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ISOLATION AND CHARACTERIZATION OF A PUTATIVE SERINE/THREONINE KINASE EXPRESSED DURING OIL PALM TISSUE CULTURE

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ABSTRACT

One of the key processes hindering the efficiency of oil palm tissue culture is its poor embryogenesis rate and therefore efforts to pursue improvements in this area remain essential. EgPK1, *a homolog of the mammalian* STK16, *was isolated from oil palm embryogenic suspension cultures through cold plaque screening. Up-regulation of* EgPK1 *transcripts was observed in embryogenic calli relative to its non-embryogenic counterpart. Quantitative PCR also demonstrated similar results. A 5'RACE fragment was found to be different from the original* EgPK1 *sequence, with the variation unique to the first 115 nucleotides at the 5'region, suggesting alternative splicing events. Quantitative PCR analyses suggested that expression profiles of the variants across different developmental stages were similar to each other. As hypothesized in the mammalian systems,* EgPK1 *may serve a similar role in disruption of the extracellular matrix surrounding the proembryogenic masses during somatic embryogenesis.*

Keywords: kinase, EgPK1, STK16, somatic embryogenesis.

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INTRODUCTION

With the high demand for palm oil, increasing the oil yield is the main priority for the oil palm industry. Although research in plant genetics and breeding has largely improved the performance of the crop, current conventional breeding and seed production methods may still be insufficient to realize the maximum potential of the selected hybrid genotypes (Paranjothy, 1989; Duval *et al*., 1995; Rajanaidu *et al*., 1997). Therefore, vegetative propagation of the oil palm via tissue culture is an attractive alternative with a huge ready market for tissue culture plantlets worldwide.

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Through tissue culture, planting materials are more uniform, leading to a potential increase in yield of up to 30% compared to seedlings (Hardon *et al*., 1987; Soh, 1986). However, one of the major obstacles to the development of an economically efficient propagation system is the low frequency of embryogenesis. Most tissue culture laboratories still report average embryogenesis rate of only 6% (Wooi, 1995). Despite its economic importance, little is known about the biology of oil palm somatic embryogenesis.

Many of the earlier studies on somatic embryogenesis of the oil palm were on the development of methodologies for the initiation and production of somatic embryos (Ahee *et al*., 1981; Jones, 1974). These groups mainly manipulated the phytohormones in their media and identified tissues with better clonability. Histological analysis of somatic embryogenesis from leaf-derived callus was able to detail the emergence of callus and the subsequent formation of somatic embryos (Schwendiman *et al*., 1988).

The tissue culture process, being very much influenced by environmental conditions, will involve

the triggering of elements that perceive these changes. At any one time, plant cells experience a mass of external signals that can arise from abiotic related influences such as mechanical, temperature, mineral, light, gaseous, wound, water, physical and electrical effects (Gilroy and Trewavas, 1990). Internally, they are subjected to signals from a stream of growth regulators, peptides, sugars and other metabolites, steroids, wall fragments, turgor-related and wall-imposed mechanical signals from other cells and further electrical information. Hence, signal transduction is used by plants to coordinate their development and to respond to endogenous or exogenous stimuli. Proteins involved in signal transduction pathways such as protein kinases are most likely activated.

In order to unravel the complex process of oil palm somatic embryo development, genetic approaches were used in an attempt to generate molecular markers. Such markers will aid in understanding plant development, and the subsequent analysis of their regulation will greatly facilitate improvements in *in vitro* systems to hopefully enhance the somatic embryogenesis process. Thus, study of protein kinases that may be involved in signal transduction pathways during oil palm somatic embryogenesis was carried out. In this study, *EgPK1*, a gene encoding the putative protein kinase homolog of the mammalian STK16, was isolated from oil palm. Expression studies were conducted and its role in embryogenesis hypothesized.

MATERIALS AND METHODS

Plant Materials

All tissue culture materials, including oil palm embryogenic suspension cultures, embryogenic and non-embryogenic cultures (clones/lines; 282, 283, 291, 293, 294, 295) were kindly provided by Applied Agricultural Research Sdn Bhd, Malaysia. Zygotic embryos were excised from oil palm fruits at two stages of growth [15 weeks after anthesis (WAA) and 16 WAA], kindly provided by the Oil Palm Research Station, Sime Darby Bhd, Malaysia.

