

The inhibition of photosynthesis of *Angelica dahurica* caused by early bolting

Yi-Jie Jiang

Sichuan Agricultural University

Ren-Lang Liu

Sichuan Agricultural University

Xuan Du

Sichuan Agricultural University

Hui-Hui Zhang

Sichuan Agricultural University

Kai Hou

Sichuan Agricultural University

Yin-Yin Chen

Sichuan Agricultural University

Dong-Ju Feng

Sichuan Agricultural University

Wei Wu (✉ ewuwei@sicau.edu.cn)

Sichuan Agricultural University

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Abstract

Background Early bolting affects the photosynthesis of plants. However, there are positive or negative impacts in different plants. The mechanism of photosynthesis pathway respond to early bolting is still a mystery. *Angelica dahurica* is a traditional Chinese medical plant that has been used as the raw material of medicine and food. Early bolting of *Angelica dahurica* occurring in the field production, which decreases quality and yield of *A. dahurica*.

Results In this research, we firstly revealed the damage of early bolting on the photosynthesis of *A. dahurica*. The photosynthetic capacity significantly decreased after bolting. The metabonomic analysis showed that the chlorophyll synthesis metabolism was repressed and the accumulation of saturated fatty acid increased after early bolting. Meanwhile, transcriptomic analysis indicated a down-regulation of photosynthetic electron transport and up-regulation of saturated fatty acid synthesis. Interestingly, an unexpected raise in Rubisco and PEPC activities of the leaves of early bolting plants was found in our research.

Conclusion The photosynthesis is repressed by early bolting in transcriptomics and metabonomics. Meanwhile, early bolting induced accumulation of saturated fatty acid and increased the activities of PEPC and Rubisco. It suggested a special mediated way responds to the inhibition of photosynthesis of *A. dahurica* by early bolting.

Background

Angelica dahurica is a perennial herb of Umbelliferae, widely cultivated in China, Japan and Korea [1, 2]. "Bai Zi" is the dried root of *A. dahurica*, which is known as a kind of traditional Chinese herbal medicine, rich in volatile oils and coumarins. It is widely used in pharmaceuticals, spices, cosmetics and for the treatment of toothache, cold, headache [3, 4]. Growth of *A. dahurica* is usually divided into three stages: vegetative stage (V-stage), bolting stage (B-stage) and summer dormancy stage (D-stage) [5]. The B-stage often occurs in the third year after sowing in southern regions of China. However, an early bolting phenomenon is always found after only half-year vegetative growth in practical production. The roots of early bolting plants get serious lignification and lose their pharmacological value. After the early bolting, it not only affects the yield of a single plant, but also affects the growth of other plants because the bolting plants compete with the normal plants for water, fertilizer and light [6].

In plant growth cycle, photosynthesis is the most critical physiological process of plant growth and development involved in two important metabolism, carbon assimilation and photosynthetic electron transport. The fixed carbon accounts for 40% of the dry matter. At present, a series of photosynthetic physiological parameters, such as net photosynthetic net rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO₂ concentration (C_i) etc. are used to evaluate the photosynthetic capacity of plants[7]. The photosynthetic capacity of plants is closely related to the structural characteristics and biochemical components of leaves [8].

Rubisco enzyme is the key rate limiting enzyme of carbon assimilation. It is a kind of bifunctional enzyme in the mesophyll. It can not only fix the CO₂ absorbed by the leaves through carboxylation, but also catalyze the oxidation of ribulose-1,5-diphosphate to participate in photorespiration, which plays an important role in photosynthesis. The rapid Rubisco accumulation that depends on sufficient PEP activity is important for normal seedling growth [9]. Phosphoenolpyruvate carboxylase (PEPC) widely exists in organisms, and its carbon assimilation efficiency and specificity are dozens of times higher than that of Rubisco. Previous studies showed that PEPCs proteins are up-regulated under low CO₂ to carry on a rearrangement of carbon metabolism to support C₄ photosynthetic carbon assimilation in *Thalassiosira pseudonana* [10]. As an important photoreceptor, chlorophyll is essential for photosynthesis of plants like affecting the photosynthetic electron transfer efficiency of plants and thus affects their photosynthesis [11]. However, the Chl content cannot fully reflect leaf age, because even completely green leaves began to senesce at the molecular level. Compared with single leaf senescence parameters, comprehensive senescence parameters at the whole plant level, such as the proportion of aged leaves, can better reflect the molecular state of plants [12]. Transcription factors regulating chlorophyll metabolism have been gradually resolved. For example, *OsMYB102* gene delays chloroplast apoptosis by down-regulating ABA accumulation signal induction pathway, thus delaying leaf senescence and prolonging photosynthesis [13].

There is no doubt that photosynthesis changed significantly before and after bolting. However, the effect of early bolting on plants is quite a controversial topic. The analysis of 48 lineages of *Arabidopsis thaliana* distributed under different environmental gradients suggested that the higher fitness was associated with earlier bolting, greater early allocation to increased numbers of inflorescences, reduction in rosette leaf photosynthesis and earlier fruit ripening [14]. A research on early flowering high-quality rice showed that its photosynthesis was higher, and the contents of carbohydrate, protein and amino acid were also higher.

In conclusion, the effect of early bolting on photosynthesis of different plants are different or even opposite. Although the early bolting is identified a harmful trait on *A. dahurica* by preliminary studies, the mechanism of occurrence and influence are still a mysterious. In this study, we suggested a novel suppressed mechanism on the photosynthesis of *A. dahurica* by early bolting.

Methods

Plant material and culture condition

The 6 varieties (strains) of *A. dahurica* named A1, A2, A3, A4, B2 and B3 were all bred and cultivated by our laboratory. A1 was a certified variety bred by professor Wu Wei (Agronomy College, Sichuan Agricultural University) and approved by Sichuan crop variety Approval Committee. A2-B3 strains were stable breeding line for more than 3 successive generations by our laboratory. The experimental strains were all planted in the same field of Modern R&D Base of Sichuan Agricultural University (103.67, 30.63).

Determination of Agronomic Characters and photosynthetic parameters

After the early bolting of *Angelica dahurica* in March 2019, the photosynthetic parameters of normal and early bolting plants were measured by Li-6400 portable photosynthesis analyzer from the 25th to 28th of each month. The light intensity was set at $1600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in 6 times repeatedly. Meanwhile, the plant height and basal culm thickness were measured by ruler and electronic vernier caliper of 10 normal and early bolting plants, respectively.

Chlorophyll Content

The 0.1g fresh plants leaves samples were stored in ice box in the field. The chlorophyll was extracted with mortar and pestle in 80% chilled acetone plus, after centrifugation at 4°C the resultant solution were determined spectro-photometrically at 663 nm and 646 nm, repeated 4 times.

Enzyme assays

The 0.5 g fresh leaves were rapidly frozen with liquid N_2 , and powdered with a pestle in a prechilled mortar under and ground in an extraction buffer containin $100\text{mmol}\cdot\text{L}^{-1}$ HEPES (pH 7.5), $5\text{mmol}\cdot\text{L}^{-1}$ MgCl_2 , $5\text{mmol}\cdot\text{L}^{-1}$ EDTA, 6% (w/w) PVP, 7% PEG 2000, $2\text{mmol}\cdot\text{L}^{-1}$ DTT, 10%(v/v) glycerol $2\mu\text{mol}\cdot\text{L}^{-1}$ PMSF, $1\mu\text{mol}\cdot\text{L}^{-1}$ gastric endostatin and $1\mu\text{mol}\cdot\text{L}^{-1}$ leucine inhibitory peptide[15]. The homogenates were centrifuged for 30 min at 4°C in a microcentrifuge (Cetrifuge 5424R; EPPENDORF, Germany) at 12,000g. Ribulose-1,5-bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase activity was measured by Rubisco ELISA Kit, and PEPC ELISA Kit, respectively (produced by YiBaiDao Biotechnology Company).

