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Differential expression of hormone related genes between extreme segregants of a *Saccharum* interspecific F2 population

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Abstract Sugarcane is a highly productive, first generation biofuel feedstock, known for its remarkable efficiency in accumulating biomass. Hormones are important regulators for many biological processes in plants, especially in plant development and plant growth, which are crucial for plant biomass traits. To understand how hormones regulatory mechanisms contribute to sugarcane lignocellulose yield, we studied the transgressive segregation on biomass yield in the F2 population derived from a cross between *Saccharum officinarum* 'LA Purple' and *Saccharum robustum* 'MOL5829'. Gene expression profiling was used to detect genes involved in three important hormone-related pathways, auxin, ethylene and gibberellin, to find out how they are differently regulated between the extreme segregants of high and low biomass yield groups. We identified seventeen differentially expressed genes in auxin, one in ethylene and one in gibberellin related signaling and biosynthesis pathways, which could potentially regulate biomass yield. Differentially expressed genes, *PIF3* and *EIL5*, involved in gibberellin and ethylene pathway could play an important role in biomass accumulation. These plant hormone-related genes could serve as candidate genes in genetic modification and breeding programs to develop high yielding energy cane.

Keywords Auxin · Biomass · Hormone regulation · Transcriptome · Gibberellin · Ethylene

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Introduction

Energy demand is expected to increase by > 50% by 2025 (Ragauskas et al. 2006). To meet this urgent need, finding and developing sustainable and environmental-friendly energy sources are important for humanity. Fossil fuels, such as gas, coal, and petroleum, have been the major energy sources for our society for centuries. The expansions of the population, the environmental problems associated with this non-renewable fossil fuel and the concerns of

unsecured foreign oil supplies have put alternative energy sources in the spotlight (Ragauskas et al. 2006). Biofuel, as a renewable energy source, has been regarded as a promising source to decrease or even replace the use of traditional fossil fuels. Also, replacing fossil fuel with fossil-carbon free biofuel will also contribute to reduce atmospheric CO_2 emission and decelerate global climate change (Pacala and Socolow 2004). Concerning about food safety and potential competition of cropland, developing an advanced biofuel from ligno-cellulosic feedstock is becoming more demanding, not only by the funding agencies but also by the industry (Somerville et al. 2010; Searchinger et al. 2008; Sticklen 2008; Energy and Bioenergy Technologies Office 2016).

Sugarcane (*Saccharum* spp.) was first domesticated and cultivated in New Guinea > 12,000 years ago based on archeological evidences (Chen and Chou 1993; Pennington and Baker 1990). As a biofuel feedstock, bioethanol derived from sugarcane has already replaced 30% of gasoline, and the techniques utilizing sugarcane in the production of sustainable jet fuel and diesel are also under rapid development in Brazil (Arruda 2011; Lam et al. 2009). As a C4 plant, sugarcane is known for its high efficiency in fixing solar energy and high biomass yield, making it an excellent crop for agricultural residues and bagasse, which have notably high cellulose content and has been used as fuel. Although previous research focuses more on the sugar content and sugar yield, the demand for efficient and high yield cellulose biofuel feedstock led to a renewed focus on high biomass yield of energy cane since the last energy crisis in late 1970s.

Plant hormones are growth regulators playing essential roles in plant growth and development. These hormones, such as auxin, ethylene, gibberellins (GAs), abscisic acid (Tabashnik 2010), brassinosteroids (BRs), and Jasmonic acid (JA), can regulate a wide range of plant physiological processes with low concentration (Gray 2004). They participate in responding to environmental and endogenous conditions and determining the plant form. Intensive studies of differential expression and modifications of hormone-related genes have been conducted in Arabidopsis and many other crops. These studies indicated that different hormone-related genes can have various regulation effects on plant growth rate, biomass accumulation rate, and yield (Jeon et al. 2016; Choe et al. 2001; Eriksson et al. 2000; Voorend et al. 2016; Sahni et al. 2016; Do et al. 2016; Dayan et al. 2010) (Fendrych 2016 #584).