Cold Plaque Screening

Total RNA extracted from oil palm embryogenic suspension cultures (Schultz *et al*., 1994) was used for poly A^+ RNA isolation with the PolyA Tract Isolation System (Promega) according to the manufacturer's instructions. The cDNA library was constructed using the ZAP-cDNA Synthesis Kit (Stratagene) according to the manufacturer's instructions. In the cold plaque screening procedure adapted from Hodge *et al*. (1992), the oil palm embryogenic suspension culture cDNA library was screened with a α–[^{32}P]dCTP (~3000 Ci m mol^{-1}; Amersham Biosciences) radiolabelled probe prepared from embryogenic suspension culture first-strand cDNA using the High Prime reaction mix (Roche) according to the manufacturer's instructions. The membranes were pre-hybridized and then hybridized with the probe in a hybridization buffer [5x SSPE, 5x Denhardt's solution, 0.5% (w/v) SDS, 100 μg ml^{-1} denatured herring sperm DNA] at 60°C. Low stringency washes (2x SSC, 0.1% SDS) at room temperature were carried out twice for 10 min each. The level of radioactivity between washes was measured using a hand-held Geiger counter. The membranes were exposed to autoradiography film (Agfa) with intensifying screen at -80°C for about two weeks. Approximately a thousand 'cold' plaques were randomly cored and *in vivo* excised. One of these clones, designated *EgPK1*, encoded a putative serine/threonine kinase.

Cloning and Sequence Analysis

For further manipulations, the PCR products of various regions derived from *EgPK1* plasmid were cloned into the pCR2.1-TOPO® vector (Invitrogen, Inc.). For nucleotide sequence analysis and further manipulation, template DNA was isolated using a standard alkaline lysis method (Sambrook *et al*., 1989). Sequencing was carried out using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, USA) with ABI Prism 377 DNA automated sequencer (Applied Biosystems, USA). Nucleotide sequences were compared to the non-redundant (NR) database using the BLAST algorithm (Altschul *et al*., 1997).

RACE Amplification of 5′ Upstream Regions

RACE PCR was performed according to the manufacturer's instructions (Marathon™ cDNA Amplification Kit, Clontech) with a starting template of approximately 4.5 μg suspension culture total RNA. The 5′RACE specific primer (5′-CCCTCACAAACTGTAACTGGTCCTCAC-3′), designed to nt. 355 to 381, was used together with the AP1 primer (Clontech) in PCR conditions of: five cycles of 94ºC, 30 s, 72ºC, 4 min; 5 cycles of 94ºC, 30 s, 70ºC, 4 min; 25 cycles of 94ºC, 20 s, 68ºC, 4 min.

Southern Analysis

For Southern hybridization, a specific probe that spans the region of 1051nt-1314nt was generated by PCR with primers 5′-TCCAGCCTCAACCTGCAGTC-3′ and 5′-GAAGATAATCTGTCATATATTTG-3′. This 263bp fragment was cloned into the pCR-II TOPO® vector (Invitrogen Inc.), verified by sequencing and used for the generation of radiolabelled probes with

α[^{32}P]dCTP for hybridization. Southern hybridization was performed as previously described (Montag *et al*., 1995).

Real Time Quantitative PCR

RACE PCR analyses suggested that there might be more than one transcript of EgPK1. Thus, real time quantitative PCR was conducted to analyse the differential expression profiles, with the assumption that there are two variants. Primers and TaqMan probe sets were designed and synthesized by Assay-By-Design® service (Applied Biosystems) for GAPDH while primers and FAM-labelled fluorogenic probes to distinguish the profiles of the EgPK1 variants were designed and synthesized by Bioneer, Korea. The primers and probe sequences were designed to the unique 5′-region of EgPK1 (nt.10-154) and the sequences were 5′-GGAGAGGGAGAGAGATAGAGAGAG-3′ (forward), 5′-CCGTTCACCGCGTTGTAGAG-3′ (reverse) and 5′-TGAGCCCTGAGAAGGAGCATCCCATC-3′ (FAM-labelled probe, designated EgPK1), respectively. Another set of primers and probe was designed to the common region (nt. 653-781) in both variants of EgPK1 and they were 5′-CGAATTTCCTTTCTTGCGGGCCGAGC-3′ (forward), 5′-TGCAATTGTAATGCCTCTGAACG-3′ (reverse) and 5′-CGAATTTCCTTTCTTGCGGGCCGAGC-3′ (FAM-labelled probe, designated EgPK); the primers for GAPDH were 5′-ACTGCTAC-TCAGAAGACTGTTGATG-3′ (forward), 5′-TGCTGCTAGGAATGATGTTAAAGCT-3′ (reverse) and 5′-ACCCCTCCAGTCCTTG-3′ (FAM-labelled probe). Contaminating DNA was removed from the RNA samples by RNAse-free DNAse1 (Roche) treatment. Subsequently, the purified RNA was analysed for its integrity and purity using the Agilent 2100 BioAnalyzer according to the manufacturer's instructions. RNA (2 μg) with a 28S:18S ratio higher than 1 was selected for cDNA synthesis using the High Capacity cDNA Archive kit (Applied Biosystems, USA) according to the manufacturer's instructions. Five microlitres of first strand cDNA (1:150 dilution with 0.1 mM EDTA) were used with all primer/probe sets for real-time PCR. PCR reactions of 25 μl were carried out according to the manufacturer's instructions. Real Time PCR was performed on the ABI Prism 7700 Sequence Detection System (Applied Biosystems, USA) in a 96-well reaction plate according to the manufacturer's recommendations for the PCR programme of 2 min at 50ºC; 10 min at 95ºC; and 40 cycles of 95ºC for 15 s and 60ºC for 1 min. Each PCR reaction was performed in triplicate with no template controls included. The Comparative C_t method of analysing the real time PCR data was conducted to compare the transcript levels of a particular variant across various tissues.

