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Sequence and Expression Analysis of EgSAPK, a Putative Member of the Serine/Threonine Protein Kinases in Oil Palm (*Elaeis guineensis* Jacq.)

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Abstract: In present study, EgSAPK (EU805512), an oil palm transcript coding for a putative SAPK protein kinase, have been molecular characterized. The cDNA for EgSAPK isolated from an oil palm cell suspension culture is 1470 bp in length with a longest Open Reading Frame (ORF) of 963 bp. No translation start codon could be identified so EgSAPK cDNA sequence is lacking the 5'-end. The deduced protein sequence shares 89% identity with the serine/threonine protein kinase SAPK9 from rice (AB125310.1). Real time PCR results showed that the expression levels of EgSAPK varied in different oil palm tissues and the EgSAPK gene shares a similar expression pattern with the SAPK gene of rice. Furthermore, the transcription of the EgSAPK gene in green embryo, white embryo and embryogenic calli tissues were higher than in non-embryogenic calli tissues. Southern blot analysis showed that the EgSAPK gene might be present as a single copy gene in the oil palm genome. These results suggest that EgSAPK may have a similar function as the SAPK gene of rice and thus can be a candidate marker for oil palm somatic embryogenesis.

Key words: Oil palm, *Elaeis guineensis* Jacq., real time PCR, serine/threonine protein kinase, SAPK

INTRODUCTION

Plants have developed various mechanisms as adaptation to the ever-changing environmental conditions such as drought, temperature extremes, hyperosmotic and high salinity stresses. To survive these challenges, plants produce proteins with phosphorylation capability called serine/threonine kinases with various functions. Phosphorylation of specific proteins is a major strategy for the regulation of protein and enzyme activity in the transduction of environmental, developmental and metabolic signals in animals and simple eukaryotes (Bray, 1997; Xiong and Zhu, 2002; Zhu, 2002). These protein kinases have been described with the majority of them falling into a dozen major groups based on their sequence relationships such as the Ca^{2+}-Dependent Protein Kinase (CDPK) subfamily, the sucrose non-fermenting-1 (SNF1)-related protein kinase (SNRK) subfamily, the receptor-like kinase subfamily, the map kinase (MAPK), the map kinase kinase (MAPKK) and the map kinase kinase kinase (MAPKKK) subfamilies, the cyclin-dependent kinase (CDK) subfamily, the casein kinase I (CK1) and the casein kinase II (CK2) subfamilies, the GSK3/SHAGGY subfamily and other subfamilies (Hardie, 1999).

The subfamily SNF1 consists of protein kinases that play major roles in regulating gene expression in eukaryotic cells. There are three types of kinases in this subfamily which is represented in eukaryotic systems such as SNF1 protein kinase in *Saccharomyces cerevisiae* (*S. cerevisiae*) (Carlson *et al*., 1981), AMP-activated protein kinases (AMPKs) in mammals and SnRKs in higher plants (Carraro *et al*., 2001). In 1981, the first protein kinase member of SNF1 was isolated and characterized from *S. cerevisiae* (Carlson *et al*., 1981; Celenza and Carlson, 1984). In this organism, the SNF1 protein is expressed in response to stress, notably in the adaptation of cells to low glucose levels (Gancedo, 1998). In mammalian cells, members of the AMP-activated/SNF1-related protein kinase subfamily are also expressed in response to a variety of stress conditions. With a higher AMP/ATP ratio, AMPK can be activated in mammalian cells (Kudo *et al*., 1995). Once activated, AMPK switches on the ATP producing pathways such as fatty acid oxidation and glycolysis. Surprisingly, the SnRK family in higher plants shares the same characteristics of a conserved domain with the SNF1 in yeast and AMPK in animals, which suggests that they might also share similar functions (Halford and Hardie, 1998). Based on the

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evolutionary relationships of their amino acid sequences, the SnRK family has been classified into three subfamilies (SnRK1, SnRK2 and SnRK3) in higher plants (Halford and Hardie, 1998). Members of the SnRK1 subfamily share an amino acid identity of 62 to 64% at the N-terminal catalytic domain to yeast SNF1 (Halford and Hardie, 1998). The SnRK2 gene subfamily is also very similar (42-45% amino acid identity) to SNF1, mostly at the N-terminal catalytic domain and have relatively short C-terminal conserved domains when compared to members of the SnRK1 subfamily while the SnRK3 subfamily is less similar in sequence to SNF1 and AMPK than SnRK1, especially at the C-terminal conserved domain (Halford and Hardie, 1998).

