Comparative Transcriptome Analysis of a Fan-shaped Inflorescence in Pineapple Using RNA-seq

Tao Xie

Foshan University <https://orcid.org/0000-0002-0373-7425>

Zhiquan Cai

Foshan University

Aiping Luan

Chinese Academy of Tropical Agricultural Sciences

Wei Zhang

South China Agricultural University

Jing Wu

South China Agricultural University

Quannv Yang

Foshan University

Yehua He (✉ heyehua@hotmail.com)

South China Agricultural University, Guangzhou <https://orcid.org/0000-0002-9317-7520>

Research article

Keywords: Pineapple, Transcriptome analysis, Fan-shaped inflorescence, RNA-Seq technique, DEGs

Posted Date: September 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-52110/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Genomics on August 1st, 2021. See the published version at <https://doi.org/10.1016/j.ygeno.2021.08.022>.

Abstract

Background: Pineapple plant usually has a capitulum. However, a fan-shaped inflorescence was evolved in an exceptional material, having multiple crown buds. In order to reveal the molecular mechanisms of the formation of the fan-shaped inflorescence, fruit traits and the transcriptional differences between a fan-shaped inflorescence (FI) and a capitulum inflorescence (CI) pineapples were analyzed in the three tissues, i.e., the flower stem apex (FIs and CIs), the base of the inflorescence (FIb and Clb), and the inflorescence axis (FIa and Cla).

Results: Except for a clear differentiation of inflorescence morphology, no significant differences in the structure of inflorescence organs and the main nutritional components (soluble solids, soluble sugar, titratable acid, and V_C) in fruits were found between the two pineapples. Between the fan- and capitulum-shaped inflorescences, a total of 5370 differentially expressed genes (DEGs) were identified across the three tissues; and 3142, 2526 and 2255 DEGs were found in the flower stem apex, the base of the inflorescence, and the inflorescence axis, respectively. Of these genes, there were 489 overlapping DEGs in all three tissue comparisons. In addition, 5769 DEGs were identified between different tissues within each pineapple. Functional analysis indicated between the two pineapples that 444 transcription factors (TFs) and 206 inflorescence development related genes (IDGs) were differentially expressed in at least one tissue comparison, while 45 TFs and 21 IDGs were overlapped across the 3 tissues. Among the 489 overlapping DEGs in the 3 tissue comparisons between the two pineapples, excluding the IDGs and TFs, 80 of them revealed a higher percentage of involvement in the biological processes relating to response to auxin, and reproductive processes. RNA-seq value and real-time quantitative PCR analysis exhibited the same gene expression patterns in the three tissues.

Conclusions: Our result provided novel cues for understanding the molecular mechanisms of the formation of fan-shaped inflorescence in pineapple, making a valuable resource for the study of plant breeding and the speciation of the pineapples.

Background

A fruit of pineapple (*Ananas. comosus* (L.) Merr.) comprises of 30–200 sessile florets, which are spirally arranged on the top of the flower axis and form a terminal capitulum with a crown bud on the top [1, 2]. However, in the pineapple plantations, we found that up to 10% inflorescence axis of some Cayenne cultivars (i.e., ‘Smooth Cayenne’ and ‘Tainong 17’) were evolved into the fan-shaped inflorescences (fruits) at the inflorescence differentiation stage. Those inflorescence mutants are good materials for studying the formation and development of inflorescence, and the related gene regulation. However, the molecular mechanisms of the transcription variation related to the fan-shaped inflorescence formation has not been illustrated.

In general, inflorescence development begins with the transformation of shoot apical meristem (SAM) into inflorescence meristem (IM) [3, 4]. In recent years, for the molecular mechanisms of inflorescence

formation, some known or potential transcription factors and transcription co-regulators (e.g., microRNAs and proteins) have been identified in the model plants (e.g., *Arabidopsis thaliana* and *Oryza sativa*), by transcriptome and gene analysis [3, 4]. In many cases, the molecular mechanisms of these regulatory factors are not clear.

Nowadays, the research about the inflorescence development in pineapple mainly focused on the expression and signal regulation of the related genes, including functional genes (e.g., ACC synthase, DELLA genes) [5] and regulatory genes (e.g., MADS box, AP2, and other transcription factor families) [6, 7]. Although some genes related to the inflorescence development were cloned and identified, whether these genes participated in the development of the fan-shaped inflorescence in pineapple remains to be further revealed. Due to the advancements of high throughput and its low cost [8, 9], the next generation sequencing (NGS) technology has been emerged as an invaluable tool to clarify the plant development [10] and senescence [11, 12], and also provided a new way to accelerate the genetic analysis of transcriptome changes in response to various abiotic stresses, such as cold [13], salt [14], drought [15], and waterlogging [16]. Some application of NGS were to use transcriptome analysis to aid in identification of genes related to flavor, appearance, and texture in pineapple plants [17]. In addition, the differentially expressed genes were analyzed in the RNA sequence libraries of mature pineapple fruits, which revealed that ethylene related genes may also be involved in the regulation of non-climacteric ripening in pineapple [18].

Recently, research on expressed sequence tags (ESTs) of various pineapple tissues, including root, fruit and aerial tissues [19], green and yellow fruits [20], and nematode infected gall [21], has been accumulated. The results showed that metallothionein was the main protein in green (unripe) and yellow (ripe) fruits libraries [20]. In terms of plant mutants, NGS has become an efficient tool for the transcriptome analysis for exploring gene expression patterns in some plants, including cabbage [22], tomato [23], *Arabidopsis* and rice [24]. However, there are few reports on the transcriptome of the genome-wide inflorescence mutants in plants. As the whole genome in pineapple has been sequenced [25], the gene expression pattern and function in the development of inflorescence in pineapple mutants is discernible.

In this work, we unraveled the transcriptome differences in three tissues (i.e., the flower stem apex, the base of the inflorescence, and the inflorescence axis) between two pineapples with different inflorescences (i.e., with a fan- and a capitulum-shaped inflorescence). Combined with the functional annotation of DEG, especially the genes that may be involving in the transformation from SAM to IM, we tried to identify the key genes that maybe involve in the formation of the fan-shaped inflorescence. Comprehensive analysis of the transcriptome can serve as a blueprint for the gene expression profile of the flowering or inflorescence development, and also be a source of molecular information for the development of molecular markers, gene identification, and the functional analysis of differentially expressed genes. Our results may substantially understand the flowering mechanisms and are helpful for plant breeding in pineapples.

Results

Inflorescence and fruit traits

Except for the inflorescence and fruit morphology, no great difference in the organ structure of the inflorescences and the contents of soluble solids, soluble sugar, titratable acid, V_C and water in fresh fruits were found between the two pineapples (Fig. 1a). Fruit weight, number of crown buds, number of small fruits and depth of fruit eye were much larger in the fan-shaped fruits than those in the capitulum-shaped fruits (Fig. 1b; Table 1). Especially, the average fresh weight of the fan-shaped fruit was 4.9 times than that of the capitulum-shaped fruit. Based on our observation, the variation of the fan-shaped inflorescence was determined by the initial stage of inflorescence.

Table 1
Comparisons of fruit traits between the fan- and capitulum-shaped fruit

Economic trait	index	capitulum	fan-shaped
fruit weight (g)	average	1441.33	7175.49a
	minimum	586.50	4446.32
	maximum	1689.10	8524.60
	CV(%)	17.35	40.26
number of crown buds	average	1	103
	minimum	None	71
	maximum	None	129
	CV(%)	0	63.74
number of small fruits	average	118.62	838.79
	minimum	85	649
	maximum	133	1013
	CV(%)	11.18	29.54
depth of fruit eye (cm)	average	0.92	1.02
	minimum	0.69	0.44
	maximum	1.34	1.63
	CV(%)	9.51	18.75
edible (%)	average	84.41	75.25
	minimum	76.97	65.50
	maximum	92.76	85.65
	CV(%)	6.56	16.46
soluble solids (%)	average	15.54	15.29
	minimum	11.00	12.20
	maximum	18.00	17.50
	CV(%)	14.09	12.53
titratable acid (mg/g)	average	4.69	4.87
CV is an abbreviation for coefficient of variation.			