RESULT

Isolation of EgPK1

Cold plaque screening of embryogenic suspension culture cDNA library with embryogenic suspension culture first-strand cDNA as a probe generated approximately 1% hybridization signals. Approximately 1000 cold plaques that did not produce signals were randomly isolated, screened and sequenced. The analysis of cold plaque ESTs from embryogenic suspension cultures will be reported elsewhere (Ho *et al*., 2007). The cold plaque ESTs with homology to regulatory genes, *e.g*. transcription factors and signal transduction genes, were randomly selected for further characterization studies and one of these was designated *EgPK1*, a putative serine/threonine kinase gene.

Sequence Analysis of *EgPK1*

EgPK1 contained a transcript of 1314 nucleotides with an ORF encoding a putative polypeptide of 346 amino acids. The nucleotide and deduced amino acid sequences of EgPK1 are shown in *Figure 1*. The EgPK1 was 75% similar to ATPK3, a serine/threonine kinase from *Arabidopsis thaliana* (Bonham-Smith, 1996). The ATPK3 was grouped with a relatively novel subfamily of serine/threonine kinases which includes the mammalian STK16/Krct/PKL12 (Ligos *et al*., 1998; Stairs *et al*., 1998; Kurioka *et al*., 1998; Berson *et al*., 1999; Ohta *et al*., 2000).

In recent years, putative ATPK3/STK16 orthologues have also been isolated from grape (Acc. CAN66918), cotton (Acc. AAZ39949) and cucumber (Acc. CAI30891). The *EgPK1* also contains a dinucleotide simple sequence repeat (SSR) as suggested by the presence of d(GA) repeats in the 5′UTR region.

Some of the subdomains are quite conserved between the mammalian and plant members (*Figure 2*). There are the characteristic 12 kinase domain residues that are recognized as being nearly invariant throughout the eukaryotic protein kinase superfamily and are therefore strongly implicated as playing essential roles in enzyme function (Hanks and Hunter, 1995). These residues are Gly50 and Gly52 in subdomain I, Lys72 in subdomain II, Gln91 in subdomain III, Asp166 and Asn171 in subdomain VIB, Asp184 and Gly186 in subdomain VII, Gln208 in subdomain VIII, Asp220 and Gly225 in subdomain IX and Arg280 in subdomain XI. Most of these residues are present in this serine/threonine kinase subfamily including EgPK1.

The more conserved subdomains among the five members were subdomains I, IV, VII, VIII and IX, and the conservation in all subdomains was notably higher amongst the plant members. So far, *EgPK1*,

