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TRANSCRIPTOMICS OF MICRODISSECTED STAMINODES AND EARLY DEVELOPING CARPELS FROM FEMALE INFLORESCENCES OF *Elaeis guineensis*

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ABSTRACT

In an abnormal female inflorescence from a mantled clonal palm, the male reproductive organs develop into pseudocarpels. Transcriptomes of microdissected male and female reproductive organs in a normal inflorescence were thus compared to identify genes that were differentially expressed during the normal development of these two reproductive organs. Besides the increased expression of stamen identity MADS-box genes EgDEF1, EgGLO1 *and* EgGLO2 *in male reproductive organs,* NAC *and homeodomain leucine-zipper type of transcription factors were also upregulated. Male reproductive organ development may be associated with increased ethylene and decreased bioactive gibberellin levels inferred from the increased expression levels of* ACC OXIDASE *and* GA 2-OXIDASE *genes. This expression pattern may be important for the impending developmental arrest of the staminodes as they do not develop into stamens. Early carpel development occurred in conjunction with increased expression of the floral meristem identity* LEAFY *and organ boundary specification* CUP-SHAPED COTYLEDON 2 (CUC2) *genes. Hence, genes important for male reproductive organ development and early carpel development were identified using precise isolation of specific reproductive organs.*

Keywords: laser capture microdissection, *EgDEF1*, *EgGLO*, carpel, staminode.

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INTRODUCTION

Clonal propagation is the only approach to vegetatively multiply elite oil palms. Although clones had demonstrated improved oil yields of 12%-15% over current *dura* x *pisifera* (DxP) hybrids (Soh, 2012), the cloning process can give rise to the mantled somaclonal variant. In the female pistillate inflorescence, the mantled phenotype affects flower development through development of pseudocarpels in place of the normally arrested staminodes. This leads to abnormal fruit development, abortion and ultimately oil yield losses.

Oil palm flower development has been anatomically defined into five key developmental stages (Adam *et al.*, 2007a; 2005). Development of the male staminodes takes place at stage three development. At this stage, floral whorls from the normal and mantled inflorescences are indistinguishable anatomically. At stage four development, arrested staminodes are seen at the third floral whorl in a normal female inflorescence but developing pseudocarpels are observed instead in a mantled inflorescence.

Previous transcriptomics or gene expression studies on oil palm flower development involved the use of the entire inflorescence for ribonucleic acid (RNA) isolation and this included other non-

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reproductive tissues such as the bracts and rachis. This reduces the precision in detection of gene expression differences while minor but important differences can also escape detection. A recent study used laser capture microdissection (LCM) to isolate only parts of the flower involved in reproductive organ development, *i.e.* the staminodes and developing carpel (Ooi *et al.*, 2019). This yielded specific transcriptomes of those reproductive parts. In this study, those specific transcriptomes from staminodes and their adjacent carpel initials (Ooi *et al.*, 2019) were further analysed in detail for their transcriptome differences. Genes involved specifically in development of either the male or female reproductive organs were identified.

MATERIALS AND METHODS

Plant Material

Oil palm inflorescence samples at stage three floral development were obtained from a previous study (Ooi *et al.*, 2019). These inflorescences were sampled from two normal nine-year old clonal palms (IDs: P273/145 and P273/86) at the Malaysian Palm Oil Board (MPOB) Keratong Research Station, Pahang, Malaysia, as previously described.

LCM

Procedures for LCM including tissue processing, cryoembedding and cryosectioning were previously described (Ooi *et al.*, 2019). Briefly, rachillae from the inflorescences were cryoembedded for cryosectioning on the Leica CM1950 cryostat. Cryosections on polyethylene naphthalate (PEN) membrane slides (Applied Biosystems) were used for LCM on the ArcturusXT™ Microdissection Instrument (Applied Biosystems). Microdissected male and female reproductive organs were captured on Arcturus® CapSure® HS LCM Caps (Applied Biosystems).

RNA-sequencing Analysis

Construction of RNA sequencing (RNA-seq) libraries, sequencing and data pre-processing were as previously conducted (Ooi *et al.*, 2019). Briefly, stranded RNA-seq libraries were constructed using SMARTer® Stranded RNA-Seq Kit (Clontech Laboratories Inc.) and sequenced on the Illumina HiSeq platform. Reads were trimmed off their first five to six bases from reads 1 and 2 respectively (as recommended by Clontech Laboratories Inc.). Using cutadapt tool, reads with more than 35 consecutive A-bases (or 35 T-bases in read 2) were also trimmed and lastly, reads shorter than 20 bases were removed. Read mapping to the *E. guineensis* P5-build (Singh *et al.*, 2013) was carried out using Tophat2 version.2.0.13, with intron length set at 30 to 50 000 nucleotides and fr-secondstrand option specified for library-type (Kim *et al.*, 2013). The *pisifera* P5 genome data can be accessed at the National Centre for Biotechnology Information (NCBI) with accession ID ASJS00000000 (BioProject PRJNA192219) or at http://genomsawit.mpob.gov.my.