Auxin regulates a variety of plant growth and development responses, including promoting cellular elongation, cell expansion and cell division (supplementary Fig. 1). The primary auxin-response gene families include auxin/indole-3-acetic acid (*Aux/IAA*), auxin-response factor (*ARF*), Gretchen Hagen3 (*GH3*), small auxin-up RNAs (*SAUR*), and lateral organ boundaries (*LBD*). *AUX/IAA, SAUR* and *GH3* genes can be induced immediately at the presence of auxin and lead to different cell and growth responses. *AUX/IAA* is a key gene family for plant growth regulation; the mechanism of auxin regulation is through the degradation of repressor genes *AUX/IAA* and the modulation of gene expressions involved in multiple physiological processes. *ARF* family contains important genes regulating the auxin-modulated gene expression (Liscum and Reed 2002). *SAUR* gene family contains early auxin-responsive genes that are important for tissue elongation, which can potentially contribute to biomass differences. *LBD* is usually regulated by exogenous IAA (indole-3-acetic acid), and are involved in lateral organ development. *GH3* gene family works in adjusting and maintaining endogenous auxin homeostasis.

Ethylene also takes part in the regulation of diverse biological aspects, including but not limited to sucrose accumulation, biomass production and stress tolerance (Abeles et al. 1992). Recently, the responses and mechanisms involved in ethylene and its potential relevant genes in sugarcane ripening and sucrose accumulation has been reported (Cunha et al. 2017). Ethylene receptor (*ETR*), ethylene response sensor (*ERS*), reversion-to-ethylene sensitivity (*RTE*), ethylene insensitive (*EIN*), *ETP* (*EIN2* targeting protein), *EIL* (*EIN3* like) and *EBF* (*EIN3*-binding F-box protein) are important gene families involved in ethylene signaling (Yang et al. 2015). However, the mechanisms of whether and how ethylene-related gene families are also involved in sugarcane biomass accumulation and effective energy cane are yet unrevealed.

Aside from auxin and ethylene, GA also holds an important position of regulating plant growth, stem elongation and many other processes of plant development (Biemelt et al. 2004). The best known function of GA is its role in stem elongation. Since the plant height and stem length are important factors affecting

sugarcane biomass, it is important to study how GA related genes are expressed in high yielding energy cane. It was previously reported that altering GA related genes can affect photosynthesis and biomass accumulation in tobacco (Daviere and Achard 2013). *GID* (GA insensitive dwarf), DELLA and various transcription factors, including various *PIF* (phytochrome-interacting factor) and *APG* (antagonist of PGL1) genes, are the major component of GA signal transduction pathway, of which *GID1* are the putative gibberellin receptor, and DELLA is the GA signaling repressors (Willige et al. 2007). The previous publication also suggests that Gibberellin 20-oxidase (*GA20ox*) and GA 2-oxidase (*GA2ox*) in the late stage of GA biosynthesis are critical genes for plant height and biomass (Biemelt et al. 2004; Qin et al. 2013).

Genome-wide transcriptome profiling can enhance the understanding of how these important hormone-related signaling pathways and regulatory components affect plant growth and the hormone-related mechanisms of biomass accumulation efficiency. We utilized extreme segregants in an F2 population to study how ethylene-related genes and auxin-related genes regulate differently in sugarcane in high and low biomass yield clones.

Materials and methods

Identification of the hormone-related genes

Genes involved in auxin, ethylene or gibberellin pathways were mainly identified from previous publications (Cunha et al. 2017; Daviere and Achard 2013; Jeon et al. 2016; Wang et al. 2010; Yang et al. 2015). Cloned or validated genes have the priority to be included in this study. The corresponding gene IDs were identified in phytozome (https://phytozome.jgi.doe.gov/pz/portal.html) based on sequence similarity. Moreover, genes collected from publication were further confirmed using basic local alignment search tool and the accessible information of Sorghum plant hormone signal transduction pathway from KEGG (http://www.genome.jp/kegg-bin/show_pathway?ko04075+K12126).

Extreme segregants collection and RNA preparation

The F2 population tested in this study was generated from the self-pollination of twenty F1 progenies with great variation in biomass yield. The F1 progenies were generated by crossing LA Purple (*Saccharum officinarum*, 2n = 80), and MOL5829 (*Saccharum robustum*, 2n = 80) (Wai et al. 2017). In this study, a total of 14 extreme high biomass segregants and 8 extreme low biomass segregants from the F2 population were tested. The range of the average dry weight of high biomass group was 78.7–106.5 metric ton per hectare per 12 months. The average dry weight of the low biomass group ranged from 18.0 to 37.0 metric ton per hectare per 12 months. The detailed procedure of population development, extreme segregants collection, RNA extraction and RNAseq library construction were described in Wai et al. (2017).

