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THESIS

DISCOVERY OF PLANT HORMONE SIGNAL TRANSDUCTION HOMOLOGS IN OIL PALM (*ELAEIS GUINEENSIS* JACQ.)

POOM PREEDAKOON

A Thesis Submitted in Partial Fulfilment of
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Poom Preedakon 2009: Discovery of Plant Hormone Signal Transduction Homologs in Oil Palm (*Elaeis guineensis* Jacq.) Master of Science (Agricultural Bitechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Mr. Hugo Volkaert, Ph.D. 129 pages.

Oil palm protein coding genes that are involved in hormone signal transduction and responses through the ubiquitin proteasome degradation pathway and genes involved in auxin transport were characterized. Genomic DNA fragments were amplified using primers targeting conserved regions for each of the *Arabidopsis thaliana* and *Oryza sativa* homologs of the auxin (TIR1, ARF1, AXR2, and AXR3), gibberellins (GID1), abscisic acid (ABI3 and ABI5), ethylene (EBF1, EBF2, ETR, ERS), jasmonate (COI1), brassinosteroid (BAK1 and BRX) and strigolactone (MAX2 and MAX4) signal transduction pathways and the auxin transporter family (PIN). In addition, HECT and MYB homologs which are shared among several signal transduction pathways were targeted. Twenty-nine gene fragments were characterized. Eighteen gene fragments included one or more introns. For eight of them, specific primer pairs were designed to study polymorphism among twelve different oil palm seedlings. TIR1, ARF1, AXR, and ABI3 specific primer pairs amplified a unique product but results from their SSCP (single strand conformation polymorphism) showed they didn’t have any polymorphism. Four PIN specific primer pairs amplified four different loci and their sequences showed some polymorphism. A phylogenetic comparison of the amino acid coding regions of each of the gene fragments indicated that the oil palm sequences usually grouped with sequences from other monocots such as the grasses *Oryza* and *Zea*.

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Poom Preedakoon

February 2009

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LIST OF ABBREVIATIONS

BLAST	=	Basic Local Alignment Search Tool
DNA	=	deoxyribo nucleic acid
dNTP	=	deoxy nucleotide triphosphase
F	=	forward
PCR	=	polymerase chain reaction
R	=	reverse
g	=	gram
M	=	Molar
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	milimeter
mM	=	millimolar
$MgCl_2$	=	Magnesium chloride
ng	=	nanogram
rpm	=	rotation per minute
sec	=	second
ssDNA	=	single-stranded DNA
AXR	=	Auxin resistant
GID1	=	*GA-insensitive dwarf1*
SLY1	=	*SLEEPY1*
ABI	=	*Abscisic acid insensitive*
ABI3	=	*Abscisic acid insensitive3*
ABI5	=	*Abscisic acid insensitive5*
ARF1	=	*Auxin response factors1*
ARF2	=	*Auxin response factors2*
ABP	=	*Auxin-binding protein*
COI1	=	coronatine-insensitive 1
DELLA	=	DELLA protein
TIR1	=	Transport Inhibitor Response1

LIST OF ABBREVIATIONS (Continued)

SIN1	=	Short intigument1
SLY1	=	SLEEPY1
PIN	=	Polar auxin transport (PIN-Form)
GAMYB	=	Gibberellin-inducible Myb
BRI1	=	Brassinosteroid insensitive
BRH1	=	BRASSINOSTEROID-RESPONSIVE RING-H2
BRX	=	Brevis radix
phor1	=	photoperiod responsive
HECT	=	HECT ubiquitin-protein ligase 3
KAK	=	KAKTUS, UBIQUITIN-PROTEIN LIGASE 3
XERICO	=	BRASSINOSTEROID-RESPONSIVE RING-H2
LRRs	=	leucine-rich repeat
MAX2	=	MORE AXILLARY BRACHING2
MAX3	=	MORE AXILLARY BRACHING3
MAX4	=	MORE AXILLARY BRACHING4
RMS	=	RAMOSUS1
ETR	=	ETHYLENE RECEPTOR
EBF	=	EIN3 (Ethylene insensitive) Binding
ERA1	=	ENHANCED RESPONSE TO ABA
CCDS	=	CAROTENOID-CLEAVING DIOXYGENASES
DAD1	=	DECREASED APICAL DOMINANCE 1
BAK1	=	BRI-associated receptor kinase1
SSCP	=	Single Strand Conformation Polymorphism

DISCOVERY OF PLANT HORMONE SIGNAL TRANSDUCTION HOMOLOGS IN OIL PALM (*ELAEIS GUINEENSIS* JACQ.)