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1     aggaagaggggagagggagagagatagagagagagagagagagatcttcttcttctcttt  62
                                                  M  G  C  S  F  S
63    tgatcgaggccgaaggggcaatcggagggagaagggaagagatgggatgctccttctca  122
      G  L  N  A  L  Y  N  A  V  N  G  G  G  D  V  L  I  N  E  N
123   gggctcaacgcgctctacaacgcggtgaacgggggcggcgatgtcttgatcaacgagaac  182
      P  F  R  I  L  R  Q  I  G  E  G  G  F  A  Y  V  Y  L  V  K
183   ccattccggattctgaggcagatcggcgaggggggattcgcctacgtctacctcgttaag  242
      E  V  V  D  D  A  P  S  R  S  G  L  A  A  K  K  S  M  H  P
243   gaggtcgtcgacgacgccccgtcccgcagcgggctcgccgccaagaagtccatgcaccct  302
      S  H  V  S  E  D  G  T  Y  S  M  K  K  V  L  I  Q  S  E  D
303   tcccatgtctcagaagatgggacatattctatgaagaaagtccttattcaaagtgaggac  362
      Q  L  Q  F  V  R  E  E  I  P  V  S  S  L  F  N  H  P  N  L
363   cagttacagtttgtgagggaggaaatccctgtttcgtctcttttaatcatccaaatctg  422
      L  S  L  L  D  H  A  I  I  S  V  K  G  P  Q  G  G  W  N  H
423   ctttctcttcttgatcatgcaattatttcagttaagggtccccaaggaggatggaaccat  482
      E  A  Y  L  L  F  P  V  H  L  D  G  T  F  L  D  N  S  K  V
483   gaagcatacctcttattcccagttcacctagatgggacttttctggacaattctaaagtt  542
      M  Q  A  K  K  E  F  F  S  T  L  T  V  L  Q  I  F  Q  Q  L
543   atgcaagcaaagaaggaattcttctcaacactcaccgttcttcaaattttccaacagctt  602
      C  A  G  L  K  H  M  H  S  F  D  P  P  Y  A  H  N  D  V  N
603   tgtgcagggctaaagcacatgcacagttttgatcctccatatgctcataatgatgtgaac  662
      L  V  I  S  H  A  H  R  K  G  Q  P  P  L  T  I  L  M  D  F
663   ctggtaatttctcatgcacatagaaaggggcaaccacctcttacaattttaatggatttt  722
      G  R  A  R  P  A  R  K  E  I  R  S  R  S  E  A  L  Q  L  Q
723   ggaagagctcggcccgcaagaaaggaaattcgttctcgttcagaggcattacaattgcag  782
      E  W  A  A  E  H  C  S  A  P  F  R  A  P  E  L  W  D  C  P
783   gaatgggctgctgagcattgttctgcaccattccgagctcctgagttatgggattgtcca  842
      S  Y  T  D  I  D  E  R  T  D  I  W  S  L  G  C  T  L  Y  A
843   agctacaccgacattgatgagaggacagatatctggtcactaggatgtactttgtatgca  902
      I  M  Y  G  V  T  P  F  E  Y  A  L  G  D  S  G  G  S  L  Q
903   ataatgtatggtgttactccttttgagtatgcacttggagattcaggaggcagcttgcag  962
      L  A  I  I  N  A  Q  I  K  W  P  P  G  P  D  P  P  Y  P  D
963   ttggccattatcaatgcacagataaaatggccccctggtcctgatccccccttatccggac  1022
      A  L  R  Q  F  V  G  W  M  L  Q  P  Q  P  A  V  R  P  H  I
1023  gctctccgacagtttgtaggatggatgctccagcctcaacctgcagtcagacctcacatt  1082
      D  D  I  I  I  H  V  D  K  L  I  S  K  Y  L  P  Q  A  K  P
1083  gatgatatcatcattcatgttgataagctcatttcaaagtacttgcctcaagccaaaccg  1142
      *
1143  taatattttaattttcttggattgaatatatgtgaatcatagaggagcttcctttacatg  1202
1203  taaatttatatgtgtttctctcttaaaagaggttcaccatggtgttacatgcaaagatga  1262
1263  ttacttttgtagagtcagcagactataatcaaatatatgacagattatcttc  1314
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Figure 1. Nucleotide and deduced amino acid sequences of EgPK1 *(Acc. No. DQ267436). "*" indicates the stop codon of the ORF. Deduced amino acid sequences are located above the codons. Translation was carried out using SIXFRAME (Biology Workbench Version 3.2, University of California San Diego, 1999).*

mPKL12, *ATPK3*, *OsSTK16* appear to be unique in a particular organism and may constitute a single gene family. Using a gene specific probe of *EgPK1* consisting of part of the 3' coding region and 3'-UTR, Southern analysis suggested that *EgPK1* might be present in more than one copy in the genome (*Figure 3*), as two bands were observed in *Hind*III digested genomic DNA. All three restriction sites used (*Bam*HI, *Xho*I, *Hind*III) were not found in the corresponding genomic fragment of the EgPK1 probe.

To verify the 5'untranslated region of *EgPK1*, 5'RACE was conducted from first-strand cDNA of suspension cultures. After cloning, two recombinants, designated EgPK1 variant 1 and variant 2, containing similar insert sizes were sequenced. Alignment of the 5'region of these clones is shown in *Figure 4*.