Sequence reading alignment and differential expression analysis

Sequence quality control tool FastaQC (Andrews 2010) and sequence pre-processing tool Trimmomatic (Bolger et al. 2014) were used to perform the quality check and to process the raw paired-end reads. Since no published sugarcane genome sequence is available yet, the *Sorghum bicolor* annotation references v3.1 were downloaded from Phytozome v12 (https://genome.jgi.doe.gov/pages/dynamicOrganismDownload.jsf?organism=Phytozome) and used as the reference for this study. Using the primary transcripts of sorghum as the reference, the alignments of processed pair-ended reads were performed using two programs, HISAT2 (https://ccb.jhu.edu/software/hisat2/index.shtml) and NovoAlign (http://www.novocraft.com/products/novoalign/) respectively. The aligned sam files were then sorted and converted to bam file through SAMtools suit. The transcript counts were then performed using htseq-count version 0.9.1. The differential expression analysis was accomplished with R package EdgeR, comparing between the extreme high biomass group and the extreme low biomass group of the F2 progenies. Due to false positive caused by multiple testing, we used false discover rate (FDR) in additional to p value to set up threshold and detect differentially expressed genes. Genes with p-value ≤ 0.01 and FDR ≤ 0.05 were considered as differentially expressed genes in this study.

Results

RNA sequencing reads alignment

Since high-quality *Saccharum* genome references are not yet available, *Sorghum bicolor* genome sequence and gene models were used as references in this study. The hormone-related gene families and pathways were also identified based on the sorghum gene models.

Although HISAT2 was a faster alignment tool, NovoAlign showed a higher alignment rate in our case. The genes were counted once it was detected from one of these 22 extreme biomass segregants. Out of the 34211 annotated sorghum genes, 28044 genes had uniquely aligned reads from the RNAseq libraries when NovoAlign was used, which was about 82.0% of the total sorghum genes. In contrast, only 60.6% of genes (20732 genes) had aligned reads using HISAT2. A total of 20679 genes was identified by both HISAT2 and NovoAlign, 7365 genes were only found with NovoAlign method, and 53 genes were unique to HISAT2. 99.7% of genes found from HISAT2 were identified by both programs (Fig. 1).

In addition, a total of 1588 genes was identified as differentially expressed using HISAT2 as the alignment tool, consisting of 430 downregulated genes and 1158 upregulated genes for high biomass group. While when NovoAlign was used as the alignment tool, we were able to detect 2905 differentially expressed genes with the same statistical analysis and threshold. Out of these 2905 genes, 2077 genes were up-regulated and 828 genes were down-regulated for the high biomass group. Since NovoAlign covered more genes and almost all genes detected by HISAT2, we used the output obtained from NovoAlign in this study.

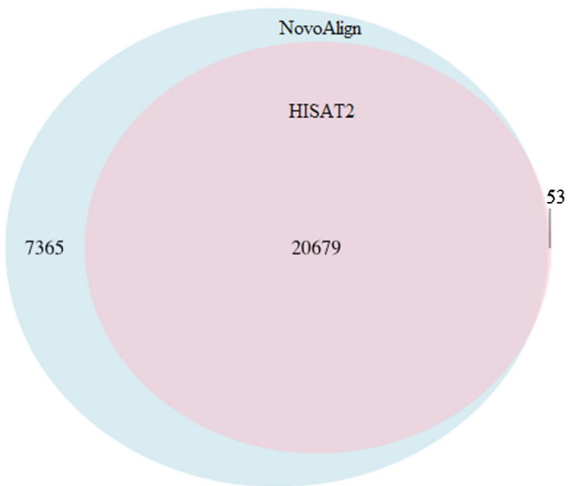

Fig. 1 The Venn diagram for uniquely aligned reads using NovoAlign and HISAT2 respectively. 28044 genes had been uniquely aligned using alignment tool NovoAlign, and 20732 genes were aligned using HISAT2. A total of 20679 genes were identified by both programs. 7365 genes were unique with NovoAlign method, and only 53 genes were unique to HISAT2

Auxin signaling genes were differentially expressed between low and high biomass sugarcane

In sorghum, the annotated ARF family consisted of 25 genes. Among these 25 genes, five genes were differentially expressed between the high and low biomass group, which was 20% of the entire gene family (Fig. 2). *SbARF16, SbARF4* and *SbARF20* were up-regulated, whereas *SbARF17* and *SbARF1* were down-regulated in the high biomass group.