INTRODUCTION

The currently recognized plant hormones are the auxins, gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, jasmonate, and strigolactones. Plant hormones are extremely important agents in the integration of developmental activities and in the response of plants to their external physical and biological environment. Environmental factors often exert inductive effects by evoking changes in hormone metabolism and distribution within the plant. Aside from participation in responses to inductive environmental effects, hormones are also the principal agents which regulate the expression of the intrinsic genetic potential of plant.

Plant hormones or phytohormones like hormones in animals are active at very low concentrations. The responses of sensitive organs, tissues, and cells are specific for each hormonal class. Hormonal signal perception is an important constituent of the hormonal regulation in plants and other multicellular organisms (Romanov, 2002). Plant hormones regulate gene expression through specific receptors and signal transduction from receptor to effector transcription factors. In animals, hormone receptors can be divided in two groups, membrane receptors and intracellular receptors. Plant hormone receptors are minor proteins occurring in the cell at very low concentrations. Their structure is generally complex and consists of different functional domains making it difficult to classify them (Romanov, 2002). However, more recent studies confirmed the correctness of the concept that receptor proteins are involved in the perception and transduction of the hormonal signals in plants. Receptors for auxin, gibberellins and ethylene have been isolated and fully characterized in *Arabidopsis* and rice.

Oil palm (*Elaeis guineensis* Jacq.) is the most important oil crop in Thailand. It's a perennial allogamous monocotyledonous tree. Palm oil, palm kernel oil and specific oils derived from them are used in the production of cooking oils, margarines, soaps, and detergents. Palm oil can be used as a combustion fuel (biodiesel) alone or in mixture with other fuels. The demand for biodiesel and other renewable fuels has increased recently and is expected to increase even more in the future.

This research aims to isolate genes that are involved in different aspects of hormone reception and signal transduction in oil palm. Plant hormone actions are directly or indirectly linked to several important aspects of the growth and development of the oil palm tree. Flower induction, determination of the sex of the unisexual inflorescence and the thickness of the kernel shell are some aspects of the oil palm development where plant hormone action is probably involved.

To isolate fragments of genes, PCR amplification was used with primers directed to conserved regions of hormone receptors and signal transduction genes in oil palm. To investigate the genetic diversity for these genes, targeted amplification of DNA fragments has been developed using specific primers to search for polymorphism.

OBJECTIVES

Obtain DNA sequence information from oil palm genes involved in hormone perception and signal transduction.

Develop assays to test for polymorphism in some of these genes.

LITERATURE REVIEW

African oil palm

The African oil palm (*Elaeis guineensis* Jacq) is placed in the Arecaceae family along with coconut and date palms. Oil palms are monoecious, producing male and female inflorescences in leaf axils. The inflorescence of both sexes is a compound spadix with 100-200 branches, initially enclosed in a spathe or bract that splits 2 weeks prior to anthesis. As in many palms, fruits are drupes, the mesocarp and endocarp vary in thickness. There are three naturally occurring forms of the oil palm fruit, termed *dura*, *tenera*, and *pisifera*.

Dura palm is thick endocarps (2-8 mm.) and less mesocarp (35-60%). *Pisifera* palm always bear large quantities of female bunches. The majority of pisiferas are more or less female-sterile, thinner shelled fruit. There was a distinct ring of fibres embedded in the mesocarp (Corley and Tinker, 2003).

Tenera is a hybrid from *dura* and *pisifera*. It has thin shelled form with a fibre ring, is a hybrid between the shell-less pisifera and the common thick-shelled dura form which has no fibre ring (Beirnaertand Vanderweyen, 1941).