For this study, read counts for 'gene' feature were obtained using HTSeq-Count version 0.6.1p1 (Anders *et al.*, 2015). The raw sequence data has been deposited in NCBI's Gene Expression Omnibus (Edgar *et al.*, 2002) and can be accessed through GEO Series Accession No. GSE115345. Differential gene expression analysis was carried out using DESeq2 (Love *et al.*, 2014), with the female group of samples designated as the reference. The differentially expressed genes (DEG) ($p_{adj}<0.1$) were annotated using BLASTX analysis to UniProtKB/Swiss-Prot database and the remaining unannotated DEG were submitted for BLASTX to the NCBI's RefSeq database, limiting the search to flowering plants. DEG are designated by their palmXplore gene IDs (http://palmxplore.mpob.gov.my). Using their corresponding orthologue's UniProt IDs, DEG were submitted for ID mapping (Huang *et al.*, 2011) at https://www.uniprot.org. Heatmaps for visualisation of gene expression data were generated using ClustVis (Metsalu and Vilo, 2015).

RESULTS

Flower Morphology at Stage 3 Development

The microdissected tissues and subsequent stranded RNA-seq data used in this study were obtained from a previous study (Ooi *et al.*, 2019). Briefly, clonal palms of normal fruit phenotypes were selected. The oil palm female inflorescence comprises approximately 150 rachillae and flowers are arranged spirally around the axis of each rachilla (Adam *et al.*, 2005). There are five key stages in oil palm flower development (Adam *et al.*, 2007a) and we were interested in stage three flower development for this study. Hence, sampled female inflorescences at frond +17 of lengths 7.5 cm and 12 cm were histologically evaluated to be at stage three flower development (*Figures 1a* to *1c*). At this developmental stage, the staminodes develop next to the female gynoecium, which consists of early developing carpels surrounding the floral meristem. The male reproductive organs in the female inflorescence will arrest in development to develop rudimentary stamens called staminodes (Adam *et al.*, 2005). The male and female reproductive organs, *i.e.* the staminodes and developing carpels respectively, were microdissected using LCM (*Figures 1d* to *1h*) (Ooi *et al.*, 2019).

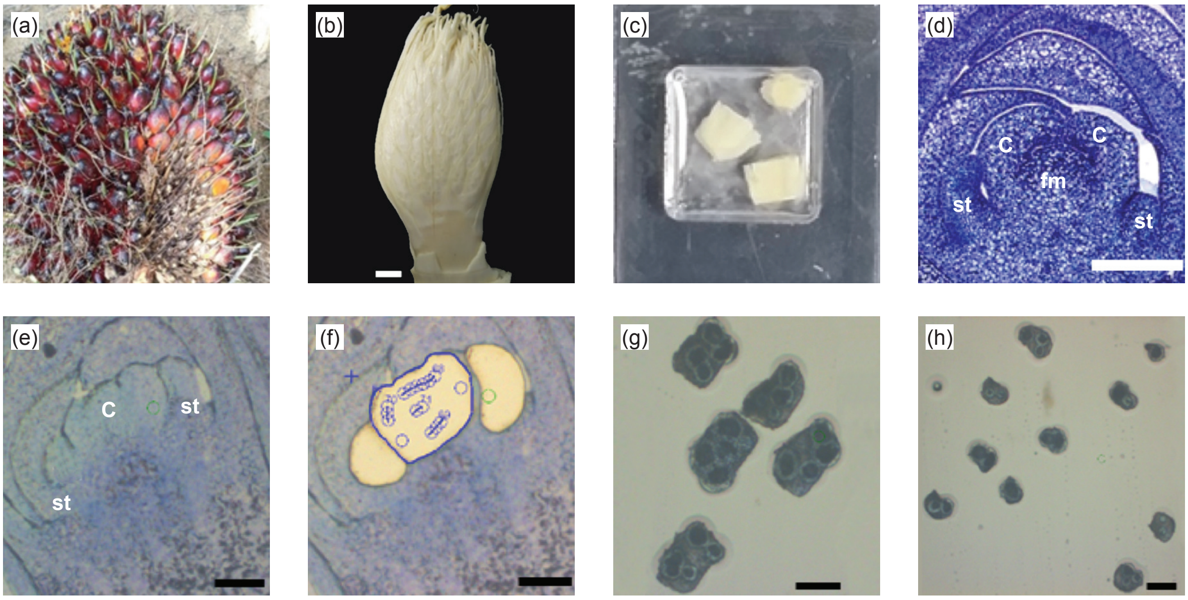

Figure 1. Oil palm fruit, female inflorescence and microdissected reproductive organs. (a) A fruit bunch from sampled palm, (b) female inflorescence of 7.5 cm length (bar = 1 cm), (c) excised and trimmed rachilla in cryomould, (d) developing flower stained with periodic acid-Schiff reagent and naphtol-blue black, st - staminodia, c - carpel, fm - floral meristem, (e) before and (f) after laser capture microdissection (LCM) of reproductive organs, (g) microdissected female reproductive organs, and (h) microdissected male reproductive organs [bar for (d)-(h) = 100 µm].

