

Transcriptome profiling provides new insights into florets number difference of inflorescence in *L. angustifolia*

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Abstract

Background Lavender flowers essential oil had been used for a variety of therapeutic and cosmetic purposes, and had been popular for centuries. The previous studies of lavender mainly focused on essential oil composition and extraction methods, ignoring the factors that affected the production of essential oils, such as the floret number. This study aims to get a deep insight into the mechanism of florets number difference.

Results 1. Hormone profile showed positive correlation between ABA content and the number of florets, while IAA was negatively correlated. 2. RNA-Seq results showed that 2848 differentially expressed genes (DEG) were screened by comparing different florets samples in one plant. By analyzing dynamic changes of differentially expressed genes, many potentially interesting genes that encoded putative regulators or key components of ABA metabolism and signaling transduction were identified, such as *NCED*, *PYL*, *PP2C*, *SnRK2*. 3. Common network analysis showed that the key genes of ABA pathway metabolism were negatively related to the DEG of IAA, especial IAA18. 4. Exogenous IAA significantly inhibited the number of lavender florets and affected the expression of ABA pathway metabolism genes, such as *NCED*, *PP2C*.

Conclusions 1. The different concentrations of ABA lead to florets number difference in the *L. angustifolia* "JX-2" clusters; 2. ABA may affect the florets number by regulating IAA transport and accumulation.

The results will be useful for a better understanding of the molecular mechanism on florets number difference, which will lay the foundation for molecular breeding of multi-florets varieties.

Background

Lavenders belonging to the family Labiatae (*Lamiaceae*) have been used as dried flowers and extracting essential oil which is used for a variety of therapeutic and cosmetic purposes for so long time [1]. The essential oil continued to be as popular as it had been down the centuries past. Lavender essential oil is extracted from both the flowers and foliage by steam distillation with varying chemical composition but most of the aromatic oil is derived from the flowers [2]. The previous studies were mainly focused on lavender essential oil composition and extraction methods ignoring factors which affected the production of essential oils, such as the floret number.

The type of *L. angustifolia* inflorescence is verticillaster where numbers of floret form a false whorl at the node. Some of whorls are borne on main floral axis forming a spike. One whorl as example, the main floral axis gives rise to two lateral branches and the succeeding branches bear only one branch each on alternate sides. Practice had shown that the number of floret and branch was positively correlated. The axillary meristems had the same developmental potential as the primary inflorescence primordium played an important role in regulating floret number, but cannot go on infinitely meristem [3, 4]. Axillary meristems develop into flower buds or form a new meristem at their base [3]. The flexibility in axillary

meristem activity made possible substantial variation in inflorescence architecture, allowing the *L.angustifolia* to adapt to the prevailing environmental conditions.

The inflorescence pattern of lavender resembled with the developmental process of plant stems, all them relied on constant branching. Previous studies showed that the shoot architecture was regulated by light, nutrition and density [5, 6]. These environmental signals were likely to be relayed through the action of plant hormones, of which auxin, cytokinin, strigolactone, ABA were particular important [4,7]. Auxin was the first hormone to be linked to regulation of shoot branching and it had been in the spotlight for more than 100 years. Cytokinin played a fundamental role in regulation of apical meristem size [8, 9, 10]. Strigolactones blocked the transport of IAA through the *Pin* protein to regulate branching[11]. ABA likewise inhibited the expression of genes associated with the bud autonomous auxin pathway and hindered the accumulation of IAA in the bud [12].

The biggest difference between the formation of inflorescences and stems was the termination signals; stems relied on dormant buds while inflorescences were dependent on flower. The difference of the termination signal may be caused by the difference of regulation mechanism.

In *L.angustifolia* 'JX-2' cluster, all inflorescences had five whole flowers but the floret number on different inflorescence was from 30 to 70 (about 2.5-fold difference) which directly affected the production of essential oils. However, the reason for florets number difference was poorly understood. In this study, we aimed to understand florets number difference in "*L.angustifolia* 'JX-2' by addressing the following issues; 1. Which hormones regulate the number of florets? 2. which are the key genes in hormonal metabolic pathways that affect floret number? 3. Possible regulatory mechanism of the difference in the number of florets.

Results

Inflorescence characteristics of lavender

The botanical characteristics of Lavender were dwarf shrubs, branch with long inflorescence and short leafy shoots. One three-year-old plant had about 100 flowering branches (Fig. 2a). Each flowering shoots borne five whorl flower (Fig. 2c). Take one side of one round flower for example, the 2nd flower had symmetry but the flowers begun to be inward hyperplasia from the third flower (Fig.2b). The inflorescence of lavender "JX-2" was 30-70 flowers per shoots, 6-14 florets per round, (Fig. 2c).

Hormone contents of different florets samples

Hormone content of different parts in LaF6, LaF10 and LaF14 samples were measured by ELISA (Table 1). Hormones content of florets and flower axis were not significantly different. In leaves and shoots, change trends of GA and ZR content were similar, while ABA and IAA content of leaves in three samples had the biggest difference (Table 1). ABA content was the lowest in LaF6 sample, followed by that in the LaF10, and highest in the LaF14. However, for IAA, leaves from LaF6 sample had highest content

followed by that in the LaF10 and lowest in the LaF14. Further evidence by GC-MS supported this result that ABA content of leaves from sample with more floret numbers was higher, but IAA content of leaves from sample with more floret numbers was lower (Fig. 3A). The ABA/IAA ratio also increase along with the increase in the florets numbers, and the ABA/IAA ratio of LaF14 is the highest among them (Fig. 3B).

Table.1 Hormone content in different parts of flowering branches in bud stage

Hormone	Samples	Shoot (ng/g.FW)	Leaf (ng/g.FW)	Flower axis(ng/g.FW)	Florets (ng/g.FW)
GA	LaF6	3.89	3.42	5.99	5.41
	LaF10	4.36	8.71	9.11	8.69
	LaF14	3.74	6.96	4.18	5.35
ABA	LaF6	42.04	27.20	35.67	51.56
	LaF10	48.88	43.84	39.68	91.17
	LaF14	70.00	68.56	39.22	56.43
IAA	LaF6	65.10	115.22	81.49	35.84
	LaF10	48.25	85.67	82.84	76.32
	LaF14	36.91	64.04	62.85	52.24
ZR	LaF6	4.21	3.66	5.90	4.02
	LaF10	3.17	3.78	3.80	6.22
	LaF14	4.38	5.51	3.50	4.18

Sequencing and de novo assembly of lavender transcriptome

The three cDNA samples were prepared from leaves of the LaF6, LaF10 and LaF14 samples and sequenced using the Illumina HiSeq 3000. After stringent quality check and data cleaning, 6.17-7.67 GB clean nucleotides were obtained for each sample, totaling 20.45 GB nucleotides for all samples (Table 2). The Q20 percentage (sequencing error rate < 1 %) and GC percentage were 96.21-96.95 % and 47.75-48.17 %, respectively (Table 2).

Table. 2. Sequence statistics of the lavender transcriptome

Sample	Raw Reads	Clean Reads	Clean Bases	Q20(%)	GC Content(%)
LaF6	45975468	44047736	6.61G	96.27	48.17
LaF10	54285374	51139942	7.67G	96.95	47.75
LaF14	43301158	41106448	6.17G	96.21	48.09

Functional annotation

All unigenes were aligned to six protein databases including Nr, KO, PFAM, Swiss-Prot, GO, KOG. A total of 103,555 unigenes were annotated which contained 72,820 (70.32%) unigenes identified from Nr, 52,114 (50.32%) from Nt, 30,814 (29.75%) from KO, 57,472 (55.50%) from Swiss-Prot, 51,664 (49.89%) from PFAM, 51,985(50.2%) from GO, 32,247(31.14%) from KOG (Table 3). E-value indicated the extent of sequence homology and if E-value is smaller, the sequence homology is higher. For E-value distribution of unigenes, blastx hits in the Nr database which had the largest number of annotated unigenes, 37% homolog sequences ranged between $1E^{-5}$ to $1E^{-60}$, while 63% sequence had a threshold E-value less than $1E^{-60}$ that showed strong homology (Fig.4a). Species distribution of the first blastx hits of each unigene in the Nr database indicated that *Sesamum indicum* provided the best blastx matches with 56.8% unigenes in lavender, and *Erythranthe guttata* was the second closest species, which had 13.8% homology with lavender (Fig. 4b).

