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Photoperiod Effected on Indole-3-acetic Acid Synthase Genes Expression of Beta Vulgaris

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Abstract. The phytohormone auxin drives plants growth, development and the transformation of morphogenesis. Indole-3-acetic acid (IAA) has been identified as the major auxin in plant species increasingly and the evolutionary biosynthesis pathway of IAA was conserved in the plant kingdom. Sugar beet (Beta vulgaris) is an important crop plant that accounts for 30% of the world's sugar production annually. Ten IAA synthetic genes such as BvAMI1, BvNIT4, Bv22D, BvIAA6, BvIAA8, BvIAA9, BvIAA13, BvIAA26, BvIAA29 and BvIAA33 were explored whose expression played critical roles in rate-limiting step of the IAA synthesis pathway in Sugar beet. Moreover the expression patterns of these genes were investigated by qRT-PCR and the IAA concentration was quantified by UPLC-MS/MS in leaves of different time points and photoperiods in order to find out the mechanism that how IAA regulated plant development under different photoperiods. These results demonstrated that BvIAA6, BvIAA9, BvIAA13, BvIAA26, BvIAA33 and Bv22D genes significantly expressed different in leaves between the long day(16h) and short day(8h), which were consistanted with the contents of IAA by UPLC/MS/MS. Collecttively, our data provided a different angle for analyzing and understanding the molecular mechanisms between photoperiods and the synthesis of IAA during plant development.

Key words: Photoperiod, IAA, synthease gene, orthology, UPLC-MS/MS

1. Introduction

Photoperiod and auxin were regarded as the important factors that controls plant growth and development. Plants perceived the day length to optimize the daily photosynthesis in order to promote developmental process [1], [2]. Indole-3-acetic acid (IAA) is a classic phytohormone that plays key role in various aspects of plant growth and development including the response to light, bend toward gravity of root and the architecture of root, shoot elongation, organ and vascular was modulated by endogenous IAA hormone [3]-[5]. However, environmental cues such as photoperiod, temperature and drought can influence the expression of IAA biosynthesis genes resulting in fluctuation of IAA quantity in plant. Photoperiod was considered as the biological calendar of plant that reminded to optimize development time point [6]. Photoperiod including long day (LD; 16h/8h) and short day (SD; 8h/16h) caused phenotypic transformation and morphology of leaf size [7], [8]. Current assays for photoperiod and IAA were very few even in model plant Arabidopsis thaliana let alone in the Beta vulgaris under diverse photoperiods, which attracted more attention to us to illuminate the regulation mechanism between different photoperiods and IAA quantity. We explored ten genes of IAA biosynthesis pathway in Beta vulgaris and investigated the diffenence of IAA expression between LDs and SDs. In addition, we quantified the IAA concentration in leaves of Beta vulgaris. Our results was the first report demonstrating the underlined molecular mechanism of IAA biosynthesis pathway genes expression under diverse photoperiods and opened up new insight into the application of agriculture and crop cultivating.

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2. Materials and Methods

2.1 Plant growth and photoperiod treatment

Cultivated Sugar beet line DY14-O was empolyed for plant materials throughout this study. Fifty Sugar beet roots were cut into two petals from the shoot apical meristems equally. Photoperiod treatment was carried out after cold exposure (vernalization; 4-5℃) 16 weeks under dark conditions, then transfered them into the land where the temperature increased to 18~28℃under different photoperiods. One parts were exposured to long day conditions (LD; 16h/8h) while the others were under short days (SD; 8h/16). Leaves were harvested from the different photoperiod samples at the three time points: seeding, bolting and flowering periods, which corresponded to the short day samples.

2.2 Identification of IAA biosynthesis pathway genes in Beta vulgaris

Multiple searches were performed in order to identify rate-limiting step of the IAA biosynthesis genes in Beta vulgaris and the sequences of the Arabidopsis thaliana IAA biosynthesis gene were used to explore the database of Beta vulgaris by local BLAST searches with a cutoff e-value ($^{-10}$) [9].