Functional studies of the SnRK2 subfamily suggest that this subfamily is involved in the response to environmental stresses. The first cloned SnRK2 subfamily member known as PKABA1 was identified from wheat (Anderberg and Walker-Simon, 1992). In this study, PKABA1 mRNA expression occurs when seedlings are water-stressed and when plants are supplied with low concentrations of abscisic acid (ABA). Studies had shown that SnRK2 subfamily members are also involved in osmotic signaling. For example, the AAPK gene from faba bean is induced by ABA in guard cells in response to drought (Li *et al*., 2000). Ten members of SnRK2 (SAPK1, SAPK2, SAPK3, SAPK4, SAPK5, SAPK6, SAPK7, SAPK8, SAPK9 and SAPK10) were identified from the rice genome, of which were induced by hyperosmotic stress. However, three out of the ten members (SAPK8, SAPK9 and SAPK10) were also activated by ABA (Kobayashi *et al*., 2004). The transient expression study of SAPKs in cultured cell protoplast showed that these kinases are involved in the gene-regulation pathway of ABA signaling (Kobayashi *et al*., 2005).

In this study, sequence analysis and molecular characterization of a previously isolated EST clone, cp 42 is reported. This clone has high homology to SAPK9 protein kinase from rice. Based on the sequence analysis and expression pattern, the possible function of this gene in oil palm is proposed.

MATERIALS AND METHODS

The cp 42 is an EST clone that was obtained from an oil palm cell suspension culture cDNA library. The oil palm *in vitro* cultures and the cDNA clone were kindly provided by the Malaysian Palm Oil Board (MPOB). For the tissues culture samples, oil palm cell suspension culture, embryogenic calli, non-embryogenic calli and embryoids were cultured following the protocol from MPOB (Rohani *et al*., 2003). Vegetative tissues of oil palm were obtained from field grown palm in Malaysia. Plant materials were cleaned and dissected before being frozen in liquid nitrogen and stored at -80°C. Primer synthesis and sequencing of DNA were done by First Base Laboratories Sdn. Bhd., Kuala Lumpur, Malaysia.

Sequence analysis: The BLAST 2.0 programme (http://www.ncbi.nlm.nih.gov.BLAST) was used for sequence analysis (Altschul *et al*., 1997). The BLASTX algorithm was used for homology searches of the EST at the protein level by comparing the translated protein sequence with other protein sequences available in the databases. Alignments of the protein sequence with several closely related genes were carried out using the CLUSTAL W tool (Thompson *et al*., 1994). A phylogenetic tree was constructed using the neighbour joining method via the MEGA4 package (Tamura *et al*., 2007). The reliability of the neighbour joining tree was estimated by a bootstrap analysis of 1000 replicates.

Real time PCR analysis: Total RNA extraction of all tissue samples (young leaves, mature leaves, meristems, roots, female flowers, male flowers, suspension cultures, white embryo, green embryo, non-embryogenic calli and embryogenic calli) were performed as per the method described by Schultz *et al*. (1994). Two micrograms of total RNA was used for reverse transcription into first-strand cDNA using the Quantitect Reverse Transcription Kit (Qiagen, German) according to the manufacturer's instructions. All oligonucleotides for TaqMan were designed by Sigma-Proligo (Sigma-Genosys, Sigma-Aldrich Co. USA). The primer sequences are shown in Table 1. The singleplex Taqman reactions for each sample were performed in quadruplicate in a 96-well optical plate (Applied Biosystems, USA) with a final volume of 20 μL each. All the reactions of each plate were prepared from a single PCR master mix containing: 100 nM forward and reverse primers, 250 nM TaqMan probe, TaqMan Universal PCR Master Mix (Applied Biosystems, USA).

Table 1: Primers and probe sequences for real time PCR analysis of the EgSAPK gene

Name	Sequences 5'-3'
PD 380 For	5'-CTGTTCTGACTTACCGACTC-3'
PD 380 Rev	5'-AAATATAAAGCATTCCTGGACTAAC-3'
PD 380 Pro	5'-(6-Fam)ACCGATCTTACCCTTCTGCT (BHQ1)
PD 569 For	5'-ATCAACCACTCAATCTTCTGG-3'
PD 569 Rev	5'-CTTCTGCGTTCATCTTTTGC-3'
PD 569 Pro	5'-(6-Fam)TGCCTCCACTGAACCAT (BHQ1)
EgSAPK For	5'-ATT ATA GCT GGG GCA ACC ATC C- 3'
EgSAPK Rev	5'- ATC AAG ATC TAT GCT GCC TGT C - 3'
EgSAPK Pro	5'-(6-Fam) AGCTGGCACTGGTGGGCTGAACCC (Tamra)