It was previously reported in Arabidopsis that *ARF19*, *ARF16* and *ARF14* were regulated by auxin (Paponov et al. 2008). *ARF19* was the most sensitive *ARF*, which can be induced by very low *IAA* level, but *ARF16* and *ARF4* are moderate *ARF*s. When *IAA* or auxin concentration is relatively high in the plant, there will be the demand for more moderate auxin signal, such as, *ARF16* and *ARF4* (Paponov et al. 2008). In our study, we found that *ARF16* and *ARF4* had a higher transcription in the high biomass group, indicating that there might be a higher level of *IAA* in the high biomass group, which need to be regulated by the less sensitive *ARFs*. *ARF1*, which is down-regulated in high biomass group, was a transcriptional repressor of *IAA2, IAA3* and *IAA7* (Ellis et al. 2005; Hagen and Guilfoyle 2002). *ARF17* regulated the expression of *CalS5*, which was responsible for callose synthesis (Nishikawa et al. 2005; Dong et al. 2005; Shi et al. 2015). Callose is important for cell duplication. It is the major component of the cell plate separating two daughter cells, which can serve as an indicator for cell duplication. Thus, ARF17 may play an important role in controlling cell division, which can contribute to cell duplication and lead to different rates of biomass accumulation in sugarcane growth.

Another critical component of the perception and signaling of auxin is *AUX/IAA* gene family. *AUX/IAA* genes are early auxin response genes that are active

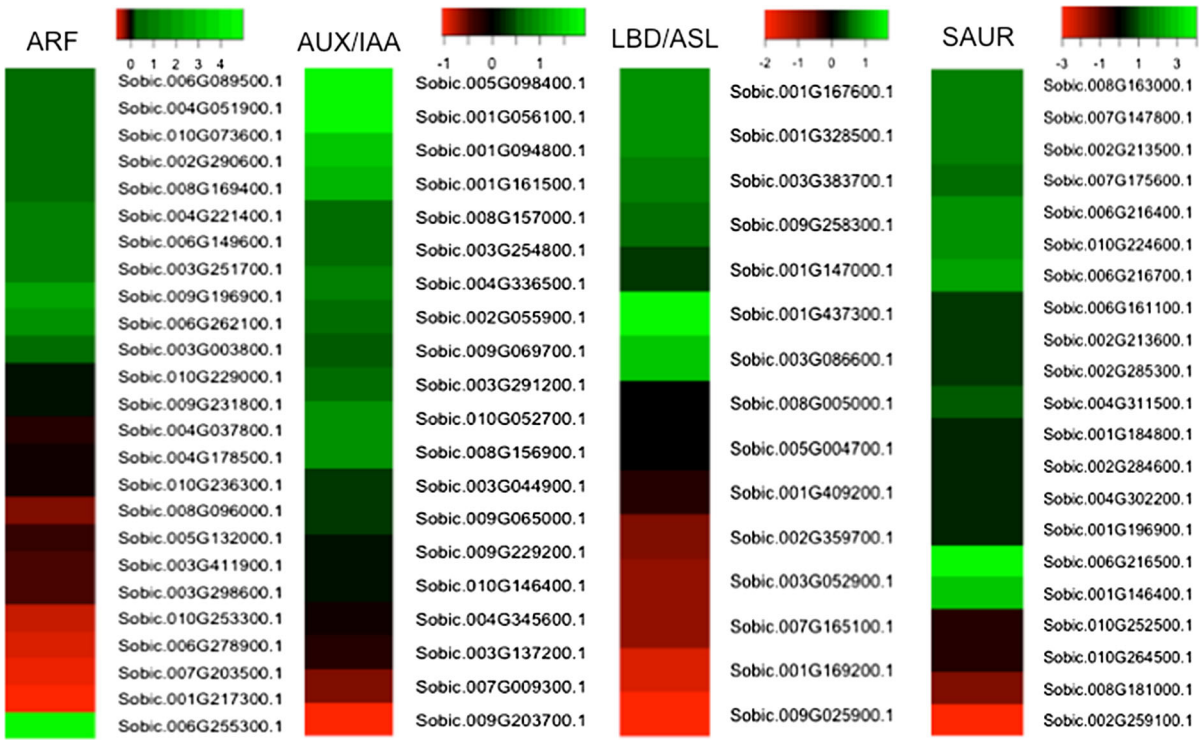

Fig. 2 Gene expression comparison of high biomass and low biomass group for auxin signaling related gene family. Heat-map of the logFC of major sugarcane ARF, IAA, LBD, and SAUR genes comparing the high biomass group with the low biomass group. The heat-map was drawn with R package gplots

repressors of transcription of auxin-regulated genes (Audran-Delalande et al. 2012; Tiwari et al. 2001). Four out of 20 annotated sorghum AUX/IAA genes were differentially expressed in the high biomass group. *SbIAA2, SbIAA*, and *SbIAA17* were up-regulated, while *SbIAA22* was down-regulated.