2.3 Quantitative RT-PCR

For each biological replicate, material from five plants were pooled to form a single sample for RNA extraction. Total RNA was isolated using MiniBEST Plant RNA Extraction Kit (Takara) and 1μg of total RNA was used to synthesize cDNA by a PrimeScript RT reagent kit (Takara) with gDNA Eraser according to the manufacturers' instructions. The final cDNA products were diluted 20-fold to use. Gene-specific primers for quantitative RT-PCR analysis were designed by Primer premier 6.0 software and were listed in Table 1. Amplification reactions were operated by SYBR Premix Ex TaqTM Kit (Takara) on a ViiATM7 System. Three independent biological replicates and the technical replicates were employed to minimize error of the assay. The relative expression levels of the IAA biosynthesis genes from the different RNA samples were calculated and normalized to the geometric mean of BvICDH[10] as the control.

2.4 Quantification of IAA content

Major plant hormones were analyzed by Ultra Performance Liquid chromatography-tandem mass spectrometry(UPLC/MS/MS). Samples were prepared in batches and three replicates of each sample was recommended in order to get statistically meaningful results. The following assays listed three periods and different photoperiods including Seeding, Bolting and flowering of LDs corresponding to 17W (weeks), 19W (weeks) and 20W (weeks) of SDs. For each biological replicate, 3g of fresh plant leaves from five plants were pooled to form a single sample using for IAA quantification on the basis of a published method[11] with minor modifications. Briefly, plant leaves were homogenized and extracted under 4 ℃ conditions. Each sample was operated on a liquid chromatography-tandem mass spectrometry system, which drove the Acquity Ultra Performance Liquid Chromatograph (Acquity UPLC; Waters) and a triple quadruple tandem mass spectrometer (Quattro Premier XE; Waters). External standard curve method was used to determine the concentration of IAA. Seven concentrations (0, 1, 5, 15, 30, 50 and 100ng mL^{-1}) of standard IAA sample were employed by this assay. It is also recommended that authentic IAA (Aldrich, cat. no. I3750) was used to optimize the UPLC/MS/MS setup before analyzing biological samples for the quantitative accuracy.

3. Results and Discussion

3.1 Identification of IAA biosynthesis genes in Beta vulgaris

A total of 15 high homologous sequences were obtained by doing BLAST against and keyword searcheing the database of Beta vulgaris. Finally, 10 IAA biosynthesis pathway genes in Beta vulgaris were identified according to the conserved domains and available primer sequences. Information about these 10 BvIAA genes were listed in Table 1 including gene name, locus ID, chromosome location, the accession, function and the primer sequences.