Differential Expression between Male and Female Reproductive Organs

Approximately five to 10 million mapped reads (Ooi *et al.*, 2019) were used for differential analysis using HTSeq read count (Anders *et al.*, 2015) to oil palm predicted genes (Chan *et al.*, 2017) and DESeq2 (Love *et al.*, 2014) packages. DEG were further annotated using BLASTX analysis to UniProtKB/Swiss-Prot and RefSeq-flowering plants databases. A total of 42 DEG ($p_{adj}<0.05$) were further analysed after Benjamini-Hochberg false discovery correction. The expression heatmap for these 42 significant DEG (*Table 1*) indicated that 12 genes were up-regulated in female reproductive organs and five of these were of unknown function (*Figure 2a*). UniProt ID mapping tool (Huang *et al.*, 2011) was applied on a larger set of DEG ($p_{adj}<0.1$), *i.e.* 48 DEG up-regulated in staminodes and 18 DEG up-regulated in early developing carpels (*Figures 2b* and *2c*, *Table 2*). Results associated with the 'developmental process involved in reproduction' gene ontology (GO) term (GO:0003006) indicated that DEG up-regulated in staminodes were enriched in the GO term associated with specification of stamen identity (GO:0010097), while majority of the DEG up-regulated in developing carpels were assigned to the GO term associated with floral meristem determinacy (GO:0010582). Several of these genes are associated with floral organ identity and development, such as the B-type MADS-box genes *EgDEF1*, *EgGLO1*, *EgGLO2*, and a gene similar to *MOSAIC FLORAL ORGANS 1* (ID p5.00_sc00002_p0446). The *DEF*/*GLO* and *MOSAIC FLORAL ORGANS 1* genes are involved in floral organ specification in other plants (Alvarez-Buylla *et al.*, 2010; Duan *et al.*, 2012; Li *et al.*, 2011).

As expected, up-regulation of the B-type MADS-box genes important for stamen development, *i.e.* *EgDEF1*, *EgGLO1* and *EgGLO2*, was detected in staminodes (*Figure 3*). This indicated that despite the contamination detected in the RNA-seq data (Ooi *et al.*, 2019), the processed transcriptome data still provides valuable information supported by the differential expression evidence of these MADS-box genes. Other flowering-related transcription factors such as a *NAC* gene (ID p5.00_sc00007_p0167) and *ATHB40*-like gene (p5.00_sc00106_p0020) were up-regulated in staminodes too. On the other hand, a higher relative expression of the flowering-related *SQUAMOSA-PROMOTER-BINDING-LIKE* gene (p5.00_sc00023_p0064), *CUC2* orthologue (p5.00_sc00050_p0193), meristem associated homeobox *SBH1*-like (p5.00_sc00080_p0036), *TERMINAL EAR1*-like (p5.00_sc01636_p0001) and a MADS-box transcription factor gene (p5.00_sc00002_p0446) was detected in early developing carpels. The MADS-box gene (p5.00_sc00002_p0446) was highly similar to *AGL6*/*13*-like (Accession No. CAE46187.1) of the AGAMOUS-like gene family through BLASTX analysis to RefSeq database and was also similar to *Oryza sativa MOSAIC FLORAL ORGANS 1* from UniProt database. In addition, important floral meristem identity genes, such as putative *LEAFY* (p5.00_sc00046_p0050) and *CAULIFLOWER* (p5.00_sc00059_p0084) genes, were highly expressed in developing carpels ($p_{adj}<0.1$) (*Figure 3*). Verification of the sequencing results using in situ RNA hybridisation analysis had been reported previously (Ooi *et al*., 2019).

A few genes associated with phytohormone biosynthesis or metabolism exhibited higher expression in the staminodes (*Figure 2*). These comprised an *ACC OXIDASE* that is involved in the final step of ethylene biosynthesis, *GA 2-OXIDASE* which inactivates endogenous gibberellins and an abscisic acid hydroxylase involved in abscisic acid degradation. A DEG encoding an ethylene responsive transcription factor *ETHYLENE RESPONSE FACTOR 12* (*ERF12*) was also up-regulated in staminodes. On the other hand, a nuclear factor *NO VEIN* (p5.00_sc00001_p0473) required for expression of *PIN* genes (Tsugeki *et al*., 2009), was up-regulated in early developing carpels. *PIN* genes are involved in auxin transport (Wisniewska *et al*., 2006) and its transcripts accumulated in the developing gynoecium during maize pistillate flower differentiation (Forestan *et al*., 2012).