To gain insight into functions of the annotated genes from the macro level, GO functional classification was performed. A total of 55 GO terms were categorized into three domains: biological process, cellular component and molecular function (Fig. S1). The term of 'cellular process', 'metabolic process' and 'single-organism process'; 'cell' and 'cell part'; 'binding' and 'catalytic activity' were the most representative of biological process, cellular component and molecular function, respectively (Fig. S1).

KEGG mapping of the unigenes was carried out to analyze pathways involved in lavender transcriptome and 131 pathways were identified. The 'translation' had the greatest members (3,432), followed by 'carbohydrate metabolism' (2,858) and folding, sorting and degradation (2,248) (Fig. S2). KOG analysis was performed to predict phylogenetic classification. In total, 36,062 unigenes were matched and grouped into 25 functional classes, the clusters for 'general function prediction only' (5,036) and 'posttranslational modification, protein turnover, chaperones' (4471) were the two largest groups (Fig. S3).

Table. 3. Annotation of unigene sequences

Database	Number of annotated unigenes	Percentage (%)
NR	72820	70.32
KO	30814	29.75
Swiss-Prot	57472	55.50
PFAM	51664	49.89
GO	51985	50.2
KOG	32247	31.14

Identification and KEGG enrichment analysis of DEGs

With q value < 0.005 and an absolute \log_2 fold change > 1 , a total of 2848 genes were found to be differently expressed among the groups LaF6 vs LaF10, LaF6 vs LaF14 and LaF10 vs LaF14. In the group LaF6 vs LaF10, 1089 genes were found, among which 522 genes were down-regulated and 567 genes were up-regulated. In the LaF6 vs LaF14 group, 1344 genes were differently expressed, of which 741 genes were up-regulated and 603 genes were down-regulated. In LaF10 vs LaF14 group, 320 genes were up-regulated and 299 genes were down-regulated (Fig. 5a). These results indicated that both up-and down-regulation of gene expression occurred, and the transcript abundance of genes changed dynamically among the difference florets samples.

To validate DEGs expression profiling obtained by RNA-seq, 7 DEGs in LaF10 vs LaF14 group, 17 DEGs in LaF6 vs LaF14 group and 12 DEGs in LaF6 vs LaF10 group were selected for qRT-PCR analysis, and their expression trend were similar to that in RNA-Seq. Their correlation coefficients (R^2) ranged from 0.94 to 0.97, with a very significant level ($P < 0.01$) (Fig. 5b,c,d). Thus, Data from RNA-Seq in this study were reliable.

To further understand the effect of florets number difference of inflorescence at the molecular level in *L. angustifolia*, we mapped the DEGs to the terms in the KEGG database, and significantly enriched metabolic pathways or signal transduction pathways were identified by comparing with the whole genome background. In this study, there were 573 DEGs in the group LaF6 vs LaF10, 656 DEGs in the group LaF6 vs LaF14, and 360 DEGs in the group LaF10 vs LaF14 were mapped to KEGG pathways. Totally, 10 pathways were significantly enriched (p -value < 0.05). Photosynthesis (ko00195), phenylpropanoid biosynthesis (ko00940) were significantly enriched among three groups, while protein processing in endoplasmic reticulum (ko04141), oxidative phosphorylation (ko00190) were significantly enriched in the group LaF6 vs LaF10 and LaF10 vs LaF14. In addition, specific enrichment was observed for flavonoid biosynthesis (ko00941) and stilbenoid, diarylheptanoid and gingerol biosynthesis (ko00945) in LaF10 vs LaF14 group (Table 4).

Table. 4. List of the enriched KEGG pathways

Pathway ID	KEGG Pathway	DEGs with pathway annotation			All genes with pathway annotation (30161)
		LaF6 vs LaF10 (573)	LaF6 vs LaF14 (656)	LaF10 vs LaF14 (360)	
ko04141	Protein processing in endoplasmic reticulum	25(4.3%)*	16(2.4%)*	21(5.8%)*	942(3.1%)*
ko00190	Oxidative phosphorylation	15(2.6%)*	10(1.5%)	20(5.6%)*	712(2.4%)*
ko00195	Photosynthesis	25(4.3%)*	13(2.0%)*	17(4.7%)*	233(0.8%)*
ko00940	Phenylpropanoid biosynthesis	13(2.2%)*	13(2.0%)*	12(3.3%)*	329(1.1%)*
ko00040	Pentose and glucuronate interconversions	7(1.2%)	10(1.5%)*	8(2.2%)	233(0.8%)*
ko00053	Ascorbate and aldarate metabolism	2(0.3%)	7(1.1%)	7(1.9)	167(0.6%)
ko00071	Fatty acid degradation	4(0.7%)	11(1.7%)*	7(1.9%)	192(0.6%)
ko00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	3(0.5%)	4(0.6%)	6(1.7%)	66(0.2%)
ko00941	Flavonoid biosynthesis	3(0.5%)	5(0.8%)	6(1.7%)	78(0.3%)
ko00920	Sulfur metabolism	6(1.0%)	2(0.3%)	4(1.1%)	98(0.3%)
ko00906	Carotenoid biosynthesis	6(1.0%)	6(0.9%)	1(0.2%)	222(0.7%)*

DEGs associated with ABA, IAA metabolism and signal transduction

Hormone content showed that the difference in the number of florets was related to ABA anabolism in the flowering shoots and leaf (Table 1, Fig.3). Here, we also found that ABA metabolism (carotenoids biosynthesis pathway) and signaling transduction, IAA signaling transduction was enriched among the different florets samples (Table 4). Thus, the DEGs involved in the entire ABA metabolism and signaling transduction were focused.

β -carotene hydroxylase (*BCH*) and 9-cis-epoxycarotenoid dioxygenase (*NCED*) were two key enzymes in the ABA biosynthesis pathway, especially the *NCED* (Fig. 6a). The expression of *BCH* gene (CL-10130.19560) in LaF6 sample was lower than that of LaF10, and that in LaF14 sample was the highest (Fig. 6c). Four DEGs encoding *NCED* were found among the florets difference samples. Based on their expression pattern, three genes (CL-10130.29693, CL-10130.42768, CL-10130.34829) had similar expression patterns with *BCH* gene, and one gene (CL-10130.40990) had lowest expression level in LaF14, and similar level in LaF6 and LaF10 samples (Fig. 6c). Above all, the expression levels of five ABA biosynthetic genes except one were higher in sample with higher ABA content.

In the ABA signaling transduction (Fig. 6b), one *PYL* gene, nine *PP2C* genes, three *SnRK2* genes and two *ABF* genes were found in response to florets difference (Fig. 6c). The expression level of *PYL* gene declined with florets number increasing. The nine *PP2C* genes all had a similar expression profile with

highest expression in LaF14, followed by that in LaF10 and lowest expression level in LaF6. However, there were complicated expression patterns for *SnRK2* and *ABF* genes. Some of genes encoding homologous products were upregulated, but others were downregulated in the same sample (Fig. 6c).

In plant hormone signal transduction pathway, three *AUX/IAA* genes, one *SAUR* genes, were found in response to florets difference (Fig.S4). The three *AUX/IAA* genes had a similar expression profile that had highest expression in LaF6, followed by that in LaF10 and lowest expression level in LaF14. The *SAUR* gene expression trend is not obvious among the samples.

Common network analysis of ABA and IAA DEGs

A common network was further analyzed by checking the shared crosstalk of DEGs among LaF6, LaF10 and LaF14 samples. The common significant correlations were examined with 27 DEGs from different florets number samples. A common crosstalk network between ABA signaling pathways with IAA was constructed by using method of two-way variance analysis (Fig.7). Positive correlations were identified between *BCH*, *NCED*, *PYL*, *PP2C*, *SnRK2* and *ABF*. Negative correlations were identified between ABA signal pathway and IAA, such as the correlations coefficients of *BCH*, *NCED*, *ABF* and IAA were -0.741,-0.759,-1.000.

The relationship between ABA and IAA was further investigated by analysis the network among key factors *NCED*, *PP2C*, *ABF* and IAA DEGs (Fig.8). The IAA DEGs, *IAA18*, *IAA16* and *SAUR* were negatively related to the key genes of ABA metabolism pathway, especially *IAA18*. The correlation coefficients of *IAA18* with *NCED*, *PP2C* and *ABF* are -0.922, -0.814 and -0.953, respectively (Fig.8). It is speculated that *IAA18* may be the key factors in the relationship between ABA and IAA.