Table 1: the information of IAA biosynthesis genes in Beta vulgaris

Gene	Locus ID	Chromosome location	Accession	Function	Primers
BvAMI1	LOC104890855	Bvchr4.sca004:2,237,513..2,237,750	XM_010676468	PREDICTED: amidase 1	F: TGACACTGGAGGAAGC R: GTTGGAAGTAGCACAT
BvNIT4	LOC104899831	Bvchr7.sca021:4,314,470..4,314,795	XM_010687111	Bifunctional nitrilase/nitrile hydratase	F: TCCGACTCTTCTGCTCCTA R: TCCTTACCCTTTGATGTCCT
BvIAA6	LOC104904637	Bvchr9.sca026:4,366,087..4,366,594	XM_010692945	PREDICTED: auxin-induced protein	F: CCACACCGAGAAAGTA R: CCTAAGGAAAGGCACA
BvIAA8	LOC104883520	0049.scaffold00172:10,300..10,749	XM_010668058	PREDICTED: auxin-responsive protein	F: GCAGAAGGCTAAGGAT R: CCAAGACAAGCACACG
BvIAA9	LOC104897812	Bvchr6.sca028:1,411,145..1,411,820	XM_010684757	PREDICTED: auxin-responsive protein	F: AAAAATCTAAGAGCCG R: ACAGACAGTAACAGGA
BvIAA13	LOC104899391	Bvchr7.sca015:376,539..377,270	XM_010686567	PREDICTED: auxin-responsive protein	F: ATGGAGACTGGATGCT R: GTTTTCCGTGAGATAA
BvIAA26	LOC104883127	Bvchr9_un.sca002:177,167..177,718	XM_010667580	PREDICTED: auxin-responsive protein	F: TGGACGGAAGTGGGG R: CGGCTTAGTGTGGGA
BvIAA29	LOC104901993	Bvchr8.sca018:1,474,750..1,475,286	XM_010689634	PREDICTED: auxin-responsive protein	F: TCATCAGCAACCTAAAAG R: CGCCGCAACCGCCGCCTC
BvIAA33	LOC104902411	Bvchr8.sca018:6,985,359..6,986,049	XM_010690186	PREDICTED: auxin-responsive protein	F: CCACCACAACCACCTA R: ATCACCACCACCACCG
Bv22D	LOC104894591	Bvchr5.sca024:1,949,307..1,950,187	XM_010680862	PREDICTED: auxin-induced protein	F: ACGCTAAGAAGCACGAAA R: GAATGAAACTCAAATCCA

3.2 Differential expression of IAA biosynthesis genes

The IAA biosynthesis pathway genes were likely to involve in Sugar beet development that quantitive RT-PCR analysis was used to assess BvAMI1, BvNIT4, Bv22D, BvIAA6, BvIAA8, BvIAA9, BvIAA13, BvIAA26, BvIAA29 and BvIAA33 expression patterns in leaves of diverse time points between LDs and SDs aim at exploring the molecular mechanism of IAA genes transcription. Quantitive RT-PCR data was obtained for all IAA genes selected. The results showed that the expression patterns of the IAA genes were seriously different in leaves under diverse photoperiods.

Quantitive RT-PCR analysis the difference of BvAMI1, BvNIT4, Bv22D, BvIAA6, BvIAA8, BvIAA9, BvIAA13, BvIAA26, BvIAA29 and BvIAA33 expression under diverse photoperiods during the development of Sugar beet. BvIAA6, BvIAA8, BvIAA13, BvIAA26, BvIAA29 and BvIAA33 (Fig. 1. D, E, F, G, H J) were highly expressed in leaves of SDs, especially BvIAA6, BvIAA26, BvIAA29 and BvIAA33 exhibited abundantly expression patterns under SDs that BvIAA6 and BvIAA33 (Fig. 1. D, F)increased to the peak at 20W time point, whereas BvIAA26 and BvIAA29 (Fig. 1. E, J) reach to the high levels at 19W time point comparing with the LDs. This significant difference might be the irritability of plant to adjust themselves to withstand the changed environmental conditions. BvAMI1 and BvNIT4A sharing similar expression patterns (Fig. 1. A and B) at all time points between LDs and SDs. However, Bv22D was the only gene with greatest expression level at Flowering periods in leaves (Fig. 1. I) under LDs(16h) in contrast to the other time points and photoperiods.

These expression observations indicated that photoperiods seriously influenced the IAA biosynthesis genes expression during the development of Beta vulgaris. All these clues implied that BvIAA9, BvIAA29 and Bv22D genes might be predominant during the development of Sugar beet. However, further investigation either gain-of-function or loss-of-function mutants will be need to confirm the function of IAA biosynthesis genes under environmental stress. Recent years, studies had indicated that auxins contributed to the bolting and flowering development of Beta vulgaris. Therefore, evidences pointed to auxin function as the key promoter of initiation and development of Beta vulgaris.