Economic trait	index	capitulum	fan-shaped
	minimum	4.03	4.02
	maximum	6.95	6.75
	CV(%)	15.35	13.55
soluble sugar (mg/g)	average	89.51	91.42
	minimum	82.48	80.78
	maximum	97.91	97.48
	CV(%)	6.03	5.59
Vc (mg/g)	average	0.35	0.35
	minimum	0.20	0.22
	maximum	0.48	0.47
	CV(%)	27.14	27.22
water content (%)	average	82.51	82.09
	minimum	80.68~	80.46
	maximum	85.44	84.44
	CV(%)	1.81	1.82
CV is an abbreviation for coefficient of variation.			

Illumina sequencing and comparison with the reference genome

For the transcriptional analysis, across the three tissues (i.e., flower stem apex, the inflorescence axis base, and the inflorescence axis; Fig. 1c) from the two pineapples, a total of 18 cDNA libraries were obtained from 18 samples, which were constructed and sequenced using the Illumina HiSeq™ 2500 platform. For the transcriptome sequencing of 18 samples, a total of 130.24 G clean data was generated. After excluding low-quality reads and trimming adapter sequences, effective data volume was ranged from 6.80 to 8.84 G; the basis distribution of Q30 was from 92.37–95.33%, and the mean of GC content was 48.37%. In a clean reading, the basic distribution of Q20 level was 100% (Table 2).

Table 2
Output statistics of the total reads from 18 libraries.

Library	Raw reads (n)	Clean data	GC percentage	Q20 percentage	Q30 percentage
Fls_1	47521996	7.13G	47.63%	100.00%	94.74%
Fls_2	58704314	8.81G	48.65%	100.00%	94.87%
Fls_3	54007018	8.10G	48.65%	100.00%	94.91%
Flb_1	51737698	7.76G	48.58%	100.00%	94.82%
Flb_2	52672706	7.90G	48.52%	100.00%	95.24%
Flb_3	49763452	7.46G	48.54%	100.00%	95.01%
Fla_1	53383244	8.01G	48.15%	100.00%	95.04%
Fla_2	51934690	7.79G	48.12%	100.00%	95.09%
Fla_3	58960564	8.84G	47.35%	100.00%	94.79%
Cls_1	52391826	7.86G	48.79%	100.00%	92.44%
Cls_2	48972286	7.35G	48.88%	100.00%	92.89%
Cls_3	57735614	8.66G	48.67%	100.00%	93.01%
Clb_1	49023720	7.35G	47.63%	100.00%	92.37%
Clb_2	45302790	6.80G	48.88%	100.00%	92.59%
Clb_3	49825744	7.47G	48.76%	100.00%	92.45%
Cla_1	48979570	7.35G	48.41%	100.00%	92.75%
Cla_2	45586200	6.84G	48.25%	100.00%	92.67%
Cla_3	52505530	7.88G	48.19%	100.00%	95.10%
Fls, Flb and Fla are the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively					
Cls, Clb and Cla are the flower stem apex, the base, and the axis of the capitulum-shaped inflorescence, respectively.					

All illumina reads (ranging from 42,949,036 to 57,318,180 reads in each sample) were mapped to the reference sequence database of reads ranged from 83.80–94.17%. The unique mapped reads accounted for approximately 79.41% – 89.81% of all mapped reads. Thus, the illumina sequencing technology was an effective tool to acquire a large portion of the transcriptome rapidly in pineapple (Additional file 1).

Gene expression annotation and statistics

The expression levels of each gene in the 18 libraries were normalized and calculated using FPKM [26] (Additional file 2). The FPKM method can eliminate the influence of protein-coding gene length and

sequencing amount on the calculation of protein-coding gene expression; the calculated protein-coding gene expression level that reflected its high or low expression. The five statistics in the data [minimum, first-quartile (25%), median (50%), third-quartile (75%), and maximum value] can also be used to describe the data (Additional file 3).

The FPKM value boxplot for each sample gene was shown in Fig. 2. Then we used ggplot2 for their visualization. The gene expression distribution of 18 samples was similar, with no obvious expression bias. These results indicated that the sequencing quality of FPKM standardization was good enough to estimate expression of the set of sequencing data.

Investigation of differentially expressed genes and functional categorization

Across the three corresponding tissues between FI vs. CI, the differentially expressed genes (DEGs) analyses resulted in a total of 5370 DEGs (Additional file 4e); 3142, 2526 and 2255 DEGs at the flower stem apex, the base of the inflorescence, and the inflorescence axis were obtained, respectively, between the two pineapples (Fig. 3b; Additional file 4a–c). Between different tissues within each pineapple, 5769 DEGs were obtained (Fig. 3a; Additional file 5a–d); of which 3634 were overlapped with the 5370 DEGs within each tissue between the two pineapples (Additional file 5f). Most DEGs (422) were only found in FIb vs. FIs comparison (Fig. 4; Additional file 5a), followed by 99 DEGs in FIs vs. CIs comparison (Fig. 4; Additional file 4a).

Between the two different pineapple inflorescences, 489 DEGs were overlapped in all three tissue comparisons (Fig. 3b; Additional file 4d). The degree of the overlapping DEGs between the different pineapple tissues was showed in Fig. 3b. In all four comparisons between different tissues within each pineapple, a total of 5767 DEGs were identified with 1916, 1035, 1063, and 897 DEGs, in the FIb vs. FIs, FIa vs. FIb, CIb vs. CIs, CIa vs. CIb, respectively (Additional file 5a–d).

The 3 top biological groups, including metabolic processes, cellular process and single-organism process, participated in the biological process category of gene ontology (GO) assignment in all 7 comparisons (Fig. 5a). In terms of the molecular function category, catalysis and binding were the most abundant subcategories (Fig. 5b). Based on the KEGG pathway annotation of DEGs between the two pineapples, 575, 386 and 389 DEGs were respectively assigned to 169, 155 and 159 KEGG pathways in the flower stem apex, the base of the inflorescence, and the inflorescence axis, respectively (Additional file 6a–c).

To examine the function of the identified DEGs, the pathway enrichment analysis indicated that phenylpropanoid biosynthesis, plant-pathogen interaction, starch and sucrose metabolism, and plant hormone signal transduction were significantly enriched in the FIs vs. CIs comparison (Additional file 6a). In the FIb vs. CIb comparison, the 4 most DEGs (i.e., phenylpropanoid biosynthesis, starch and sucrose metabolism, plant hormone signal transduction, and biosynthesis of amino acids) were represented (Additional file 6b). Moreover, with comparison of FIa vs. CIa, there were 4 prevailing categories (i.e.,

phenylpropanoid biosynthesis, starch and sucrose metabolism, cell cycle, and plant hormone signal transduction) (Additional file 6c).

Between the two pineapples, 890 DEGs were allocated to 180 KEGG pathways in the Flb vs. Fls comparison (Additional file 6d), while 273 were allocated to 134 KEGG pathways in the Fla vs. Flb comparison (Additional file 6e). Additionally, 255 were allocated to 131 KEGG pathways in the Clb vs. Cls comparison (Additional file 6f), and 49 were allocated to 43 KEGG pathways in the Cla vs. Clb comparison (Additional file 6g).