The sequence information of EgPK1 variant 1 verified the cDNA sequence of the original *EgPK1*, with the exception of some mismatches which could be due to PCR errors. The second clone, EgPK1 variant 2, was only identical to *EgPK1* from the second codon of the ORF onwards but, in addition to not having a start codon at the same location as in *EgPK1*, its 5' upstream region was also completely different. BlastN analysis of this unique region generated 100% identity to 18S small subunit ribosomal RNA from various species, including *Elaeis oleifera* (Acc. AY012395). This entire nucleotide region was also found in cDNA clones of other species at approximately 95% identity, *e.g.* in rice

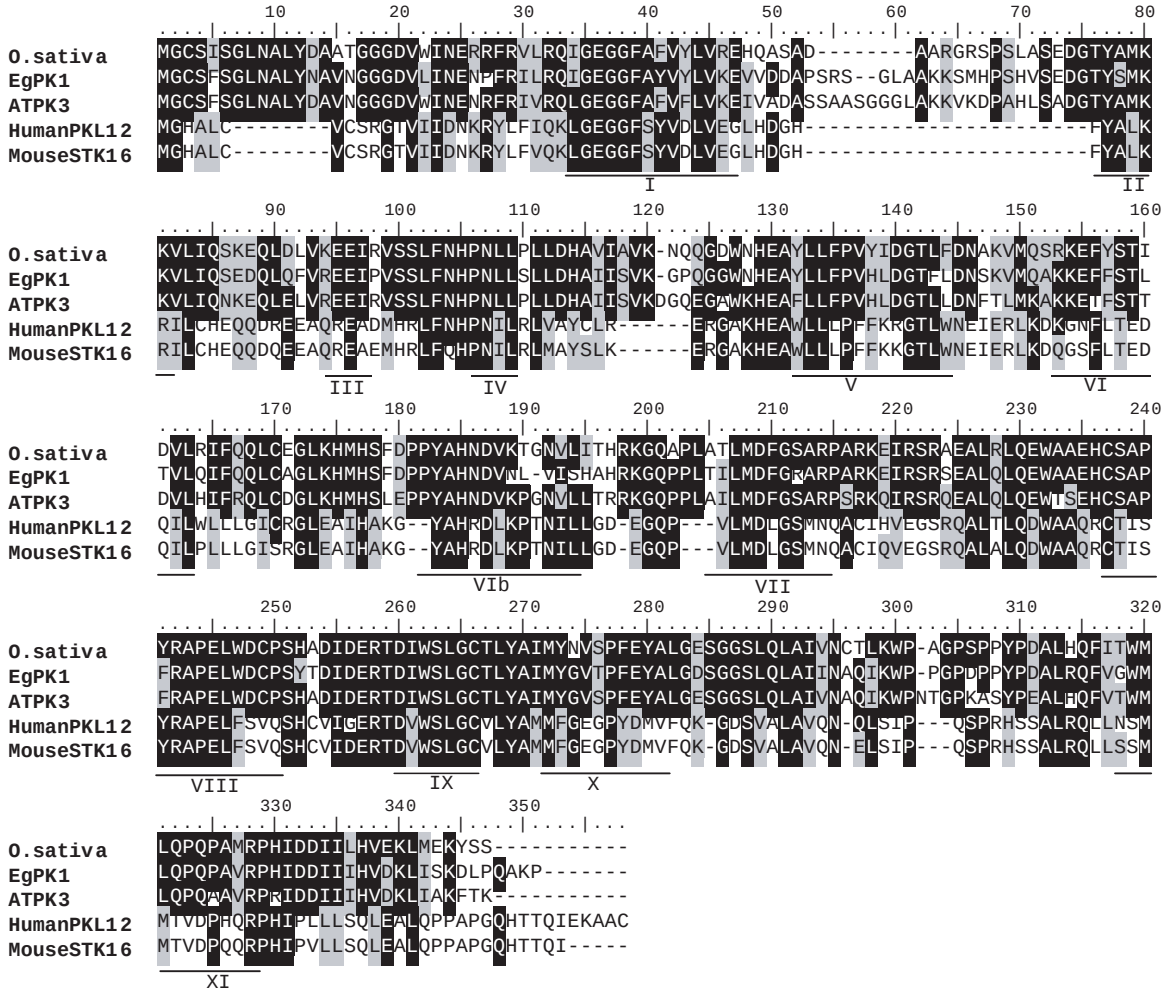

Figure 2. Sequence alignment of EgPK1 *with other related members from the same serine/threonine kinase subfamily. Amino acids in black boxes and in grey boxes are identical and similar, respectively, in at least three of the five members. Subdomains (I-XI) are shown. Accession numbers:* Oryza sativa *putative serine/threonine-protein kinase 16 (XP_450718),* Elaeis guineensis *EgPK1 (DQ267436),* Mus musculus *STK16 (AF089869),* Homo sapiens *PKL12 (AJ005791),* Arabidopsis thaliana *ATPK3 (AAB69123). Alignment was conducted using CLUSTALW (Biology Workbench Version 3.2, University of California San Diego, 1999) and BioEdit version 7.0.5.3 (Hall, 1999).*

