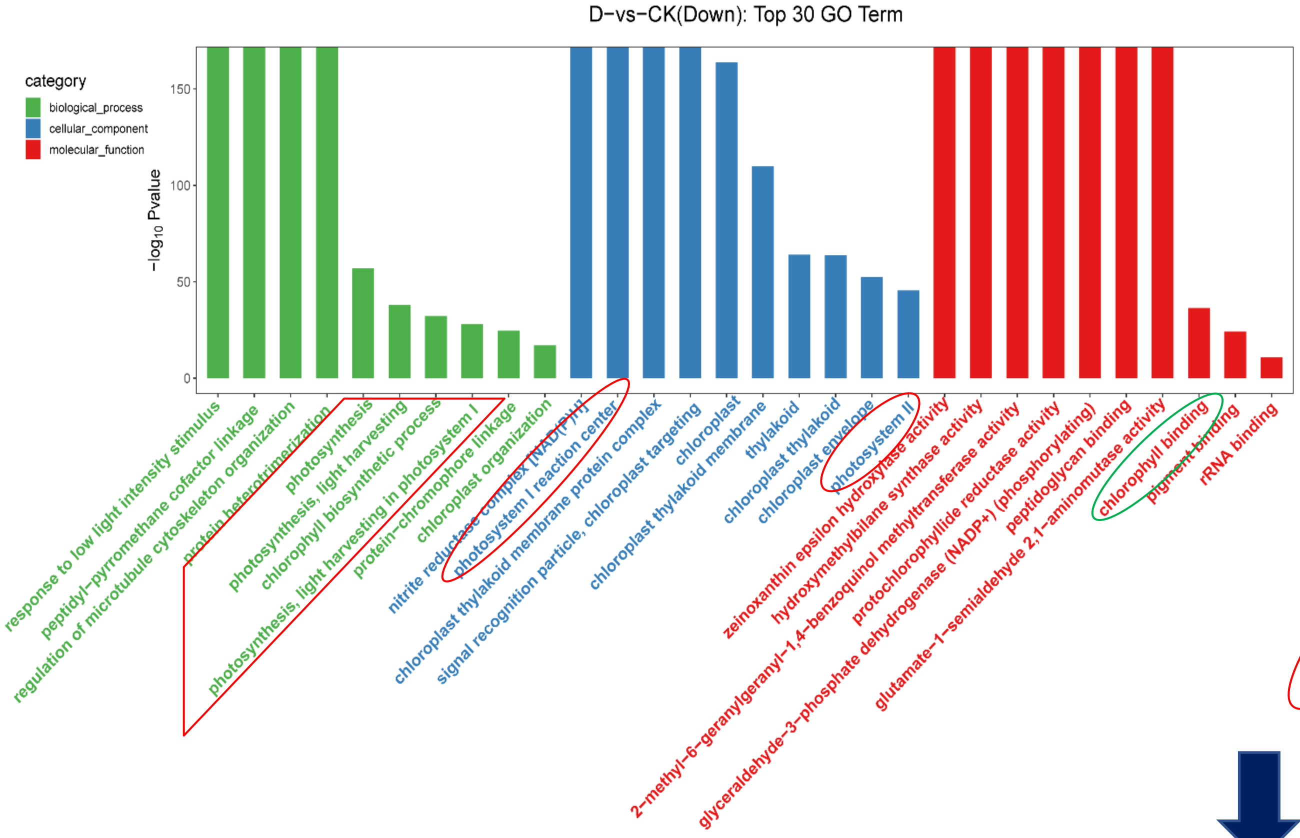
The further analysis on key nitrogen metabolism enzyme activities and nitrate content revealed that SA spraying enhanced the ability of nitrogen metabolism. And the nitrate reductase and glutamine synthetase activities increased by 47.08 % and 23.63 %. In addition, the nitrate content decreased by 12.98 % over the drought stress control.

