

Pod Photosynthetic

Subjects: **Biophysics**

Contributor: Hui Wang , Qingping Zhou , Peisheng Mao

Increasing photosynthetic ability as a whole is essential for acquiring higher crop yields. Nonleaf green organs (NLGOs) make important contributions to photosynthate formation, especially under stress conditions. In the present study, the experiment is designed for alfalfa (*Medicago sativa*) under drought stress to explore the photosynthetic responses of pod walls after 5, 10, 15, and 20 days of pollination (DAP5, DAP10, DAP15, and DAP20) based on ultrastructural, physiological and proteomic analyses. Stomata were evidently observed on the outer epidermis of the pod wall. Chloroplasts had intact structures arranged alongside the cell wall, which on DAP5 were already capable of producing photosynthate. The pod wall at the late stage (DAP20) still had photosynthetic ability under well-watered (WW) treatments, while under water-stress (WS), the structure of the chloroplast membrane was damaged and the grana lamella of thylakoids were blurry. The chlorophyll a and chlorophyll b concentrations both decreased with the development of pod walls, and drought stress impeded the synthesis of photosynthetic pigments. Although the activity of ribulose-1,5-bisphosphate carboxylase (RuBisCo) decreased in the pod wall under drought stress, the activity of phosphoenolpyruvate carboxylase (PEPC) increased higher than that of RuBisCo. The proteomic analysis showed that the absorption of light is limited due to the suppression of the synthesis of chlorophyll a/b binding proteins by drought stress. Moreover, proteins involved in photosystem I and photosystem II were downregulated under WW compared with WS. Although the expression of some proteins participating in the regeneration period of RuBisCo was suppressed in the pod wall subjected to drought stress, the synthesis of PEPC was induced. In addition, some proteins, which were involved in the reduction period of RuBisCo, carbohydrate metabolism, and energy metabolism, and related to resistance, including chitinase, heat shock protein 81-2 (Hsp81-2), and lipoxygenases (LOXs), were highly expressed for the protective response to drought stress. It could be suggested that the pod wall in alfalfa is capable of operating photosynthesis and reducing the photosynthetic loss from drought stress through the promotion of the C4 pathway, ATP synthesis, and resistance ability.

pod wall

nonleaf green organs

ultrastructure

proteomic

alfalfa

1. Introduction

Photosynthesis is considered as the most important chemical reaction and provides over 90% of dry matter for crop yield formation [1]. Increasing crop yield by promoting photosynthesis has been the research hotspot until now [2]. Green leaves are commonly focused as the main source for producing photosynthate. However, nonleaf green organs (NLGOs) have been proven to be practically or potentially capable of assimilating CO₂. Many scientists have previously reported that the silique shell of oil rape (*Brassica napus*) [3]; the boll shell of castor (*Ricinus communis*) [4]; the pod wall of legume crops, including chickpea (*Cicer arietinum*) [5], soybean (*Glycine max*) [6],

and alfalfa (*Medicago sativa*) [7]; ears of cereal including rice (*Oryza sativa*) [8], barley (*Hordeum vulgare*) [9], and wheat (*Triticum turgidum*) [10]; flowers [11], stems [12], and roots [13] in some plants could photosynthesize and make an important contribution to yield formation. In addition, under drought conditions, the photosynthetic contribution of NLGOs turn greater, and NLGOs even become the primary photosynthetic organs for grain-filling [8,14].

Alfalfa is widely cultured around the world to produce high-quality hay for feeding livestock, especially dairy cows. Seed producers have long focused on alfalfa seed yield increase. Moderate drought contributes to achieving higher seed yield during the flowering and seed maturation period. Nevertheless, little is known on the physiological response and the photosynthetic contribution of the pod wall in alfalfa under drought stress. Investigating and increasing the photosynthetic ability of NLGOs, especially under stress conditions, is a novel way to increase the photosynthetic ability of the whole plant and finally increase the grain yield. In this study, physiological, ultrastructural, and proteomic analyses were carried out to (1) investigate the photosynthetic characteristics of the pod wall in alfalfa, and (2) research the response mechanism of photosynthesis in the pod wall to drought stress.