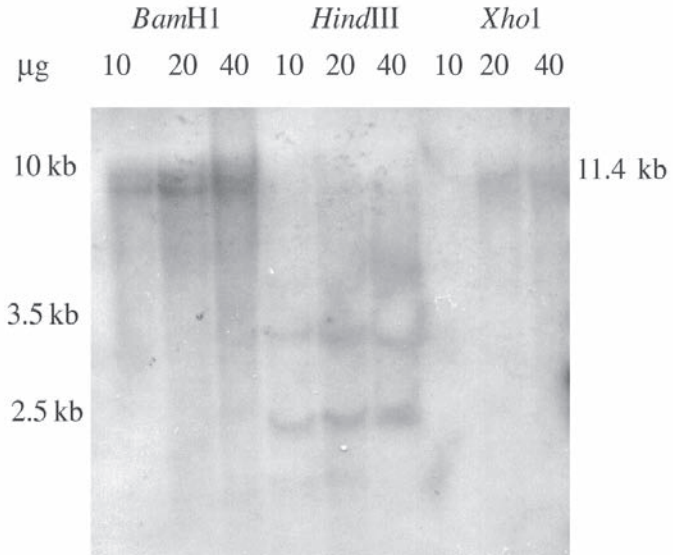

Figure 3. Southern analysis of EgPK1. *Genomic DNA (10 µg, 20 µg, 40 µg) was digested with three different restriction enzymes, blotted onto nylon membrane and hybridized with the 3'UTR of* EgPK1.

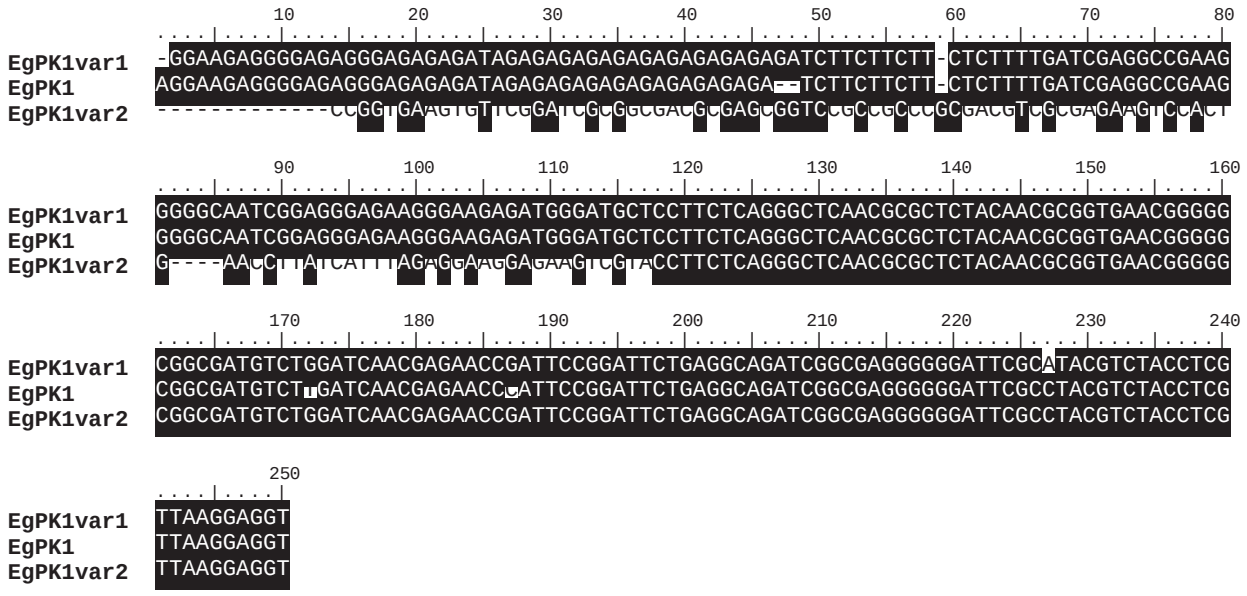

Figure 4. Comparison of EgPK1 *transcript variants from oil palm. Only the variable 5'region is shown. Shaded residues are identical in at least two out of the three transcripts. Accession numbers: EgPK1 (DQ267436), EgPKvar1 (DQ267438), EgPKvar2 (DQ267439).*

(Acc. CT830426 and AK109445.1) and Arabidopsis (Acc. AY056114.1). As of now, we are not clear on a possible reason for the existence of this transcript variant, but it could be due to splicing or recombination events. In humans, two spliced variants have also been isolated which encodes the same protein but differ at their 5′ UTR (hSTK16 variant 1 (Acc. NM_003691) and hSTK16 variant 2 (Acc. NM_001008910)). Two spliced variants were also found in Arabidopsis, whereby one variant retained an intron in the 5′ region of its mRNA (Acc. NM_120899 and NM_180461). However, transcript variants have not been identified in other species, *e.g.* in rice and mouse.