For: Forward primer, Rev: Reverse primer; Pro: Probe

Thermal cycling conditions included a pre-run of 2 min at 50°C, followed by 10 min at 95°C and 45 cycles of 15 sec at 95°C and 1 min at 60°C according to the TaqMan Universal PCR user's manual (Applied Biosystems, USA). The quantity of the gene expression levels in every experimental tissue was expressed relative to the calibrator, i.e., suspension culture. The quantification of the relative transcript levels was performed using the comparative C_T method and normalized as described in Livak and Schmittgen (2001). The tissue-specific mRNA expression analysis, fold changes were finally normalized to the two most stably expressed house keeping genes, superoxide manganese dismutase (PD569: Acc: EL682210.1) and one unknown protein (PD380: Acc:EL684405.1), determined using GeNorm (Vandesompele *et al*., 2002).

Statistical analysis: The data of expression fold change of tissues culture samples from the real time PCR experiment of relative analysis were subjected to an analysis of variance for a Completely Randomized Design (CRD) using SAS statistic of analysis software (Version 8.0, SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was used to compare the mean values among the treatments at 95% probability.

Southern blot: Genomic DNA was isolated from young leaves of oil palm using the CTAB method (Doyle and Doyle, 1991). Approximately 30 μg of DNA was digested with the appropriate restriction enzymes *Eco*R I, *Hind* III, *Not* I and *Taq* I, respectively (New England Biolabs, UK). The fragments were separated by electrophoresis on 0.8% (w/v) agarose gel (Sigma, USA) and transferred onto a positively-charged nylon membrane Hybond N^+ (Amersham Biosciences) by capillary transfer using the protocol recommended by the manufacturer. The hybridization was carried out with a 300 bp fragment of purified 3'UTR PCR product that had been radioactively labeled using High Prime (Roche, German) reaction mix according to the manufacturer's instructions. The hybridization protocol used for the Southern analysis was based on the method by Church and Gilbert (1984). The membrane was exposed to an imaging plate (FujiFilm) and scanned using a PhosphorImager (FujiFilm FLA5100).

RESULTS AND DISCUSSION

Sequence analysis: The sequence of cp 42 is shown in Fig. 1. This cDNA is 1470 bp in length with a longest Open Reading Frame (ORF) of 963 bp, but no translation start codon could be identified. Therefore, the cp 42 cDNA sequence was deemed to be of partial length. The BLASTX results showed that the deduced amino acid (aa) sequence of cp 42 is highly homologous to the serine/threonine-protein kinase SAPK9 from rice, also known as osmotic stress/abscisic acid-activated protein kinase 9 (Kobayashi *et al*., 2004). Here after, the cp 42 clone is designed as EgSAPK. The CLUSTALW analysis result showed that EgSAPK is lacking about 40 amino acids at the 5'-end (Fig. 1, 2). According to Hanks and Quinn (1991), all serine/threonine protein kinases contain 12 highly conserved sub-domains designated as sub-domain I-V, VIa, VIb and VII-XI and these were characteristically shared in EgSAPK (Fig. 2). However, the deduced amino acid sequence of EgSAPK was lacking of sub-domain I in comparison to the serine/threonine protein kinases of other plants. Based on the 3-D structure of several protein kinases in eukaryotes, the 12 conserved sub-domains are divided into three separate roles: the first four sub-domains (I-VI) play a role as the N-terminal nucleotide binding domains, docking of the substrate to be phosphorylated and phosphate transfer occurs at sub-domains VIb to XI and the intervening linker contains sub-domain V (Hanks and Hunter, 1995; Kennelly, 2003). In the recent years, another key aspect defined in many protein kinases is the phosphorylation site on the threonine (Thr) residue(s) located in between two conserved motifs (DFG and APE) of sub-domains VII and VIII, which is termed the activation segment (Johson *et al*., 1996). The activation segment including Thr_{137} was found in the deduced peptide of EgSAPK (Fig. 1). Also, the predicted amino acid sequence of EgSAPK also contains the S_TKc domain at the N-terminus (Fig. 3) which is known to be the main catalytic domain of the phosphotransferase of the serine or threonine-specific kinase (Marchler-Bauer and Bryant, 2004). All the above findings strongly suggest that EgSAPK is a member of the serine/threonine protein kinases.