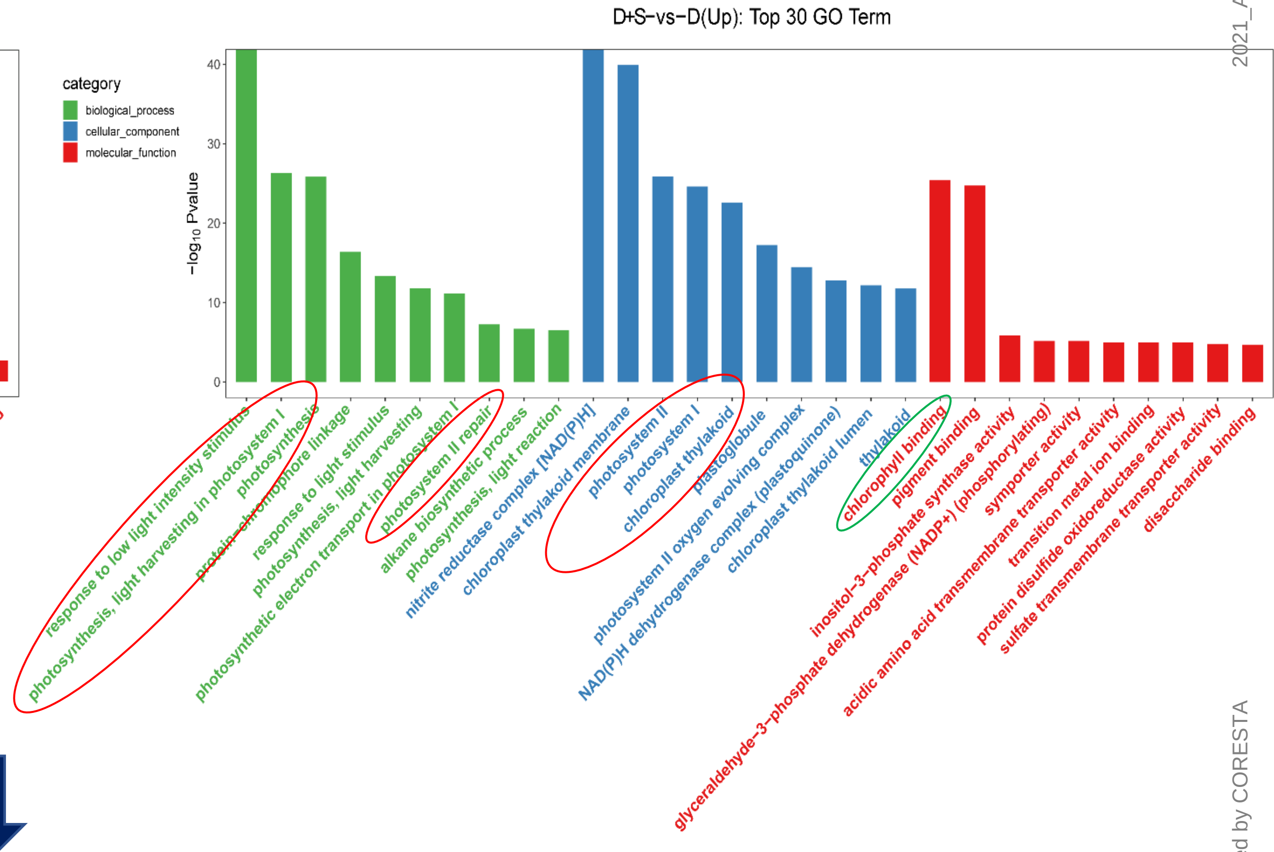
GO enrichment, and KEGG pathway analysis of differentially expressed genes under PEG drought stress





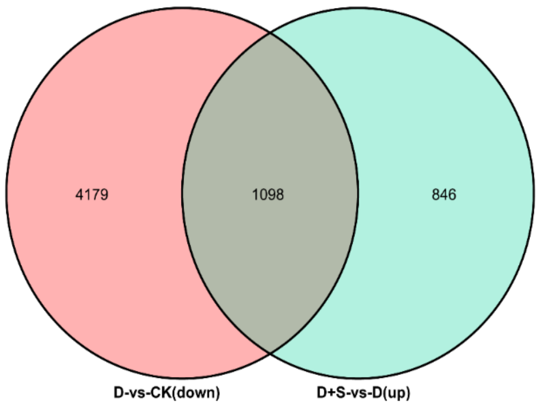
GO enrichment, and KEGG pathway analysis of differentially expressed genes under PEG drought stress