2. Changes of the Surface Characteristics and Ultrastructure of Pod Wall under Drought Stress

Stomata and epidermal hair were distinctly observed in the outer surface of the pod wall (Figure 1A,B). Cells of the inner surface of the pod wall were tightly arranged together (Figure 1C).

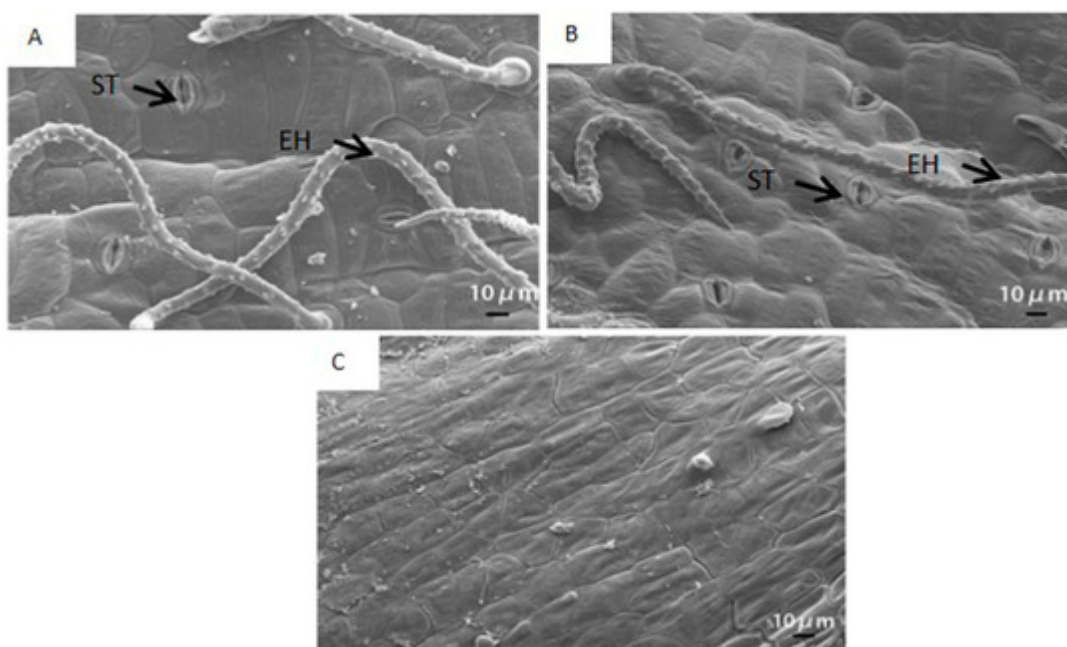


Figure 1. The scanning electron micrograph of the outer (A,B) and the inner surface (C) of the pod wall on the 10th day after pollination (DAP10) under well-watered (A) and water-stressed treatments (B). ST, stoma; EH, epidermal hair; D, dots; H, hump.

Under WW, chloroplasts in the pod wall had the ability to photosynthesize from DAP5 to DAP20. Chloroplasts on DAP5 existed with the intact structure and were arranged close to the cell wall ([Figure 2A](#)). Chloroplast membrane structure was intact, and grana lamella was arrayed along the long axis of the chloroplast, some of which had already produced starch grains. More and bigger starch grains were produced in the chloroplasts on DAP10 ([Figure 2B](#)) and DAP15 ([Figure 2C](#)). The pod wall on DAP20 still had photosynthetic activity, while the cells had started to age and the nuclei were degrading. Few osmiophilic granules were found in cells ([Figure 2D](#)).