3.3 Quantification of IAA content

To determine whether IAA levels correlated with the expression profiles of their biosynthesis pathway genes or not under different photoperiods that we quantified IAA amounts in leaves of different time points by Ultra Performance Liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Fig. 2. D). The MS/MS spectrogram of 1ng/mL of indole-3-acetic acid (IAA) standard (Fig. 2. A). The MS/MS spectrogram of fragmentation of indole-3-acetic acid (IAA) standard (Fig. 2. B). UPLC chromatogram of one sample (Fig. 2. C). The concentration of IAA in seeding (LDs) was the highest level (0.078ng/g). The concentration at

19W (SDs) (0.061ng/g) time point was higher than others. Taken together, athough high IAA accumulation in seeding (LDs) during the development did not associate with the expression of their corresponding hormone biosynthesis genes (Fig. 2. D) except the Bv22D at Flowering time point. By contrast, BvIAA8, BvIAA13, BvIAA26 and BvIAA29 (Fig. 2. E, G, H, J) transcription levels closely associated with the concentration of IAA by UPLC at 19W time point (SDs).

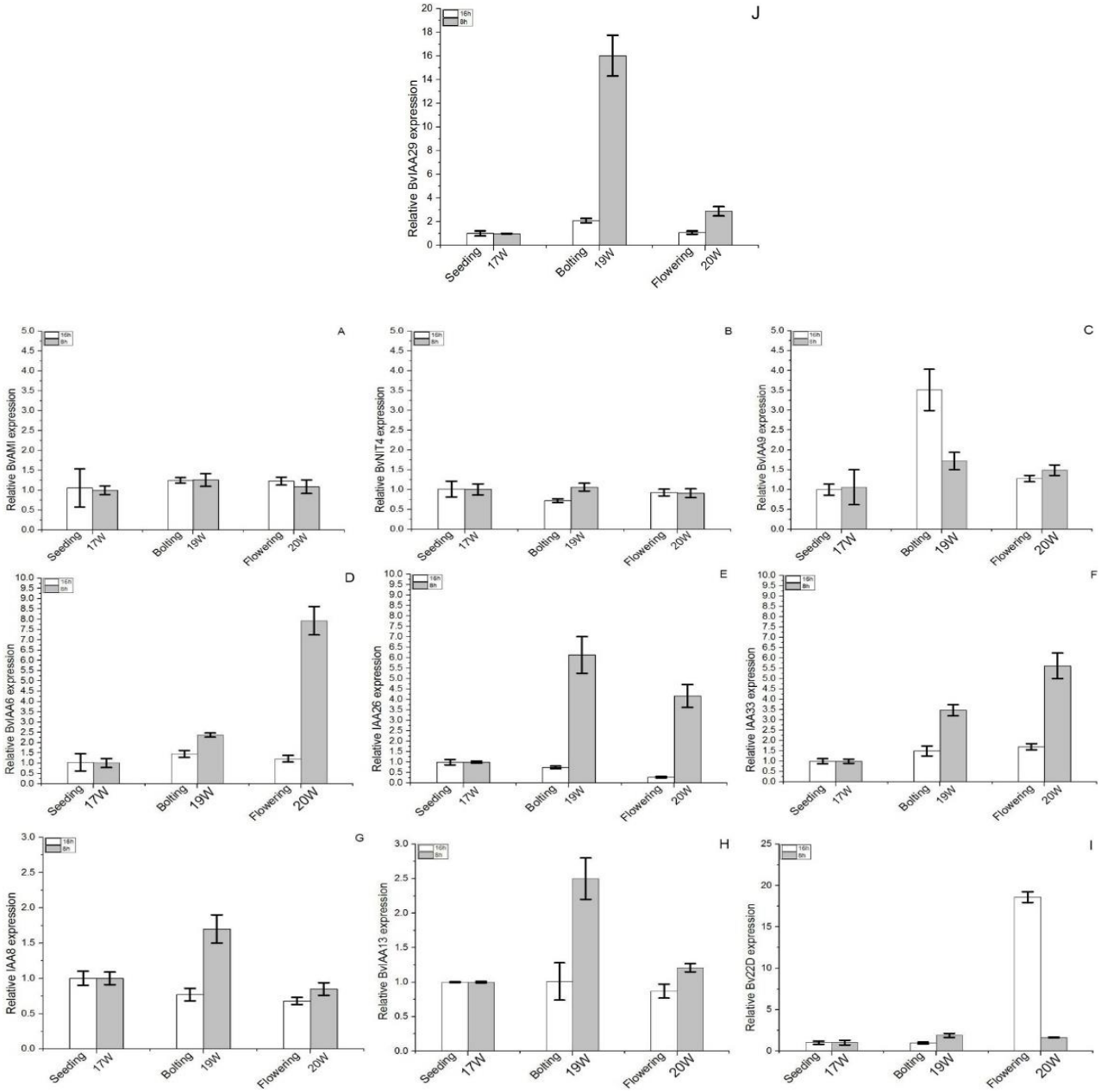

Fig. 1: Quantitive RT-PCR analysis the expression of IAA biosynthesis pathway genes. Relative expression levels of these genes were normalized by the BvICDH (Pin et al. 2010). Expression patterns of BvAMI1, BvNIT4, Bv22D, BvIAA6, BvIAA8, BvIAA9, BvIAA13, BvIAA26, BvIAA29 and BvIAA33 in leaves of Seeding, Bolting and Flowering under LDs corresponding to the SDs. Error bars were calculated on the basis of independent biological replicates (n =3).

4. Conclusions and Perspectives

Consequently, our results demonstrated that the photoperiods contributed to the difference of IAA accumulation and biosynthesis genes expression levels. The most biosynthesis genes were not correlated to the accumulation of IAA in leaves (LDs), which might be not the key biosynthesis genes of the IAA biosynthesis pathway except the Bv22D. We speculated the Bv22D gene promoting the development by upregulating the concentration of IAA and we also deduced that the IAA9 gene associated with the bolting of

Beta vulgaris under LDs. However, photoperiods effected on plant growth by changing the irritability of plant such as the IAA genes expression patterns under SDs. The molecular mechanisms of irritability were ambiguous, so the next step to better understand the molecular mechanisms, all of investigations might be imposed on either gain-of-function or loss-of-function mutants of IAA biosynthesis genes in order to shed light on determining the mechanism of auxin IAA responding to photoperiods. Furthermore, new insight into the application of agriculture and crop cultivating will be opened up.