Effects of exogenous IAA on the number of florets and the expression of key genes in ABA metabolism

We also examined the effect of exogenous IAA on the number of florets and the expression of key genes in the ABA metabolism. The results showed that exogenous IAA significantly inhibited the number of florets, the inhibitory effects was concentration-dependent. The florets number of IAA300 treatment was significantly higher than that of IAA800 treatment (Fig.9 A).

The qPCR results showed that exogenous IAA significantly affected the expression of DEGs in the ABA metabolic pathway, such as CL36222, CL38198 and CL40990 (Fig.9B,C,D,E).

Discussion

Transcriptome and regulatory hormones of different florets number

Increasing the florets number is a desired target of lavender breeding. To explore the intrinsic mechanism or influencing factors underlying the differences in the number of florets were the basis for cultivating multi-flowering varieties. 2848 DEGs were revealed that 10 metabolic pathways and signal transduction pathways were significantly enriched by deep sequencing and function analysis (Table 4). These

pathways were involved in a wide range of molecular processes from gene expression to protein, hormone signal and secondary metabolites synthesis, and from signaling transduction to transportation regulation. Thus, it was speculated that a variety of molecular processes in response to florets number may lead to different morphological and physiological responses branching number in the flowering shoots.

Previous studies showed that axillary buds outgrowth can respond to different light quality, ABA regulated stem branching by affecting the accumulation of IAA in the buds [21, 22]. In our experiment, the IAA contents and DEGS (IAA18, IAA16 and SAUR) expression was negatively correlated with the florets number (Table.1, Fig.S4). Auxin had been proved to be directly linked to regulation of shoot branching in many plants, such as *Oryza.sativa* L, *Arabidopsis thaliana* (L.) Heynh [11, 12]. The ABA contents and ABA/IAA ratio gradually increased with the florets number increases (Table.1, Fig.3), which was regulated by the genes involved in the ABA biosynthesis pathway. Four ABA biosynthetic genes expressed increasingly with the florets number increases. The *NCED3* gene was a key gene in ABA metabolism process, and high expression of *AtNCED3* or *BgNCED1* in *Arabidopsis* can significantly increase the level of endogenous ABA [23, 24, 25, 26]. Our result also indicated that the expression level of three *NCED3* or *NCED1* genes was in accord with endogenous ABA content. It was speculated that ABA was involved in florets number regulation in *L.angustifolia*.

Like ABA metabolic pathways, some components of ABA signaling transduction were also different among the different florets samples, such as the protein *PYR/PYL*, *PP2C*, *SnRK2* and *ABF*. These core component proteins constituted a dual negative regulatory system that regulates ABA signaling [27,28,29]. The previous studies showed that seed germination, vegetative growth, gene expression and stomatal movement were more sensitive to ABA in the plant in which *PYL9/RCAR1*, *PYL5/RCAR8* or *PYL8/RCAR3* genes over-expressed [28,29]. In the experiment, *PYR/PYL*, *PP2C*, *SnRK2* and *ABF* proteins were aggregated among differential genes (Fig.6). Molecular mechanisms study further showed that ABA is involved in the regulation of florets number in *L.angustifolia* inflorescence.

Possible mechanism of ABA regulating the number of florets

ABA has been known as the plant stress hormone. However, it also plays a role in non-stress-related plant development and growth processes. ABA may turn out to be one of the earliest upstream factors when shoot branching response to environment signal [30]. In our experiment, ABA content is positively correlated with the number of florets, while IAA is the opposite (Fig.3A). The IAA and ABA contents in the LaF6 were 369.2, 452.6 ng/g, respectively and in the LaF14 sample was 90.3, 4534.5 ng/g, respectively (Fig. 3). Pot experiment also confirmed that exogenous IAA significantly inhibited the number of florets, and the inhibitory effect was positively related to IAA concentration (Fig.9 A). The change trend of ABA/IAA ratio also correlated with the florets number (Fig.3B). According to phenotypic data, it is speculated that the ABA regulated the florets number may be related to the hormone IAA.

DEGs correlation analysis showed that the key genes of ABA metabolism pathway, such as *NCED*, *PYL*, *PP2C*, *ABF* and *SnRK2* were all negative correlation with IAA genes (Fig.7). In pot experiments, exogenous

IAA can also affect the expression of DEGs in the ABA metabolic pathway (Fig.9). Further correlation analysis between the ABA rate-limiting factors and the IAA DEGs (IAA18, IAA16 and SAUR) showed that IAA18 and the ABA rate-limiting factors (*NCED*, *PP2C*, *ABF*) all have a negative correlation, with the correlation coefficients > 0.8 (Fig.8). The previous studies showed that IAA18 was mainly involved in the regulation of plant axillary meristems development [34,35]. The characteristics of verticillaster inflorescence indicated that the key factors affecting the number of "JX-2" florets were the axillary meristems and their breaching (Fig.2). IAA18 may be a keystone in the interaction between ABA and IAA.

Previous studies also showed that ABA and auxin are interacted with each other in many developmental processes, especially, in part of regulating root growth, seed vigour regulation [31, 32, 33, 36]. ABA also regulated lateral root formation and elongation by suppressing the expression of PIN-FORMED1 through ABA-insensitive (*ABI4*) [33]. In *Populus*, *PdNF-YB21* positively regulates root growth and drought resistance by ABA mediated IAA transport [37].

Comprehensive analysis of previous research and our experimental results, we speculated that ABA may affect the IAA transport or accumulation which leads to axillary meristems increase and the number florets difference.

Conclusion

1. the reason for the difference of florets number in the lavender clusters may be due to different ABA concentrations in the flower bud stage. 2. ABA may affect the florets number by regulating IAA transport and accumulation.

Materials And Methods

Study site

The inflorescence samples of experiment were collected from Qing Shui He town (44°11' N, 80°43'E, 715m a.s.l) Huo Cheng county, Yi Li Kazakh autonomous prefecture, Xin Jiang uygur autonomous region, China. The area is located in temperate semi-arid climate zone where the mean annual temperature is 8.2-9.4°C and long-term annual precipitation is 460 mm. Lavender essential oil production in this area account of 95 % of the national total, and known as the hometown of lavender in China [13].

Plant materials

In the lavender planting area of 65th group farm in Xin Jiang, 667m² was selected as experimental area. In the experimental area, 3-year-old lavender plants were selected as the sample plants making 3 groups with 10 sample plants in a single group. The flowering branches with 6 florets (LaF6), 10 florets (LaF10) and 14 florets (LaF14) per round in bud stage of each sample plants were used as experimental materials. The flowering branches with different florets were divided into four parts: floral axis 1, leaves,

floral axis 2 (between red lines), florets, respectively (Fig.1). The sample materials were cut and put into the ziplock bag, then placed in a crisper filled with ice bag at 9 a.m.

Exogenous IAA spraying experiment

In late May 2019, select growth robust and consistent “JX-2” cutting seedlings, transplant them in pots. The substrate is peat:river=7:3, after 1 week, performed IAA spray treatment, IAA concentration was 0, 300 and 800mg/L, the control was 0.1% ethanol + distilled water solution, there was 5 pots of seedlings for each treatment, the interval between treatments was 2 meters, and the PC board was used to shield when spraying to prevent mutual interference. Spray once every 7 days, 5 times continuously. After the flower buds are formed, take the leaves from the flower branches, and put into the ziplock bag which filled with ice bag, bring it into the laboratory in time, quick-freeze with liquid nitrogen and store at -80°C. Then perform RNA extraction and qTPCR verification of ABA metabolism pathway genes.

Hormone determination

Enzyme-linked immunosorbent assay (ELISA): GA, ABA, IAA, ZR of four parts (floral axis 1, leaves, floral axis 2 and florets) for LaF6, LaF10 and LaF14 samples were measured. Firstly, hormones were extracted and purified according to modified method described by [14]. Secondly, the extracted hormone elution was analyzed according the ELISA method described by [15]. The mouse monoclonal antigens and antibodies against GA, ABA, IAA, ZR, and immunoglobulin G-horseradish peroxidase (IgG-HRP) used in the ELISA were produced at the Phytohormones Research Institute, China Agricultural University, China. In order to exclude the influence of the difference in leaf water status on GA, ABA, IAA, ZR content, and all hormone contents were expressed on a dry leaf weight basis.