Metabolomic and transcriptomic analysis

The results came from our previous metabolomics and transcriptomics data of normal and early bolting A1 cultivar leaves.

Statistical analysis

All statistical analysis were conducted using SAS software (*Pro 9.4 SAS Institute Inc., Cary, NC, USA*). Student's t-test was used for comparison of two means, and linear regression analysis was used for comparison of two variables.

Results

Analysis of Agronomic Characters

In March, after the early bolting occurred, the different strains of early bolting *A. dahurica* exhibited 6.04% ~ 13.06% higher height than the normal one, while their stem base diameter were 17.41 ~ 55.94% larger than that of NB plants (Fig. 1a).

In April, the higher height and wider stem were also showed in NB plants (Fig. 1b). However, in May, the plant heights of BT plants were 8.82% ~ 30.05% significantly higher than that of NB plants, while the stem base diameter were 9.55% ~ 45.43% smaller in BT plants compared with NB plants (Fig. 1c).

Determination of Chl content and Photosynthetic Characteristics

In March, Chl contents of different BT plants were 3.44-31.83% lower compared with NB ones, the difference was not significant except for in A2 strains ($P < 0.05$). Meanwhile, the P_N also differed much higher in NB plants (7.11% ~ 23.84%) (Fig. 2a).

In April, the plunge of Chl contents occurred in the NB plants, while Chl contents of BT plants decreased slowly. Among them, the Chl contents were higher in A3, B2 and B3 BT plants, but significant lower in NB plants of A1 and A4. Except for A2, the P_N of all BT plants were significantly higher than that of NB plants (Fig. 2b). In May, the leaves of BT plants turned yellow and even withered, the P_N as well as the Chl content decreased rapidly. Therefore, Chl content and P_N were significantly higher in all NB plants compared with BT plants (Fig. 2c). Meanwhile, the NB plants showed higher photosynthetic parameters involved in g_s , Tr and C_i in March and especially in May (Table 1). But there was no consistent difference between NB and BT plants from different strains in April.

Table1 Stomatal conductance, transpiration rate and intercellular carbon dioxide concentrations of the leaves of different cultivars (strains) of normal and early bolting *Angelica dahurica*.

Mean values \pm standard error; statistically significant differences between genotypes are indicated with asterisks (* - $P < 0.05$; ** - $P < 0.01$).

	Cultivars\Strains	g_s	E	C_i
March	NBA1	0.234±0.013 **	3.596±0.272 **	402.852±17.733**
	BTA1	0.216±0.008	2.713±0.139	279.155±1.902
	NBA2	0.225±0.014 **	2.477±0.077 **	287.385±21.049 *
	BTA2	0.154±0.011	2.290±0.067	269.551±12.636
	NBA3	0.176±0.005	2.123±0.043 **	340.583±28.138 **
	BTA3	0.167±0.011	2.104±0.038	278.706±6.492
	NBA4	0.273±0.023 **	3.324±0.806	271.624±1.185
	BTA4	0.257±0.018	3.435±0.399	284.916±4.68 *
	NBB2	0.287±0.021	3.614±0.094 **	248.221±5.425
	BTB2	0.255±0.024	2.730±0.187	285.755±14.141*
	NBB3	0.299±0.018 **	2.369±0.15 **	305.056±7.897
	BTB3	0.250±0.020	2.199±0.071	294.157±8.278
	April	NBA1	0.234±0.013 **	3.596±0.272 **
BTA1		0.216±0.008	2.713±0.139	279.155±1.902
NBA2		0.225±0.014 **	2.477±0.077 **	287.385±21.049 *
BTA2		0.154±0.011	2.290±0.067	269.551±12.636
NBA3		0.176±0.005	2.123±0.043 **	340.583±28.138 **
BTA3		0.167±0.011	2.104±0.038	278.706±6.492
NBA4		0.273±0.023 **	3.324±0.806	271.624±1.185
BTA4		0.257±0.018	3.435±0.399	284.916±4.68 *
NBB2		0.287±0.021	3.614±0.094 **	248.221±5.425
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NBB3		0.299±0.018 **	2.369±0.15 **	305.056±7.897
BTB3		0.250±0.020	2.199±0.071	294.157±8.278
May		NBA1	0.249±0.011 **	4.322±0.196 **
	BTA1	0.224±0.028	2.955±0.335	266.129±1.198
	NBA2	0.27±0.016	3.466±0.65	230.503±17.904 *
	BTA2	0.293±0.005*	3.489±0.239	216.324±6.963
	NBA3	0.298±0.012	4.257±0.243	234.522±16.475
	BTA3	0.290±0.029	4.606±0.333	254.033±15.142 *
	NBA4	0.333±0.009 *	4.691±0.27 *	239.224±4.704

BTA4	0.280±0.02	3.488±0.183	261.58±11.477 *
NB	0.286±0.014 **	3.652±0.382 **	248.162±6.651
BTB2	0.210±0.006	2.092±0.104	260.699±19.23 *
NBB3	0.331±0.008 **	4.127±0.448	281.056±10.594
BTB3	0.206±0.009	4.045±0.442	330.818±5.384 **
NBA1	0.196±0.013 **	2.636±0.127 **	332.852±17.733 **
BTA1	0.066±0.007	0.693±0.063	130.129±5.896
NBA2	0.152±0.027 **	2.133±0.083 *	217.82±13.105 **
BTA2	0.059±0.003	0.382±0.034	124.91±11.519
NBA3	0.197±0.018 **	1.617±0.061 **	238.813±12.826 **
BTA3	0.079±0.005	0.56±0.028	112.699±3.519 I
NBA4	0.17±0.016 **	2.191±0.279 **	239.624±4.464 **
BTA4	0.065±0.004	0.818±0.051	123.914±6.539
NBB2	0.167±0.019 **	1.419±0.021 **	236.221±20.353**
BTB2	0.049±0.008	0.467±0.05 F**	124.699±7.901
NBB3	0.198±0.029 **	2.339±0.174 **	302.621±7.421 **
BTB3	0.068±0.006	0.662±0.115	138.485±1.848

Table 2 Correlation analysis on the chlorophyll content and photosynthetic parameters of the leaves of different cultivars(strains) of *Angelica dahurica*. Mean values ± standard error; statistically significant differences between genotypes are indicated with asterisks (** - P<0.01).

	Chl	g_s	E	C_i	P_N
Chl	1.000	0.212	0.171	0.408**	0.693**
g_s	\	1.000	0.895**	0.580**	0.065
E	\	\	1.000	0.217	0.100
C_i	\	\	\	1.000	0.305
P_N	\	\	\	\	1.000

In order to confirm whether the enzyme activity was influenced under early bolting, we examined the PEPC and Rubisco enzyme activities of all strains. Interestingly, PEPC/Rubisco enzyme activity of all

strains of BT plants leaves was significantly higher than that of normal plants in May (Fig. 3). It suggested a higher carbon assimilation efficiency in the early bolting plants.

The Analysis of Metabolome

We selected the A1 variety with stable genetic characters which was bred by our laboratory for metabolomic and transcriptomic analysis. Enrichment map indicated the photosynthesis pathway was significant difference between the NB and BT plants (Fig. 4).

Furthermore, it was found that uroporphyrinogen, coproporphyrin and protochlorophyllide contents in the Chl synthesis pathway significantly decreased in the leaves of BT plants compared with NB plants in March. (Fig. 5a, 5b, 5c). However, both of the contents of octadecanamide and palmitic acid, representing the accumulation of saturated fatty acid, significantly increased in leaves of BT plants (Fig. 5d, 5e)

The transcriptomic analysis of carbon assimilation, electron transport and chlorophyll metabolism

In the photosynthetic carbon assimilation pathway, phosphoglycerate kinase (PGK) in chloroplast mainly regulates plant metabolism and optimal growth, and the down-regulation of *AdPGK* would reduce the photosynthetic capacity of plant leaves. The function of RPI isomerase is to catalyze the conversion of xylose-5-phosphate to ribose-5-phosphate.