The previous publication had reported the importance of *IAA2* in the auxin accumulation and distribution in plant tissue (Abel et al. 1994). Like *ARF1*, the *IAA2* identified from sorghum gene model was also differentially expressed in the high biomass sugarcane progenies. Since *ARF1* could repress the transcription of *IAA2*, the down regulation of *ARF1* should lead to the upregulation of *IAA2* in higher biomass group. In our study, the upregulation of *IAA2* was observed, confirmed that the same mechanism existed in sugarcane, and this mechanism could possibly regulate growth related pathways and lead to the biomass differences in the F2 extreme segregants. It was also found that the expression of *IAA1* and *IAA2* were highly up-regulated under external IAA treatment in sorghum (Wang et al. 2010); although some sorghum IAA genes were down-regulated with IAA treatment, *IAA22* is not one of those. This regulation of *IAA22* could potentially be unique for sugarcane auxin regulation and developed after the speciation of sugarcane ancestor.

LBD genes were the targets of *ARF*, which were involved in plant growth and development, especially in the lateral organ formation and development (Reinhardt et al. 2003; Shuai et al. 2002). Two genes among 15 annotated *LBD* genes were down-regulated in the high biomass group. These two genes were identified as *LBD4* and *SbLBD28* based on sorghum gene model. *SbLBD* genes were naturally expressed in low level, and were up-regulated with BR, salt and drought treatments (Wang et al. 2010). These treatments were all stress related, therefore, the lower expression in high biomass might indicate less stress responses were triggered in them. *LBD4* was reported as with low transcription without stresses in sorghum (Wang et al. 2010). It was also reported that plant under stress would grow differently, which could cause less biomass production. Since the expression of

LBD4 and *LBD28* were higher in the low biomass group, it was possible that stress-related hormone regulations were more active in the extreme low biomass plants, leading to less biomass production.

SAUR gene family is another major player in the auxin-signaling pathway. *SAUR*s are usually expressed in elongating tissues and are likely to be involved in the regulation of cell elongation. They are also very sensitive to auxin, and auxin is likely to play an important role in their transcriptional regulation. The expression of *SAUR*s can be activated within the couple of minutes in presence of auxin (Chen et al. 2014; Franco et al. 1990). Five out of 21 *SAUR* genes were up-regulated in the group with high biomass, and one was down-regulated. The five up-regulated genes were previously reported as SbSAUR9, *SbSAUR39, SbSAUR45* and *SbSAUR46*. There is only one down-regulated *SAUR*, namely, *SbSAUR13*.

SAUR genes were known for their roles in promoting cell expansion and plant growth, which are important for biomass traits in plant (Spartz et al. 2012; Chae et al. 2012; Li et al. 2015). *SAUR9*, up-regulated in the high biomass group of sugarcane, was known for its interaction with *PP2Cs* (Spartz et al. 2014). The interaction of *PP2C* could lead to the regulation of cell wall modifying enzymes, and cell expansion (Spartz et al. 2014). Thus the upregulation of these five *SAUR*s could be a major contributor to the higher dry weight observed in the extreme segregants.

In rice, *SAUR39* was reported as negatively regulate the transportation and synthesis of auxin; as the result, the repression growth in root and shoot were observed in plants that overexpressed *SAUR39*, which lead to lower biomass production (Kant et al. 2009). However, *SAUR39* was observed as up-regulated in sugarcane with higher biomass instead of down-regulated. This controversial might be caused by the differences in the amount of expression of *SAUR39* gene. In Kant et al.'s study, the overexpression of *SAUR39* was > 40 fold higher in the transgenic line. But it is only 2.08 fold difference in the high sugarcane biomass group. It is possible that the effects of *SAUR39* could vary at different expression level.

GH3 gene family is involved in auxin signaling pathway as well. However, unlike other gene families related to auxin, the *GH3* genes were not significantly differentially expressed in the high biomass group. *GH3* genes were reported as the major component in regulating the endogenous auxin homeostasis through conjugating excess auxin with amino acids (Ludwig-Muller et al. 2009; Singh et al. 2014). Although many different aspects of auxin signaling pathways were differentially regulated in the higher biomass group, the mechanisms involved in maintaining endogenous auxin homeostasis stayed the same. This indicates that these mechanisms are not involved in biomass differences. It was also previously reported that although *IAA* is critical for regulating plant growth and development, a higher concentration of *IAA* can be toxic to plants (Bandurski et al. 1995). Thus, the expression and transcription of genes that are involved in maintaining this *IAA* homeostasis are necessary for sugarcane, in spite of biomass.