Differently expressed transcription factors

Totally, we identified 1277 TFs in this work (Additional file 7a); 444 TFs were significantly differentially expressive between the two pineapples (Fig. 6a; Additional file 7b). Among 239 differentially expressed TFs (DETFs) between Fls and Cls, 107 and 132 of them were up- and down-regulated, respectively (Fig. 6e; Additional file 8a–b). The prevailing family was the MYB family with 46 DETFs (22 were up-regulated), followed by the bHLH family with 33 DETFs (21 were up-regulated), the NAC family with 19 DETFs (5 were up-regulated), the WRKY family with 16 DETFs (2 were up-regulated), the C2H2 family with 15 DETFs (10 were up-regulated), and the ERF family with 12 DETFs (2 were up-regulated; Fig. 6b).

Among the 238 differentially expressed TFs between Flb and Clb, 100 and 138 TFs were up- and down-regulated, respectively (Fig. 6e; Additional file 8a–b). The regulated TFs included the MYB family with 39 genes (25 were down-regulated), followed by the bHLH family with 32 genes (17 were up-regulated), the NAC family with 18 genes (15 were down-regulated), the WRKY family with 17 genes (15 were down-regulated), and the ERF family with 15 (12 were down-regulated) (Fig. 6c). There were 187 differentially expressed TFs between Fla and Cla; 32 and 155 of them were up- and down-regulated, respectively (Fig. 6d–e; Additional file 8a–b). The predominant TF families included 32 MYB (28 were down-regulated), followed by 21 bHLH (15 were down-regulated), 18 NAC (15 were down-regulated), 12 WRKY (all were down-regulated), and 10 C2H2 (all were down-regulated; Fig. 6d).

Across the three tissues between the two pineapples, there were totally 457 highly differentially expressed TFs. 362, 120, 114, and 45 TFs were obtained in Flb vs. Fls, Fla vs. Flb, Clb vs. Cls, and Cla vs. Clb, respectively; and the number of up-regulated genes were 203, 53, 60, and 19, respectively (Fig. 6e; Additional file 8a–b). Additionally, 311 of the 444 differentially expressed TFs in Fls vs. Cls, Flb vs. Clb, and Fla vs. Cla comparisons were overlapped with the 457 differentially expressed TFs in Flb vs. Fls, Fla vs. Flb, Clb vs. Cls, and Cla vs. Clb comparison, respectively (Additional file 8a). Likewise, 45 of the 444 differentially expressed TFs were overlapped across the three tissues between the two pineapples (Additional file 8c). To verify the precision and repetitiveness of the transcriptome analysis results, 12 of the 45 TFs were randomly selected for the expression level validation using qRT-PCR. The consistent results between qRT-PCR and RNA-seq data manifested that the RNA-seq was highly reliable (Fig. 9).

Candidate genes related to inflorescence development

With respect to the inflorescence formation, except for TFs, there were 206 DEGs in at least one of comparison between the two pineapples including auxin related genes or homologues to the previously annotated genes that were potentially inflorescence development related genes. The development related genes, such as CLAVATA1 (CLV1) [27], auxin transporter-like (LAX) [28], cytokinin riboside 5'-monophosphate phosphoribohydrolase LOG [29, 30], cytokinin oxidase/dehydrogenase (CKX) [31, 32], FLOWERING LOCUS T protein [33], terminal flower (TFL) [34], LOB domain-containing protein [35], TEOSINTE BRANCHED1 (TB1) [36], and other auxin related genes [37, 38] (Fig. 7a; Additional file 9a). Among the 206 development-related genes of inflorescence (IDGs), 21 of them were overlapped across the three tissue comparisons between the two pineapples; 8 overlapping IDGs were randomly selected for qRT-PCR. The qRT-PCR results for 8 genes (Fig. 9) were agreed with the expression data generated from RNA-Seq. Moreover, 62 DEGs (57 from the 206 IDGs and 5 from the 444 TFs) were associated with auxin response; there were 19 auxin-responsive proteins, 12 auxin efflux carrier components, 6 auxin transporter-like proteins, 5 ARFs, 13 SAUR family proteins, and 7 GH3 auxin response promoters in FI vs. CI comparisons (additional file 9a). The expression of 206 IDGs was shown in Fig. 7b. Interestingly, 159 of them were overlapped with the comparisons within each tissue between the two pineapples (Additional file 9b).

Between the different tissues in the two pineapples, the KEGG pathways were associated with plant signal transduction. With regards to auxin related genes, AUX1 and SAURs were down-regulated at the core; AUX1 was up-regulated at both the top of the inflorescence axis and the base of the inflorescence. GH3 was mix regulated (both up and down) at the top of inflorescence axis, the base of the inflorescence and the core; SAUR was mix regulated (up and down) at the top of the inflorescence axis and the base of the inflorescence; Aux/IAA was mix regulated (up and down) at top of the inflorescence axis (Additional file 10). ARF was up-regulated at the base of the inflorescence and the core, but Aux/IAA was down-regulated. Between the different tissues in FI, SAUR was down-regulated in Flb vs. FIs and in Fla vs. Flb, while GH3 was up-regulated in the Flb vs. FIs comparison. Most auxin-related genes were down-regulated during the inflorescence formation from FIs initiation to Flb of visible inflorescence initiation, and to the fan-shape inflorescence formation at FI (Additional file 10). This implied that the most auxin related genes were regulated in the process of inflorescence formation.

Expression and GO analysis of the common DEGs in FI vs. CI comparisons

Further analysis between the two pineapples showed that 489 of all 5370 DEGs were simultaneously differentially expressed in all the three tissues comparisons. Of these 489 overlapping DEGs, 21 of them were overlapped in the above-mentioned 206 IDGs, while 45 of them were overlapped in the above-mentioned 444 TFs. Using GO functional analysis, 489 overlapping DEGs were classified into the three major functional categories, including cellular component, molecular function, and biological process. For the biological process category, DEGs were involved in cellular process (194 genes), metabolic process (175 genes), and response to stimulus (111 genes; Fig. 8). The DEGs related to processes of biological regulation (95 genes), developmental process (66 genes), multicellular organismal process (59 genes),

and the positive regulation of biological process (27 genes) were significantly higher across 489 overlapping DEGs (Fig. 8). 93 DEGs related to response to biological regulation included 8 of the 21 IDGs and 29 of the 45 TFs, indicating that inflorescence formation was closely related to the response to biological regulation. Therefore, the remaining 56 genes may also be involved in the inflorescence formation. With regards to the developmental process (66 DEGs) and growth (18 DEGs) in biological processes these genes were further merged into 66 DEGs because of their multiple functions. Five of the merged 66 DEGs belonged to 21 IDGs and 17 TFs, and the remaining 44 genes may also be involved in the inflorescence formation. Twelve of 44 genes were concurrently involved in both developmental and growth processes. Furthermore, the 56 and 44 genes that potentially involved in inflorescence formation were merged into 80 genes by the bio-process terms of the GO analysis (Additional file 11). Six of 80 bioprocesses-involved genes (BPGs) were screened to analyses expression level by qRT-PCR (Fig. 9). The results indicated that their expression levels were highly matched with the RNA-Seq value (Fig. 9).

Across all three tissues between the two pineapples, there were 489 overlapping DEGs. Among them, 16 containing 8 annotated genes (3 from the 21 IDGs, 4 from the 45 TFs, and 1 from the 80 BPGs mentioned above) and 8 unknown genes (Table 3) showed remarkable differences between the different tissues of FI; while, no significant difference was identified between the different tissues in CI. That suggested that 16 overlapping genes might be related to the inflorescence formation. Moreover, the above-mentioned genes, including 45 TFs, 21 IDGs, 80 BPGs and 8 unknown DEGs, might contribute to the formation of the fan-shaped inflorescence in pineapple by the expression comparative and function analysis.