Based on the protein prediction results, the EgSAPK kinase contained only 11 out of 12 conserved kinase sub-domains that are typical of protein kinases (Hanks *et al*., 1988). In addition, multiple alignments of the predicted amino acids of EgSAPK with the most similar and relevant protein sequences of other plants from the GENEBANK database indicates significant homology between the incomplete ORF of EgSAPK (321 amino acid polypeptide) to serine/threonine protein kinase SAPK9 from *Oryza sativa* (AB125310.1) (89% homology), SnRK2 protein kinase from *Arabidopsis thaliana* (AT4G33950.1) (85% homology) and serine/threonine protein kinase SAPK8-like protein from *Solanum tuberosum* (AAM47602.1) (81% homology) (Fig. 2). The BLASTX search showed that EgSAPK has high homology to SAPK

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1     CAT GAA AAA ACC CGT GAA CTG GTG GCG GTG AAA TAT ATT GAA CGT   45
1      H   E   K   T   R   E   L   V   A   V   K   Y   I   E   R   15
46    GGC GAA AAA ATT GAT GAA AAC GTG CAG CGT GAA ATT ATT AAC CAT   90
16     G   E   K   I   D   E   N   V   Q   R   E   I   I   N   H   30
91    CGT AGC CTG CGT CAG CCG AAC ATT ATT CGT TTT AAA GAA GTG ATT   135
31     R   S   L   R   Q   P   N   I   I   R   F   K   E   V   I   45
136   CTG ACC CCG ACC CAT CTG GCG ATT GTG atg GAA TAT GCG AGC GGC   180
46     L   T   P   T   H   L   A   I   V   M   E   Y   A   S   G   60
181   GGC GAA CTG TTT GAA CGT ATT TGC AAC GCG GGC CGT TTT AGC GAA   225
61     G   E   L   F   E   R   I   C   N   A   G   R   F   S   E   75
226   GAT GAA GCG CGT TTT TTT TTT CAG CAG CTG ATT AGC GGC GTG AGC   270
76     D   E   A   R   F   F   F   Q   Q   L   I   S   G   V   S   90
271   TAT TGC CAT AGC atg CAG GTG TGC CAT CGT GAT CTG AAA CTG GAA   315
91     Y   C   H   S   M   Q   V   C   H   R   D   L   K   L   E   105
316   AAC ACC CTG CTG GAT GGC AGC ATG GCG CCG CGT CTG AAA ATT TGC   360
106    N   T   L   L   D   G   S   M   A   P   R   L   K   I   C   120
361   GAT TTT GGC TAT AGC AAA AGC AGC GTG CTG CAT AGC CAG CCG AAA   405
121    D   F   G   Y   S   K   S   S   V   L   H   S   Q   P   K   135
406   AGC ACC GTG GGC ACC CCG GCG TAT ATT GCG CCG GAA GTG CTG CTG   450
136    S   T*  V   G   T   P   A   Y   I   A   P   E   V   L   L   150
451   AAA AAA GAA TAT GAT GGC AAA ATT GCG GAT GTG TGG AGC TGC GGC   495
151    K   K   E   Y   D   G   K   I   A   D   V   W   S   C   G   165
496   GTG ACC CTG TAT GTG ATG CTG GTG GGC GCG TAT CCG TTT GAA GAT   540
166    V   T   L   Y   V   M   L   V   G   A   Y   P   F   E   D   180