Gas chromatography-mass spectrometry (GS-MS): ABA and IAA of leaves for LaF6, LaF10 and LaF14 samples were measured more accurately using modified GS-MS according to the previous described method [16].

The internal standard [$^2\text{H}_6$] ABA was purchased from OIChemIm Company (OIChemIm cat. No. 034 2723) and [$^{13}\text{C}_6$] IAA was purchased from Cambridge Isotope Laboratories (Cambridge Isotope Laboratories, Inc. cat. No. CLM-1896-0).

RNA isolation and cDNA library construction for Illumina sequencing

Leaves of LaF6, LaF10 and LaF14 were immediately frozen in liquid nitrogen until use. Total RNA of each sample was obtained using an RNAPrep Pure Plant kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. The quality of RNA was determined using the NanoDrop 1000 spectrophotometer and Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, USA), and the RNA integrity number (RIN) value of all sequencing samples was more than 8.0. The cDNA libraries were constructed as described below. Total RNA of each sample was treated with DNase I, and poly (A) mRNA was then isolated with oligodT beads, followed by reverse-transcription into first-strand cDNA using

reverse transcriptase. Second-strand cDNA was synthesized using DNA polymerase I and RNaseH, and then ligated with an adaptor of index adaptor using T4 DNA ligase. Adaptor-ligated fragments were separated and excised by agarose gel electrophoresis. PCR was performed to selectively enrich and amplify the cDNA fragments. Finally, the cDNA libraries were sequenced using and Illumina HiSeq 3000 at Novogene Tech Solutions Co., Ltd, Tianjin, China.

De novo assembly and functional annotation

Adaptor sequences, reads with unknown sequences and low quality reads were removed and the clean reads were then assembled using Trinity software [17]. Data obtained for each sample were separately assembled and the assembly sequences were called unigenes. The unigenes from all samples were further subjected to sequence splicing and redundancy removal with sequence clustering software to acquire non-redundant unigenes as long as possible. These unigene sequences were aligned and annotated to protein databases like Nr, Swiss-Prot, PFAM, COG [18], GO [19], KO[20], and nucleotide database Nt with a threshold of $E < 10^{-5}$. The best aligning results were used to decide sequence direction of unigenes. If results from different databases conflicted with each other, a priority order of Nr, Swiss-Prot, KEGG and COG should be followed when deciding sequence direction of unigenes.

Analysis of differentially expressed genes (DEGs)

Expression of the unigenes was calculated using the FPKM (Fragments Per Kb per Million reads) method which eliminated the influence of lengths and sequencing discrepancies of different genes on the gene expression calculations. DEGs were selected on condition of $qvalue < 0.005$ and an absolute value of $\log_2 foldchange > 1$.

Quantitative real-time PCR (qRT-PCR) validation

Total RNA was separately extracted from leaves for LaF6, LaF10 and LaF14 samples as described earlier. First-strand cDNA was synthesized using the PrimeScript® RT reagent kit (Takara) according to the manufacturer's instructions. The primers for the selected genes were designed using Primer Premier 5.0 software and listed in Table. S1. qRT-PCR was performed using the Power SYBR Green PCR Master Mix on the StepOne Plus Real time PCR Platform (Applied Biosystems). This experiment were carried out with the following protocol: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and at 60°C for 60s. For each sample, reactions were performed in triplicate, and the gene expression level obtained by RNA-seq and qRT-PCR were evaluated using R^2 value.

Statistical analysis

ANOVA and Dennett's tests were used to determine the ABA content among different florets samples. The DEGs correlation between ABA and IAA was assessed by two-way variance analysis using spass 11.0.

Declarations

Author Contribution

All authors discussed the results and commented on the manuscript. HP-H, HT-B designed the project. HP-H, HT-B and JG collected the samples. HP-H, L-H and L-S performed the molecular biology analyses. HP-H, YM-D, L-S and XP-Zhu analyzed the metadata. HP-H and YM-D conduct of pot experiment, HP-H, YM-D, L-S and XP-Zhu wrote the manuscript while A-F revised the manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Competing interests

None

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

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References

- [1] **Zai X.** Study on the dynamic growth and components accumulation of *Lavandula angustifolia*. Master's thesis, 2011; Henan Agricultural University, Zhengzhou
- [2] **McGimpsey JA, Porter NG,** Lavender, A grower's guide for commercial production. New Zealand institute for Crop & Food Research Ltd. **1999**; New Zealand.
- [3] **Barazesh S, McSteen P.** Hormonal control of grass inflorescence Development. Trends Plant Sci. 2008;13: 656-662. Doi: 10.1016/j.tplants.2008.09.007
- [4] **Ongaro V, Leyser O.** Hormonal control of shoot branching. J Exp Bot. **2008**;59: 67-74.
Doi:10.1093/jxb/erm134
- [5] **Cline MG.** Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. Ann Bot. 1996;78: 255–266.doi:10.1006/anbo.1996.0119
- [6] **Snowden KC, Napoli CA.** A quantitative study of lateral branching in petunia. Funct Plant Biol. 2003;30: 987–994.
- [7] **McSteen P.** Hormonal regulation of branching in grasses. Plant Physiol. 2009;149:46-55.
- [8] **Shani E, Yanai O, Ori N.** The role of hormones in shoot apical Meristem function. Curr Opin Plant Biol. 2006; **9**: 484–489.
- [9] **Kyojuka J.** Control of shoots and root meristem function by Cytokinin. Curr Opin Plant Biol. 2007;10: 442–446.
- [10] **Zhao Y.** The role of local biosynthesis of auxin and cytokinin in plant development. Curr Opin Plant Biol. 2008;11: 16–22.
- [11] **Ferguson BJ, Beveridge CA.** Roles for auxin, Cytokinin, and Strigolactone in regulating shoot branching, Plant physiol. 2009;149:1929-1944.
- [12] **Yao C, Finlayson SA.** Abscisic acid is a general negative regulator of Arabidopsis axillary bud growth. Plant Physiol. 2015;169: 611-626.DOI: 10.1104/pp.15.00682
- [13] **Tong H, Tang J, Zhang ZF.** Study on the research development of Lavender and its essential oil. Flavor Fragrance Cosmetics. 2013;**6**:55-58.
- [14] **He Z.** Guidance to experiment on chemical control in crop Plants.**1993**; Beijing Agricultural University publication, Beijing.
- [15] **Yang J, Zhang J, Wang Z, Zhu Q, Liu L.** Water deficit induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. Agron J. 2001;**93**:196–206.

- [16] Müller A, Düchting P, Weiler EW. A multiplex GC-MS/MS technique for the sensitive and quantitative single-run analysis of acidic phytohormones and related compounds, and its application to *Arabidopsis thaliana*. *Planta*. 2002;216:44–56. doi: 10.1007/s00425-002-0866-6
- [17] Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol*. 2011;29: 644–652. Doi:10.1038/nbt.1883
- [18] Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV. The COG database: new development sinphylogenetic classification of proteins from complete genomes. *Nucleic Acids Res*. 2001;29:22–28. doi:10.1093/nar/29.1.22
- [19] Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M, Montserrat R. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*. 2005;21:3674–3676. doi: 10.1093/bioinformatics/bti610
- [20] Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and interpretation of large-scale molecular datasets. *Nucleic Acids Res*. 2012;40:109–114. doi:10.1093/nar/gkr988
- [21] Reddy SK, Holalu SV, Casal JJ, Finlayson SA. Abscisic acid regulates axillary bud outgrowth responses to the ratio of red to far-red light, *Plant Physiol*. 2013;163:1047–1058.
- [22] Holalu SV, Finlayson SA. The red light:far red light alters *Arabidopsis* axillary bud growth and abscisic acid signaling before stem auxin changes. *J Exp Bot*. 2017.67:943–952.
- [23] Thompson AJ, Jackson AC, Parker RA, Morpeth DR, Burbidge A, Taylor IB. Abscisic acid biosynthesis in tomato: Regulation of zeaxanthin epoxidase and 9-cis-epoxycarotenoid dioxygenase mRNAs by light/dark cycles, water stress and abscisic acid, *Plant Mol Biol*. 2000;42:833-845.
- [24] Iuchi S, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K. A stress-Inducible gene for 9-cis-epoxycarotenoid dioxygenase involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. *Plant Physiol*. 2000;123:553-562.
- [25] Qin XQ, Zeevaart JAD. The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water stressed bean. *PNAS*. 1999;96:15354-15361.
- [26] Boyd J, Gai YZ, Nelson KM, Lukiwshi E, Talbot J, Loewen MK, Owen, Cutler AJ, Abrams SR, Loewen MC. Sesquiterpene-like inhibitors of a 9-cis-epoxycarotenoid dioxygenase regulating abscisic acid biosynthesis in higher plants. *Bioorgan Med Chem*. 2009; 17:2902-2912. DOI.10.1016/j.bmc.2009.01.076
- [27] Raghavendra AS, Gonugunta VK, Christmann A, Grill E. ABA perception and signaling. *Trends Plant Sci*. 2010;15:395–401.