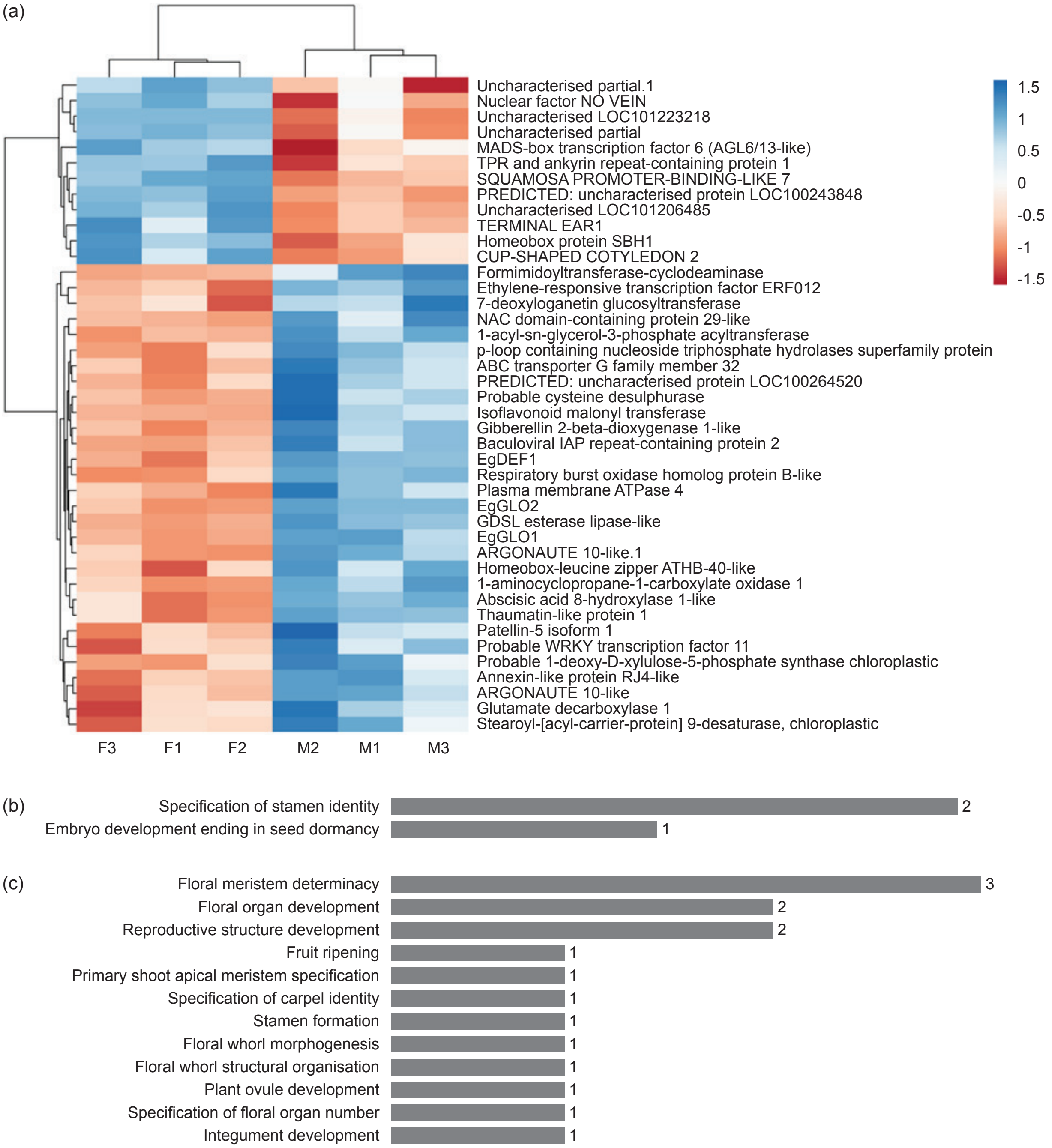

Figure 2. Differentially expressed genes (DEG) in male and female reproductive organs. (a) Expression heatmap and hierarchical clustering of all six samples based on the DEG (p_{adj} <0.05). Rows were centred and unit variance scaling was applied to rows. Both rows and columns were clustered using Euclidean distance and average linkage. Genes are labelled by their palmXplore annotations (Table 1). *Colour key refers to processed regularised log values. Biological replicates of male reproductive organs are designated as M1, M2 and M3; female organs are designated as F1, F2, F3, (b) functional categorisation of up-regulated DEG (p_{adj}<0.1) in staminodes, and (c) early developing carpels based on Gene Ontology (GO) annotations.*