Gene Expression Analyses

In order to distinguish the expression patterns among the *EgPK1* variants, real time quantitative PCR was conducted utilizing probes designed to the unique regions in the variants (*Figure 5*). Thus, specific primers and dual labelled fluorogenic probes (*EgPK1*) were designed to the unique 5′region of *EgPK1* and a second set of primers and probe (EgPK) was also designed to the common region present in the two variants isolated so far.

In most tissue culture samples analysed, the levels of *EgPK1* and its variants (EgPK) were generally higher, relative to their respective levels in the calibrator sample, 293NEC. In tissue culture materials, higher levels of *EgPK1* were observed in embryogenic cultures. However, these levels varied when comparing among different clones of embryogenic calli, suggesting genotype or clonal differences. Levels in non-embryogenic cultures were generally lower, with as much as a 14-fold difference in the EgPK levels (both transcript variants) between embryogenic and non-embryogenic calli observed in clone 295.

Subsequently, in oil palm tissue culture, friable embryogenic callus on solid media are occasionally used to initiate suspensions (de Touchet *et al*., 1991; Wong *et al*., 1999). The proliferating embryonic aggregates do not have an epidermis and are composed of actively dividing meristematic cells (de Touchet *et al*., 1991). Here, the *EgPK1* transcript levels decreased from its earlier levels in embryogenic calli on solid media. Later on, embryos were obtained by spreading the proliferating nodules on hormone-free medium plates (de Touchet *et al*., 1991; Wong *et al*., 1999). The embryonic aggregates converted to embryoids, which subsequently turned green and eventually developed shoots (Wong *et al*., 1999). At these maturing stages, the transcript levels of *EgPK1* and those of its variants increased again, as observed in both the maturing somatic (293WE and GE) and zygotic embryos (15WAA and 16WAA).

Leaf tissues are normally used as explants in oil palm tissue culture. We were curious to know whether EgPK1 expression differences were already apparent during callus initiation. Thus, we investigated the transcript levels of EgPK1 and its variants in the leaf explants from which the embryogenic and non-embryogenic calli were respectively derived from, that is, after callogenesis had occurred and embryogenic calli and non-embryogenic calli observed to have formed from their leaf explants. After detaching the embryogenic and non-embryogenic calli, respectively, from their leaf explants, RNA was also extracted from these