- [28] **Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR.** Abscisic acid: emergence of a core signaling network. *Ann Rev Plant Biol.* 2010;**61**:651–679.
- [29] **Hu S, Wang FZ, Liu ZN, Liu YP, Yu XL.** ABA signaling mediated by PYR/PYL/RCAR in plants. *Hereditas.* 2012;**34**:560-572.
- [30] **Rameau C, Bertheloot J, Leduc N, Andrier B, Foucher F, Sakr S.** Multiple pathways regulate shoot branching. *Front Plant Sci.* 2015; 5:1-15. doi:10.3389/fpls.2014.00741
- [31] **Ephritikhine G, Fellner M, Vannini C, Lapous D, Barbier-Brygoo H.** The sax1 dwarf mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. *Plant J.* 1999;**18**:303–14.
- [32] **Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen H.** Root growth maintenance during water deficits: Physiology to functional genomics. *J Exp Bot.* 2004;**55**: 2343–2351.
- [33] **Brady SM, Sarkar SF, Bonetta D, McCourt P.** The ABSCISIC ACID INSENSITIVE 3 (ABI3) gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in *Arabidopsis*. *Plant J.* 2003;**34**:67–75.
- [34] **Uehara T, Okushima Y, Mimura T, Tasaka M, Fukaki H.** Domain II mutations in CRANE/IAA18 suppress lateral root formation and affect shoot development in *Arabidopsis thaliana*. *Plant Cell Physiol.* 2008;**49**(7): 1025–1038. DOI. 10.1093/pcp/pcn079
- [35] **Bustillo-Avendano E, Ibáñez S, Sanz O, Sousa Barros JA, Gude I, Perianez-Rodriguez J, Micol JL, Del Pozo JC, Moreno-Risueno MA, Pérez- Pérez JM.** Regulation of hormonal control, cell reprogramming and patterning during De Novo root organogenesis. *Plant Physiol.* 2018;**176**:1709-1727. DOI: 10.1104/pp.17.00980
- [36] **Zhou YY, Zhang Y, Wang XW, Han X, An Y, Lin SW, Chao S, Wen JL, Liu C, Yin WL, Xia XL.** Root-Specific NF-Y family transcription factor, PdNF-YB21, positively regulates root growth and drought resistance by abscisic acid-mediated indoylactic acid transport in *Populus*. *New Phytol.* 2020, **227**(2):407-426.doi: 10.1111/nph.16524.
- [37] **He YQ, Zhao J, Yang B, Sun S, Peng LL, Wang ZF.** Indole-3-acetate beta-glucosyltransferase OsiAGLU regulates seed vigour through mediating crosstalk between auxin and abscisic acid in rice. *Plant Biotechnol J.* 2020;**18**(9):1933-1945. doi: 10.1111/pbi.13353.