541   CCG GAA GAA CCG AAA AAC TTT CGT AAA ACC ATT CAG CGT ATT CTG   585
181    P   E   E   P   K   N   F   R   K   T   I   Q   R   I   L   195
586   GGC GTG CAG TAT AGC ATT CCG GAT TAT GTG CAT ATT AGC CCG GAA   630
196    G   V   Q   Y   S   I   P   D   Y   V   H   I   S   P   E   210
631   TGC CGT CAG CTG ATT AGC CGT ATT TTT GTG GGC AAC CCG GCG atg   675
211    C   R   Q   L   I   S   R   I   F   V   G   N   P   A   M   225
676   CGT ATT ACC ATT CCG GAA ATT CAG AAC CAT GAA TGG TTT CTG AAA   720
226    R   I   T   I   P   E   I   Q   N   H   E   W   F   L   K   240
721   AAC CTG CCG GCG GAT CTG ATG GAT GAT AAC ACC atg AGC AAC CAG   765
241    N   L   P   A   D   L   M   D   D   N   T   M   S   N   Q   255
766   TAT GAA GAA CCG GAT CAG CCG atg CAG AGC ATT GAT GAA ATT atg   810
256    Y   E   E   P   D   Q   P   M   Q   S   I   D   E   I   M   270
811   CAG ATT ATT GCG GAA GCG ACC ATT CCG GCG GCG GGC ACC CGT GGC   855
271    Q   I   I   A   E   A   T   I   P   A   A   G   T   R   G   285
856   CTG AAC CCG TAT CTG ACC GGC AGC ATT GAT CTG GAT GAT GAT ATG   900
286    L   N   P   Y   L   T   G   S   I   D   L   D   D   D   M   300
901   GAA GAT CTG GAT AGC GAT CCG GAA CTG GAT GTG GAT AGC AGC GGC   945
301    E   D   L   D   S   D   P   E   L   D   V   D   S   S   G   315
946   GAA ATT ATT TAT GCG ATG TAA GTG TGG CTG GCG TGG CTG CCG CGT   990
316    E   I   I   Y   A   M   *
991   TGC ACC GTG ATT TTT AGC ATT CGT TGG AAA CAT CGT ATT CGT ATG   1035
1036  TTT GAT ATT CCG GGC TGG CAG CGT CAG AGC ATT CCG CTG ACC AGC   1080
1081  TTT CTG AGC CTG AAA GGC GCG TGC ATT TAT CTG GAA TAT ACC ACC   1125
1126  CTG atg CTG TAT GCG TGC CAT GTG TGC CGT TTT GGC GAA GTG CTG   1170
1171  AGC CTG CTG GTG CTG TTT ATT TAT AGC CCG GCG TGC TGC CTG CTG   1215
1216  AGC GTG ATT ATG GTG CTG ATT ACC CTG GCG TTT CAG TTT TTT AGC   1260
1261  GGC AAA CAG GGC atg TAA AGC CCG ATT GCG GGC TTT TAT GCG GCG   1305
1306  CTG AAA CTG CAT AGC CTG CAG CCG TAT TTT TAA CGT AAA TTT CTG   1350
1351  CTG ACC GTG GTG GCG CTG TGC AAA AAC TTT CTG ATT AAA AAA AAA   1395
1396  AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA   1440
1441  AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA   1470
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Fig. 1: The nucleotide and deduced amino acid sequences of EgSAPK. Serine/threonine protein kinases active-site (aa_{97-109}) is boxed. DFG and APF conserved motifs are indicated by gray boxes and the conserved T between these motifs is indicated by star (*). The polyadenylation signal is shaded