No previous publication has found that different regulation of *GH3* could lead to increase in biomass-related traits. In Arabidopsis, multiple *GH3*-overexpressing mutants were known for responses like reduced plant growth (Takase et al. 2004; Nakazawa et al. 2001). The knockout of *GH3* genes could also lead to higher sensitivity to auxin, and growth repression (Ludwig-Muller et al. 2009). Thus, it was possible neither increases nor decreases in *GH3* gene expression could lead to better growth and higher biomass accumulation. Since *GH3* regulated auxin homeostasis and auxin play important roles in many plant physiological processes, the change in *GH3* genes could lead to reduced fitness, causing similar *GH3* gene expression levels in all plants, regardless of biomass differences.

Ethylene-related gene families were differentially expressed

Ethylene regulates a wide range of plant responses, including fruit ripening, sugar accumulation, leaf epinasty, and leaf senescence (Abeles et al. 1992). *ETR, ERS, RTE, EUB, ETP, EIL* and *EBF* are important gene families participating in Ethylene signaling. Most members of the ethylene family did not show signs of differential expression in the high biomass group, except for *SbEIL5* (Fig. 3). EIL genes were EIN3-like genes, which were previously reported as functionally redundant positive regulators of various ethylene responses, especially in ripening (Chen et al. 2004).

The gene function of *EIL5* had not been revealed yet in plant (Guo and Ecker 2004). It was previously reported that some EILs identified from tobacco,

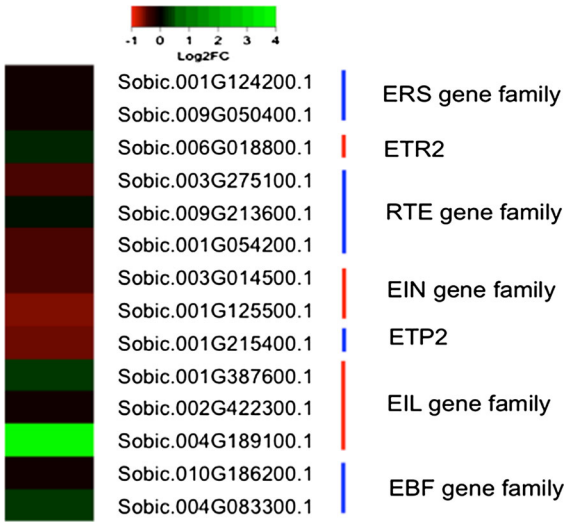

Fig. 3 Gene expression comparison of high biomass and low biomass group for Ethylene signaling related gene family. Heat-map of the logFC of major sugarcane ERS, ETR, RTE, EIN, EIL, ETP, and EBF genes comparing the high biomass group with the low biomass group. The heat-map was drawn with R package gplots

tomato and mung bean had similar functions as *EIN3* (Rieu et al. 2003; Tieman et al. 2001; Lee and Kim 2003). *EIN3* gene and some *EIL* genes were the master transcription factors that can regulate hundreds of ethylene-related genes and functions (Guo and Ecker 2004). Thus, *EIL5* could possibly function as a master regulator involved in biomass-related traits in sugarcane.

GA related gene families were differentially expressed

Gibberellin is commonly known as an important hormone regulating plant growth, stem elongation and many other processes of plant development (Daviere and Achard 2013). These growth and elongation related genes are main factors contribute to lignocellulose yield. Among the six genes identified based on Gibberellin hormone signaling pathway, only one of them, transcription factor *PIF3* was significantly down-regulated (Fig. 4). As transcription factors involved in gibberellin signaling pathway, *PIF*s were the integrators of photomorphogenic development that involved in promoting stem growth (Leivar and Monte 2014). Since *PIF3* concentration can regulate the stem growth, its differential expression may lead to a different level of biomass accumulation in sugarcane.

It was reported that the *GA20ox* and *GA2ox* from GA biosynthesis pathway can lead to plant height and biomass differences (Biemelt et al. 2004; Qin et al. 2013). However, in this study, we were not able to identify significantly differentially expressed genes from this category. This might be due to the fact that there is no variation in the regulation of these genes in the F2 population that we worked with. It was also possible that since we used sorghum as our reference, we were unable to capture all the *GA20ox* genes and *GA2ox* gene.