Figures

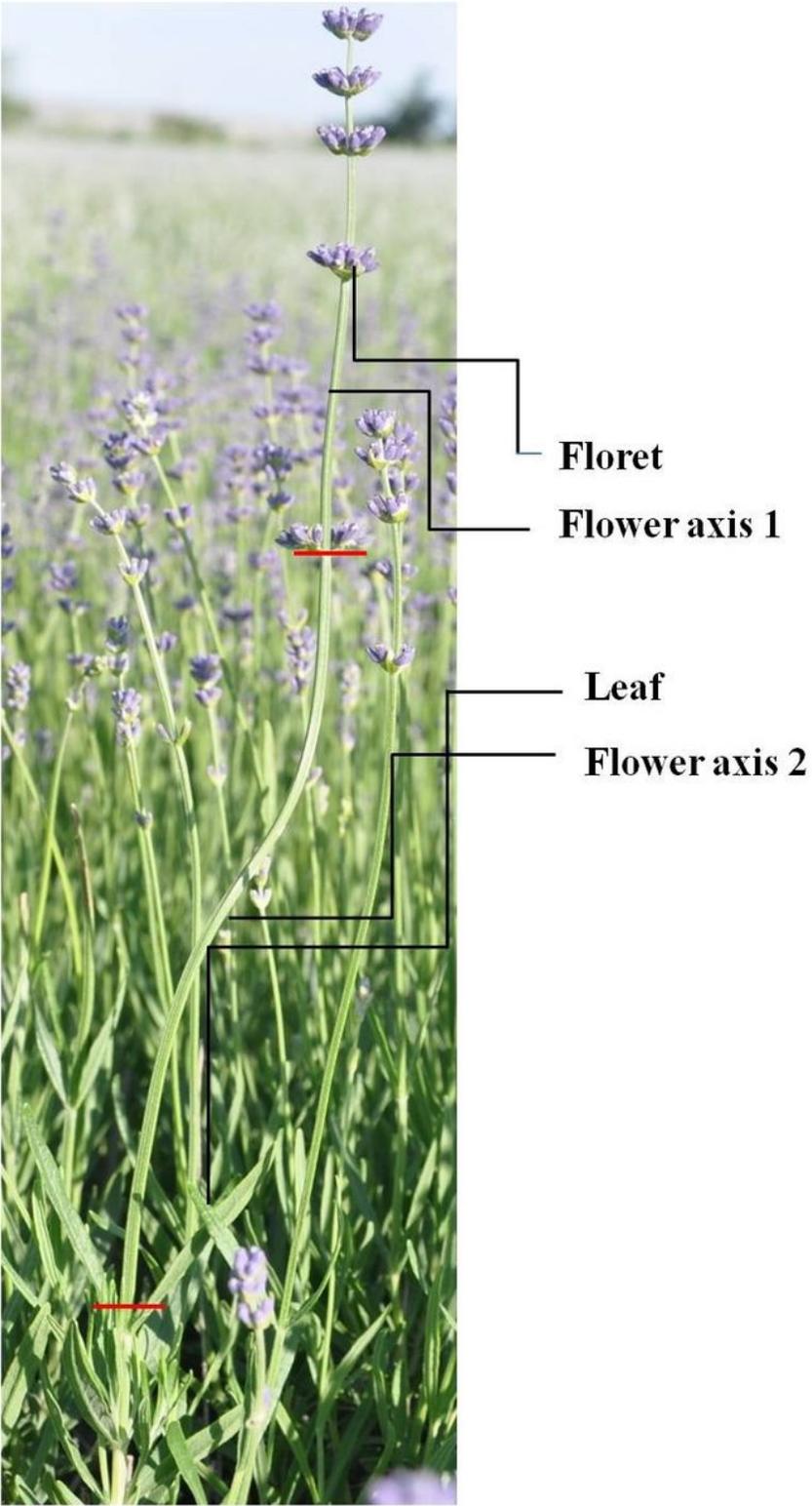


Figure 1

Inflorescence sample classification diagram

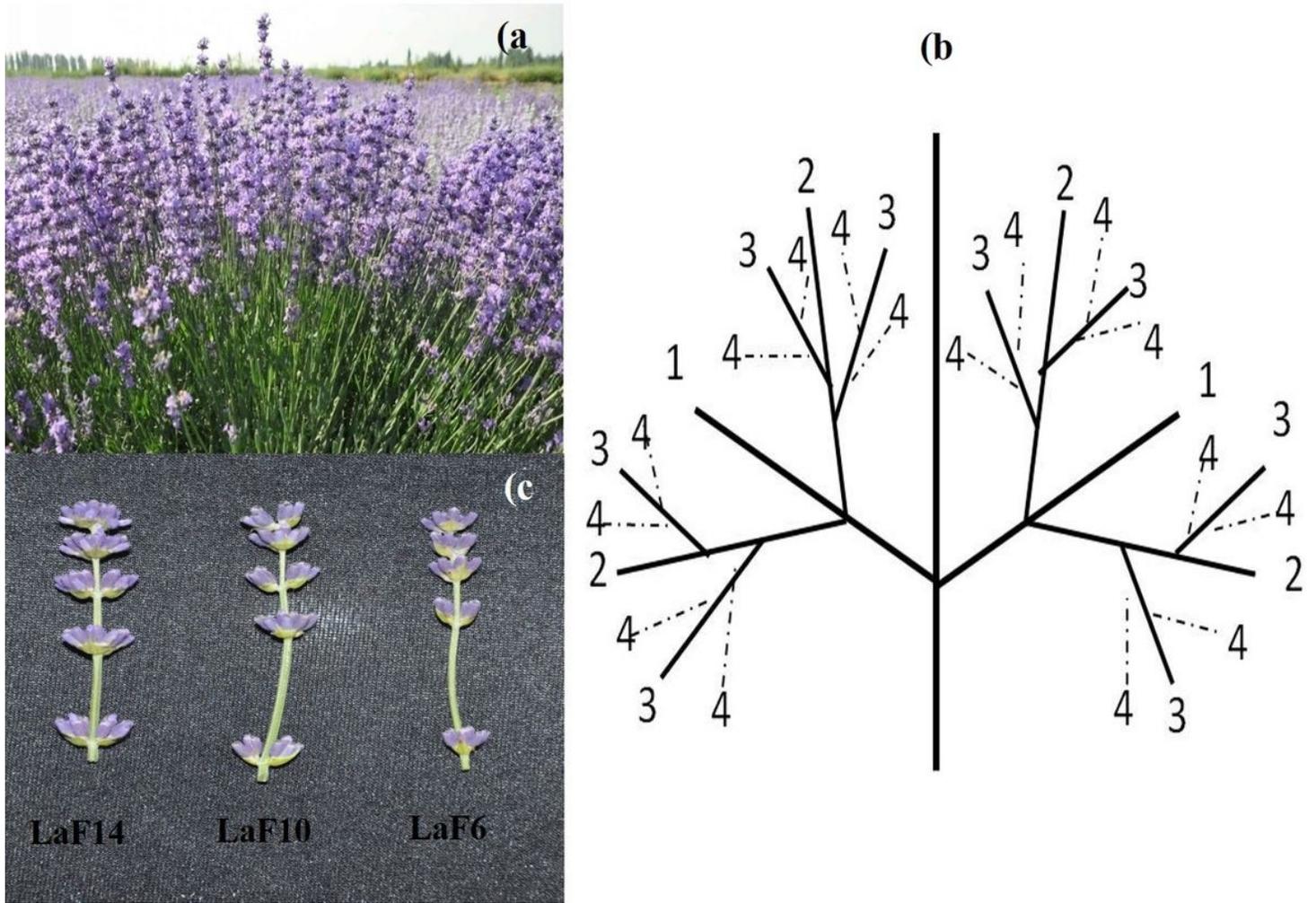


Figure 2

The lavender inflorescence botanical characteristic and schematic diagram of florets. (a: Inflorescence clusters of lavender; (b: Schematic line diagram of florets in a completely whorl, the dotted line indicates the theoretical number of florets, the solid line indicates the number of florets that can be birthed in production; (c: inflorescence of different florets;

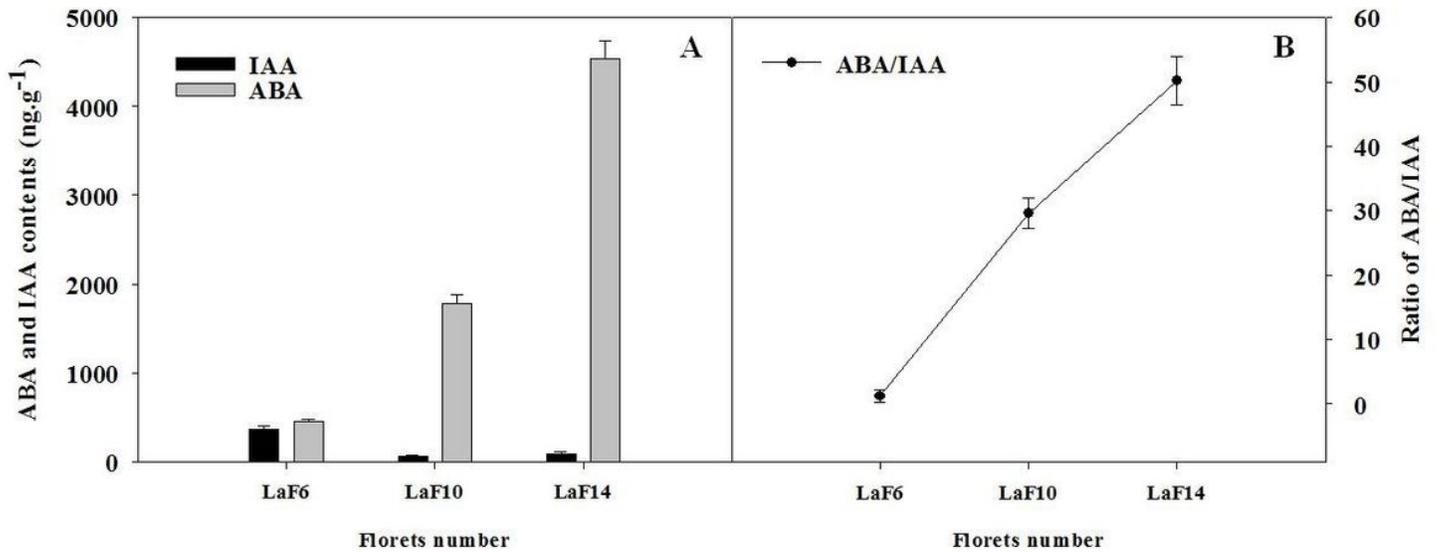


Figure 3

ABA, IAA content of leaves from flowering branches in bud stage by GC-MS

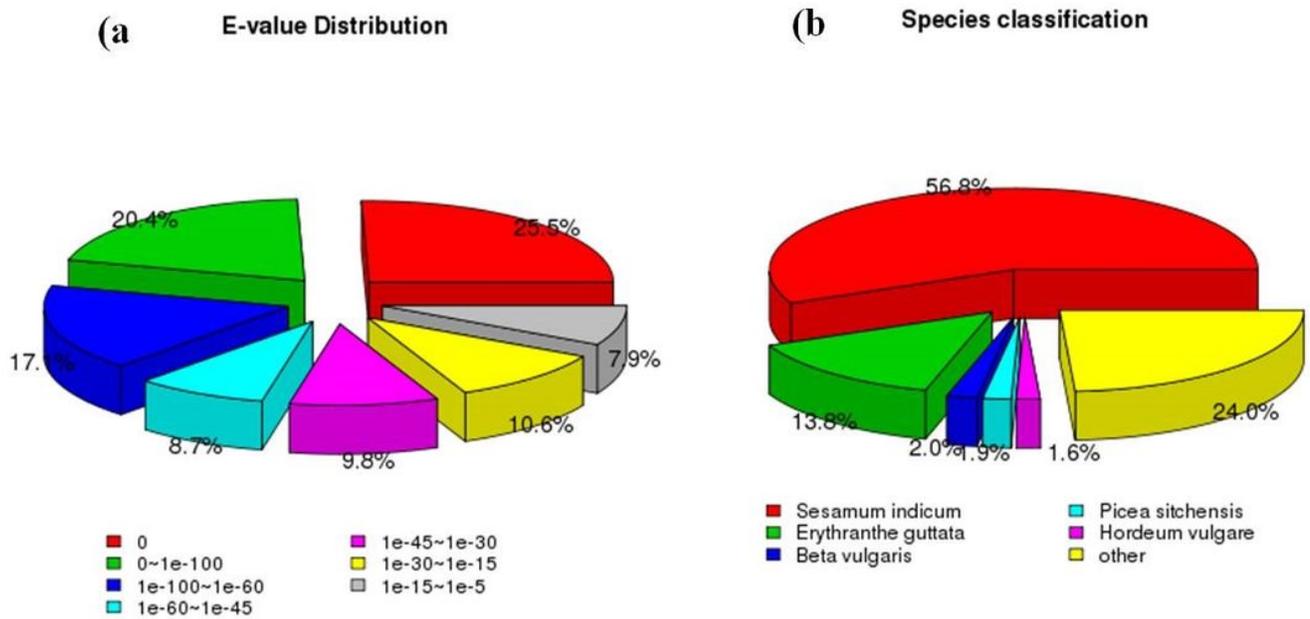


Figure 4

Characteristics of homology search of unigenes aligned by BLASTx to the Nr database. (a): E-value distribution of unigenes annotated in the Nr database. (b): Species distribution of the first BLAST hits for each sequence with a cut-off E-value .1.0E-5