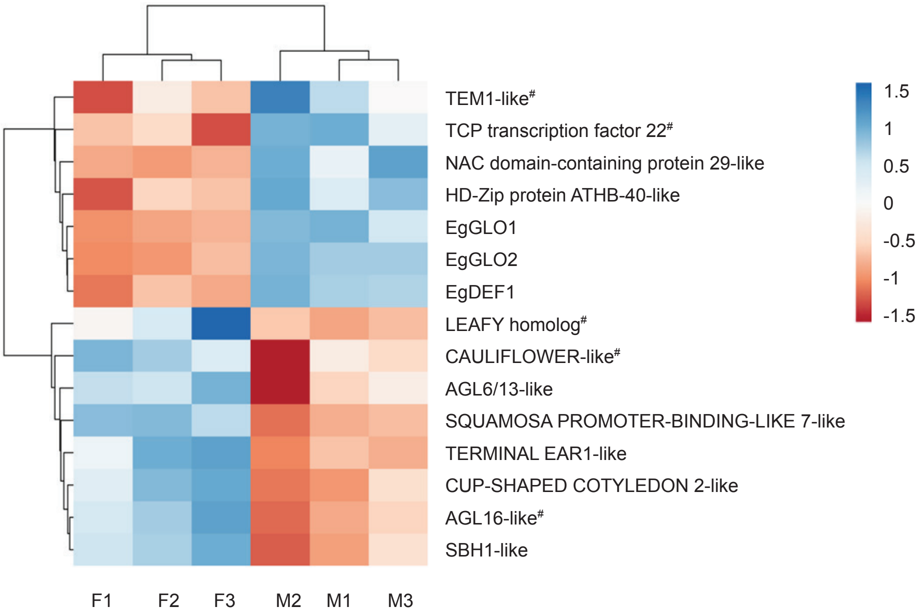

Figure 3. Expression heatmap of differentially expressed flowering-related genes only (p_{adj} <0.1). Rows were centred and unit variance scaling was applied to rows. Both rows and columns were clustered using Euclidean distance and average linkage. Genes are labelled by their simplified annotations (refer to Table 1*) and genes labelled with # symbol can be referred to in* Table 2*. Colour key refers to processed regularised log values. Biological replicates of male reproductive organs are designated as M1, M2 and M3; female organs are designated as F1, F2, F3.*

TABLE 1. SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES (DEG) (P_{ADJ}<0.05) IN MALE *vs.* FEMALE REPRODUCTIVE ORGANS

No.	PalmXplore Gene ID	Annotation	Log_2FC	p_{adj}
1	p5.00_sc00243_p0006	MADS-box transcription factor 2-like (*EgGLO2*)	2.21	7.58E-20
2	p5.00_sc00051_p0055	MADS-box transcription factor 2-like (*EgGLO1*)	2.17	1.03E-21
3	p5.00_sc00009_p0237	annexin-like protein RJ4-like	2.03	1.13E-10
4	p5.00_sc00322_p0006	MADS-box transcription factor DEF-like protein (*EgDEF1*)	1.78	1.58E-18
5	p5.00_sc00147_p0002	abscisic acid 8-hydroxylase 1-like	1.61	6.10E-07
6	p5.00_sc00011_p0146	ethylene-responsive transcription factor ERF012-like	1.54	2.83E-04
7	p5.00_sc00027_p0086	probable 1-deoxy-D-xylulose-5-phosphate synthase chloroplastic-like	1.43	8.49E-04
8	p5.00_sc01187_p0001	baculoviral IAP repeat-containing protein 2-like	1.42	3.16E-04
9	p5.00_sc00014_p0139	GDSL esterase lipase at5g55050-like	1.40	4.91E-07
10	p5.00_sc00124_p0013	protein ARGONAUTE 10-like	1.40	1.49E-06
11	p5.00_sc00009_p0022	respiratory burst oxidase homolog protein b-like	1.32	2.66E-06
12	p5.00_sc00154_p0049	formimidoyltransferase-cyclodeaminase-like	1.22	1.20E-02
13	p5.00_sc04517_p0001	7-deoxyloganetin glucosyltransferase	1.16	4.90E-02
14	p5.00_sc00085_p0048	isoflavounoid malonyl transferase	1.15	1.60E-02
15	p5.00_sc00007_p0167	NAC domain-containing protein 29-like	1.14	1.42E-04
16	p5.00_sc00106_p0020	homeobox-leucine zipper protein *ATHB*-40-like	1.11	1.42E-04
17	p5.00_sc00014_p0059	thaumatin-like protein 1-like	1.11	4.34E-02
18	p5.00_sc00135_p0079	gibberellin 2-beta-dioxygenase 1-like (*GA 2-OXIDASE*)	1.07	6.10E-07
19	p5.00_sc00147_p0015	stearoyl-[acyl-carrier-protein] 9-desaturase, chloroplastic	1.01	4.28E-02
20	p5.00_sc00133_p0062	glutamate decarboxylase 1-like	0.93	9.79E-03
21	p5.00_sc00141_p0018	probable cysteine desulphurase-like	0.93	2.07E-04
22	p5.00_sc00074_p0015	protein ARGONAUTE 10-like	0.86	7.64E-03
23	p5.00_sc00045_p0152	patellin-5 isoform 1	0.81	2.63E-02
24	p5.00_sc00019_p0190	probable WRKY transcription factor 11-like	0.79	4.28E-02
25	p5.00_sc00039_p0126	1-aminocyclopropane-1-carboxylate oxidase 1-like (*ACC OXIDASE*)	0.79	3.16E-04
26	p5.00_sc00871_p0002	1-acyl-*sn*-glycerol-3-phosphate acyltransferase-like	0.79	3.16E-04
27	p5.00_sc00104_p0023	ABC transporter G family member 32-like	0.77	5.27E-03
28	p5.00_sc00036_p0056	PREDICTED: uncharacterised protein LOC100264520	0.76	6.29E-03
29	p5.00_sc00042_p0103	plasma membrane ATPase 4-like	0.68	8.83E-03
30	p5.00_sc00018_p0053	p-loop containing nucleoside triphosphate hydrolases superfamily protein	0.67	1.55E-02

