Transcriptional regulators of oil palm somatic embryogenesis

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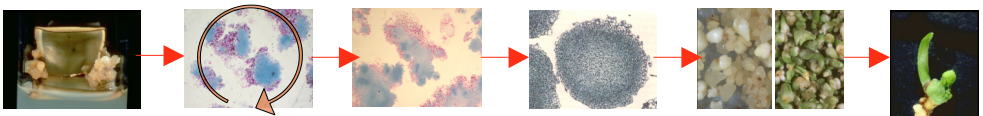
Introduction

Oil palm is a monocotyledonous perennial plant, which is the first source of edible vegetable oil worldwide. The elite genotypes of this species are propagated by *in* somatic embryogenesis (SE) (Fig.1). To understand the developmental regulation during SE and improve the quality of embryo production, we are investig transcriptional regulators (TRs) and chromatin-related (CR) genes associated with embryogenic potential and embryo development (Fig.2). We have isolated a number palm cDNAs with similarity to a range of transcription factor family genes and characterised their expression (Fig. 3-6). However to elucidate further the developm regulation that controls embryogenesis, we have constructed a series of suppression subtractive hybridisation (SSH) libraries corresponding to key stages of embryoge and selected putative TRs and CR ESTs to be used in macroarray based gene expression (Fig. 7).

Indirect Somatic Embryogenesis Culture of Oil Palm (*Elaeis guineensis* Jacq.)



[+ 2,4-D] Callogenesis (Initiation of embryogenic cells)

[+ 2,4-D] Embryogenic cell maintenance (SC) (Simultaneous multiplication of embryonic cells and inhibition of cell differentiation)

[- 2,4-D] Initiation of the embryogenic pathway (redifferentiation)

Protoderm formation (SEI) (axial polarization)

Embryo development (SE II and III) (Meristem organization and organogenesis) ES II

Shoot formation

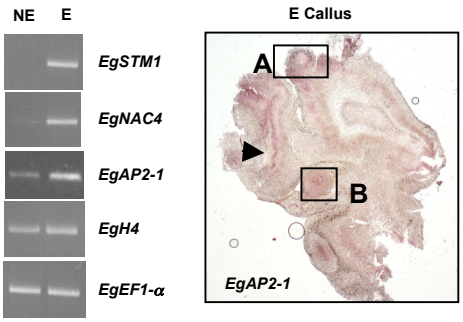
Fig. 1. The key stages of somatic embryogenesis (SE) of oil palm. The formation of embryogenic cells requires a callogenesis step prior to the initiation of embryo development. Embryogenic cells are maintained in culture in a developmentally arrested state in the presence of 2,4-D. The embryo developmental pathway is initiated by the removal of 2,4-D from the culture medium.

Oil palm	Arabidopsis	Rice
EgSTM	*STM*	*OSH1*
EgNAC3	*CUC3*	*OSJNBa0016N23*
EgNAC4	*CUC1-CUC2*	*ONAC072*
EgNAC5	*ANAC101*	*ONAC056*
EgAP2/1	*BBM / PLT*	*OS BNM3lk*
EgbZIPA1	*AREB3*	*AREB3*
EgAP2D2	-	*DBF2*
EgCHD3	*PICKLE/GYMNOS*	*CHD3*

Fig. 2. Oil palm cDNAs encoding KNOX, NAC, AP2, bZI CHD3 TRs and closest-related *Arabidopsis* and rice genes.

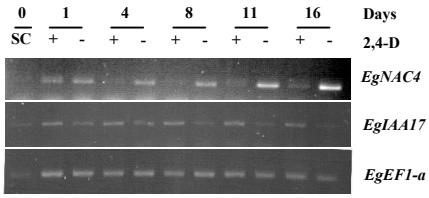
Differential Gene Expression associated with embryogenic potential

Fig. 3. RT-PCR gene expression analysis of TF candidates in non-embryogenic (NE) and embryogenic (E) calli from the same line. The transcripts for *EgSTM*, *EgNAC4* and *EgAP2-1* preferentially accumulate in callus with embryogenic potential. *In situ* hybridisation with *EgAP2-1* reveals a higher mRNA accumulation in the **E** callus within the inner dense zone (↓) from which embryos will emerge (A, B).