The results indicated that the transcription levels of *AdRPI* (Fig. 6a) and phosphoglycerate kinase gene 1 *AdPGK* (Fig. 6b) in the calvin cycle pathway of BT plants were significantly down-regulated compared with the NB plants.

Only *AdhemH* was found to be significantly up-regulated in the leaves of BT plants in Chl transcription pathways (Fig. 6c). The PSI and PSII are the main pathways of photosynthesis electron transport in the higher plants. It was found the transcription level of *AdPsaB* in the leaves of BT plants was significantly up-regulated (Fig. 6d). The main function of *PSbW* is to maintain the stability of supramolecular structure of PSII photoreaction center. The expression of the *AdPsbW* encoding the PSII reaction center was significantly down-regulated as shown in (Fig. 6e) revealed that the stability of PSII protein complex was damaged, which affected its normal function. In addition, the *AdFdrA*, which regulated the important electron carrier in the photosynthetic electron transport chain, was also significantly down-regulated in the BT plants leaves, indicated that the photosynthetic electron transfer efficiency of BT plants was inhibited, resulting in the decline of photosynthesis (Fig. 6f). The transcriptional levels of *AdACACA*, *AdKCS* and *AdFATB* were significantly up-regulated, while the transcriptional levels of *AdFAdA* and *AdACSL* were significantly down-regulated. The results showed that the synthesis pathway of fatty acid in early mossy plants was up-regulated and fatty acid degradation pathway was down-regulated which was basically consistent with the results of metabolome (Fig. 7).

Discussion

Previous studies have shown that the quality and yield of *A. dahurica* in the production field are affected by the early bolting [16]. In this research we further describe the inhibition of photosynthesis pathway caused by early bolting, this is probably a straight correction with production loss. At the initial stage of the early bolting, the P_N and Chl contents of NB plants were higher than the early bolting plants. Although the difference became less obvious in April, the photosynthetic characteristics of BT plants finally plunged in May. The result of correlation analysis suggested the decrease of P_N be positively correlated with the Chl content of *A. dahurica*. The Chl content of early bolting *A. thaliana* lines decreased faster than the normal ones, indicating that the senescence of early bolting plants was accelerated during the process flower bud differentiation to bolting [17]. Interestingly, we found the activities of PEPC and Rubisco enzyme in BT plants of all varieties were higher than that in NB. *A. dahurica* owned both higher PEPC activity and lower intercellular CO_2 concentration in the late stage of early bolting. This feature is the reminiscent of the phenotype of cauliflower under low concentration CO_2 treatment. It was found that PEPC enzyme activity was nearly 2 times higher compared with the control [18]. The same phenomenon was also found in *Hydrilla verticillata* treated with low concentration CO_2 [19]. The improvement of Rubisco enzyme activity could rescue the delayed greening of FLN2 mutant [9]. We assumed that the increase of Rubisco activity in BT plants of *A. dahurica* be a response to the sharp decrease of Chl content, which could alleviate the chlorosis of leaves to a certain extent, so as to maintain the basic photosynthesis. The higher activity of PEPC could provide more organic acids for the development of carbon assimilation products such as α -keto acid for reproductive growth under the condition of leaf senescence and significant decrease of P_N of *A. dahurica*. Previous studies have shown that inhibition of photosynthesis was consistent with senescence and the decrease of carbon assimilation enzyme activity [20]. However, the results of our study showed that the repressed photosynthesis did not induce the decrease of carbon assimilation enzyme activity, but increased it. It referred this mechanism might relate to the lower concentration intercellular CO_2 and Chl contents.

Our studies showed that Chl synthesis was one of the important factors affecting photosynthetic capacity of *A. dahurica* under early bolting. The metabolic analysis revealed that prochlorophyllate, copolyporogen and urea porphyrinogen III contents were down-regulated in BT plants leaves. The Chl synthesis pathway was inhibited when early bolting began. Therefore, it is assumed that Chl synthesis metabolic be significantly repressed by the early bolting and finally cause serious damage to the process of photosynthesis.

The uroporphyrinogen III is the common precursors of siroheme (synthesized in bacteria, yeast and plants) and coproporphyrinogen III. It should be noted that the down-regulation of uroporphyrinogen III did not cause the down-regulation of sirohemon synthesis, but downstream coproporphyrinogen III and protochlorophyllide showed similar trend in BT plants.

Despite significantly different Chl precursors content, no genes were to be found down-regulated in Chl synthesis pathway in BT plants. However, the transcription level of *AdhemH* was up-regulated in BT plants. The hemH catalyzes the chelation of protoporphyrin IX with Fe to form heme, which essentially

competes with chlorophyll synthesis pathway for protoporphyrin IX [21]. In cucumber seedlings under salt stress, the transcription level of protoporphyrin iron chelatase increased, while the transcription level of several genes in chlorophyll synthesis pathway was down-regulated. Exogenous 5-ALA treatment could significantly reduce the transcription level of *feme* and increase the transcription level of chlorophyll synthesis pathway [22].

In *A. thaliana*, inhibition of *feme* transcription level could improve the Chl content and photosynthetic capacity in PS II damaged mutants. The transcription level of protoporphyrin iron chelatase gene significantly increased in the leaves of early bolting *A. dahurica*. Therefore, it was speculated that the up-regulated of *Feme* may affected the photosynthesis of early bolting *A. dahurica* plants. In addition, the expression levels of *AdPGK* and *AdRPI* were significantly down-regulated in the Calvin cycle pathway of early bolting *A. dahurica* leaves. Phosphoglycerate kinase gene in chloroplast mainly regulates plant metabolism and optimizes plant growth. The decrease of *AtPGK1* expression level could reduce the photosynthetic capacity of plant leaves. It was found that the photosynthetic capacity and starch content decreased in the *pgk A. thaliana*, which was due to the impaired glycolysis caused by mutation of PGK enzyme [23].

In *A. thaliana*, *AtRPI* contains a chloroplast transport sequence, so it plays a regulatory role in plant chloroplast function. The activity of RPI enzyme did not differ significantly, chloroplast structure was to be found destroyed in *rpi2* resulting in decreased photosynthetic capacity and promoted premature cell senescence [24]. The down-regulation of *AdRPI* might lead to the inhibition of ribose-5-phosphate regeneration and chloroplast function, which was consistent with the inhibition of Chl synthesis shown by metabolome.

Ferritin is an important electron transport carrier of PSI. The down-regulation of *AdFdrA* transcription level indicated the decrease of electron transfer efficiency of PSI, indicating that plants are vulnerable to photoinhibition and affect photosynthesis. P700 protein is the main electron donor of PSI [25]. In studies on *A. thaliana*, *AtPsaB* regulated P700 protein. The expression of *AtPsaB* was related to the damage of photosynthetic organs by reactive oxygen species [26]. In this study, the significantly up-regulated *AdPsaB* was in the leaves of early bolting *A. dahurica* might reduce the destructive effect of photoinhibition to the BT plants.

PsbW is a low molecular weight protein unique to photosynthetic eukaryotes, which is related to the composition of PSII 680 protein complexes [27]. The results showed that the loss of PsbW in *A. thaliana* could destroyed the supramolecular structure of PSII. In the absence of PsbW protein, PSII-LHCII hypercomplex could not be detected or isolated, and the phosphorylation of PSII core protein decreased significantly, and the redox state of plastid quinone (PQ) in dark adapted leaves changed. Compared with wild type, PsbW protein deletion leads to a rapid transition state 1 to state 2 of PQ, that is, the redox change is faster. The down regulation of PsbW transcription level in early bolting *A. dahurica* leaves might also distructe the stability of PSII protein complex. In this research, the photosynthetic electron transport pathway appeared to be down-regulated at the transcriptional level in BT plants. It is widely

believed that unsaturated degree of membrane lipid could significantly affect the photosynthesis of the plants [28].