TABLE 1. SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES (DEG) (P_{ADJ}<0.05) IN MALE *vs.* FEMALE REPRODUCTIVE ORGANS (continued)

No.	PalmXplore Gene ID	Annotation	Log_2FC	p_{adj}
31	p5.00_sc00023_p0064	*SQUAMOSA PROMOTER-BINDING-LIKE* protein 7-like	-0.78	3.16E-04
32	p5.00_sc00014_p0039	PREDICTED: uncharacterised protein LOC100243848	-0.84	1.02E-02
33	p5.00_sc00050_p0193	protein *CUP-SHAPED COTYLEDON* 2-like	-0.87	1.20E-02
34	p5.00_sc00076_p0054	uncharacterised LOC101206485	-0.98	1.43E-02
35	p5.00_sc00001_p0475	uncharacterised partial	-1.04	1.02E-02
36	p5.00_sc00001_p0474	uncharacterised partial	-1.06	6.49E-03
37	p5.00_sc00080_p0036	homeobox protein SBH1-like	-1.08	6.42E-03
38	p5.00_sc00001_p0473	nuclear factor *NO VEIN*	-1.13	7.74E-03
39	p5.00_sc01636_p0001	protein *TERMINAL EAR1*-like	-1.13	4.28E-02
40	p5.00_sc01260_p0002	TPR and ankyrin repeat-containing protein 1-like	-1.22	1.66E-03
41	p5.00_sc00002_p0446	MADS-box transcription factor 6-like (AGL6/13-like)	-1.26	6.29E-03
42	p5.00_sc00050_p0129	uncharacterised LOC101223218	-1.30	3.16E-04

Note: DEG are arranged in descending order based on Log_2 fold-change (Log_2FC) values.
Positive Log_2FC value indicates higher relative expression in male reproductive organs.

TABLE 2. SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES (DEG) (0.05<P_{ADJ}<0.1) IN MALE *vs.* FEMALE REPRODUCTIVE ORGANS

No.	PalmXplore Gene ID	Annotation	Log_2FC	p_{adj}
1	p5.00_sc00223_p0016	homeobox-leucine zipper protein *ATHB*-40-like	1.11	5.10E-02
2	p5.00_sc00002_p0113	pyruvate kinase isozyme chloroplastic-like	1.10	5.24E-02
3	p5.00_sc00606_p0010	UDP-glycosyltransferase 85A2-like	1.10	7.69E-02
4	p5.00_sc00031_p0147	TCP transcription factor 22	1.08	8.40E-02
5	p5.00_sc00002_p0190	homeobox-leucine zipper protein HAT4-like	1.06	8.73E-02
6	p5.00_sc00218_p0013	UPF0481 protein At3g02645-like	1.04	8.40E-02
7	p5.00_sc00015_p0026	U-box domain-containing protein 16-like	0.98	6.37E-02
8	p5.00_sc00342_p0001	cyclin-U1-1	0.98	7.80E-02
9	p5.00_sc00047_p0003	vacuolar-sorting receptor 3-like	0.96	7.28E-02
10	p5.00_sc00001_p0930	transcription factor bHLH145-like	0.92	9.73E-02
11	p5.00_sc00018_p0049	probable membrane-associated kinase regulator 2-like	0.91	9.98E-02
12	p5.00_sc00008_p0129	AP2 ERF and B3 domain-containing transcription repressor TEM1-like	0.87	9.98E-02
13	p5.00_sc00096_p0048	transcription factor bHLH48-like	0.82	8.40E-02
14	p5.00_sc05725_p0001	cysteine proteinase inhibitor 1-like	0.78	6.46E-02
15	p5.00_sc00016_p0210	GATA transcription factor 5-like	0.74	6.37E-02
16	p5.00_sc00010_p0141	copper-transporting ATPase RAN1-like	0.73	6.46E-02
17	p5.00_sc00018_p0155	diacylglycerol *o*-acyltransferase 1-like	0.71	6.37E-02
18	p5.00_sc00121_p0010	pyruvate decarboxylase isozyme 2-like	0.70	8.40E-02
19	p5.00_sc00008_p0030	LOB domain-containing protein 41-like	0.68	7.56E-02
20	p5.00_sc00054_p0104	sucrose transport protein SUT1-like	0.68	6.61E-02
21	p5.00_sc00332_p0022	WEB family protein chloroplastic-like	0.57	8.73E-02
22	p5.00_sc00099_p0082	PREDICTED: uncharacterised protein LOC101765505	-0.60	6.46E-02
23	p5.00_sc00010_p0123	leucine-rich repeat-containing protein DDB_G0290503-like isoform X1	-0.63	9.98E-02
24	p5.00_sc00049_p0017	protein SET-like	-0.70	9.28E-02
25	p5.00_sc00001_p0136	auxin-responsive protein IAA26-like	-0.71	7.56E-02
26	p5.00_sc00722_p0001	protein IQ-domain 1-like	-0.78	6.81E-02
27	p5.00_sc00069_p0129	auxin-regulated protein	-0.97	7.56E-02
28	p5.00_sc00075_p0103	lysosomal alpha-mannosidase-like	-1.00	8.40E-02
29	p5.00_sc00313_p0009	protein LHCP translocation defect-like	-1.02	6.46E-02
30	p5.00_sc00123_p0017	1-aminocyclopropane-1-carboxylate synthase 3-like	-1.06	5.24E-02
31	p5.00_sc00001_p0607	MADS-box transcription factor 27-like (AGL16-like)	-1.09	5.24E-02
32	p5.00_sc00059_p0084	MADS-box transcription factor 32 (CAULIFLOWER-like)	-1.12	6.37E-02
33	p5.00_sc00048_p0029	MYB family transcription factor APl-like	-1.12	6.46E-02
34	p5.00_sc00071_p0128	protein AFG1-like	-1.14	5.73E-02
35	p5.00_sc00046_p0050	floricaula *LEAFY* homolog	-1.15	5.35E-02