Table 3
DEGs for qRT-PCR

Category	ID	Annotation	Overlapped between 3 tissues in FI not in CI
Bio-processes involved genes (BPGs)	Aco003366	response regulator 4	
	Aco025401	Auxin response factor	
	Aco010456	otein phosphatase 2C family protein	
	Aco004216	Auxin-responsive protein	Yes
	Aco020531	CYCLIND3	
Overlapped TFs	Aco003777	HD-ZIP	
	Aco006017	MIKC_MADS	Yes
	Aco006706	RAV	
	Aco010917	bHLH	
	Aco011886	ARF	
	Aco012028	bHLH	
	Aco012353	LBD	Yes
	Aco012619	ZF-HD	Yes
	Aco013194	G2-like	
	Aco013198	MYB	
	Aco019379	NAC	Yes
	Aco022238	LBD	
Overlapped IDGs	Aco006318	cytokinin oxidase/dehydrogenase 1	
	Aco009169	SAUR-like auxin- responsive protein family	
	Aco004216	Auxin-responsive protein	Yes
	Aco003045	Peroxidase superfamily protein	Yes

Category	ID	Annotation	Overlapped between 3 tissues in FI not in CI
	Aco016519	Peroxidase superfamily protein	
	Aco008390	germin-like protein 2	Yes
	Aco026249	germin-like protein 4	
	Aco021042	Dormancy/auxin associated family protein	
Unknown genes that overlapped between 3 tissues in FI not in CI	Aco000147	hypothetical protein	Yes
	Aco000656	uncharacterized protein	Yes
	Aco001260	Glutathione S-transferase family protein	Yes
	Aco017829	Glycerophosphodiester phosphodiesterase GDE1	Yes
	Aco024341	Glucan endo-1%2C3-beta-glucosidase	Yes
	Aco031729	Plant protein of unknown function DUF247	Yes
	Aco007556	Protein of unknown function%2C DUF538	Yes
	Aco012680	Glycosyltransferase family 61 protein	Yes
DEGs not overlapped	Aco000039	golgin 5	
	Aco007412	Protein of unknown function DUF1218	
	Aco013183	Transmembrane 9 superfamily member	
	Aco024025	Nucleobase ascorbate transporter	
	Aco029040	ovate family protein 5	
	Aco031807	hypothetical protein	

Discussion

Plant mutant, one of the basic phenomena in plant evolution, is highly valuable for crop breeding. With the rapid development of modern sequencing technology, we can provide support for the development of molecular breeding, and speed up the breeding process of new varieties. The fan-shaped inflorescences (fruits) in pineapple had both high ornamental value and higher fruit yields (Table 1). Therefore, clarifying the molecular mechanism of the formation of the fan-shaped inflorescence is highly valuable in plant breeding in pineapples.

No differences in the gene and signal transduction were found between the fan- and capitulum-shaped inflorescences using NGS. The comparisons across the three tissues and two pineapples provided us with 5370 DEGs; more DEGs were recognized between Fls vs. Cls and Flb vs. Clb than those between Fla vs. Cla. In addition, 5769 DEGs were identified between different tissues within each pineapple, indicating that the regulatory pathway existed in the formation of the fan-shaped inflorescence in pineapple.

Previous studies have identified genes that may be involved in the inflorescence development in plants, including some genes related to auxin and auxin processes [39, 40]; auxin has recently been reported to be associated with inflorescence development [41]. Across the three tissues and two pineapples, 206 IDGs were identified, including LAX, LOG, CKX, PAP, FLOWERING LOCUS T, LOB, and Aux / IAA, which may participate in the formation of the fan-shaped inflorescence in pineapple. Moreover, there exist 21 overlapping genes of them in all 3 comparisons within each tissue between the two pineapples; they were also crucial for regulating development of fan inflorescence in pineapple.

CLAVATA (CLV), which is related to the inflorescence development by regulating the transformation of SAM into IM, played an essential role in the transition from SAM into IM [42]. It has been proposed that LOG was involved in various induction of inflorescence formation, some transcriptional regulators and cytokinin regulation through the complex interactions [29, 30]. TERMINAL FLOWER and FLOWERING LOCUS T play an important role in the inflorescence formation [6, 43]. In our study, five differently expressed genes related to TERMINAL FLOWER (1 gene) and FLOWERING LOCUS T (4 genes) were identified within each tissue between the two pineapples (Additional file 9a). Cytokinin, auxin and ethylene are necessary for inflorescence formation [44, 45]. For example, PIN in Arabidopsis was found to control meristem activity, and was regulated in turn by three redundant PLETHORA (PLT) proteins [46].

As a plant growth hormone, auxin is considered as a necessary condition for plant growth, development [47, 48], flower bud differentiation [45], as well as transition from SAM to IM [42]. Auxin was accumulated during inflorescence development in Arabidopsis, and the endogenous auxin concentration was higher in IM tissue in developing inflorescence [37]. Auxin was also involved in the induction of flower bud differentiation in *Juglans sigillata* [49]. It was previously found that the free and bound auxin levels are highly regulated by inflorescence formation [45]. In pineapples, we found that the majority of genes were down-regulated across the three different tissues between the two pineapples (Fig. 8a). Therefore, we speculated that the formation of fan-shaped inflorescence may be related to the epigenetic modification (i.e., DNA methylation). Research of inflorescence development identification and characterization indicated that the auxin-related genes and proteins were related to inflorescence development in auxin-

induced acquisition [37]. Thus 206 IDGs, especially the 21 overlapping IDGs, might be involved in the formation of the fan-shaped inflorescence in pineapple.

TFs have great potential value in plant growth and development. Previous studies on the inflorescence development showed that the complex transcription regulation networks played an integral part in tissue differentiation and hormone mediated signaling [47]. In this study, we obtained 271 TFs showing significantly different expression across the three different tissues between the two pineapples; 45 of them were simultaneously differentially expressed in across all three different tissue comparisons between the two pineapples. Of these 444 TFs, MYB, AP2/ERF, and MADS-box showed highly representative expression.

Cytokinin plays an integral part during regulating the inflorescence structure of different inflorescence types by regulating the activity of meristem [50, 51]. Cytokinin promoted inflorescence meristem development and affected inflorescence structure by promoting Arabidopsis meristem gene WUSCHEL (WUS) and by inhibiting meristem inhibitors CLAVATA1 (CLV1) and CLV3 [52]. The expression of WUS can be directly induced by cytokinin. WUS enhanced cytokinin signal transduction and formed a positive feedback loop [53, 54]. At the same time, the transcription of CLV3 gene can also be promoted by WUS expression. WUS is not only an important target gene of CLV negative control signal, but also a positive regulatory gene. WUS and CLV constitute a regulatory feedback loop [53, 55]. In our research, the up-regulated genes of WUS had 3, 4 and 4 genes, respectively, within each tissue comparison between the two pineapples (Additional file 7). Compared with the capitulum-shaped inflorescences, the proportion of down-regulated DEGs at FIs tissue was higher than that Flb and Fla tissues, indicating that they had a regulatory role in the formation of the fan-shaped inflorescence in pineapple.

AP1, a member of AP2/ERF domain family, shows a significant effect on inflorescence configuration regulation [56]. *AP1* plays the role of transcriptional inhibition to inhibit flower development at the beginning, and can promote flower development when combined with SEP3 protein [57, 58]. *LFY* is a flower tissue differentiation gene, which is mainly expressed in leaf primordium in vegetative growth stage, and rapidly increased to the highest level in the flower induction stage [57]. *LFY* can regulate the transformation from the vegetative growth to the reproductive growth of plants, and can also regulate the special morphology of flower organs [57]. *LFY* and *AP1* can positively promote each other's expression. *LFY* can bind to the promoter of *AP1* gene and promote the expression of corresponding gene, while *TFL1* gene can inhibit the expression of *LFY* and *AP1* gene [59]. These interactions among the three genes play a unique role in the regulation of inflorescence configuration and inflorescence evolution. In addition, AP1 protein and its homologues CAL and FUL in Arabidopsis can inhibit *TFL1* gene expression [59]. In this study, there were 6 AP2/ERF genes differentially expressed across three tissues between the two pineapples (Additional file 8a); among them, 2 genes (Aco008597, Aco013345) were expressed differently among 3 tissues within FI, but not within CI (Additional file 8a). Therefore, these AP2/ERF genes may be related to the formation of the fan-shaped inflorescence in pineapple.