The contents of octadecanamide and palmitic acid in the leaves of early bolting *A. dahurica* leaves were significantly higher than those in the normal one. The results showed that the accumulation of saturated fatty acids in leaves increased after early bolting. Previous study indicated that the increasing accumulation of saturated fatty acids in the early senescence stage could improve the sensitivity to ethylene and promote the flowering of *Petunia hybrida* [29]. And long-chain fatty acids were involved in the transport of polar auxin in *A. thaliana* [21]. In addition, studies on membrane lipids of photosynthetic organs in leaves showed that the increase of unsaturated fatty acid ratio helps to protect plant PSII damage under stress, maintain the stability of thylakoid membrane, and improve the photosynthetic heat resistance of plants. The saturated fatty acid content of old leaves was also higher than that of new leaves [30, 31]. IAA can induce the accumulation of palmitic and stearic while decreasing the synthesis of linoleic and α -linolenic in *Chlorella vulgaris* [32]. In our previous research, we found that the accumulation of auxin in early bolting *A. dahurica* also increased, which might be related to the accumulation of long-chain saturated fatty acids in leaves. The transcriptional levels of several genes involved in the biosynthesis and metabolic of long-chain saturated fatty acids significantly increased, and the down-regulation of long-chain acyl CoA synthase and acetylcoa acyltransferase was also found in the fatty acid degradation pathway, indicating that early bolting could increase the accumulation of long-chain saturated fatty acids in leaves of *A. dahurica* at the molecular level. At present, we do not know the mechanism of this phenomenon, but many studies have shown that the accumulation of saturated fatty acids is not conducive to maintaining the stability of various organelle membranes, and plants are vulnerable to various stress environment damage, which may lead to early bolting of *A. dahurica* leaf photosynthetic organs vulnerable to strong light damage and accelerate aging.

Early bolting could decrease both the quality and yield of lettuce. However, a comparative proteomics analysis suggested a high temperature enhances the function of photosynthesis to promote the process of bolting of lettuce [33]. This conclusion contradicted our findings. Therefore, we speculate that the effect of early bolting on plant photosynthesis be related to species differences.

Conclusion

These present findings confirmed a negative effect of early bolting on photosynthetic pathway of *A. dahurica*. The repression of Chl synthesis at the early stage of bolting seems not directly related to the change on transcript level of genes in Chl synthesis pathway. Furthermore, a special response mechanism on inhibited photosynthesis under early bolting by increasing activities of PEPC and Rubisco increased at the late stage of bolting of *A. dahurica*. It provides a novel way to explore the different effect on photosynthesis of early bolting among different plants.

Abbreviations

Chl: Chlorophyll; P_N : Photosynthesis net rate; NB – Normal; BT: Early bolting; PEPC: Phosphoenolpyruvate carboxylase; *Ad*: *Angelica dahurica*; ACACA: Acetyl coenzyme A carboxylase; KCS: 3-keto-yl-coa synthetase; ACSL: Long chain acyl CoA synthetase; FATB: Fatty acyl transporter protein B; ACCA: Acetyl CoA acyltransferase; PGK: phosphoglycerate kinase; RPI: 5-phosphate isomerase; heMH: ferrochelatase gene; PsaB: photosystem I P700 A ester protein A2 gene; PsbW: PSII W subunit gene; Fdr: iron oxide reducing protein gene.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WW, YJJ conceived and designed research, and wrote and revised the manuscript. YJJ, RLL XD, HHZ, DJF conducted experiments. KH and YYC analyzed the data. All authors read and approved the manuscript.

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References

1. Wang GH, Chen CY, Tsai TH, Chen CK, Cheng CY, Huang YH, Hsieh MC, Chung YC: Evaluation of tyrosinase inhibitory and antioxidant activities of *Angelica dahurica* root extracts for four different

- probiotic bacteria fermentations. *J Biosci Bioeng* 2017, 123(6):679-684. <http://dx.doi.org/10.1016/j.jbiosc.2017.01.003>.
2. Lechner D, Stavri M, Oluwatuyi M, Pereda-Miranda R, Gibbons S: The anti-staphylococcal activity of *Angelica dahurica* (Bai Zhi). *Phytochemistry* 2004, 65(3):331-335. <http://dx.doi.org/10.1016/j.phytochem.2003.11.010>.
 3. Li X, Zeng X, Sun J, Li H, Wu P, Fung KP, Liu F: Imperatorin induces Mcl-1 degradation to cooperatively trigger Bax translocation and Bak activation to suppress drug-resistant human hepatoma. *Cancer Lett* 2014, 348(1-2):146-155. <http://dx.doi.org/10.1016/j.canlet.2014.03.017>.
 4. Xu H, Cui J, Zhou R, Fu J, Hao N: First report of leaf spot disease in *Angelica dahurica* caused by phoma bellidis in China. *J Phytopathol* 2016, 164(7-8):448-454. <http://dx.doi.org/10.1111/jph.12470>.
 5. Liang WH, Chang TW, Charng YC: Influence of harvest stage on the pharmacological effect of *Angelica dahurica*. *Bot Stud* 2018, 59(1):14. <http://dx.doi.org/10.1186/s40529-018-0230-1>.
 6. Hou K, Chen JW, J.Y. Z, H. S, L. C, Wei. W: Effect of different plant growth regulators on yield and quality of *Angelica dahurica* var. formosana development. *Chin J Chin Mater Med* 2013, 38:2082-2085. <http://dx.doi.org/10.4268/cjcmm20131309>.
 7. Ye ZP, Yu Q: A coupled model of stomatal conductance and photosynthesis for winter wheat. *Photosynthetica* 2008, 46(4):637-640. <http://dx.doi.org/10.1007/s11099-008-0110-0>.
 8. Zhang Y, Wang J, Gong S, Xu D, Sui J: Nitrogen fertigation effect on photosynthesis, grain yield and water use efficiency of winter wheat. *Agric Water Manage* 2017, 179:277-287. <http://dx.doi.org/10.1016/j.agwat.2016.08.007>.
 9. Huang C, Yu QB, Yuan XB, Li ZR, Wang J, Ye LS, Xu L, Yang ZN: Rubisco accumulation is important for the greening of the fln2-4 mutant in *Arabidopsis*. *Plant Sci* 2015, 236:185-194. <http://dx.doi.org/10.1016/j.plantsci.2015.04.005>.
 10. Kustka AB, Milligan AJ, Zheng H, New AM, Gates C, Bidle KD, Reinfelder JR: Low CO₂ results in a rearrangement of carbon metabolism to support C4 photosynthetic carbon assimilation in *Thalassiosira pseudonana*. *New Phytol* 2014, 204(3):507-520. <http://dx.doi.org/10.1111/nph.12926>.
 11. Jenkins GI, Woolhouse HW: Photosynthetic Electron Transport During Senescence of the Primary Leaves of *Phaseolus vulgaris* L. III. KINETICS OF CHLOROPHYLL FLUORESCENCE EMISSION FROM INTACT LEAVES. *J Exp Bot* 1981, 32:467-478. <http://dx.doi.org/10.1093/jxb/32.3.467>.
 12. Balazadeh S, Parlitz S, Mueller-Roeber B, Meyer RC: Natural developmental variations in leaf and plant senescence in *Arabidopsis thaliana*. *Plant Biol* 2010, 10 Suppl 1(s1):136-147. <http://dx.doi.org/10.1111/j.1438-8677.2008.00108.x>.
 13. Piao W, Sakuraba Y, Paek NC: Transgenic expression of rice MYB102 (OsMYB102) delays leaf senescence and decreases abiotic stress tolerance in *Arabidopsis thaliana*. *BMB Rep* 2019, 52(11):653-658. <http://dx.doi.org/10.5483/BMBRep.2019.52.11.071>.
 14. Wolfe MD, Tonsor SJ: Adaptation to spring heat and drought in northeastern Spanish *Arabidopsis thaliana*. *New Phytol* 2014, 201(1):323-334. <http://dx.doi.org/10.1111/nph.12485>.