The exocarp color is green changing to orange at maturity in virescens types, and orange with brown or black cheek colors in the nigrescens types. The mesocarp, from which palm oil is derived, is fibrous and oily, and the seed is opaque white, encased in a brown endocarp; palm kernel oil is derived from seeds. The female infructescence contains 200-300 fruit, and fruit set is 50-70%. Fruit ripen about 5-6 months after pollination. Individual oil palm fruit are drupes with an oily, fibrous mesocarp.

The demand for oil palm has risen dramatically in recent years and, as a result, there has been substantial interest in increasing production efficiency by selective breeding. Identification of the individual genetic factors underlying quantitative traits or QTLs will provide a potential way for improving oil production in breeding program. However, improvement of oil palm is a slow and difficult process because of the large size of the plants requiring large areas for field testing, the long time between pollination and reliable observations of the trait phenotype in the progeny. Marker assisted selection would be able to shorten breeding cycles by giving higher confidence in early selection. Determination of the linkage and associations between oil yield and particular loci responsible for variation in oil yield of oil palm may be able to help to identify the better parents for further seedling production with accumulate high oil content in breeding program.

Plant hormones

Plant growth regulators or plant hormones are organic compounds that regulate plant growth and development. Plant hormones, which are active in very low concentrations, are produced in certain parts of the plants and are usually transported to other parts where they elicit specific biochemical, physiological, or morphological responses. They may also be active in the tissues where they are produced. Plant hormones can evoke many different responses. The effects of different hormones may be either stimulatory or inhibitory. Each hormone performs its specific functions, though nearly all of the measurable responses of plants are controlled by interaction between two or more hormones. Such interactions may occur at various levels, including the synthesis of hormones, hormone receptors, and secondary messengers, as well as at the level of ultimate hormone action. Furthermore, hormonal interactions may be cooperative, antagonistic, or balancing. About 400 genes have been identified to be involved in aspects of plant hormone biosynthesis, transport, signal transduction or action (Davies, 2004). Some of these genes play together in the same pathway.

In plants, the ubiquitin-mediated protein degradation by the ubiquitin/26S proteasome contributes significantly to development by affecting a wide range of processes, including embryogenesis, hormone signaling, light regulated responses and senescence. In *Arabidopsis thaliana* more than 1400 genes (~5% of the proteome) are thought to encode components of the ubiquitin/26S proteasome (Ub/26S) complexes (Smalle and Vierstra, 2004). Approximately 90% of these genes encode subunits of the E3 ubiquitin ligases, which confer substrate specificity to the ubiquitination pathway. The roles and functions that some of these genes play during development have been studied in plants such as rice and / or *Arabidopsis*.

The role of SCFs in plant development

The SCF complex is one of the 4 known types of E3 ubiquitin ligases and was first identified in yeast (Itoh *et al*., 2005). The name is derived from three of its four subunits including SKP1 (or its homolog ASK1 in *Arabidopsis*), CDC53 (or Cullin), and an F-box protein. The fourth subunit is the RING finger protein RBX1. In this complex, the scaffold-like cullin binds both RBX1 and the linker protein ASK1 (Zheng *et al*., 2002b). The ASK1 protein in turn binds to an F-box protein that confers the substrate specificity. F box proteins belong to a large array of very diverse proteins that share a more or less conserved sequence of amino acids, called the F-box (Pickart, 2001). The F-box is a protein motif of approximately 50 amino acids that functions as a site of protein-protein interaction. F-box proteins were first characterized as components of SCF ubiquitin - ligase complexes, in which they bind substrates for ubiquitin-mediated proteolysis. The F-box motif links the F-box protein to other components of the SCF complex. However, F-box proteins have more recently been discovered to function also in non-SCF protein complexes in a variety of cellular functions. These complexes catalyse the covalent addition of ubiquitin molecules to proteins, targeting them for destruction. The ubiquitin pathway is highly conserved among species.

The participation of SCFs and F-box proteins in plant development is extensive, affecting processes such as hormone response, photomorphogenesis, circadian rhythms, floral development, and senescence. Nearly 700 F-box proteins have been identified in the *Arabidopsis* genome (Gang *et al*., 2002). At present, information exists on the functions of only a relatively small number of them. Most of these are involved in regulation of hormone signaling pathways. For some responses, the role of the SCF is to degrade repressors of hormone action (auxin, GA), whereas in response to ethylene, the SCF degrades regulators of gene expression that are active in the absence of the hormone. The following sections will discuss some of the recent progress in determining the role of the SCF in these signaling pathways.