Note: DEG are arranged in descending order based on Log_2 fold-change (Log_2FC) values.
Positive Log_2FC value indicates higher relative expression in male reproductive organs.

DISCUSSION

Through detailed anatomical analysis, Adam *et al.* (2005) showed that the androecium and gynoecium forms concomitantly at stage three development. In pistillate flower development at this stage, the primordia of reproductive organs rapidly lose their meristematic potential after initiation, leading to the final formation of a gynoecium of three carpels, surrounded by staminodes, *i.e.* rudimentary stamens (Adam *et al.*, 2007a; 2005).

While Ooi *et al.* (2019) mainly reported on the transcriptome differences associated with the mantling abnormality of the reproductive organs, this study explored in further detail on the differences between normal male and female reproductive organs and identified genes involved in normal development of the third and fourth floral whorls. The staminode transcriptome was compared with the transcriptome of the adjacent early developing carpels at stage three flower development, a stage which essentially involves initiation and early development of the reproductive organs (Adam *et al.*, 2007a). As expected, the B-type MADS-box genes, *EgDEF1*, *EgGLO1* and *EgGLO2* were highly expressed in staminodes. These MADS-box genes, together with *SEPALLATA* and *AGAMOUS* genes are important for stamen specification and development (Sablowski, 2015). The oil palm B-type MADS-box genes have been extensively characterised previously (Adam *et al.*, 2007b; Beulé *et al.*, 2011; Jaligot *et al.*, 2014). Hypomethylation of the *karma* transposon in *EgDEF1* is associated with the mantled flowering/fruit phenotype, which exhibits feminisation of the staminodes in mantled female inflorescences (Ong-Abdullah *et al.*, 2015). Lower expression of *EgDEF1* has also been detected in abnormal staminodes at stage three floral development (Ooi *et al.*, 2019).

Orthologues of the *DEFICIENS/GLOBOSA* (*DEF/GLO*) genes, the *AP3/PI* complex in *Arabidopsis*, indirectly or directly regulates 47 target genes, comprising only two transcription factors (Zik and Irish, 2003). *NAP*, a member of the *NAC* gene superfamily, is a target of the *AP3/PI* complex (Sablowski and Meyerowitz, 1998). A *NAC* gene (p5.00_sc00007_p167) was expressed relatively higher in staminodes and it may be worth to further investigate in future whether this is the *NAP* orthologue target of *EgDEF1/EgGLO*. *NAP* is involved in the transition between cell division and cell expansion during stamen and petal development (Sablowski and Meyerowitz, 1998). In addition, the *ATHB-40* homeobox leucine zipper orthologue was expressed higher in staminodes. Expression of the *ATHB-40* gene was found up-regulated in open flowers of almond plants, together with other homeobox-leucine zipper genes (Hosseinpour *et al.*, 2018).