15. Berveiller D, Damesin C: Carbon assimilation by tree stems: potential involvement of phosphoenolpyruvate carboxylase. *Trees* 2007, 22(2):149-157. <http://dx.doi.org/10.1007/s00468-007-0193-4>.
16. Pu SC, Shen ML, Deng CF, Zhang WW, Wei ZQ: Effects of N,P and K rates and their proportions on curtail earlier bolting. *J of Cent South Univ* 2011, 33(11):168-172. <http://dx.doi.org/10.1038/cmi.2011.4>.
17. Nozue H, Oono K, Ichikawa Y, Tanimura S, Shirai K, Sonoike K, Nozue M, Hayashida N: Significance of structural variation in thylakoid membranes in maintaining functional photosystems during reproductive growth. *Plant Physiol* 2017, 160(1):111-123. <http://dx.doi.org/10.1111/ppl.12528>.
18. Zhang Y, Yin L, Jiang HS, Li W, Gontero B, Maberly SC: Biochemical and biophysical CO₂ concentrating mechanisms in two species of freshwater macrophyte within the genus *Ottelia* (*Hydrocharitaceae*). *Photosynthesis Res* 2014, 121(2-3):285-297. <http://dx.doi.org/10.1007/s11120-013-9950-y>.
19. Li ZB, W. L, Gu SP, Cao Y, Yin LY: Effects of low CO₂ on photosynthetic characteristics of *Ottelia alismoides* and *Hydrilla verticillata*. *Plant Sci J* 2011, 29:226-233. <http://dx.doi.org/0.3724/SP.J.1142.2011.20226>.
20. Zhang LT, Gao HY, Zhang ZS, Xue ZC, Meng QW: Multiple effects of inhibition of mitochondrial alternative oxidase pathway on photosynthetic apparatus in Rumex K-1 leaves *Biol Plantarum* 2012, 56:365-368. <http://dx.doi.org/10.1007/s10535-012-0100-8>.
21. Ambrosini V, Issawi M, Leroy LS, Riou C: How protoporphyrinogen IX oxidase inhibitors and transgenesis contribute to elucidate plant tetrapyrrole pathway. *J Porphyrins Phthalocyanines* 2019, 23(04n05):419-426. <http://dx.doi.org/10.1142/s1088424619300076>.
22. Wu Y, Jin X, Liao W, Hu L, Dawuda MM, Zhao X, Tang Z, Gong T, Yu J: 5-aminolevulinic acid (ALA) alleviated salinity stress in Cucumberseedlings by enhancing chlorophyll synthesis pathway. *Front Plant Sci* 2018, 9:635. <http://dx.doi.org/10.3389/fpls.2018.00635>.
23. Rosa TS, Anoman AD, Flores TM, Toujani W, Alseek S, Fernie AR, Nebauer SG, Munoz BJ, Segura J, Ros R: Phosphoglycerate kinases are co-regulated to adjust metabolism and to optimize growth. *Plant Physiol* 2018, 176(2):1182-1198. <http://dx.doi.org/10.1104/pp.17.01227>.
24. Howles PA, Birch RJ, Collings DA, Gebbie LK, Hurley UA, Hocart CH, Arioli T, Williamson RE: A mutation in an *Arabidopsis* ribose 5-phosphate isomerase reduces cellulose synthesis and is rescued by exogenous uridine. *Plant J* 2006, 48(4):606-618. <http://dx.doi.org/10.1111/j.1365-313X.2006.02902.x>.
25. Santabarbar S, Bombell P, Purton S, Russell C, Heathcote P: The physiological relevance of electron transfer involving redox-active centres bound to the PsaA and PsaB subunits of Photosystem I. *Photosynthesis Res* 2007, 91:155-156. <http://dx.doi.org/10.1016/j.clnesp.2015.07.001>.
26. Qian H, Yu S, Sun Z, Xie X, Liu W, Fu Z: Effects of copper sulfate, hydrogen peroxide and N-phenyl-2-naphthylamine on oxidative stress and the expression of genes involved photosynthesis and

- microcystin disposition in *Microcystis aeruginosa*. *Aquat Toxicol* 2010, 99(3):405-412. <http://dx.doi.org/10.1016/j.aquatox.2010.05.018>.
27. Garcia CG, Kovacs L, Toth T, Kereiche S, Aseeva E, Boekema EJ, Mamedov F, Funk C, Schroder WP: The PsbW protein stabilizes the supramolecular organization of photosystem II in higher plants. *Plant J* 2011, 65(3):368-381. <http://dx.doi.org/10.1111/j.1365-313X.2010.04429.x>.
 28. Koichi, Kobayashi: Role of membrane glycerolipids in photosynthesis, thylakoid biogenesis and chloroplast development. *J Plant Res* 2016. <http://dx.doi.org/10.1007/s10265-016-0827-y>.
 29. Yang Z, Ohlrogge JB: Turnover of fatty acids during natural senescence of *Arabidopsis*, *Brachypodium*, and *Switchgrass* and in *Arabidopsis* beta-oxidation mutants. *Plant Physiol* 2009, 150(4):1981-1989. <http://dx.doi.org/10.1104/pp.109.140491>.
 30. Rozentsvet OA, Bogdanova ES, Ivanova LA, Tabalenkova GN, Zakhochiy IG, Nesterov VN: Structural and functional organisation of the photosynthetic apparatus in halophytes with different strategies of salt tolerance. *Photosynthetica* 2016, 54:405-413. <http://dx.doi.org/10.1007/s11099-015-0182-6>.
 31. Sui N, Li M, Li K, Song J, Wang BS: Increase in unsaturated fatty acids in membrane lipids of *Suaeda salsa* L. enhances protection of Photosystem II under high salinity. *Photosynthetica* 2010, 48:623-629. <http://dx.doi.org/10.1007/s11099-010-0080-x>.
 32. Jusoh M, Loh SH, Chuah TS, Aziz A, Cha TS: Indole-3-acetic acid (IAA) induced changes in oil content, fatty acid profiles and expression of four fatty acid biosynthetic genes in *Chlorella vulgaris* at early stationary growth phase. *Phytochemistry* 2015, 111:65-71. <http://dx.doi.org/https://doi.org/10.1016/j.phytochem.2014.12.022>.
 33. Hao JH, Zhang LL, Li PP, Sun YC, Li JK, Qin XX, Wang L, Qi ZY, Xiao S, Han YY *et al*: Quantitative proteomics analysis of Lettuce (*Lactuca sativa* L.) reveals molecular basis-associated auxin and photosynthesis with bolting induced by high temperature. *Int J Mol Sci* 2018, 19(10). <http://dx.doi.org/10.3390/ijms19102967>.