Discussion

Many gene families involved in Auxin signaling pathway are differentially expressed in the high biomass group. From *ARF, IAA* and *SAUR* gene families, three, three, and five genes are up-regulated in the high biomass group, respectively. There are also two *ARF*s, two *LBD*s and one *SAUR* genes are down-regulated in the high biomass group. Since auxin is a major regulator of plant growth and development, which are determining factors for biomass, it is expected to detect differential expression of these genes between the high and the low biomass groups. It was reported that the transcriptions of *ARF* genes vary at different *IAA* concentrations in Arabidopsis. Since different growth related regulations in various tissue types are likely to be the main factor affecting the biomass accumulation, *IAA* concentrations are possibly different between the high and low biomass groups, causing the activation and inhibition of different *ARF* genes. It was also reported that members of *ARF* family could serve as either activator or inhibitor of transcription (Ulmasov et al. 1999). Thus, the *ARF* genes could be either up regulated or down regulated according to their functions and *IAA* concentration to regulate the plant growth and biomass related traits. The binding of *ARF* proteins and *IAA* proteins were reported to be specifically based on previous yeast two-hybrid assays (Tatematsu et al. 2004). Thus the downstream and interacting *IAA* genes, *LBD* genes and *SAUR*s of specific *ARF* genes could be either up or down-regulated based on their functions and interactions.

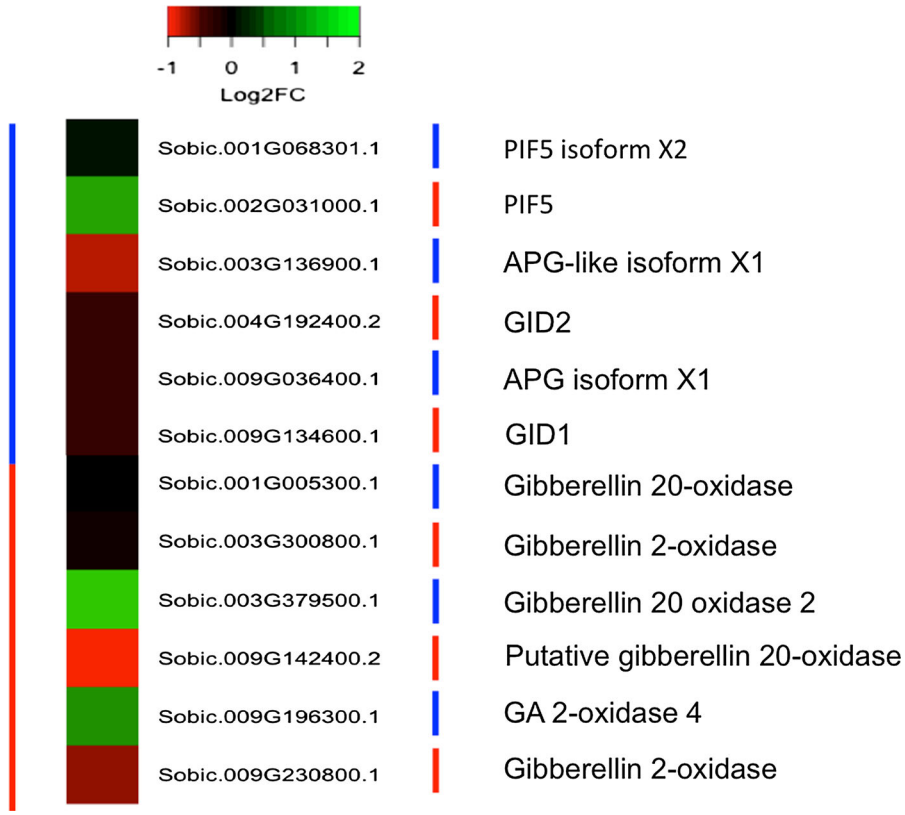

Fig. 4 Gene expression comparison of high with low biomass group for GA related gene family. Heat-map of the logFC of major sugarcane PIF, APG, GID, and GA oxidase genes comparing the high biomass group with the low biomass group. The heat-map was drawn with R package gplots

Among the five auxin-related gene families we studied, *GH3* genes are the only ones that are not differentially expressed. It was previous reported that although auxin and *IAA* is critical for regulating plant growth and development, a higher concentration of auxin or *IAA* can be toxic to plant (Bandurski et al. 1995). It was possible that the differentially expressed genes in the auxin hormone pathway was not caused by the concentration of *IAA* or auxin, but caused by auxin sensitivity; whether moderate or sensitive transcription factors were involved in auxin regulation might be more important to biomass trait compare to actual auxin concentration. Thus, the expression and transcription of genes that are involved in maintaining *IAA* homeostasis are likely to be necessary for sugarcane, in spite of the biomass differences, which explains why *GH3* expressions are similar between the two different biomass groups.