Increased expression of *GA 2-OXIDASE* suggested increased gibberellin metabolism (Sakamoto *et al.*, 2004) in staminodes, thus inferring that decreased endogenous bioactive gibberellins is associated with male reproductive organ development in oil palm female inflorescences. Gibberellic acid (GA) treatment promotes male flower development in several plants including cucumber and can rescue developmentally arrested stamens (Jacobsen *et al.*, 1994; Pimenta Lange *et al.*, 2012). GA is known to negatively regulate several genes in rice including *TEOSINTE BRANCHED 1* (*TB1*) (Lo *et al.*, 2008). Incidentally, a putative *TB1* oil palm orthologue was highly expressed in staminodes ($p_{adj}<0.1$), supporting the hypothesis that bioactive gibberellin levels are lower in this organ at this developmental stage. In addition, increased expression of *ACC OXIDASE* which catalyses the final step of ethylene biosynthesis was detected in staminodes. Therefore, the inferred low gibberellin and high ethylene levels may be required leading to the impending arrest of staminode development in female inflorescence, as ethylene has been shown to promote carpel development but arrests stamen development in cucumber and melon (Chen *et al.*, 2016). Moreover, overexpression of the cucumber *ACC OXIDASE*, *CsACO2*, arrested stamen development (Duan *et al.*, 2008). Sex determination of female flowers in melon, cucumber and zucchini, require not only a higher ethylene production at early developmental stages, but also for the maintenance of stamen arrest for the proper development of female flowers (Manzano *et al.*, 2014).

LEAFY, a key floral meristem identity gene, was up-regulated in developing carpels ($p_{adj}<0.1$), similar to the expression pattern detected in pseudocarpel initials (Ooi *et al.*, 2019). *LEAFY* expression in *Arabidopsis* is high in the floral meristem during flower development and its expression gradually diminishes to the centre of the flower (Blázquez *et al.*, 1997; Wagner *et al.*, 2004). Although *LEAFY* and *UNUSUAL FLORAL ORGANS* (*UFO*) initially induces *AP3* and *PI*, *AP3* and *PI* later maintain their expression in a self-regulatory loop (Honma and Goto, 2000). This may explain the high expression of *EgDEF1* and *EgGLO1/2* in the staminodes while *LEAFY* expression is instead relatively higher in the developing carpels and the floral meristem, located at the center of the flower.

Other than *LEAFY*, putative orthologues of *SOYABEAN HOMEOBOX-CONTAINING GENE 1* (*SBH1*), *CUC2* and *SQUAMOSA PROMOTER-BINDING-LIKE 7* (*SPL7*) were also highly expressed in developing carpels ($p_{adj}<0.05$). The *Glycine max SBH1* belongs to the *KNOTTED-LIKE HOMEOBOX* (*KNOX)* family and is highly similar in sequence to *SHOOT MERISTEMLESS* (*STM*) of *Arabidopsis thaliana* (Tioni *et al.*, 2003). *CUC* genes and *STM* are important regulators of meristem and organ boundary specification and play a pivotal role at both vegetative and reproductive stages (Spinelli *et al.*, 2011). These genes have been associated with cytokinin for gynoecium development in persimmon (Yang *et al.*, 2019). In rice, *SPL* genes are involved in regulating inflorescence structure by controlling spikelet meristem identity (Jiao *et al.*, 2010; Miura *et al.*, 2010).

In rice and several grass species, the homeotic function for carpel specification is attributed to *DROOPING LEAF* (*DL*), a YABBY-domain protein (Alvarez-Buylla *et al.*, 2010; Nagasawa *et al.*, 2003). It is possible that carpel specification genes such as *DL* are not differentially expressed yet at this stage in the developing carpels though a *DL* orthologue was up-regulated in pseudocarpel initials (Ooi *et al.*, 2019). This may infer that carpel specification was taking place in pseudocarpel initials at this flower developmental stage, but not likewise in normal carpel initials.

CONCLUSION

LCM technique was able to provide specific transcriptomes of the reproductive organs, thereby increasing sensitivity in the detection of DEG. Although previous studies have implicated the involvement of the B-type MADS-box genes, *EgDEF1* and *EgGLO1/2*, in floral development, specific transcriptomes of the reproductive organs themselves strengthens the previous evidence that *EgDEF1* and *EgGLO1/2* are highly expressed in staminodes at the stage whereby male floral organ development takes place. These stamen specification genes are expressed in the staminodes at this developmental stage even though development will halt soon after this. In addition, the impending arrest in the development of the staminodes may be accompanied by increased ethylene biosynthesis and decreased levels of bioactive gibberellins. Evaluation of the hormone levels themselves in the staminodes should be conducted in future for verification. It may also be useful to further investigate the involvement of these two phytohormones in stamen development of the male inflorescence. The differentially expressed transcription factors identified in this study also provide candidate genes involved in reproductive organ development for further studies. As some of these genes are members of gene superfamilies such as *NAC*, Homeodomain Leucine Zipper (HD-Zip) and MADS-box, the DEG identified allows investigation into the specific gene member involved in floral organ development.

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