The transcription regulators, e.g., *SVP*, *SOC1* and *AGL24* of MADS domain have dual effects [60]: preventing the reverse of floral meristem to inflorescence structure [61], and maintaining a moderate level of expression at a specific stage of development. On the one hand, they have redundant functions with *SEP4* and can interact with *AP1* to inhibit the expression of *TFL1*; *SEP4* directly suppresses *TFL1* expression without *SOC1*, *AGL24* and *SVP* [60]. On the other hand, *SVP*, *SOC1* and *AGL24* also directly inhibit the expression of *SEPALLATA3* (*SEP3*), a flower organ characteristic gene, to prevent flower meristem from early differentiation, so as to ensure the timely formation and development of flower organs in Arabidopsis [62]. Between our two studied pineapples, 8 MADS genes were differentially expressed in the three tissue comparisons; 2 genes (Aco006017, Aco018015) were expressed differently in the comparison of tissues in FI, but not in the comparison of tissues in CI (Additional file 8a).

Members of MYB are involved in growth, development and regulation of phenylpropane secondary metabolic pathways in plants [63]. They control the cell morphology and participate in the development and regulation of floral organs [64]. *MYB38* and *MYB84* are important regulatory factors that affect the formation of axillary meristem in Arabidopsis [65]. *AtMYB38* affects the formation of auxiliary lateral branches during inflorescence development [65]. In our study, up-regulated MYB in the flower stem apex, the base of the inflorescence, and the inflorescence axis were 22, 39 and 4, respectively (Fig. 6), indicating that there was a complex regulation in the formation of the fan-shaped inflorescence in pineapple. In addition, most of MYB genes were up-regulated in the fan-shaped inflorescence tissues (Fig. 6), indicating that these family members played an important role in the formation of the fan-shaped inflorescence.

Plant inflorescence development is a complex process. In the morphogenesis of higher plants, inflorescence development is the basic step to determine the final yield and reproductive characteristics. Auxin is essential for flower development [37]. In this study, many auxin related genes and TFs were expressed differently in each comparison. Therefore, the auxin related genes and TFs regulate the formation of the fan-shaped inflorescence in pineapple. In addition to IDGs and TFs, 80 genes were involved in the GO biological process and reproduction of auxin signal in the three tissue comparisons between the two pineapples, and they were perhaps related to the formation of the fan-shaped inflorescence.

Conclusions

Between the two pineapples, there are a clear differentiation of inflorescence morphology; and no differences in the structure of inflorescence organs and the main nutritional components were found. Using expression and functional analysis, a total of 5370 DEGs were identified across the three tissues between the two pineapple inflorescences; Of them, there were 489 overlapping DEGs in all three tissue comparisons. Besides, 5769 DEGs were identified between different tissues within each pineapple. The obtained 444 DETFs (45 overlapped across the three tissues between the two pineapples), 206 IDGs (21 overlapped across the three tissues between the two pineapples), 80 BPGs showed significant differences across three different tissues between the two pineapples. Therefore, the functional analysis of these genes will promote to address underlying molecular regulatory mechanism for the formation of the fan-

shaped inflorescence. Due to the formation of fan-shaped inflorescence needs to be formed under specific conditions, further research at the gene functional level is desired to understand the perturbed biological system of the formation of the fan-shaped inflorescence in pineapple.

Methods

Plant material and RNA extraction

Pineapple cultivar 'Tainong17' were collected in the field in Zhanjiang (20°12'N 110°24'E), Guangdong province, South China), and no permission was required to collect them. We performed formal identification of the plant samples. They were planted in South China Agricultural University garden, Guangdong Province. The collection of samples and the performance of experimental research on such plant were complied with the national guidelines of China. It is worth to note that pineapple is not endangered or protected species, and experimental research was environmentally friendly and had no negative effects on the local ecosystem. The fan-shaped inflorescence (FI) and the capitulum-shaped inflorescence (CI) were selected for RNA sequencing. It is considered that FI and CI have identical genetic backgrounds. In April 2019, three tissues (i.e., the flower stem apex, the base of the inflorescence, and the inflorescence axis) of red young inflorescence appeared in the center of both the fan- and capitulum-shaped inflorescences, were immediately frozen in liquid nitrogen and were stored in at -80 °C refrigerator until RNA extraction.

With 3 replications, each pineapple tissue was dissected. The total RNA was extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and then were analyzed by one 1% agarose gel electrophoresis. The integrity of RNA was quantified using Nanodrop ND-1000 spectrophotometer. All RNA samples with Integrity Number (RIN) ≥ 7 were subjected to the subsequent analysis.

Determination of fruit traits

Fresh fruits of both the fan- and capitulum-shaped pineapple plants were harvest; over thirty fruit samples were measured in each pineapple. Soluble reducing sugar was determined by the Anthrone method [66]. The titratable acid content was determined by acid-base titration (based on citric acid) [67], and vitamin C content was determined by 2,6-dichloroindophenol titration [66]. Soluble solid content was measured by LB50T sugar refractometer.

RNA-seq library construction and Illumina sequencing

After the total RNA of the sample was digested with DNase, the eukaryotic mRNA was enriched and purified by magnetic beads with oligo (dT). The mRNA was broken into short fragments by adding interruption reagent, and the interrupted mRNA fragments were used as the templates and reverse transcribed into cDNA. The purified cDNA was repaired at the end. A-tail was added and connected with sequencing connector, then these cDNA fragments were selected, and finally PCR amplification was carried out. The constructed library was qualified by Agilent 2100 Bioanalyzer, and then the Illumina

sequencing platform (HiSeqTM 2500) was used for sequencing to generate 125 bp/150 bp paired-end reads. The RNA-seq data have been deposited in the NCBI Sequence Read Archive under Accession no. SRR123467.

Sequence alignment to the pineapple reference genome

The pineapple reference genome and corresponding gene annotation files were downloaded directly from the pineapple Genome website [25] (<http://pineapple.angiosperms.org/pineapple/html/index.html>). The htseq-count [68] software was used to count the number of reads in each sample which was mapped to each gene with in the gene annotation file, and the cufflinks [69] software was used to calculate the FPKM (fragments per kilobase per million reads) value of each gene expression.

A comparative analysis of the transcriptome and the reference genome were used to reveal the fan- and capitulum-shaped inflorescence characteristics of pineapple. Raw reads were processed using Trimmomatic [70], and the reads which contain ploy-N and the low-quality reads were removed to obtain the clean reads. The clean reads were mapped to the pineapple reference genome using hisat2 [71].

Identification of differentially expressed genes

The DESeq [72] software was used to normalize expression values FPKM of each gene using the DESeq R package. For significantly differential genes (DEGs), we used the false discovery rate (FDR) statistical method and compared the normalized expression values FPKM using a threshold value of $P \leq 0.05$ and $\text{foldchange} \geq 2$ or $\text{foldchange} < 0.5$ based on the $\text{FDR} < 0.05$. Firstly, the expression level of the detected genes was compared in parallel between libraries from the two pineapples. Then, the common DEGs were obtained from seven comparisons. For DEGs with similar expression patterns, a hierarchical cluster analysis of DEGs was carried out to explore genes expression pattern. GO enrichment and KEGG [73] pathway enrichment analysis of DEGs were respectively performed for the functional analysis of DEGs using R based on the hypergeometric distribution.

