In vitro somatic embryogenesis (SE) is the only viable approach to multiply elite genotypes of oil palm, a tropical perennial monocot species that is the leading source of vegetable oil worldwide. The molecular basis of SE even in model plant species is not well understood, and no single techniques with individual crop species need to be developed for comparison. To begin to determine the molecular basis of SE in oil palm, we have initiated complementary approaches including the construction of suppression subtractive hybridization libraries corresponding to key developmental stages, the development of an oil palm EST database, and macroarray expression analysis. In the present study we have selected from our database 227 ESTs that encode putative regulatory related products (e.g. transcription and chromatin remodelling factors and other proteins related to gene regulation, hormone metabolism and development) and examined their corresponding transcript abundance during the initiation of somatic embryo development. We have selected and validated the expression of a subset of these genes by RT-PCR and their possible significance in somatic embryo development will be discussed.

**Somatic Embryo Development is Triggered by the Depletion of 2,4-D**

Differential Gene expression during the initiation of oil palm (Elaeis guineensis Jacq.) somatic embryo development

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**Introduction** In vitro somatic embryogenesis (SE) is the only viable approach to multiply elite genotypes of oil palm, a tropical perennial monocot species that is the leading source of vegetable oil worldwide. The molecular basis of SE even in model plant species is not well understood, and no single techniques with individual crop species need to be developed for comparison. To begin to determine the molecular basis of SE in oil palm, we have initiated complementary approaches including the construction of suppression subtractive hybridization libraries corresponding to key developmental stages, the development of an oil palm EST database, and macroarray expression analysis. In the present study we have selected from our database 227 ESTs that encode putative regulatory related products (e.g. transcription and chromatin remodelling factors and other proteins related to gene regulation, hormone metabolism and development) and examined their corresponding transcript abundance during the initiation of somatic embryo development. We have selected and validated the expression of a subset of these genes by RT-PCR and their possible significance in somatic embryo development will be discussed.

**Differential Gene Expression During the Initiation of Somatic Embryo Development**

**Developmental Regulator Macrocarray Approach**

- **Oil Palm EST Database** (sequences available: 5149)
- **BLAST/Key Word Search**
- **Regulatory Related ESTs** (e.g. transcription factors, hormones, chromatin remodeling etc... 227 total)
- **EST cDNA Amplification by PCR**
- **PCR Product Spotted on Nylon Membranes**
- **Macrocarray Hybridization** (Hybridization with labeled cDNAs)

**Data Analysis**

**RT-PCR expression analysis Validation**

**Macroarray Construction Strategy:** To identify regulatory genes differentially expressed during the initiation of embryogenesis and development from oil palm that could be regulated by the depletion of 2,4-D, we selected from the oil palm EST database which contained 5149 ESTs from cDNA libraries constructed from different key developmental stages of somatic and zygotic embryogenesis, shoot apex and flowers. For the hybridizations, two biological replicates were done with cDNAs made from T0, T16+ and T16- total RNA. Images were analyzed using ArrayVision software and the significance of differential expression was evaluated using the analysis of variance (ANOVA) test.

**Conclusions and Perspectives**

Physiological (decrease in free IAA) and molecular (differential transcript accumulation) changes occur by 16 days in cells initiated to develop somatic embryos. 27.8 sporulation frequency occurred prior to 2,4-D removal (T16+). Several of the genes found to be differentially expressed indicate changes in auxin, gibberelin, and ABA response or metabolism are occurring in cells initiated to develop embryos. The combination of macroarray and RT-PCR analysis enables the selection of candidate genes differentially expressed during the initiation of embryo development for functional studies related to changes in hormone physiology and coordinated gene expression.

**Free IAA Content Decreases During the Initiation of Embryo Development**

**Fig. 1. The initiation of somatic embryo development.** Oil palm embryogenic cells are maintained in liquid culture for 30 days (A, T0 cells) in a developmentally arrested state in the presence of 2,4-D (2,4-Dichlorophenoxyacetic acid) and remain undifferentiated when plated on solid medium for 24 days without 2,4-D (B). Cells grown subsequent to 16 days in liquid culture without 2,4-D (T16-) cells show no noticeable morphological differences from T0 cells, however, at the end of the 30 day cycle when plated on solid medium for 24 days without 2,4-D, embryogenic (D).

**Fig. 2. Gas chromatography-mass spectrometry analysis of free IAA in different cell types.** Free IAA amounts decrease in cells grown in liquid culture without 2,4-D for 16 days (T16- cells) while remain unchanged in cells grown in the presence of 2,4-D (T16+ cells).