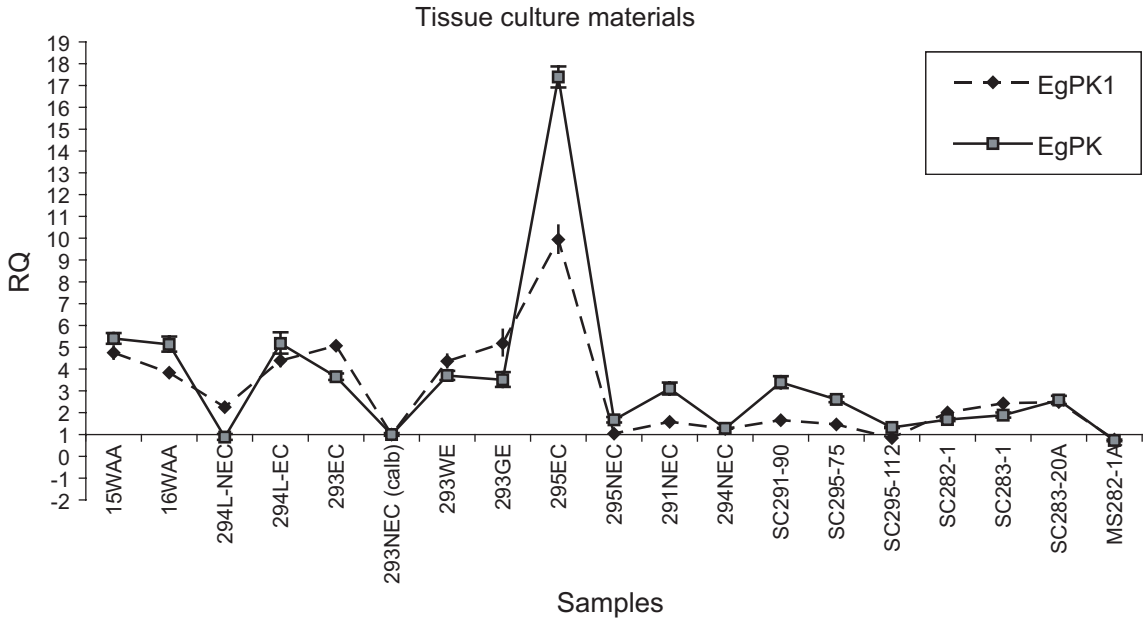

Figure 5. Expression analysis of EgPK1 variants in various tissues by real time quantitative PCR analysis. EgPK1 expression levels were normalized to GAPDH levels. The ddC_t values were normalized to the calibrator (calb), 293NEC. Note that comparisons can only be carried out across the different tissues for each variant but not between the variants in a particular tissue. Abbreviations: zygotic embryos 15 weeks after anthesis (15WAA), zygotic embryos 16WAA (16WAA), leaf explants detached from non-embryogenic solid cultures (L-NEC), leaf explants detached from embryogenic solid cultures (L-EC), embryogenic calli (EC), non-embryogenic calli (NEC), white embryoids (WE), green embryoids (GE), embryogenic suspension cultures (SC), suspension cultures in MS-basal medium (MS). Numbers next to sample type (tissue cultured samples only) are clone numbers.

explants (the explants from which embryogenic calli were derived were designated L-EC; the explants from which non-embryogenic calli were derived were designated L-NEC). Interestingly, the *EgPK1* and *EgPK* levels were observed to be approximately two-fold and five-fold higher, respectively, in L-EC tissues than in L-NEC. The higher levels of *EgPK1* in L-EC itself suggested that up-regulation of *EgPK1* either occurred before or simultaneously with the formation of embryogenic calli from the explants. This may prove useful in the early detection of this marker during the tissue culture process; however, more detailed studies need to be conducted.

Limited characterization studies have been carried out on the plant members of the STK16 protein kinase familiy. However, the mammalian *STK16* was found to be widely distributed throughout murine fetal development and adult tissues (Ligos *et al*., 1998; Stairs *et al*., 1998; Kurioka *et al*., 1998). In oil palm, EgPK1 was observed to be differentially expressed throughout early oil palm development during tissue culture, although the expression levels did not differ significantly. Non-radioactive *in situ* RNA hybridization failed to detect the localization of transcripts in embryogenic calli and suspension cultures, even after several attempts. It is possible that the transcript levels were very low as Northern hybridization previously conducted also required a long exposure time (two weeks) for the detection of faint bands (data not shown).

A regulatory role has been hypothesized for PKL12/STK16, the mammalian homolog, in the control of extracellular matrix-cell adhesion, mediating the dynamic equilibrium of organization or disorganization of focal adhesion structures and actin cytoskeleton (Ligos *et al*., 2002). As transient overexpression of PKL12 causes disruption of the extracellular matrix-cell adhesion due to disorganization of the actin cytoskeleton, a similar scenario may also occur during early oil palm somatic embryogenesis. This is because the formation of proembryogenic masses (PEMs) occurs in conjunction with the isolation of these embryogenic cells from the surrounding cells (Ong, 2001). The isolation is believed to be a result of auxin-induced response of cell wall metabolism as structural proteins like hydroxyproline-rich glycoproteins, glycine-rich and proline-rich proteins have been found to be up-regulated after auxin induction (Ebener *et al*., 1993; Suzuki *et al*., 1993; Vera *et al*., 1994). Under the influence of auxin on oil palm calli, changes occur in the cell walls that result in the formation of a thickened wall that surrounds the PEMs with several layers of highly vacuolated cells (Ong, 2001). As the *EgPK1* mRNA levels were higher in embryogenic cultures compared to in non-embryogenic and suspension cultures, we hypothesized that EgPK1 may have caused a similar disruption of the extracellular-matrix around the PEMs in order to isolate these proembryogenic

clusters from the neighbouring vacuolated cells. In suspension cultures, the constant agitation of the cultures aids in the dispersion or isolation of the developing proembryos and embryos, which may correlate to the decrease in *EgPK1* levels. Overexpression studies in oil palm may help to shed some light on this.

CONCLUSION

Somatic embryogenesis is an intricate biological puzzle. In oil palm, this is aggravated by the asynchronous developmental characteristics of individual genotypes (clones). One of the bottlenecks during oil palm tissue culture remains the low embryogenesis rate. From this study, EgPK1 may be utilized as a potential marker for embryogenesis. However, to have a better understanding of the role(s) this protein plays during embryogenesis, functional elucidation of EgPK1 is needed, which is currently in progress. This may lead to a venue for manipulation at the early selection stages of tissue culture explants in future.

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