Most members of the ethylene family did not show signs of differential expression. This might be because the ethylene regulations are more relevant to ripening and sweetness instead of cellulose biomass traits. The F2 extreme segregants we used in this study showed extraordinary 30 folds difference on biomass yield, but sugar content was similar between the two extremes. This lack of variation in sugar content is a possible reason of the less number of differentially expressed genes in ethylene related gene families. There is one exception that one *EIL* gene is up-regulated in the high biomass group. *EIL* genes have similar sequences as *EIN3,* and function as a major regulator that can activate *ERF* genes and control a vast number of ethylene-related processes (Chao et al. 1997; Yang et al. 2015). Since ethylene-related genes regulate many aspects of different plant physiological processes, some of these processes, such as sugar partition, are relevant to biomass yield. Further research on the potential targets and downstream gene networks can help improve the understanding of how biomass relevant traits are regulated by ethylene

signaling pathway and potentially reveal more genes to modify or breed for to increase the biomass accumulation efficiency in energy cane and other C4 grasses.

GA regulates many important plant biomass related processes. Nevertheless, most GA related genes are not differentially expressed in the sugarcane high biomass group. Gene families that are involved in GA signaling and biosynthesis pathways were analyzed, but only one transcriptional factor, *PIF3*, was differentially expressed. Previous research had shown that mutations in GA related genes could cause dwarf and semi-dwarf in plants and the expression of GA related genes could improve biomass (Biemelt et al. 2004). Since GA played an important role for plant growth and biomass traits, more differences in GA related genes were expected to be detected. One possible reason for not detecting more GA related genes could be that our RNA-seq data was only collected at one-time point, and this specific life stage might not be when GA was most differentially expressed. If multiple life stages of samples were collected and compared between the high and low biomass groups, we might have been able to detect more differences in GA related genes. Another possible reason was the lack of high quality sugarcane reference genomes and gene models. Although hormone genes were conserved, it was still likely that the sorghum gene model could not cover all sugarcane GA genes. We might be able to identify more differentially expressed GA related genes once high quality sugarcane reference genomes and gene models are available.

The *PIF3* detected in the GA signaling pathway can regulate stem growth, which is a key component of biomass difference. Thus, the differential expression of this gene might be a key factor behind the different biomass accumulation between F2 extreme segregants. *PIF3* is also an important gene contributing to the crosstalk between Ethylene and GA pathways. In the dark, Ethylene would suppress hypocotyl length, and in the day time, it would promote hypocotyl elongation. *PIF3* expression is an important component in this process, which need to be activated by *EIN3* (Solano et al. 1998; Kosugi and Ohashi 2000; Leivar and Monte 2014). The *EIL5* gene observed as differentially expressed in higher biomass group in ethylene pathway was similar to EIN3 and the *PIF3* was observed as differentially expressed in GA pathway. Considering that *PIF3* could interact and be regulated by *EIN3*, and *EIL5* were similar to *EIN3*, it was possible that they were the crosstalk points of ethylene signaling and GA signaling pathway contributing to the biomass differences. This could potentially act as a scheme to for engineering and breeding for a more effective tissue elongation system is in the high biomass group, leading to more effective energy cane.

Conclusions

Gene expression profiling of hormone-related genes was analyzed among extreme segregants of high and low biomass yield from an *Saccharum* interspecific F2 population. We have identified potentially important genes in biomass regulation of auxin ethylene, and GA signaling and biosynthesis pathways. In this study, seventeen genes involved in Auxin related signal pathways were differentially expressed, but only one gene was differentially expressed in the other two pathways. Further study on these gene families once a high quality sugarcane genome is available can enhance the understanding of how these important hormones work together. However, these identified genes and regulatory mechanisms can still potentially serve as candidates in genetic modification and breeding programs to help develop effective sugarcane for bioenergy.

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Author contributions RM and FZ conceived the study, RM coordinated all research activities, TJ and CN conducted field trials, FZ, CMW, and JZ carried out RNAseq experiment and bioinformatics analysis. FZ and RM wrote the manuscript; all authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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