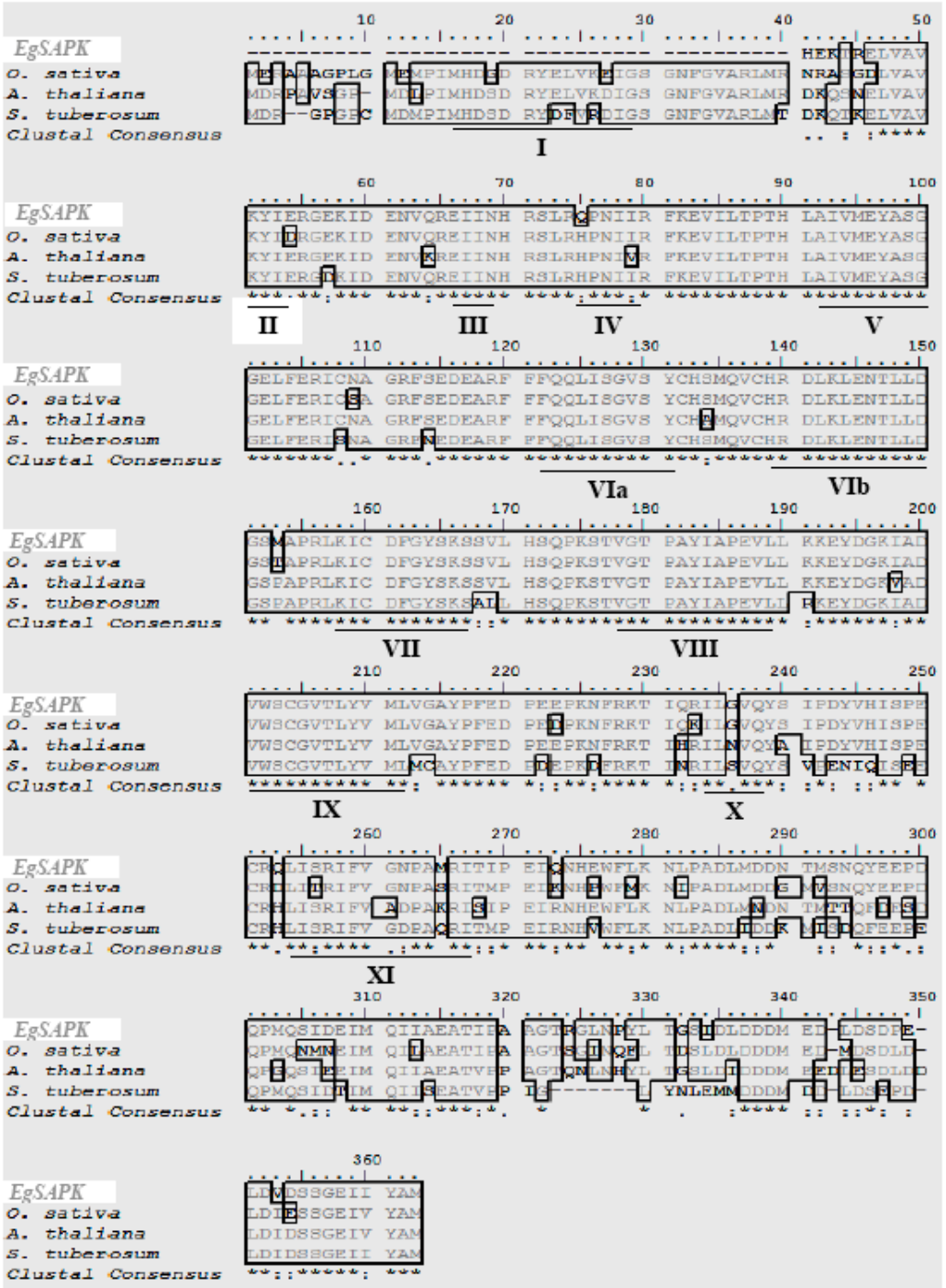

Fig. 2: Amino acid sequence alignment of EgSAPK with a serine/threonine protein kinase SAPK9 from *Oryza sativa* (BAD18005; second sequence from top), an open stomata 1 protein kinase (NP_567945; third sequence) from *A. thaliana* and a serine/threonine protein kinase SAPK8-like protein from *S. tuberosum* (ABA40436). Dashed lines are gaps introduced to maximize alignments. The conserved residues are highlighted. *Represents those that are highly conserved. The positions of the catalytic subdomains according to Hanks and Quinn (1991) are indicated under the sequences with roman numerals

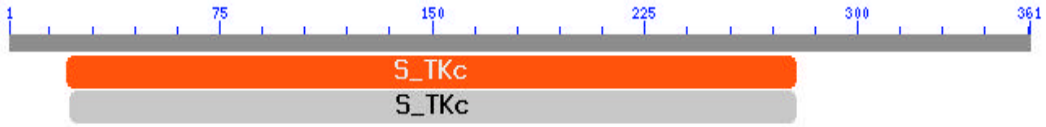

Fig. 3: Location of Serine/threonine protein kinases, catalytic domain, S-TKc (from amino acids 1 to 238) that was recognized based on the deduced amino acid sequence of EgSAPK using the NCBI-CDD programme

protein from rice and SnRK2 from *Arabidopsis*. The result suggests that EgSAPK is a member of the SnRK2 family of protein kinases. Analysis of a phylogenetic tree consisting of all the SAPK members and other closely related and functionally characterized SnRK2 family members is shown in Fig. 4. According to Kobayashi *et al*. (2004), rice SAPK proteins belong to the SnRK2 family of protein kinases that can be divided into three subclasses. EgSAPK is grouped with subclass III of the SnRK2 family of protein kinases, which contain SAPK8, SAPK9 and SAPK10 in *Oryza sativa* (Kobayashi *et al*., 2004), SnRK2.6 (OST1/SRK2E), SNRK2-3 and SPK-2 in *Arabidopsis* (Mustilli *et al*., 2002; Boudsocq *et al*., 2004) and AAPK in *Vicia faba* (Li *et al*., 2000). Subclass III of SnRK2 has been reported to be activated by hyperosmotic stress and ABA (Kobayashi *et al*., 2004). According to Michu (2007), bootstrap values of 70% or higher indicate reliable groups. The tree suggests that EgSAPK can be clustered into subclass III of SnRK2 and has the closest relationship with SAPK9 and SAPK10 from rice.