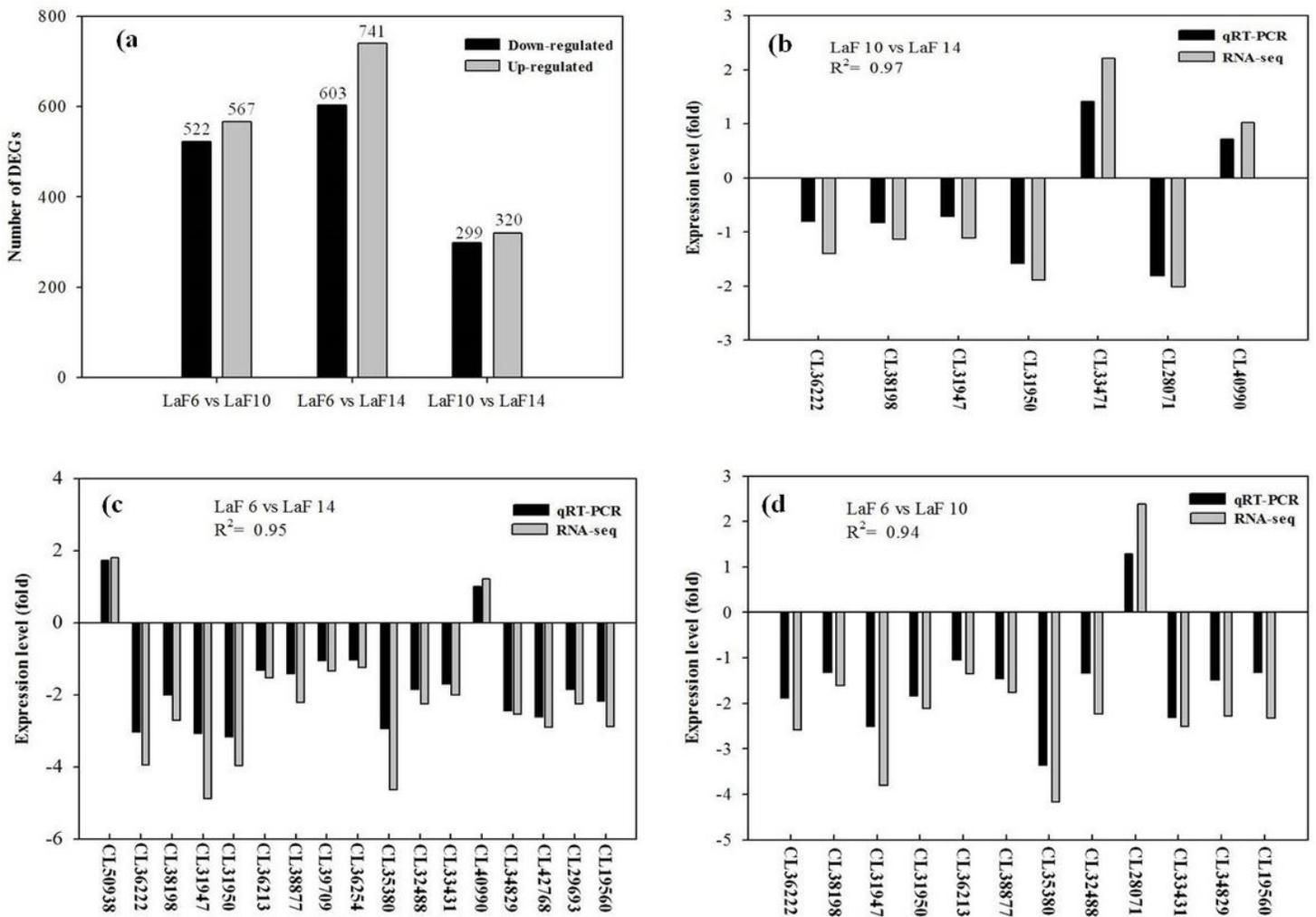


Figure 5

Gene expression comparisons and qRT-PCR verification. (a: changes in gene expression profile, the number of up-regulated and down-regulated genes between LF6, LF10, LF14. (b: DEG qRT-PCR verification between LaF10 and LaF14, (c: DEG qRT-PCR verification between LaF6 and LaF14, (d: DEG qRT-PCR verification between LaF6 and LaF10.

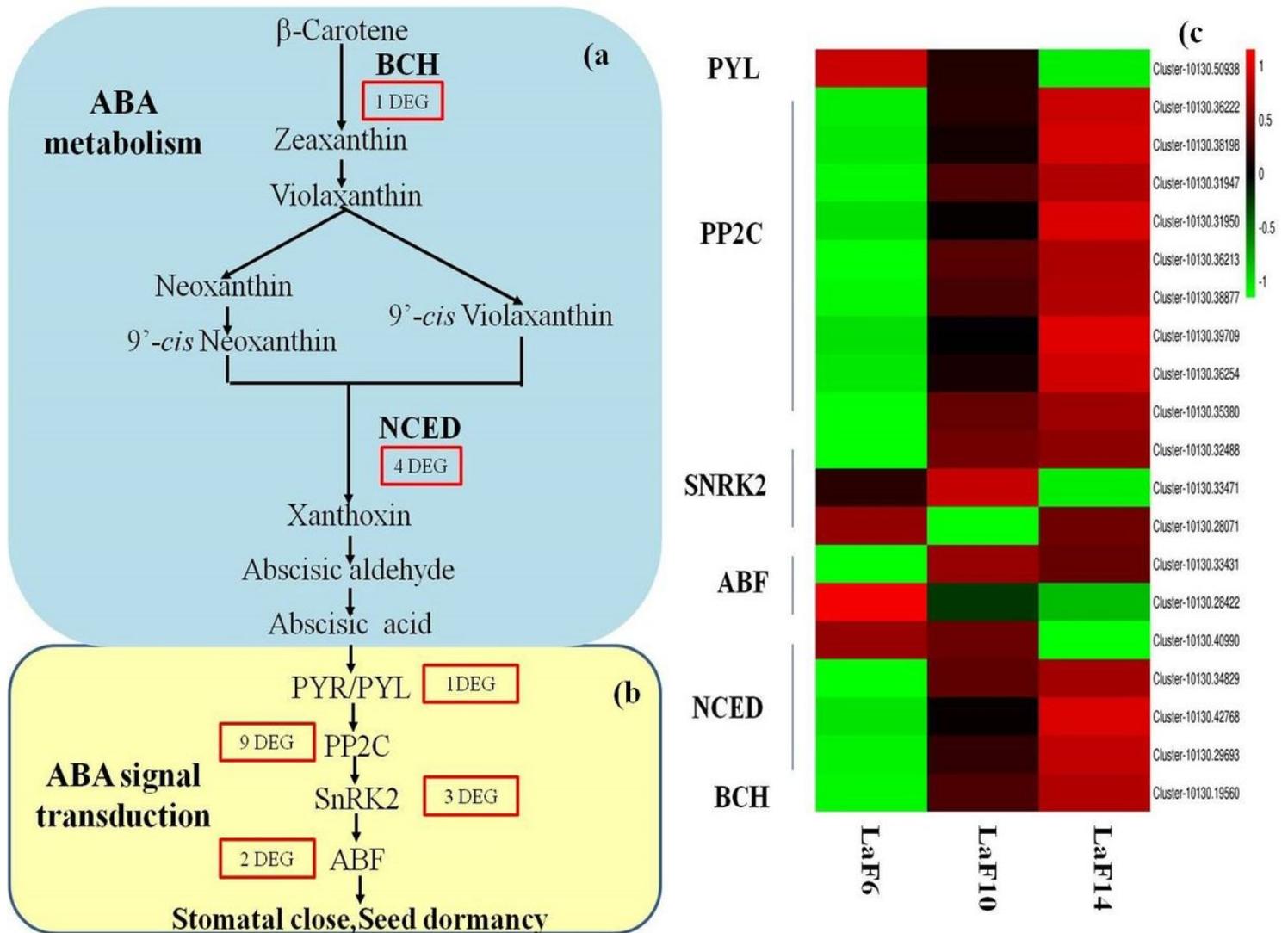


Figure 6

ABA anabolic pathway map and heat map of DEG gene expression in different florets sampls. (a: the principal pathway of ABA metabolism, (b: ABA signal transduction, c: Expression profiles of DEGs involved in different florets.