The ubiquitin-proteasome pathway in auxin response

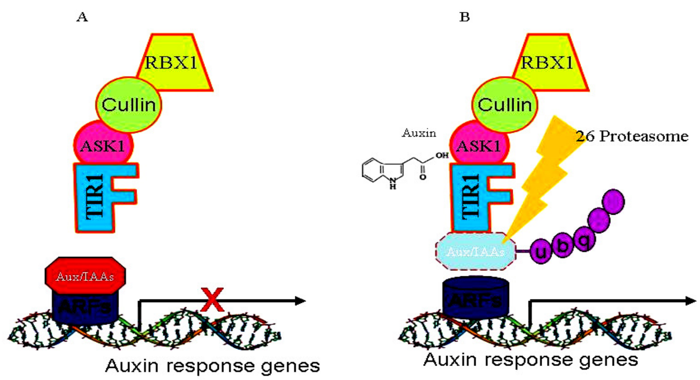

Figure 1 The auxin signaling pathway.

(A) Under low auxin concentrations, the transcription of auxin response genes is blocked by the hormone transcription repressor proteins (Aux/IAAs). When auxin is bound by the F-box protein TIR1 (B) it stimulates the interaction of AUX/IAA with SCF^{TIR1}, which induces the ubiquitination of AUX/IAA, targeting them for destruction by the 26S proteasome. This in turn releases the hormone transcription factor (ARFs) from their inhibitors and hormone response gene can be expressed.

Auxins were the first plant hormones discovered and believed to be essential for plant life because, to date, no plant unable to synthesize auxin has been found. They elicit a diverse array of responses and are involved in the regulation of growth and development throughout the plant life cycle. Auxins induce cell elongation and cell specialization. Indole acetic acid (IAA), the most common naturally occurring form of auxin (Taiz and Zeiger, 2006), is a simple molecule similar to tryptophan. The ability of auxin to bring about such diverse responses appears to result partly from the existence of several independent mechanisms for auxin perception. Auxin signaling is assumed to start with the perception of auxin by its interaction with some kind of receptor. Evidence suggests that there are multiple sites for auxin perception, and appears to be transduced through various signaling pathways. The transport inhibitor response 1 protein (TIR1) was previously isolated in a genetic screen looking for mutant plants tolerant to auxin transport inhibitors. Ruegger *et al*. (1998) reported that TIR1 was involved in auxin action, not in auxin transport. The function of TIR1 was suggested by the presence of an F-box motif in its protein sequence (Callis, 2005). Cloning of TIR1 showed it to be an F-box protein with a set of leucine-rich repeats (LRRs) linking it functionally to the important ubiquitination protein degradation pathway (Dharmasiri *et al*., 2005; Kepinsky and Leyser, 2005). SCF^{TIR1} was the first such complex characterized in plants (Gray *et al*., 2003).

Loss-of-function mutations in SCF components confer resistance to auxin, suggesting that targets of SCF^{TIR1} are negative regulators of auxin response (Gray *et al*., 2001; Hellmann *et al*., 2003). This hypothesis was confirmed when members of the AUX/IAA family of proteins, short-lived transcriptional repressors of auxin response, were shown to be substrates of SCF^{TIR1} (Gray *et al*., 2001). A direct auxin-dependent interaction between the F-box protein TIR1 and several AUX/IAA proteins has been demonstrated, and two AUX/IAA proteins (IAA7 and IAA17) are stabilized in the *tir1* mutant (Gray *et al*., 2001; Dharmasiri 2003).

Auxins cause rapid changes in gene expression, and two families of proteins have been identified in this response: the AUX/IAA proteins and auxin response factors. AUX/IAA proteins contain four conserved regions called domains I through IV (Abel *et al*., 1995). Domains III and IV are necessary for dimerization with other AUX/IAA proteins and with members of another family of transcriptional regulators called AUXIN RESPONSE FACTORS (ARFs) (Guilfoyle *et al*., 1998). The phytohormone auxin plays critical roles during plant growth, many of which are mediated by the auxin response transcription factor (ARF) family. Earlier studies showed that AUX/IAA proteins repress ARF function in a way that requires dimerization between the two proteins. More recently, it has been shown that domain I of the AUX/IAA proteins contains a Leu-rich region that can act as a general transcriptional repressor. In response to auxin, the AUX/IAA proteins are ubiquitinated and degraded, allowing ARFs to function.