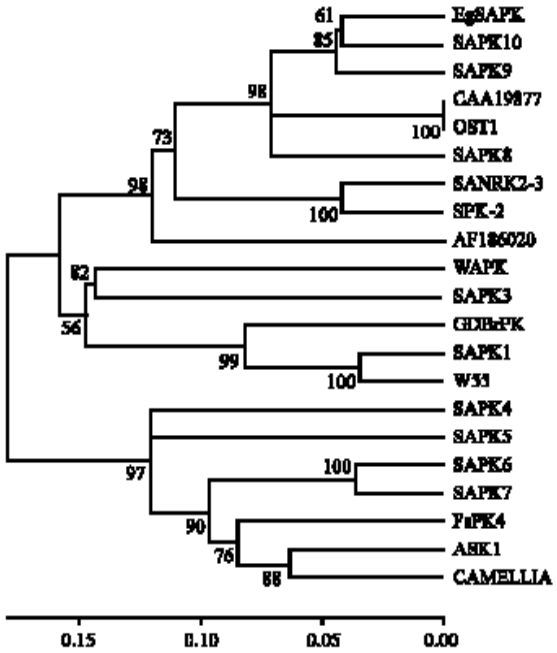

Fig. 4: A neighbour-joining tree displaying the phylogenetic relationship of EgSAPK (EU805512) with all SAPK members and other closely related and functionally characterized SnRK2 family: SAPK10 from rice (BAD18006.2), SAPK9 from rice (BAD18005.1), SAPK8 from rice (Q7Y0B9.1), OST1 from *A. thaliana* (NP_567945.1), CAA8758 from *A.thaliana* (CAA18758.1), SPK-2 from *A. thaliana* (AAM65501.1), SNRK2-3 from *A. thaliana* (NP_201489.1),AF186020.1 abscisic acid-activated protein kinase from *V. faba* (AAF27340.1), W55 from *Triticum aestivum* (ABD37624.1), SAPK1 from rice (Q75LR7.1), GDBrPK from *Vitis vinifera* (AAG31326.1), WAPK from *Nicotiana tabacum* (AAC69450.1), SAPK3 from rice (BAD17999.1), SAPK5 from rice (BAD18001.1), SAPK7 from rice (BAD18003.1), SAPK6 from rice (BAD18002.1), SAPK4 from rice (BAD64101.1), FsPK4 from *Fagus sylvatica* (CAE54075.1), Camellia Serine/threonine protein kinase from *Camellia sinensis* (ABG81507.1) and ASK1 from *A. thaliana* (NP_172563.1)

Real time PCR analysis of EgSAPK: Real time PCR has become a popular method for high-throughput and accurate expression profiling of selected genes. Therefore, accurate normalization of gene expression levels is an absolute prerequisite for reliable results. We have evaluated eight house keeping genes with differences in the abundance of their expressions in various oil palm tissues using the geNorm software (Vandesompele *et al*., 2002) (data not shown). Then, the two most stable house keeping genes were chosen to normalize nine types of oil palm tissues for the EgSAPK expression study.

In this study, we examined the expression of EgSAPK in 9 types of tissues using real time PCR (Fig. 5A, B). When a comparison was made between cell suspension culture and vegetative plant materials, EgSAPK transcripts were down-regulated in all the above ground-organs but were up-regulated in the root (Fig. 5A). Kobayashi *et al*. (2004) reported that SAPK8, SAPK9 and SAPK10 were expressed in blades and sheaths of rice seedling as well as in the root. The expression patterns of EgSAPK are similar to SAPK8, SAPK9 and SAPK10 in

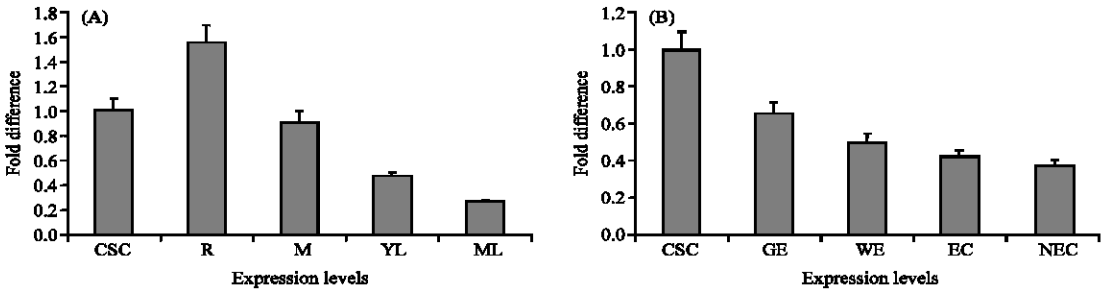

Fig. 5: Fold difference in expression levels of EgSAPK between suspension culture and other tissues. (A) The fold change of the EgSAPK gene between suspension culture and vegetative tissues and (B) the significant fold change of EgSAPK gene expression between suspension culture and green embryoids, white embryoids, embryogenic calli and non-embryogenic calli. CSC: Cell Suspension Cultures, R: Root, M: Meristems, YL: Young Leaves, ML: Mature Leaves, GE: Green Embryoids, WE: White Embryoids, EC: Embryogenic Calli, NEC: Non-Embryogenic Calli