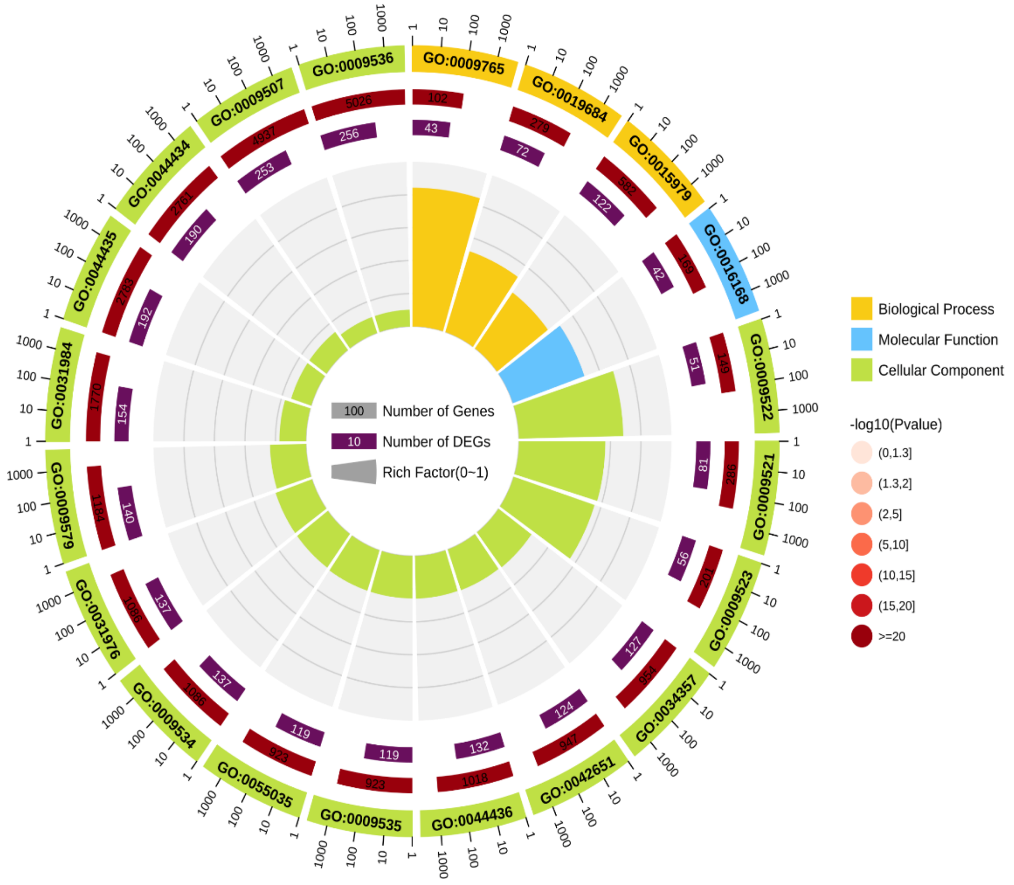


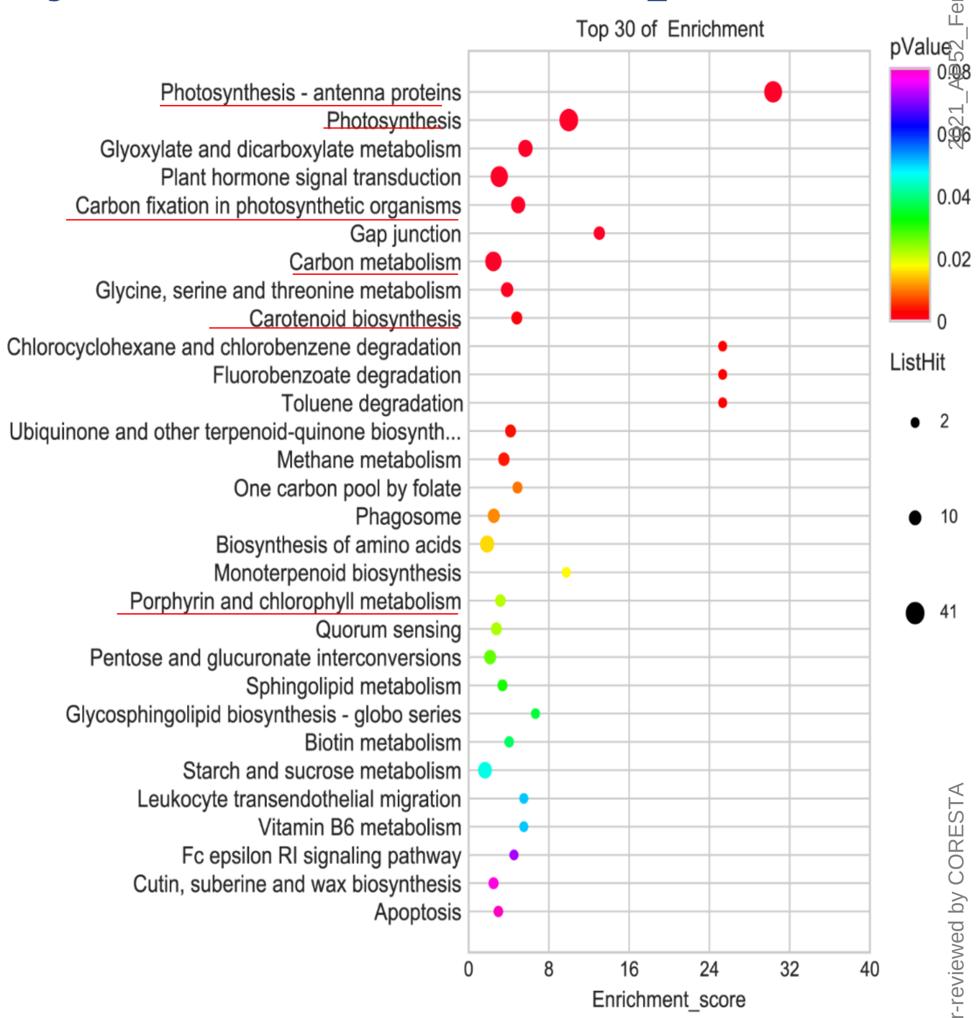


GO enrichment and KEGG pathway analysis showed that most of DEGs involved in photosystem I, photosystem II, photosynthesis, chlorophyll binding and carbon fixation were down-regulated in PEG-treated tobacco seedlings while up-regulated in SA-treated tobacco seedlings.

GO enrichment and KEGG pathway analysis of DEGs in response to SA under PEG drought stress