It is still unclear at present how auxin regulates the interaction between the SCF and its substrates. In animal and fungal systems, SCF-substrate recognition typically requires phosphorylation of the substrate (Pickart, 2001). By contrast, several studies indicate that the SCF^{TIR1} – AUX/IAA interaction is not regulated by phosphorylation. In addition, auxin promotes the interaction in plant extracts that have been cleared of membranes, indicating that the auxin receptor and other signaling proteins required for this response are soluble. Most recently, Kepinski and Leyser (2004) present data suggesting that auxin acts on TIR1 or a closely associated protein to promote substrate recognition. Tan *et al*. (2007) used crystallographic analysis to provide more details about auxin perception and the auxin receptor. The crystal structure showed that auxin fits into a surface pocket of TIR1 and enhanced the binding of Aux/IAA repressors to TIR1. They found that the TIR1-ASK1 complex had a mushroom shape, with the leucine-rich-repeat domain of TIR1 forming the cap, and the F-box of TIR1 along with ASK1 forming the stem. A pocket on the top of the TIR1 leucine-rich-repeat domain functions in both auxin binding and substrate recruitment (Tan *et al*., 2007; Guilfoyle, 2007). Some aspects of the auxin response appear to be controlled indirectly by COP1. Constitutive Photomorphogenesis1 (COP1) is known as a negative regulator of the light response (Moon *et al*., 2004). Dark-grown cop1 mutant seedlings display characteristics of light-grown seedlings, including short hypocotyls, leaf development, and photosynthetic activity (Deng *et al*., 1991). COP1 represses light-regulated development by targeting activators of light response for degradation (Osterlund *et al*., 2000). In the light, COP1 is depleted from the nucleus so these activators are no longer degraded (von Arnim and Deng, 1994; Osterlund *et al*., 2000). An analysis of *Arabidopsis* gene expression by microarray showed that more than 20% of the transcriptome representing more than 28 pathways, are regulated by COP1 in the dark (Me *et al*., 2002)

The identification of a plant auxin-binding protein (ABP1) marked a major advance in understanding auxin perception in plants. Developing plants that lacked ABP1 showed defective cell elongation, failed to organize the basic plant body plan, and subsequently degenerated (Napier *et al*., 2002). However, cell division still occurs in these plants, indicating that auxin pathway to regulate cell division is still working

Auxin transport, the energy-requiring, polar movement of IAA from the shoot apex to the root tip, and the subsequent redistribution of auxin from the root tip to the portion of the root is an essential process in plant development. The molecular mechanism of polar IAA transport has been elucidated by the discovery of membrane-located carrier proteins, namely AUX1, PIN and MDR1. PIN is efflux facilitator of IAA (Tanimoto, 2005), intimately connects plant cell polarity and multicellular patterning. Through the transport of the small molecule IAA, plant cells integrate their polarities and communicate the degree of their polarization. PIN proteins form a small gene families implicated in the cellular efflux of IAA. Consist of protein-mediated auxin efflux from cells driven by the membrane potential and auxin uptake into cells driven by the total proton motive force. In *Arabidopsis*, a family of at least six transporters, the PIN proteins, catalyzes auxin export from cells (Petrasek *et al*., 2006; Paponov *et al*., 2005)