rice. A study with the OST1 (SnRK2.6) promoter fused with GUS as a reporter gene showed promoter activity in leaves and root of transgenic *Arabidopsis* plants (Mustilli *et al*., 2002). On the other hand, expression of the AAPK gene from *Vicia faba* was not detected in leaves but it was only expressed in mesophyll cell protoplasts (Li *et al*., 2000). The findings of the above mentioned studies indicated that subclass III of SnRK2 genes have various expression patterns in different plants, suggesting various roles in different plants. Expression analysis showed that the EgSAPK also varied in different oil palm tissues, showing similarities in expression pattern with those belonging to the subclass III of the SnRK2 gene of rice. This result suggests that EgSAPK may have a similar function with the subclass III of SnRK2 gene of rice. Further investigations aimed at studying the biological function of the oil palm EgSAPK gene in response to osmotic stresses and ABA should be conducted to provide more insights into the mechanism of signal transduction or metabolism regulation in plant cells.

In the oil palm industry, the propagation of oil palm by tissues culture has been applied to produce pure line seedlings with superior genotypes, considerable yield increase and disease resistance. However, the tissue culture method is limited by the low success rate at callus formation and embryogenesis. Due to the fact that embryogenesis is unpredictable and is rather sporadic in occurrence, the mean percentage of embryogenesis from callus cultures is only about 6%, with rates ranging from 1.7 to 17.1% (Wooi, 1995). In this study, we also examined the expression level of EgSAPK in green embryo tissues, white embryo tissues, embryogenic calli and non-embryogenic calli to determine whether EgSAPK can be a marker for embryogenic calli. Using cell suspension as a calibrator, we found that the transcription of the EgSAPK gene in green embryoid tissues, white embryoid tissues and embryogenic calli tissues are significantly higher than in non-embryogenic calli (Fig. 5B). This is the first study to report the expression profile of a SAPK gene in the tissue culture materials. This result suggests that EgSAPK can be a candidate as a marker for oil palm somatic embryogenesis. However, the result serves as a preliminary characterization of the EgSAPK gene from oil palm. More experiments on the EgSAPK gene should be carried to the its utility as an embryogenic calli marker for the oil palm industry.

Southern analysis of EgSAPK: EgSAPK was similar in protein sequence to SAPK9 from *Oryza sativa* (Kobayashi *et al*., 2004), a member of the SnRK2 family. To examine the copy number of the EgSAPK gene in the oil palm, Southern analysis was done. As shown in Fig. 6, genomic DNA from oil palm was individually digested with four restriction enzymes. A 312 bp fragment of EgSAPK 3'-UTR was used as the probe. The restriction sites for *Eco*R I, *Hind* III, *Not* I and *Taq* I are not present in this probe sequence. The hybridization results showed that a single signal was detected with DNA digested by *Eco*R I, *Hind* III and *Not* I. However, two *Taq I*-digested fragments were observed to hybridize with the probe. The additional band could be attributed to an intron region having a *Taq I* restriction site at the 3-end of the genomic DNA. Therefore, this banding pattern shown in Fig. 6 suggests that the EgSAPK gene might exist as a single copy in the oil palm genome.

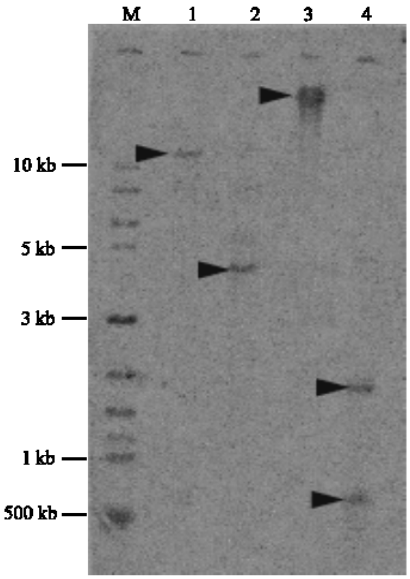

Fig. 6: Southern blot analysis with the 3'UTR region of EgSAPK as a probe on oil palm genomic DNA digested with *Eco*R I (1), *Hind* III (2), *Not* I (3) and *Taq* I (4), M: 2-log marker

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