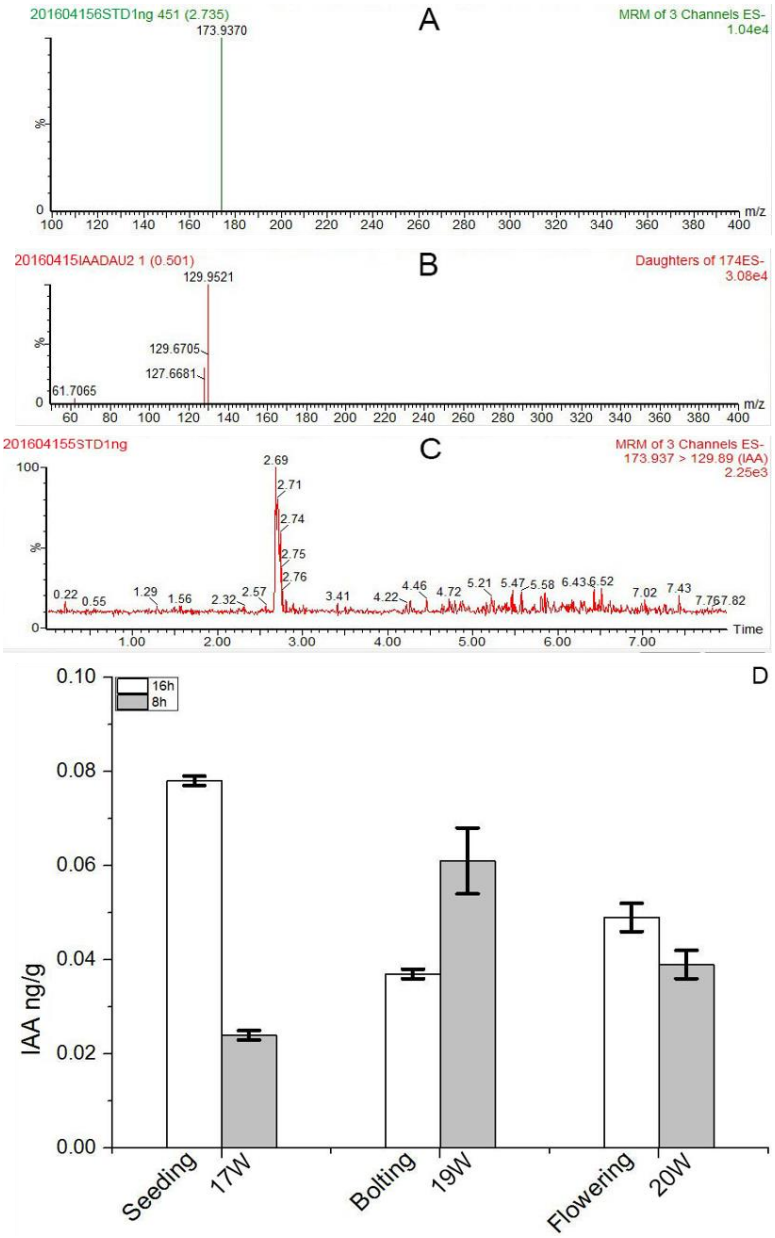

Fig. 2: The Acquity Ultra Performance Liquid Chromatograph and a triple quadruple tandem mass spectrometer quantification of IAA in leaves of different photoperiods. (A) MS/MS spectra of 1ng/mL of indole-3-acetic acid (IAA) authentic standards. (B) MRM chromatogram of fragmentation pathways of IAA (transition of 174.0→129.95). (C) MRM chromatogram of one sample detected plant hormones in 3g of fresh beet tissues crude extracts. (D) Data presented are mean values of 3 biological repeats.

5. Acknowledgments

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6. Reference

[1] A. Graf, AM Smith. Starch and the clock: the dark side of plant productivity. *Trends Plant Sci*. 2011, 16:169-175.

[2] A. Sulpice, A. Flis, F. aIvakov, N. Apelt, B. Krohn, Encke, et al. Arabidopsis coordinates the diurnal regulation of carbon allocation and growth across a wide range of photoperiods. *Mol Plant*. 2014, 7:137-155.

[3] C. Darwin. The power of movement in plants. London: John Murray.1880.

[4] T. Ciesielski. Untersuchungen über die Abwärtskrümmung der Wurzel. *Beitrage zur Biologie der Pflanzen*. 1872, 1:1-30

[5] PJ. Davies. Plant hormones. Dordrecht: Kluwer Academic Publishers. 1995.

[6] JC. Walton, ZM Weil and RJ Nelson. Influence of photoperiod on hormones, behavior, and immune function. *Frontiers in neuroendocrinology*. 2011, **32**(3): 303-319.

[7] SJ. Cookson, K. Chenu, C. Granier. Day length affects the dynamics of leaf expansion and cellular development in Arabidopsis thaliana partially through floral transition timing. *Ann Bot*. 2007, 99: 703-711.

[8] N. Wuyts, C. Massonnet, M. Dauzat, C. Granier. Structural assessment of the impact of environmental constraints on Arabidopsis leaf growth: a 3D approach. *Plant Cell Env*. 2012, 35: 1631-1646.

[9] Y. Mano and K. Nemoto. The pathway of auxin biosynthesis in plants. *Journal of Experimental Botany*. 2012, 1-20.

[10] PA. Pin, R. Benlloch, D. Bonnet, E. Wremerth-Weich, et al. An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. *Science*. 2010, 330:1397-1400

[11] XQ. Pan, R. Welti and XM. Wang. Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry. *Nature*. 2010, **5**(6):986-992