The ubiquitin-proteasome pathway in gibberellin response

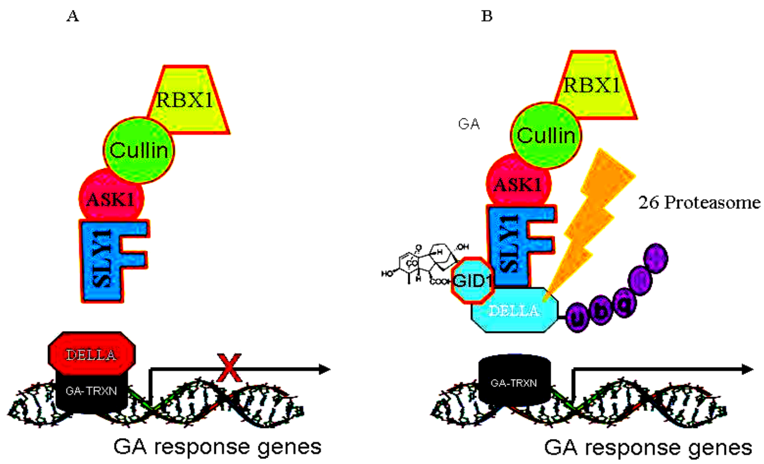

Figure 2 The gibberellin signaling pathway**.**

(A) In the absence of GA, the transcription of GA response genes is blocked by the GA transcription repressor proteins (DELLA). (B) GA is bound by its receptor protein (GID1) which then interacts with the F-box protein (SLY1 / GID2) in the SCF^{SLY1} complex. The binding of GA loaded GID1 stimulates the interaction of SCF^{SLY1} with the DELLA proteins which are then ubiquitinated, targeting them for destruction by the 26S proteasome and releasing the activator protein. Subsequently, GA response genes could be expressed.

Gibberellins (GAs) are phytohormones that are essential for many developmental processes and growth in plants, such as seed germination, stem elongation, flowering and fruit development (Ross *et al.*, 1997). They are a large family of tetracyclic diterpenoid plant growth regulators. Up to 2000, 126 GAs have been identified in higher plants, fungi and bacteria (Hedden and Phillips, 2000). Recent molecular biology and genetical studies have identified several positive and negative regulators of gibberellin signaling pathways in higher plants. The GA signaling pathway, like auxin signaling, relies on the ubiquitin-proteasome pathway to control expression through protein degradation. A conserved F-box protein of an SCF E3 ubiquitin ligase is a positive regulator of GA signaling in *Arabidopsis* and rice. GA stimulates stem elongation by causing this SCF complex to ubiquinate a family of negative regulators of GA response, called DELLA proteins, which leads to their degradation (Dill *et al*., 2001). The DELLA proteins function as negative regulators of GA signaling and their degradation through the ubiquitin/proteasome pathway is a key event in the regulation of GA stimulated processes (Shinozaki and Dennis, 2003). Recent studies indicate that the degradation of the DELLA proteins GAI and RGA occurs via the SCF^{SLY1} E3 ligase complex (McGinnis *et al*., 2003; Fu *et al*., 2004). Mutations in the F-box gene, SLEEPY1 (SLY1), result in stabilization of RGA and GAI even in the presence of GA (Silverstone *et al*., 2001). Furthermore, loss-of-function *rga* and *gai* mutants partially suppress the *sly1-10* phenotype (Dill *et al*., 2004). These results suggest that SLY1 recruits RGA to the SCF^{SLY1} E3 ligase complex for ubiquitination and subsequent degradation by the 26S proteasome. This removes the DELLA-mediated inhibition of GA-regulated growth responses. The DELLA proteins are members of the GRAS family. GA-signal-related DELLA proteins also contain a unique motif in their amino-terminal region called DELLA domain. This domain is absent from other GRAS proteins. There are 5 known DELLA proteins in *Arabidopsis*. Three of these have been shown to be involved in GA response (Fleck and Harberd, 2002).

Characterization of a GA-insensitive dwarf mutant in rice led to the identification of an F-box gene, GID2 (for GA-insensitive dwarf 2), which is the putative ortholog of *Arabidopsis* SLY1 (Sasaki *et al*., 2003; McGinnis *et al*., 2003). Dwarf phenotypes suggest that the GID2 and SLY1 genes encode positive regulators of GA response (Taiz and Zeiger, 2006). Their conserved F-box domains are component of the E3-ubiquitin ligase complexes (Itoh *et al*., 2003).

The involvement of SLY1 in the SCF complex and ubiquitination pathway is supported by experiments in *Arabidopsis* showing that SCF^{SLY} interacts more strongly with the phosphorylated DELLA proteins. Although phosphorylation of substrates is typically required for SCF recognition in animals, this would be the first example of such a mechanism in plants (Pickart, 2001).