**Fig. 3. RT-PCR expression analysis of genes during the first 16 days of the initiation of somatic embryo development.** Previous results showed that the EgIAA2 putative transcription factor gene increased during the initiation of embryogenesis while the transcript for EgIAA2 encoding an AUX/IAA transcriptional regulator similar to IAA17 from Arabidopsis thaliana, decreased during the first 16 days after removal of 2,4-D.

**Fig. 4. Macroarray construction strategy:** To identify regulatory genes differentially expressed during the initiation of embryogenesis and development from oil palm that could be regulated by the depletion of 2,4-D, we selected from the oil palm EST database which contained 5149 ESTs from cDNA libraries constructed from different key developmental stages of somatic and zygotic embryogenesis, shoot apex and flowers. For the hybridizations, two biological replicates were done with cDNAs made from T0, T16+ and T16- total RNA. Images were analyzed using ArrayVision software and the significance of differential expression was evaluated using the analysis of variance (ANOVA) test.

**Fig. 5. RT-PCR expression analysis of selected candidates.** Validation of macroarray data was based on cDNAs isolated from two biological replicates (RT-PCR1 and 2). The transcript profile of 11 genes selected for validation, in addition to EgEF1-α1 as a housekeeping control, could be divided into five groups based on their transcript accumulation in T0, T16- and T16+ cells. In profile A, EgEF1-α1 accumulated in T0 and T16+ cells in both replicates. In contrast, the transcript abundance of EgEF1-α1 was either undetected or less abundant in T16- cells. In profile B, a total of six transcripts including EgSAP1, EgGPI, EgPGC, EgDRH2, EgABC1 and EgGACO1 accumulated preferentially in T16- cells in both replicates. Four of these genes, EgSAP1, EgGPI, EgPGC and EgDRH2, had higher expression in T16- than in T0 and T16+ cells in both RT-PCR1 and 2. In contrast, transcript amounts for EgABC1 and EgGACO1 were variable in the two biological replicates in T16- cells (profile C). The EgSAM1 transcript was unique (profile D), with similar accumulation in T16- and T16+ cells and low or undetectable in T0 cells. Finally, profile E included constitutively expressed genes EgGSH1, EgERF1 and EgEF1-α1 in T0, T16- and T16+ cells. The transcript abundance of EgGSH1 and EgEF1-α1 was as expected, similar in all the treatments, whereas expression profiles of the genes EgGSH1 and EgERF1 were different from those observed in the macroarray analysis. RT-PCR analysis validated the macroarray transcript profiles for 9 of the 11 genes examined with EgGSH1 and EgERF1 the exceptions. Both the macroarray and RT-PCR results indicate that the expression profiles for T0 and T16- cells are less variable than with T16+ cells.

**Table 1. Functional categories of ESTs corresponding to genes with reproducible expression profiles in T0 and T16- cells.** The identification of genes differentially expressed after removing suspension cells (T0) into medium without 2,4-D for 16 days (T16- cells) may provide candidates involved in the initiation mechanisms of somatic embryo development. A total of 27 transcripts increased in T16- cells (P<0.05 and ratio > 2), while 54 transcripts decreased (P<0.05 and ratio < 0.5). The majority (112) of the transcripts had similar abundance in T0 and T16+ cells. Several of these genes differentially expressed were related to hormone response or metabolism including auxin, gibberellin (GA) and abscisic acid (ABA). From the 227 ESTs initially selected, 115 corresponded to transcripts with inconsistent abundance between the two biological replicates and were eliminated from further analysis. Only the results for genes with expression patterns that were confirmed in both replications are presented.

**Table 2. Functional categories of ESTs corresponding to genes with reproducible expression profiles in T0 and T16- cells.** The identification of genes differentially expressed after removing suspension cells (T0) into medium without 2,4-D for 16 days (T16- cells) may provide candidates involved in the initiation mechanisms of somatic embryo development. A total of 27 transcripts increased in T16- cells (P<0.05 and ratio > 2), while 54 transcripts decreased (P<0.05 and ratio < 0.5). The majority (112) of the transcripts had similar abundance in T0 and T16+ cells. Several of these genes differentially expressed were related to hormone response or metabolism including auxin, gibberellin (GA) and abscisic acid (ABA). From the 227 ESTs initially selected, 115 corresponded to transcripts with inconsistent abundance between the two biological replicates and were eliminated from further analysis. Only the results for genes with expression patterns that were confirmed in both replications are presented.