Analysis of transcription factors

All transcription factors were performed by BLASTx against the Plant Transcription Factor Database (PlnTFDB; <http://planttfdb.cbi.pku.edu.cn/>) [74], the pineapple Transcription Factor Database (<http://planttfdb.cbi.pku.edu.cn/index.php?sp=Acm>), and the alignment results of orthologous in the NCBI. Subsequently, the differentially expressed TFs were selected from the DEGs in between the two pineapples comparisons, and between the different tissues within fan-shaped inflorescence or capitulum-shaped inflorescence comparisons in pineapples.

Validation of DEG expression by quantitative real-time PCR (qRT-PCR)

Forty DEGs, including 12 TFs, 8 inflorescence development and flowering-related genes, 6 BPGs, 8 unknown DEGs, and 6 DEGs not overlapped were selected for validation using quantitative real-time PCR (qRT-PCR), which was performed on the Bio-Rad IQ5 Real-Time PCR System (Bio-Rad, USA) using SYBR

Green PCR mix (Vazyme) as per manufacturer's recommendations. Sequences of the primers used in this study were presented in Additional file 12. Details about the qRT-PCR were described in our previous work [75]. Each reaction was performed with three replicates, and the data from qRT-PCR amplification was calculated using $2^{-\Delta\Delta CT}$ method. CT value of the flower stem apex (Cl_s) tissues was selected as the calibrator and was set at 1.0. The relative expression levels of the same genes in the corresponding fan-shaped inflorescence tissues were then compared.

Abbreviations

FI: fan-shaped inflorescence; CI: capitulum-shaped inflorescence; FI_s, FI_b and FI_a: the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively; CI_s, CI_b and CI_a: the flower stem apex, the base, and the axis of the capitulum-shaped inflorescence, respectively; DEGs: Differentially expressed genes; IDGs: inflorescence development related genes; SAM: shoot apical meristem; IM: inflorescence meristem; AGL: AGAMOUS-like; ARF: Auxin-responsive factor; WUS: WUSCHEL; BPGs: bio-processes-involved genes; NGS: New generation sequencing; ESTs: Expression sequence tags; FDR: false discovery rate; ERF: Ethylene-responsive transcription factor; GO: Gene ontology; IAA: Indole-3-acetic acid; KEGG: Kyoto encyclopedia of genes and genomes; TF: transcription factor; DETF: differentially expressed transcription factor; TFL: terminal flower; CKX: cytokinin oxidase; TB1: TEOSINTE BRANCHED1; CLV1: CLAVATA1.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The raw sequence data of this article have been deposited as BioProject ID [PRJNA648693] in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number [SRR123467] (<https://www.ncbi.nlm.nih.gov/sra/SRR123467>).

Competing interests

The authors declare that they have no competing interests.

Funding

This research was financially supported by the National Key Research and Development Program (2018YFD1000500), National Natural Science Foundation of China (31572089), Guangdong Key Research and Development Program (2018B020202011), and Modern Agricultural Industry Technology System of Guangdong Province (2016LM1128). These funding bodies were not involved in the experimental design of the study, data collection, analysis and interpretation as well as in writing the manuscript.

Authors' contributions

QY and YH designed the experiments. AL and TX performed the experiments. WZ and JW participated in the bioinformatics analysis. TX, ZC and YH wrote the paper. All authors have read and approved the manuscript.

Acknowledgements

We thank Prof. Yun-Bo Wang (Department of Horticulture, Foshan University, Foshan 528231, China) for helpful suggestions on an earlier version of this paper.

Authors' information

¹Department of Horticulture, Foshan University, Foshan 528231, China. ²Key Laboratory of Biology and Germplasm Enhancement of Horticultural Crops in South China, Ministry of Agriculture, South China Agricultural University, Guangzhou 510642, China. ³Tropical Crops Genetic Resources Institute of Chinese Academy of Tropical Agricultural Science, Haikou 571101, China.

References

1. Py C, Lacoëuilhe JJ, Teisson C. Pineapple, its culture, its products. In: Culle J, editor. *Techniques agriculture et productions tropical*. Paris: Academic; 1987. p. 16-23.
2. Py C, Lacoëuilhe JJ, Teisson C. The pineapple cultivation and uses. In: Robert EP, editor. *The plant and the environment*. Paris: Academic; 1987. p. 68-72.
3. Smyth DR, Bowman JL, Meyerowitz EM. Early flower development in *Arabidopsis*. *Plant Cell*. 1990; 2:755-67.
4. Wang Y, Jiao YL. Axillary meristem initiation-a way to branch out. *Current Opinion in Plant Biology*. 2018; 41:61-66.

5. Trusov Y, Botella JR. Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple [*Ananas comosus* (L.) Merr. *Journal of Experimental Botany*. 2006; 57(14):3953-3960.
6. Lv LL, Duan J, Xie JH, Wei CB, Liu YG, Liu SH et al. Isolation and characterization of a FLOWERING LOCUS T homolog from pineapple (*Ananas comosus* (L.) Merr). *Gene*. 2012; 505(2):368-373.
7. Zhang X, Fatima M, Zhou P, Ma Q, Ming R. Analysis of MADS-box genes revealed modified flowering gene network and diurnal expression in pineapple. *BMC Genomics*. 2020; 21(1):1-16.
8. Mardis ER. Next-generation DNA Sequencing methods. *Annual Review of Genomics and Human Genetics*. 2008; 9:387-402.
9. Schuster SC. Next-generation sequencing transforms today's biology. *Nat Methods*. 2008; 5(1):16-28.
10. Fan Z, Li J, Li X, Wu B, Wang JY, Liu ZC, et al. Genome-wide transcriptome profiling provides insights into floral bud development of summer flowering Camellia Azalea. *Scientific Reports*. 2015; 5:9729.
11. Tsanakas GF, Manioudak ME, Economou AS, Kalaitzis P. De novo transcriptome analysis of petal senescence in Gardenia Jasminoides Ellis. *BMC Genomics*. 2014; 15:554.
12. Li L, Zhang W, Zhang L, Li N, Peng JZ, Wang, YQ, et al. Transcriptomic insights into antagonistic effects of gibberellin and abscisic acid on petal growth in Gerbera Hybrida. *Frontiers Plant Science*. 2015; 6:168.
13. Xu W, Li R, Zhang N, Ma FL, Jiao YT, Wang ZP, et al. Transcriptome profiling of Vitis Amurensis, an extremely cold-tolerant Chinese wild Vitis species, reveals candidate genes and events that potentially connected to cold stress. *Plant Mol Biol*. 2014; 86:527-41.
14. Peng Z, He S, Gong W, Sun JL, Pan ZE, Xu FF, et al. Comprehensive analysis of differentially expressed genes and transcriptional regulation induced by salt stress in two contrasting cotton genotypes. *BMC Genomics*. 2014; 15:760.
15. Zhai L, Xu L, Wang Y, Huang DQ, Yu RG, Limera C, et al. Genome wide identification of embryogenesis-associated microRNAs in radish (*Raphanus Sativus* L.) by high-throughput sequencing. *Plant Mol Biol Report*. 2014; 32(4):900-15.
16. Qi B, Yang Y, Yin Y, Xu M, Li HG. De novo sequencing, assembly, and analysis of the Taxodium 'Zhongshansa' roots and shoots transcriptome in response to short-term waterlogging. *BMC Plant Biol*. 2014; 14:201.
17. Ong WD, Voo L-YC, Kumar VS. 2012. De novo assembly, characterization and functional annotation of pineapple fruit transcriptome through massively parallel sequencing. *PLOS ONE*. 2012; 7(10): e46937.
18. Redwan RM, Saidin A, Vijay Kumar S. 2016. The draft genome of MD-2 pineapple using hybrid error correction of long reads. *DNA Research*. 2016; 23(5):427-439.
19. Neuteboom LW, Kunimitsu WY, Webb D, Christopher DA. Characterization and tissue-regulated expression of genes involved in pineapple (*Ananas comosus* L.) root development. *Plant Science*. 2002; 163:1021-1035.