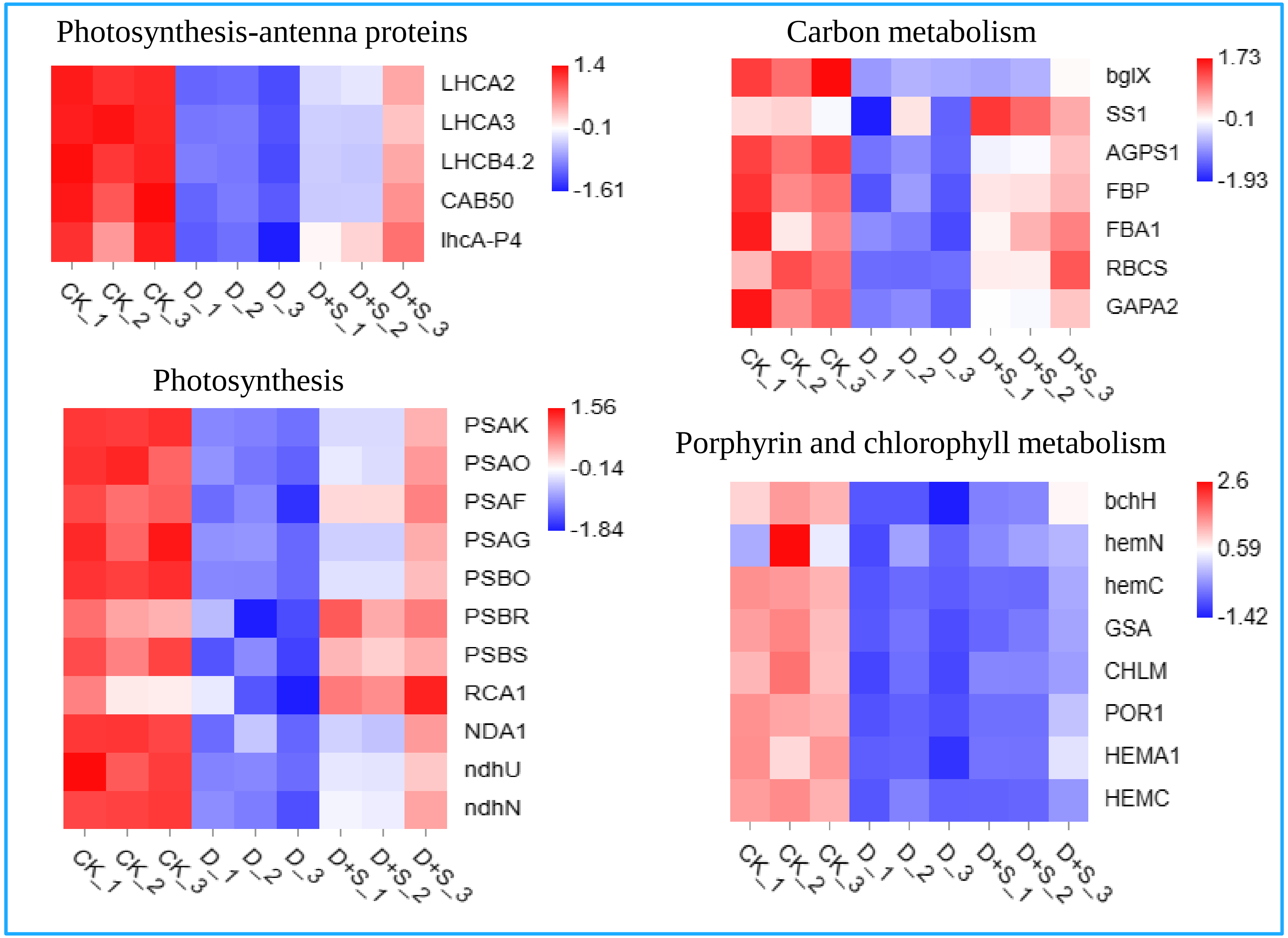






GO and KEGG pathway analysis showed that the DEGs in tobacco seedlings improved by SA are mostly enriched in photosynthesis-antenna proteins, photosynthesis, carbon fixation in photosynthetic, carbon metabolism, starch and sucrose metabolism, porphyrin and chlorophyll metabolism and carotenoid biosynthesis.

DEGs involved in photosynthesis and carbon metabolism under PEG drought stress



The important genes involved in photosynthesis (RCA1, PSBO, PSBR, PSAO), photosynthesis-antenna proteins (lhcA-P4, LHC4.2, CAB50), carbon metabolism (FBP, FBA1, RBCS, SS1), porphyrin and chlorophyll metabolism (POR1, hemC, HEMA1, CHLM) and starch and sucrose metabolism (SS1, bglX, AGPS1) were all up-regulated in SA-treated tobacco.

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Conclusion

Conclusions

- SA-treated tobacco was more drought-tolerant with better **photosynthetic performance**, more powerful **antioxidant system**, and higher ability of **photosystem repair** of tobacco under drought conditions.

- Spraying SA up-regulated the expression of genes involved in carbon metabolism (**FBP**), photosystem II, photosystem I, photosynthesis-antenna proteins (**LHC4.2**), photosynthesis(**RCA1**), porphyrin and chlorophyll metabolism (**POR1**).

- SA application effectively improved the **photosynthesis** of tobacco under drought conditions, thus enhanced the ability of carbon and nitrogen metabolism, increased nitrogen use efficiency and reduced accumulation of nitrate which is the precursor of TSNAs.


Thank You!