Figures

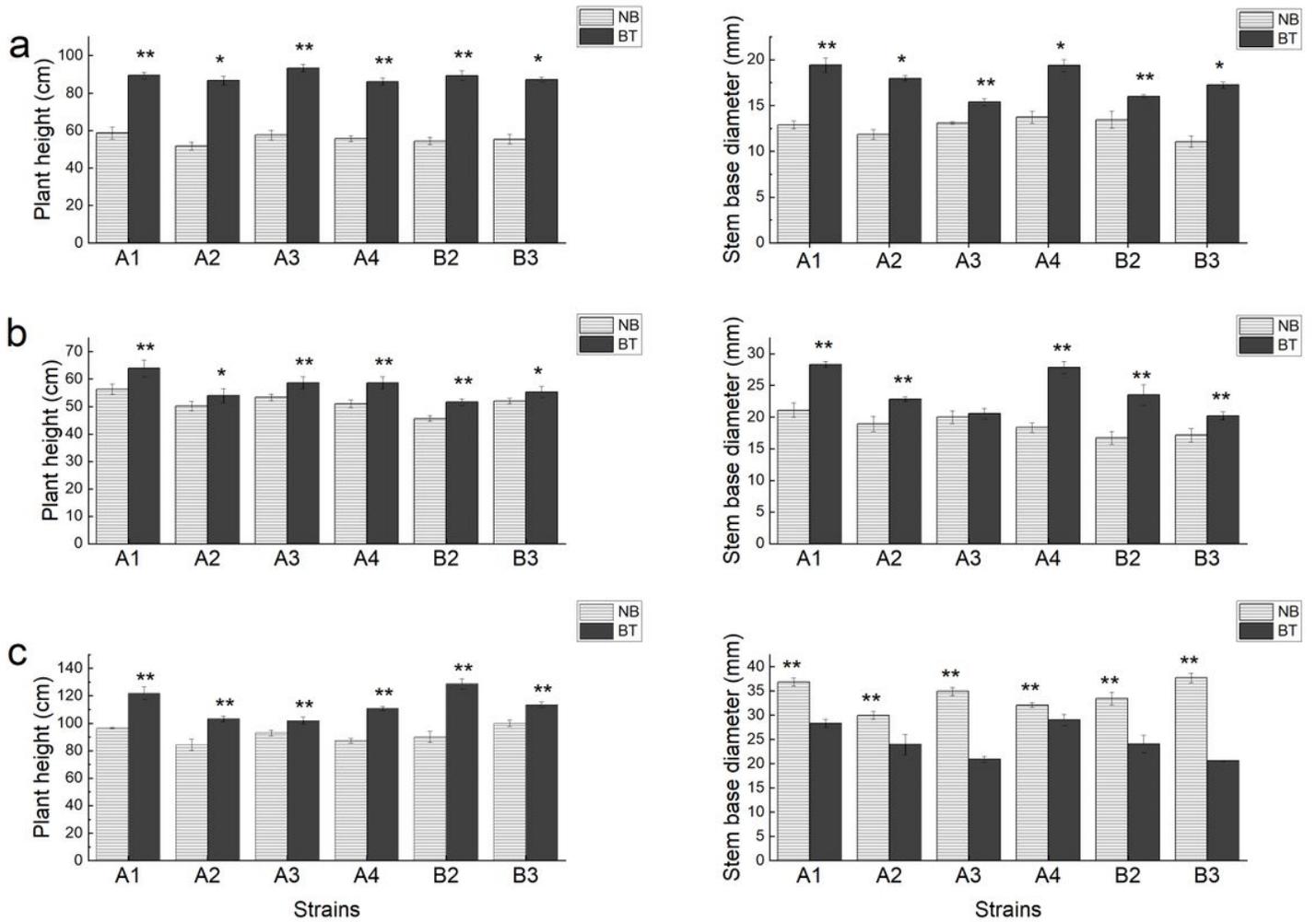


Figure 1

The plant high (cm) and stem base diameter(mm) of different strains *Angelica dahurica* in March (a), April (b) and May (c). Mean values \pm standard error; statistically significant differences between genotypes are indicated with asterisks (* – P<0.05; ** – P<0.01).

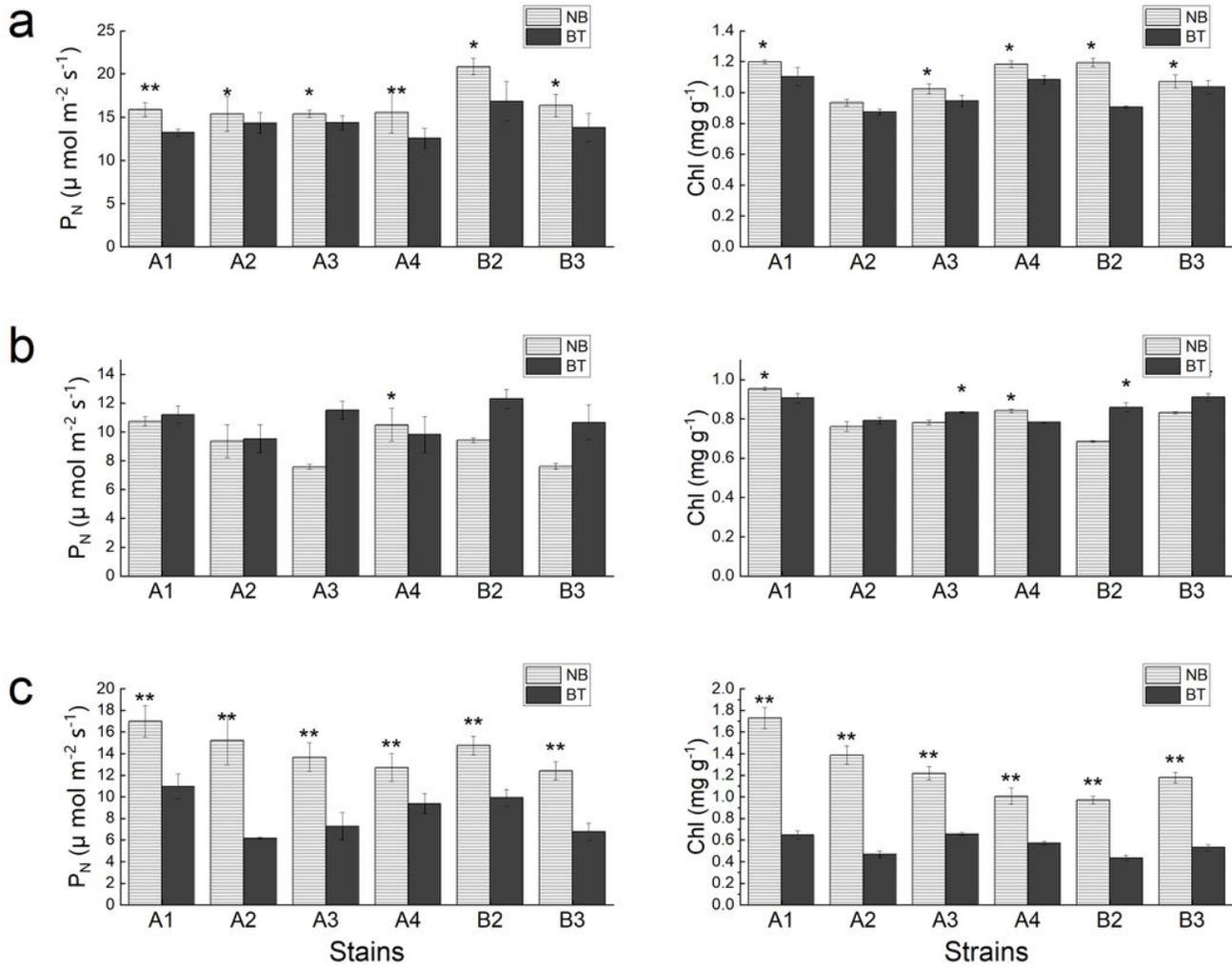


Figure 2

The plant high (cm) and stem base diameter(mm) of different strains *Angelica dahurica* in March (a), April (b) and May (c). Mean values ± standard error; statistically significant differences between genotypes are indicated with asterisks (* – P<0.05; ** – P<0.01).