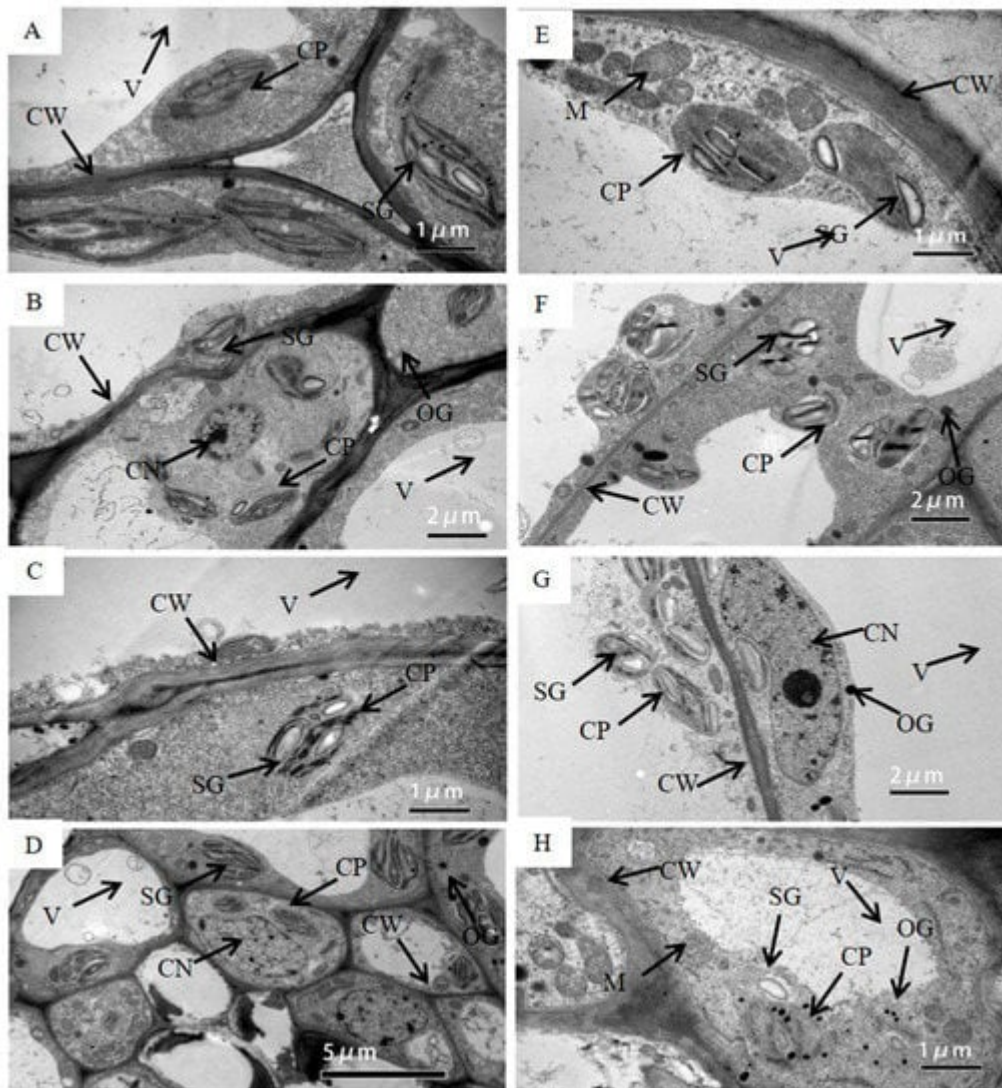


Figure 2. The transmission electron micrograph of cells in the pod wall on DAP5 ([A,E](#)), DAP10 ([B,F](#)), DAP15 ([C,G](#)), and DAP20 ([D,H](#)) under well-watered ([A–D](#)) and water-stressed treatment ([E–H](#)). CW, cell wall; CP, chloroplast; SG, starch grain; OG, osmiophilic granules; V, central vacuole; CN, cell nucleus; M, mitochondrion; T, thylakoid.