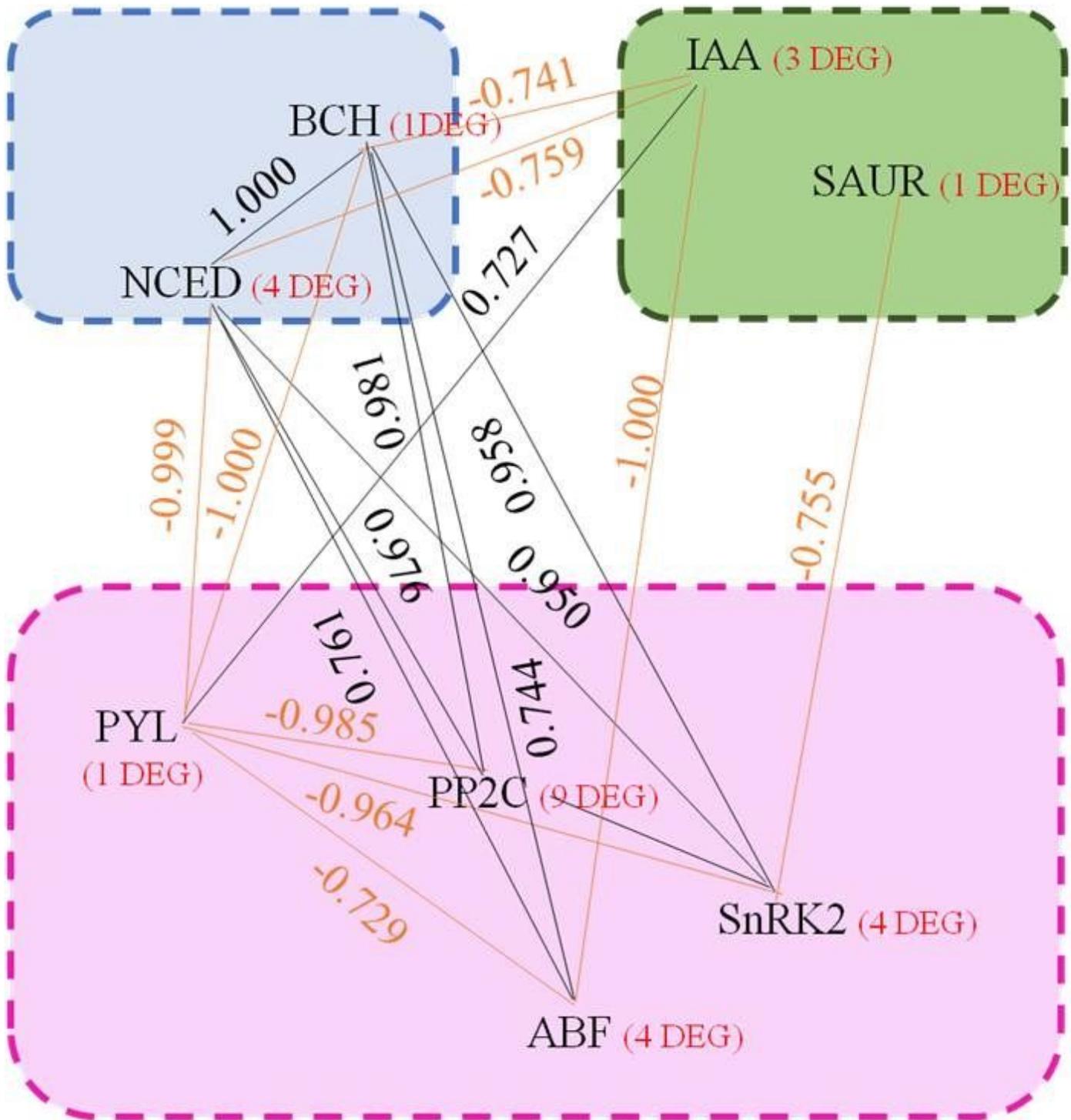


Figure 7

Common regulatory network of hormone signaling pathway.

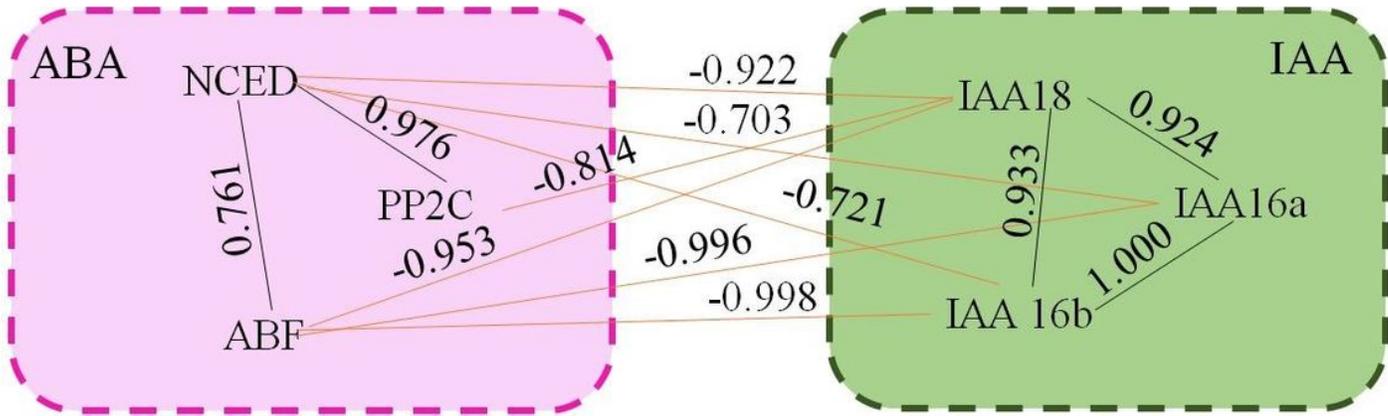


Figure 8

Correlation analysis between the ABA rate-limiting factors and the IAA DEGs

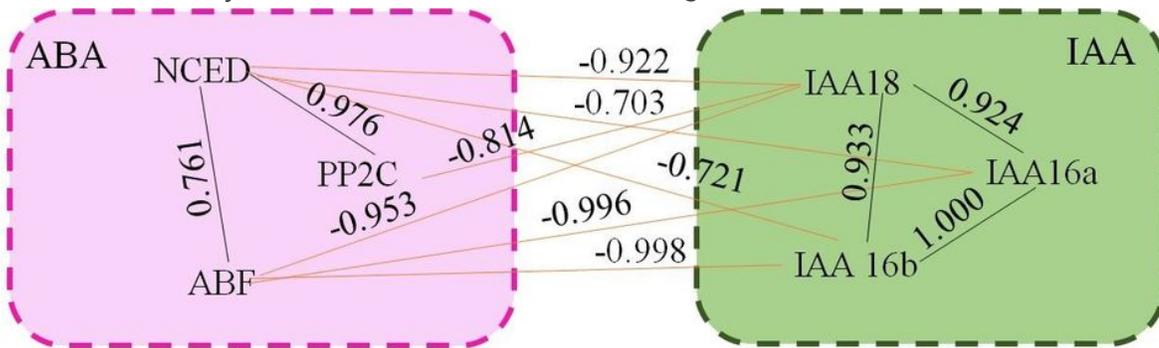


Figure 9

Exogenous IAA affected florets number and ABA metabolism key genes expression

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