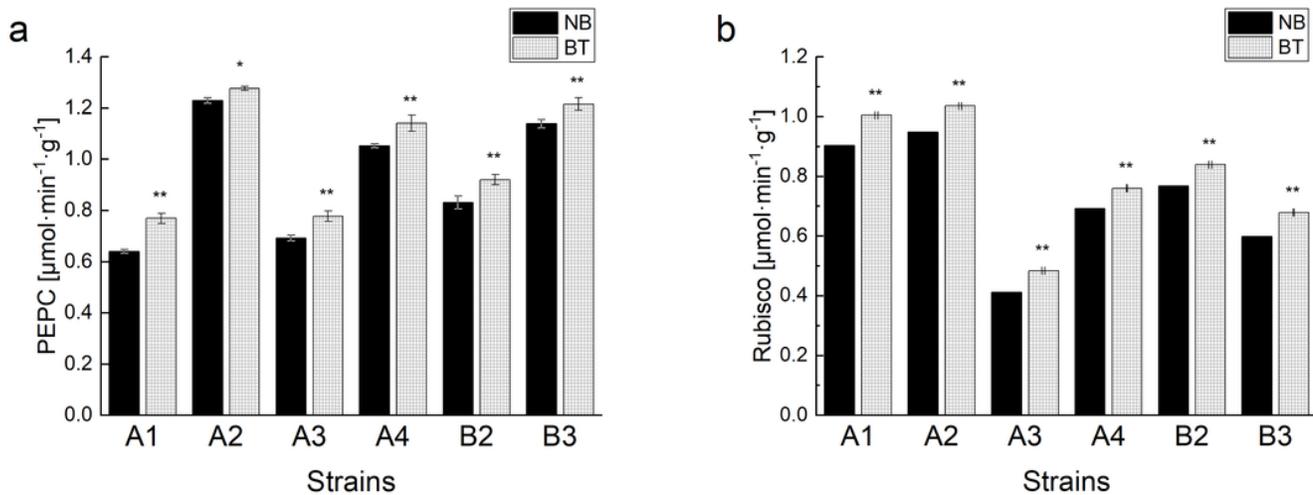


Figure 3

The PEPC and Rubisco activities of normal and early bolting *Angelica dahurica*. (a) PEPC activities of different strains of normal and early bolting *Angelica dahurica*. (b) Rubisco activities of different strains of normal and early bolting *Angelica dahurica*.

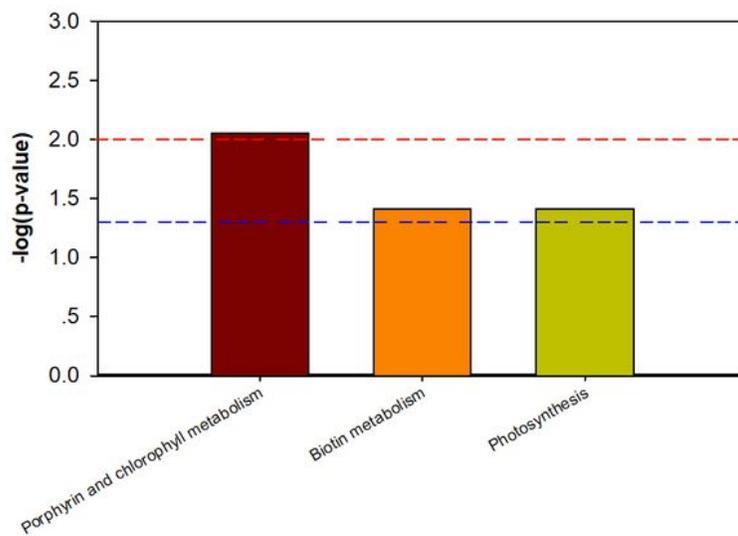


Figure 4

Enrichment map of differential metabolism.

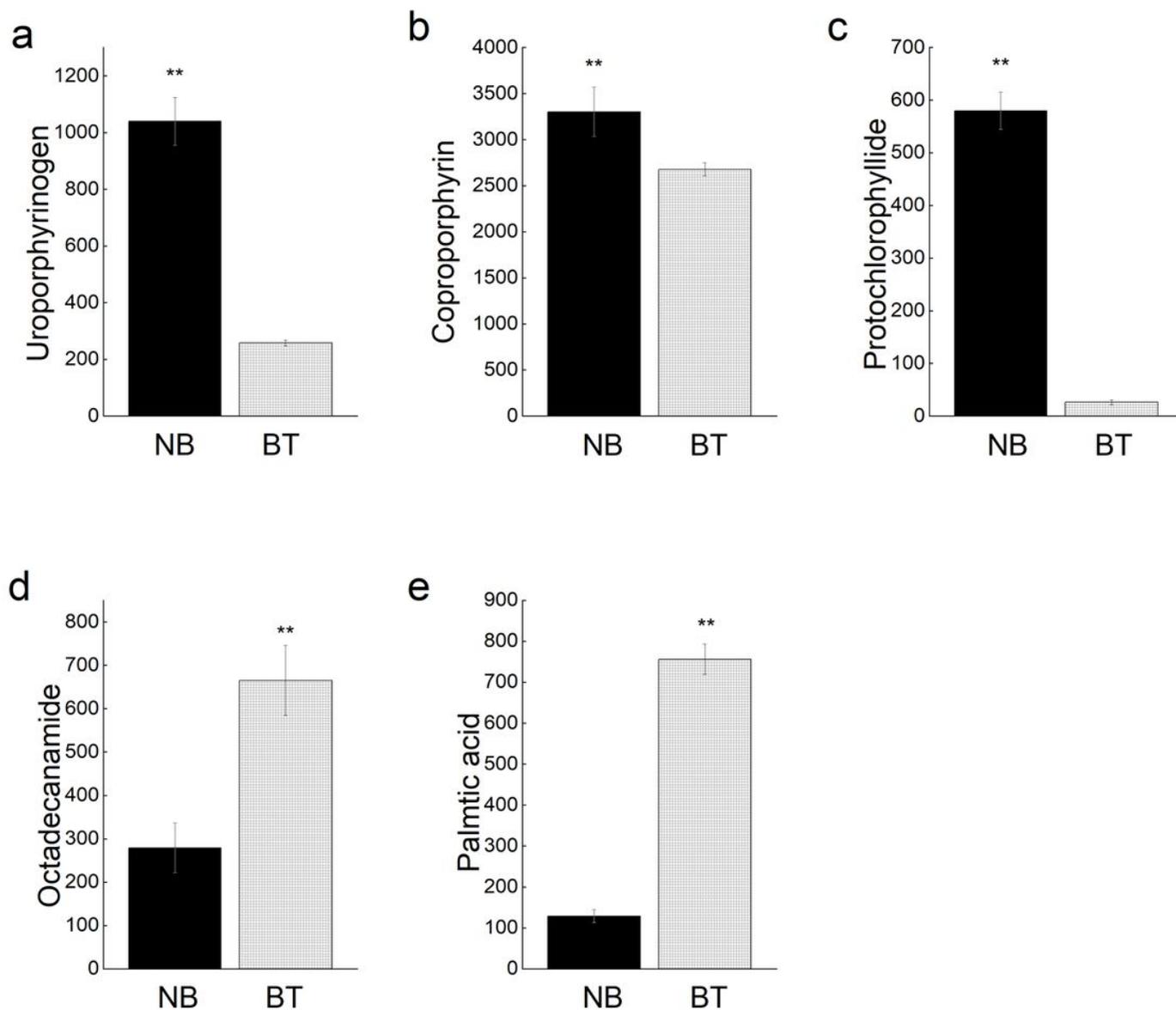


Figure 5

The differential metabolites of chlorophyll synthesis and fatty acid metabolism of normal and early bolting *Angelica dahurica*. (a) Uroporphyrinogen; (b) Coproporphyrin; (c) Protochlorophyllide; (d) Octadecanamide; (e) Palmitic acid.