Under WS, chloroplasts were able to produce photosynthate on DAP5 and DAP10, while the structure of chloroplasts was gradually damaged from DAP15 to DAP20. The chloroplasts had intact membrane structures and had already started to produce starch grains on DAP5 ([Figure 2E](#)), and they produced more and bigger starch grains on DAP10 ([Figure 2F](#)). Lots of starch grains could still be observed on DAP15, while the evident changes

occurred in the structure of chloroplasts, i.e., the membrane was partly broken, and the grana lamellae of thylakoids became blurry (Figure 2G). Few starch grains existed on DAP20, while lots of osmiophilic granules were presented. The membrane of chloroplasts was seriously broken, and the structure of thylakoids was blurring (Figure 2H).

Except for chloroplasts, the structure of other organelle or tissues changed under drought stress as well. The central vacuole was bigger in the cell under WW (Figure 2D), while the gap between the central vacuole and the cell wall become wider under WS (Figure 2H). The membrane structure of the mitochondrion was intact and clear on DAP5 under WS (Figure 2E), while it was broken and blurred on DAP20 (Figure 2H).

3. Changes of Chlorophyll Concentration in Pod Wall under Drought Stress

With the development of the pod wall, the concentration of chlorophyll a, chlorophyll b, and total chlorophyll decreased under both WW and WS treatments. Furthermore, the concentration of chlorophyll a, chlorophyll b, and total chlorophyll in the treatment of WS decreased significantly ($p < 0.05$) compared with WW (Table 1).

Table 1. Effect of drought stress on the concentration of chlorophyll in the pod wall.

Days after Pollination	Chlorophyll a (mg g ⁻¹)		Chlorophyll b (mg g ⁻¹)		Total Chlorophyll (mg g ⁻¹)	
	WW	WS	WW	WS	WW	WS
DAP5	0.314 ^{aA}	0.141 ^{aB}	0.137 ^{aA}	0.055 ^{aB}	0.451 ^{aA}	0.196 ^{aB}
DAP10	0.138 ^{bA}	0.085 ^{abB}	0.064 ^{bA}	0.044 ^{abA}	0.202 ^{bA}	0.128 ^{bB}
DAP15	0.094 ^{bA}	0.041 ^{bcB}	0.055 ^{bA}	0.025 ^{bcB}	0.149 ^{bA}	0.066 ^{cB}
DAP20	0.024 ^{cA}	0.012 ^{cB}	0.019 ^{cA}	0.010 ^{cB}	0.043 ^{cA}	0.022 ^{dB}

WW, well-watered; WS, water-stressed. Different small letters in the same column and different capitals in the same row meant a significant difference at the 0.05 probability level.

4. Changes of Photosynthetic Enzyme Activities in Pod Wall under Drought Stress

PEPC and RuBisCo activities in pod walls both present a declining trend with the pod development (Figure 3). However, there were different responses for PEPC and RuBisCo in the treatment of WS. As a comparison with WW, PEPC activities could be increased significantly ($p < 0.05$) in the treatment of WS, while RuBisCo activities were decreased.