20. Moyle R, Crowe M, Ripi-Koia J, Fairbairn DJ, Botella J R. Pineapple DB: an online pineapple bioinformatics resource. *BMC Plant Biology*. 2005; 5:21.
21. Moyle R, Fairbairn DJ, Ripi J, Crowe M, Botella JR. Developing pineapple fruit has a small transcriptome dominated by metallothionein. *Journal of experimental botany*. 2005; 56:101-112.
22. Huang SN, Liu ZY, Yao RP, Li DY, Feng H. Comparative transcriptome analysis of the petal degeneration mutant pdm in Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) using RNA-Seq. *Molecular genetics and genomics*. 2015; 290:1833-1847.
23. Liu XX, Huang YM, Qiu ZK, Gong H. Comparative transcriptome analysis of differentially expressed genes between the fruit peel and flesh of the purple tomato cultivar 'Indigo Rose'. *Plant signaling & behavior*. 2020; 15(6):1752534.
24. Campbell MA, Haas BJ, Hamilton JP, Mount SM, Buell CR. Comprehensive analysis of alternative splicing in rice and comparative analyses with *Arabidopsis*. *BMC Genomics*. 2006; 7:327.
25. Ming R, Van BR, Wai CM, Tang HB, Schatz M C, Bowers JE, et al. The pineapple genome and evolution of CAM photosynthesis. *Nature Genetic*. 2015; 47:1435-42.
26. [Roberts A](#), [Trapnell C](#), [Donaghey JL](#), [Rinn JL](#), [Pachter L](#). Improving RNA-Seq expression estimates by correcting for fragment bias. *Genome Biology*. 2011;12(3):R22.
27. Clark SE, Running MP, Meyerowitz EM. *CLAVATA1*: a regulator of meristem and flower development in *Arabidopsis*. *Development*. 1993; 119: 397-418.
28. Boot KJM, Hille SC, Libbenga KR, Peletier LA, van SPC, van DB, et al. Modelling the dynamics of polar auxin transport in inflorescence stems of *Arabidopsis thaliana*. *Journal of Experimental Botany*. 2016; 67(3):649-666.
29. Kurakawa T, Ueda N, Maekawa M, [Kobayashi K](#), [Kojima M](#), [Nagato Y](#), et al. Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature*. 2007; 445:652-655.
30. Tokunaga H, Kojima M, Kuroha T, Ishida T, Sugimoto K, Kiba T, et al. *Arabidopsis* lonely guy (LOG) multiple mutants reveal a central role of the LOG-dependent pathway in cytokine in activation. *Plant Journal*. 2012; 69:355-365.
31. Werner T, Motyka V, Laucou V, Smets R, Van OH, Schmölling T. Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell*. 2003; 15: 2532-2550.
32. Li, S, Zhao B, Yuan D, Duan MJ, Qian Qi, Tang L, et al. Rice zinc finger protein DST enhances grain production through controlling Gn1a/OsCKX2 expression. *Proceedings of the National Academy of Sciences of the USA*. 2013; 110:3167-3172.
33. Lv LL, Duan J, Xie JH, Wei CB, Liu YG, Liu SH, et al. Isolation and characterization of a FLOWERING LOCUS T homolog from pineapple (*Ananas comosus* (L.) Merr). *Gene*. 2012; 505(2):368-373.
34. Prusinkiewicz P, Erasmus Y, Lane B, [Harder LD](#), [Coen E](#). Evolution and development of inflorescence architectures. *Science*. 2007; 316:1452-1456.

35. Koppolu R, Anwar N, Sakuma S, Tagiri A, Lundqvist U, Pourkheirandish M, et al. Six-rowed spike4 (Vrs4) controls spikelet determinacy and row-type in barley. *Proceedings of the National Academy of Sciences of the USA*. 2013; 110(32):13198-13203.
36. Dixon LE, Greenwood JR, Bencivenga S, Zhang P, Cockram J, Mellers G, et al. TEOSINTE BRANCHED1 Regulates Inflorescence Architecture and Development in Bread Wheat (*Triticum aestivum*). *Plant Cell*. 2018; 30(3):563-581.
37. Yamaguchi N, Wu MF, Winter CM, Berns MC, Nole-Wilson S, Yamaguchi A, et al. A molecular framework for auxin-mediated initiation of flower primordia. *Development Cell*. 2013; 24:271-282.
38. Wu MF, Yamaguchi N, Xiao J, Bargmann B, Estelle M, Sang Y, et al. Auxin-regulated chromatin switch directs acquisition of flower primordium founder fate. *eLife*. 2015; 4:e09269.
39. William MG. Hormonal regulation of plant growth and development. *PLoS Biology*. 2004; 2(9):1270-311.
40. Edelbluth E, Kaldewey H. Auxin in scapes, flower buds, flowers, and fruits of daffodil (*Narcissus pseudonarcissus* L.). *Planta*. 1976; 131(3):285-91.
41. Feng N, Song GY, Guan JT, Chen K, Jia ML, Huang DH, et al. Transcriptome profiling of wheat inflorescence development from spikelet initiation to floral patterning identified stage-specific regulatory genes. *Plant physiology*. 2017; 174(3):1779-1794.
42. Somssich M, Je BI, Simon R, Jackson D. CLAVATA-WUSCHEL signaling in the shoot meristem. *Development*. 2016; 143:3238-3248.
43. Zhu BT, Li H, Hou YF, Zhang PC, Xia XZ, Wang N, et al. AGAMOUS and TERMINAL FLOWER controls floral organ identity and inflorescence development in *Medicago truncatula*. *Journal of Integrative Plant Biology*. 2019; 230:917-923.
44. Han YY, Yang HB, Jiao YL. Regulation of inflorescence architecture by cytokinins. *Frontiers in Plant Science*, 2014; 5:669.
45. Wils CR, Kaufmann K. Gene-regulatory networks controlling inflorescence and flower development in *Arabidopsis thaliana*. *Gene Regulatory Mechanisms*, 2017; 1860(1): 95-105.
46. Prasad K, Grigg SP, Barkoulas M, Yadav RK, Sanchez-Perez GF, Pinon V, et al. Arabidopsis PLETHORA transcription factors control phyllotaxis. *Current Biology*. 2011; 21:1123-1128.
47. Su YH, Zhang XS. Auxin gradients trigger de novo formation of stem cells during somatic embryogenesis. *Plant Signal Behavior*. 2014; 4(7):574-6.
48. Reinhardt D. Phyllotaxis – a new chapter in an old tale about beauty and magic numbers. *Current Opinion in Plant Biology*. 2005; 8:487-493.
49. Lu B, Chen LN, Hao JB, Zhang Y, Huang JC. Comparative transcription profiles reveal that carbohydrates and hormone signaling pathways mediate flower induction in *Juglans sigillata* after girdling. *Industrial Crops & Products*. 2020; 153:112556.
50. Han YY, Yang HB, Jiao YL. Regulation of inflorescence architecture by cytokinins. *Frontier in Plant Science*. 2014; 5:669.