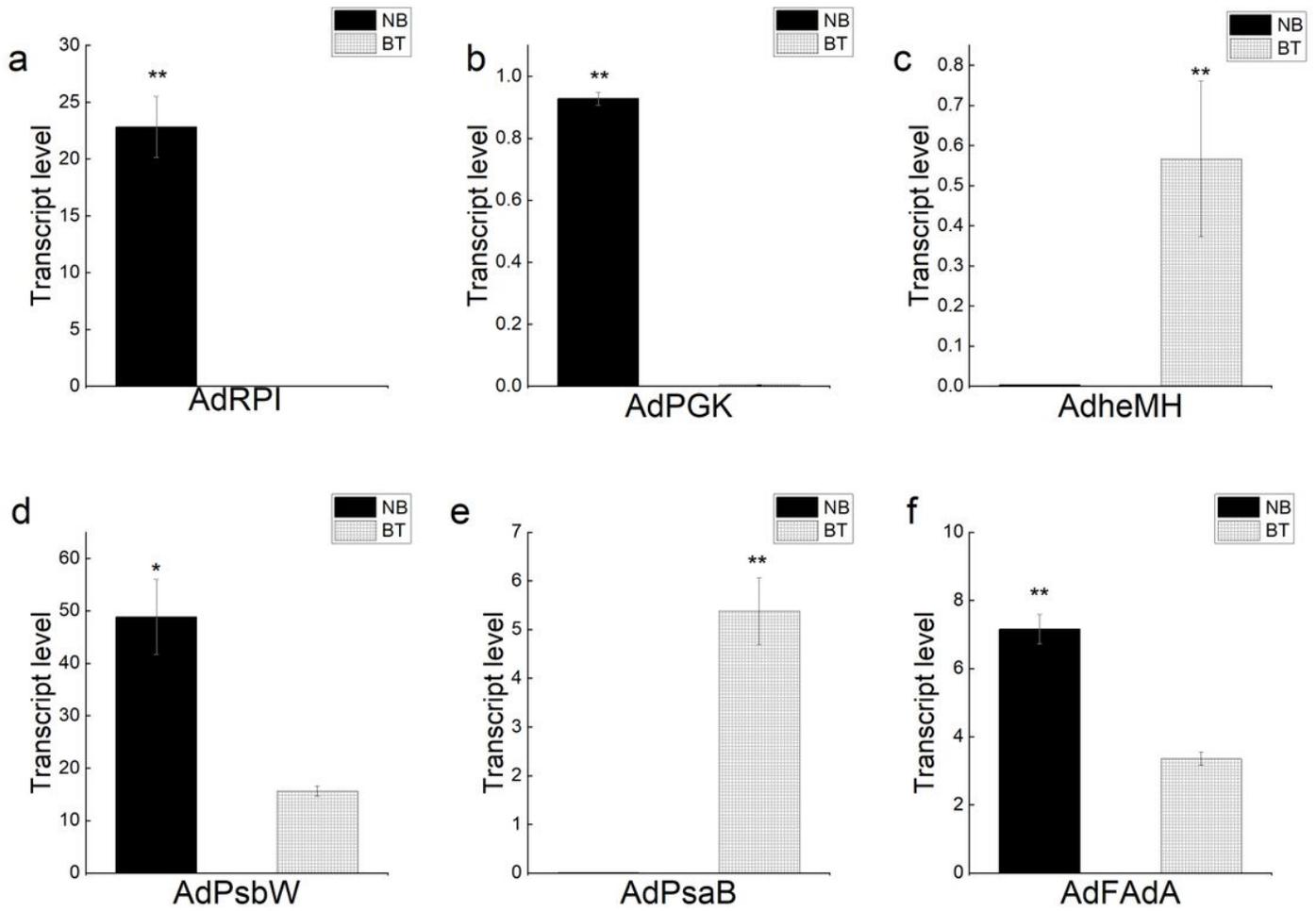


Figure 6

Transcriptomic analysis on photosynthesis related pathways in leaves between early bolting and normal *Angelica dahurica*. (a) AdRPI; (b) AdPGK; (c) AdheMH; (d) AdPsbW ; (e) AdPsaB; (f) AdFdrA.

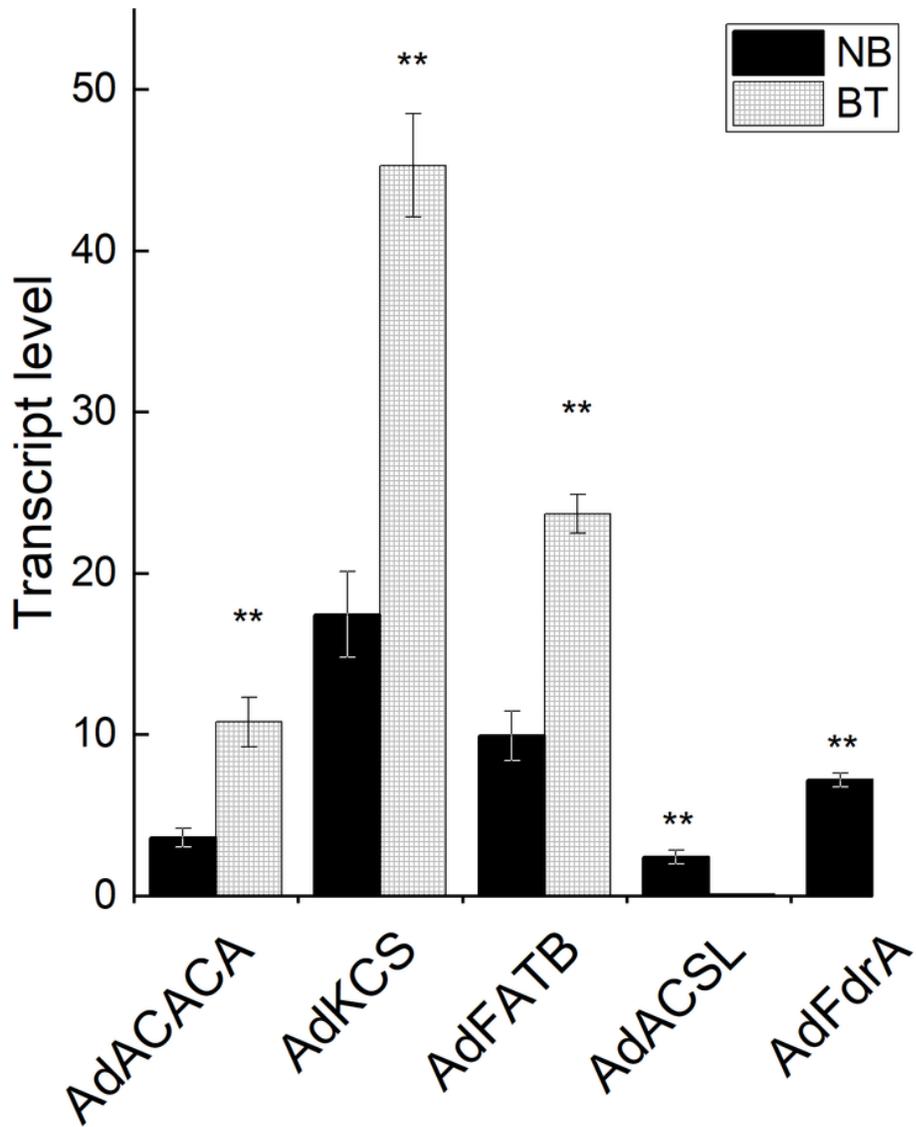


Figure 7

Transcriptomic analysis on fatty acid metabolism of the leaves of early bolting and normal *Angelica dahurica*.