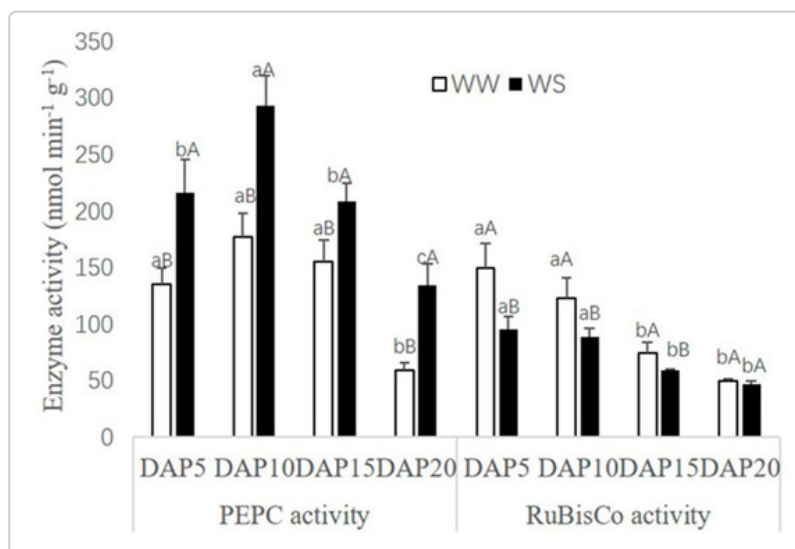


Figure 3. Effect of drought stress on photosynthetic enzyme activity ($\text{nmol min}^{-1} \text{g}^{-1}$) in the pod wall. Different small letters up the white bar and different capitals up the black bar within one photosynthetic enzyme mean a significant difference at the 0.05 probability level. WW, well-watered; WS, water-stressed.

5. Proteomic Analysis on the Response of Pod Wall to Drought Stress

The synthesis of some proteins involved in photosystem I, photosystem II, and the regeneration period of RuBisCo in the pod wall at the early stage and TCA cycle at the late stage are impeded under drought stress (Figure 4). Nevertheless, drought stress can induce the activity of PEPC and promote the synthesis of some proteins participating in the pathway of the C4 cycle and energy metabolism at the early stage and the reduction period of RuBisCo at the late stage.

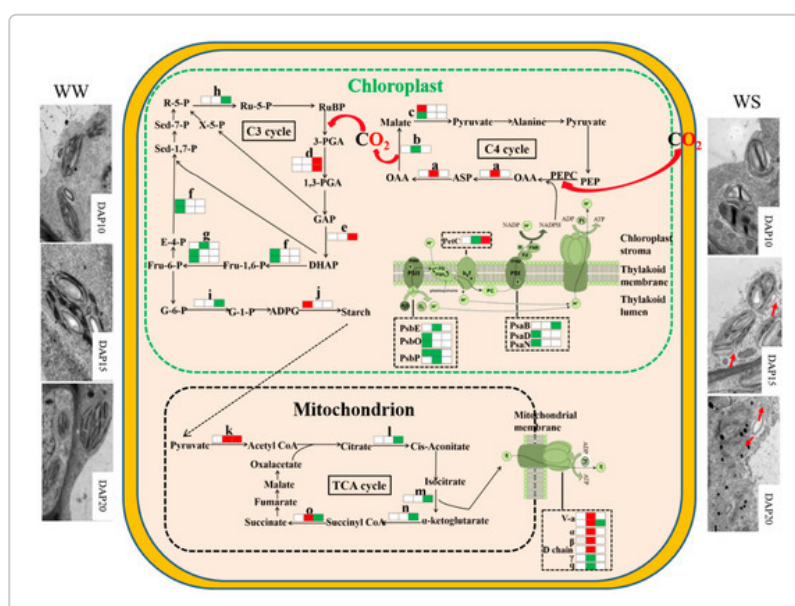


Figure 4. The pathways of proteomic mechanisms in the pod wall under drought stress. a, Aspartate aminotransferase; b, malate dehydrogenase; c, malic enzyme; d, phosphoglycerate kinase; e, triosephosphate isomerase; f, fructose-1,6-bisphosphate aldolase; g, fructose-1,6-bisphosphatase; h, ribose-5-phosphate isomerase; i, glucose-6-phosphate isomerase; j, starch synthase; k, pyruvate dehydrogenase E1 beta subunit; l, aconitate hydratase; m, isocitrate dehydrogenase (NADP); n, E1 subunit-like 2-oxoglutarate dehydrogenase, o, ATP-citrate lyase/succinyl-CoA ligase. Three squares from left to right represent WS10 vs. WW10, WS15 vs. WW15, and WS20 vs. WW20, respectively. The red squares represent significant upregulation at the 0.05 probability level. The green squares represent significant low-regulation at the 0.05 probability level. The white squares represent no significance. The red arrows show the damaged part of the chloroplast membrane in the pod wall under WS.

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