51. Yamburenko MV, Kieber JJ, Schaller GE. Dynamic patterns of expression for genes regulating cytokinin metabolism and signaling during rice inflorescence development. *PloS one*. 2017; 12(4):e0176060.
52. Lindsay DL, Sawhney VK, Bonham-Smith PC. Cytokinin- induced changes in CLAVATA1 and WUSCHEL expression temporally coincide with altered floral development in *Arabidopsis*. *Plant Science*. 2006; 170:1111-1117.
53. Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM. Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. *Proceedings of the National Academy of Sciences the USA*. 2009; 106:16529-16534.
54. Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laux T. The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell*. 2000; 100:635-644.
55. Leibfried A, To JP, Busch W, [Stehling S](#), [Kehle A](#), [Demar M](#), et al. WUSCHEL controls meristem function by direct regulation of cytokinin- inducible response regulators. *Nature*. 2005; 438: 1172-1175.
56. Ma Q, Liu X, Franks RG, Xiang QJ. Alterations of CorTFL1 and CorAP1 expression correlate with major evolutionary shifts of inflorescence architecture in Cornus (*Cornaceae*) - a proposed model for variation of closed inflorescence forms. *New Phytologist*. 2017; 216(2):519-535.
57. Wellmer F, Riechmann JL. Gene networks controlling the initiation of flower development. *Trends in genetics*. 2010; 26:519-527.
58. Kobayashi K, Yasuno N, Sato Y, Yoda M, Yamazaki R, Kimizu M, et al. Inflorescence meristem identity in rice is specified by overlapping functions of three AP1/FUL-like MADS box genes and PAP2, a SEPALLATA MADS box gene. *Plant Cell*. 2012; 24:1848-1859.
59. Blümel M, Dally N, Jung C. Flowering time regulation in crops – what did we learn from *Arabidopsis*? *Current opinion in biotechnology*. 2015; 32:121-129.
60. Liu C, Teo ZW, Bi Y, Song SY, Xi WY, Yang XB, et al. A conserved genetic pathway determines inflorescence architecture in *Arabidopsis* and rice. *Development Cell*. 2013, 24 (6):612-622.
61. Liu C, Zhou J, Bracha-Drori K, et al. Specification of *Arabidopsis* floral meristem identity by repression of flowering time genes. *Development*. 2007; 134 (10):1901-1910.
62. Wu MF, Sang Y, Bezhani S, Yalovsky S, Ito T, Yu H. SWI2/SNF2 chromatin remodeling ATPases overcome polycomb repression and control floral organ identity with the LEAFY and SEPALLATA3 transcription factors. *Proceedings of the National Academy of Sciences, USA*. 2012; 109 (9):3576-3581.
63. Riechmann JL, Heard J, Martin G, [Reuber L](#), [Jiang CZ](#), [Keddie J](#), et al. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science*. 2000; 290(5499):2105-10.
64. Cheng H, Song SS, Xiao LT, Soo HM, Cheng ZW, Xie DX, et al. Gibberellin acts through jasmonate to control the expression of MYB21, MYB24, and MYB57 to promote stamen filament growth in *Arabidopsis*. *PLoS Genetics*. 2009; 5:e1000440.

65. Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L. MYB transcription factors in *Arabidopsis*. Trends in Plant Science. 2010, 15:573-581.
66. Zhang ZA, Zhang MS, Wei HR. Experimental guidance of plant physiology. Beijing: China Agricultural Science and Technology Press, 2004. (in Chinese)
67. Yan CM, Zhang JZ, Liu YN, Krivankova M, Valasek P, Adamek M, et al. Comparison and analysis of fruit quality of five pineapple varieties. Guangdong Agricultural Science. 2012, 19:42-44. (in Chinese)
68. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics. 2015; 31(2):166-9.
69. Roberts A, Pimentel, Trapnell C, Pachter L. Identification of novel transcripts in annotated genomes using RNA-Seq. Bioinformatics. 2011; 27(17):2325-9.
70. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014, 30(15):2114.
71. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nature Methods. 2015; 12(4):357.
72. Anders S, Huber W. Differential expression of RNA-Seq data at the gene level—the DESeq package. Heidelberg, Germany: European Molecular Biology Laboratory (EMBL), 2012.
73. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, et al. KEGG for linking genomes to life and the environment. Nucleic Acids Research. 2008; 36:480-484.
74. Guo AY, Chen X, Gao G, Zhang H, Zhu QH, Liu XC, et al. PlantTFDB: a comprehensive plant transcription factor database. Nucleic Acids research. 2008; 36:966-9.
75. Xie T, Chen CJ, Li CH, Liu JR, Liu CY, He YH. Genome-wide investigation of WRKY gene family in pineapple: evolution and expression profiles during development and stress. BMC Genomics. 2018; 19(1):490.

Additional Files

Additional file 1 Statistical results of comparison rate between reads and reference genome.

Additional file 2 Expression of genes in the 18 libraries in pineapple based on the results of RNA-Seq.

Additional file 3 Statistical table of FPKM distribution of genes.

Additional file 4 Information of DEGs between the two pineapples.

Additional file 5 Information of DEGs between different tissues within each pineapple.

Additional file 6 KEGG enrichment analysis of all DEGs.

Additional file 7 List of all TFs and DETFs.

Additional file 8 List and statistics of overlapped TFs between the two pineapples.

Additional file 9 Differentially expressed and overlapped IDGs between the two pineapples.

Additional file 10 KEGG pathways of plant hormone signal transduction between the two pineapples.

Additional file 11 Differentially expressed BPGs.

Additional file 12 Sequences of the primers used in this study for qRT-PCR.

Figures

Figure 1

Morphological characteristics of inflorescence and fruit. (a) A capitulum-shaped inflorescence; (b) a fan-shaped inflorescence; (c) capitulum- and fan-shaped fruit; (d) anatomical map of initial fan-shaped inflorescence.

Figure 2

Box-plot of FPKM across all samples. Fls, Flb and Fla: the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively; Cls, Clb and Cla: the flower stem apex, the base, and the axis of the capitulum-shaped inflorescence, respectively.

Figure 3

Histogram and Venn diagram of the differentially expressed genes (DEGs) in comparisons between the two pineapples and between different tissues within each pineapple. (a) Histogram showing the number of DEGs up- or down-regulated in each comparison. (b) Venn diagrams showing similarly or distinctly regulated genes within each tissue between the two pineapples. Fls, Flb and Fla: the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively; Cls, Clb and Cla: the flower stem apex,

the base, and the axis of the capitulum-shaped inflorescence, respectively. Up and down represent up- and down-regulated gene, respectively.

Figure 4

Common and specific differentially expressed genes among different comparisons. Fls, Flb and Fla: the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively; Cls, Clb and Cla: the flower stem apex, the base, and the axis of the capitulum-shaped inflorescence, respectively.

Figure 5

Molecular functions and biological processes of DEGs in comparisons of FI vs. CI and between tissues in FI and CI based on gene ontology categories. Fls, Flb and Fla: the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively; Cls, Clb and Cla: the flower stem apex, the base, and the axis of the capitulum-shaped inflorescence, respectively.

Figure 7

Analyses of differentially expressed inflorescence development related genes (IDGs) in pineapple. (a) The distribution of 206 IDGs in comparisons of FI vs. CI at the 3 tissues. (b) Expression of 206 differentially expressed IDGs. Numbers represent the percentage of genes out of the differentially expressed genes in each comparison. Fls, Flb and Fla: the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively; Cls, Clb and Cla: the flower stem apex, the base, and the axis of the capitulum-shaped inflorescence, respectively.

Figure 8

GO Functional categorization of 206 common differentially expressed genes in FI vs. CI at three tissues between the two pineapples based on gene ontology.

Figure 9

Relative mRNA levels of 40 DEGs in pineapple were determined by quantitative RT-PCR analyses. Data were normalized to the β -actin gene (GenBank: HQ148720) and error bars indicate standard deviation (SD). FIs, FIb and FIa: the flower stem apex, the base, and the axis of the fan-shaped inflorescence, respectively; CIs, CIb and CIa: the flower stem apex, the base, and the axis of the capitulum-shaped inflorescence, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.xls](#)
- [Additionalfile2.xls](#)
- [Additionalfile3.xls](#)
- [Additionalfile12.xls](#)
- [Additionalfile4.xls](#)
- [Additionalfile11.xls](#)
- [Additionalfile5.xls](#)
- [Additionalfile10.pdf](#)
- [Additionalfile6.xls](#)
- [Additionalfile9.xls](#)
- [Additionalfile7.xls](#)
- [Additionalfile8.xls](#)