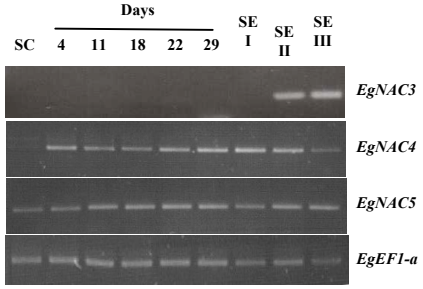
Differential Gene Expression During the Initiation of the Embryonic Pathway

Fig. 4. RT-PCR gene expression analysis of auxine regulated genes during the first stage of somatic embryo differentiation. The transcripts for the *EgNAC4* gene increase upon initiation of the embryogenic pathway once 2,4-D is removed. In contrast, the transcript for the *EgIAA17* gene, that encodes a protein similar to the auxin induced IAA17 transcription factor, decreases after removal of 2,4-D.



Differential Gene Expression of NAC Genes During *In Vitro* Culture

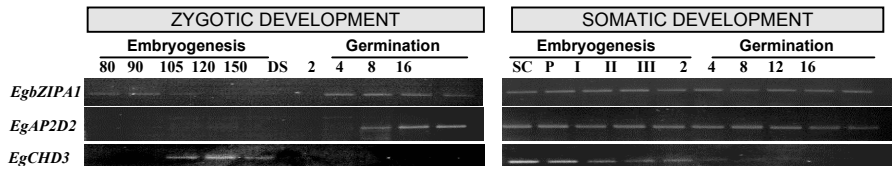
Fig. 5. RT-PCR gene expression analysis of NAC genes during oil palm SE. The *EgNAC3* gene transcript is not detected in suspension cells grown in the presence of 2,4-D (SC), while the *EgNAC4* gene transcript is barely detectable. In contrast, the *EgNAC5* gene transcript is detected in suspension cells grown with or without 2,4-D and throughout SE.



Legend

SC:	suspension cells grown with 2,4-D	
4-29:	4 to 29 days after elimination of 2,4-D	Liquid media
SEI:	somatic embryos stage I	
SEII:	somatic embryos stage II	Solid media
SEIII:	somatic embryos stage III	

Deregulation of TF expression during SE and germination



Putative TRs and CR gene familie represented in oil palm embryogene derived cDNA libraries

Transcription and Chromatin-Related Gene or Domain Family	# of ESTs	Funct Cate
C2H2 (Zn)	11	TF
SW12/SNF2	8	CR
WD-40	6	TR/C
SET	6	CR
Homeobox	4	TF
NAC	4	TF
AUX/IAA	3	TR
MYB	3	TF
C3HC4 RING finger (Zn)	3	TF/C
bZIP	2	TF
AP2/ERF	1	TF
B3	1	TF
CAMTA (calmodulin)	1	TF
GRAS	1	TF
LIM	1	TF/C
RAM	1	TF
Bromodomain	1	CR
Other CR genes (e.g. helicases, DNA repair, replication, condensation, nucleolar-related)	15	CR
other undefined TF or TR genes	4	TF
Total	76	-

Fig. 7. The total of 76 cDNAs were selected f approximately 1,000 ESTs. The cDNA libraries were constru at the following stages: cell suspension with 2,4-D; cell suspens without 2,4-D for 14 days; somatic embryos stage I; mature zyg embryos. TF: Transcription Factor with DNA binding domain ; : Transcription Regulators with Protein-Protein interaction dom CR : Chromatin-related proteins.

Conclusions and Perspectives

As a first step towards determining the molecular of oil palm SE, we investigated the expression palm putative TFs, several of which play a role i initiation of embryonic development and formati the shoot apical meristem. Gene expression ana revealed that the expression of *EgNAC4* is cor with the first stage of embryo differentiation and be associated with changes in auxin re transcriptional activity. *EgSTM EgNAC4* and *EgA* expression appears to be associated with embryogenic potential of the callus. The expressi several TR genes normally expressed d germination are deregulated during SE. deregulation of TR expression may be linked t premature germination of somatic embryos. In