One of the most interesting recent developments in the GA field is the discovery that the DELLA proteins are a point of intersection for several hormone-signaling pathways. Auxin promotes GA-dependent degradation of the DELLA proteins in the root (Fu and Harberd, 2003) whereas ethylene inhibits DELLA protein degradation (Achard *et al*., 2003). Furthermore, *axr1* plants also have a defect in GA-mediated degradation. However, in this case it is not clear if this effect is related to auxin signaling or to the likely role of AXR1 in SCF^{SLY1} function. Regardless, these results indicate the beginning to our understanding of the molecular basis for the diverse hormone interactions that occur during plant growth and development.

The GA-insensitive dwarf1 (gid1) rice mutant has a GA insensitive dwarf phenotype. This gene was shown to encode a gibberellin receptor that is a member of the serine hydrolase family, which includes esterases, lipases, and proteases (Sasaki *et al*., 2001). Variants of GID1 that cause loss-of-function phenotypes produce plants that cannot respond to gibberellins, indicating that GID1 protein acts as a positive regulator of gibberellin signaling (Bonetta and McCourt, 2005). The enzymatic function of GID1 has not yet been identified. When GA is bound to GID1, it interacts with another protein (DELLA-protein repressor) that represses the expression of gibberellin-dependent transcription factors (GA-TRXN). Following its formation, the GA-GID1-SLR1 (the DELLA-domain protein repressor in rice) complex in rice is believed to interact with GID2, the F-box protein component of an SCF^{GID2} ubiquitin ligase complex (Taiz and Zeiger, 2006). The interaction leads to destruction of the repressor protein by the plant's protein-degrading machinery and release of the transcription factors. Liberated, the transcription factors activate certain genes required for plant development. Xiangdong *et al*. (2002) investigated the properties of SLN1, a DELLA protein from barley that is destabilized by GA treatment. The results showed that proteasome-mediated protein degradation is necessary for GA-mediated destabilization of SLN1.

Abscisic acid signaling pathway

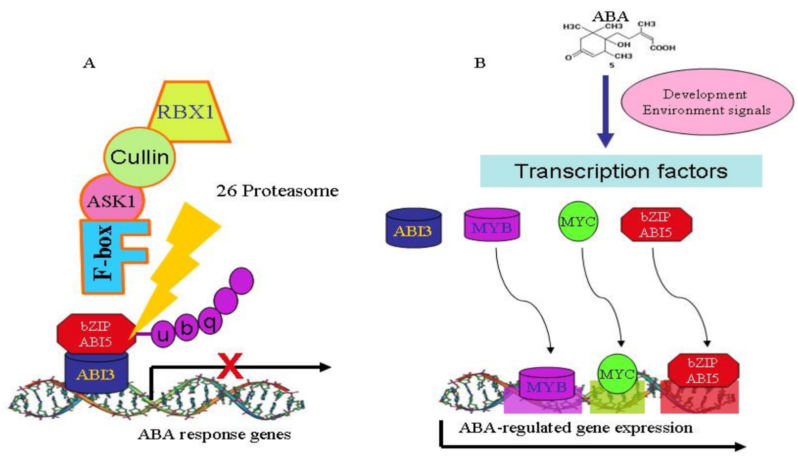

Figure 3 Regulatory mechanism of ABA-regulated gene expression

(A) When ABA is absent, ABA-insensitive transcription factors such as ABI3 and ABI5 are degraded by the 26S proteasome. (B) On the other hand, when the ABA concentration is increased due to a developmental or environment signal such as embryo maturation, drought or nutrient stress, these transcription factor are produced for response to those signals. Transcription factor bound directly to ABA regulated gene promoter for activate transcription.

The plant hormone abscisic acid (ABA) is the major player in mediating the adaptation of plants to various stresses and unfavourable environmental conditions through processes such as bud and seed dormancy, leaf abscission, and the closing of stomata. Though ABA is generally thought to play mostly growth inhibitory roles, it has many promoting functions as well. Biological phenomena such as inhibition of growth and maintenance of the dormancy of buds are the most striking effects of ABA. ABA activity alone is not enough to maintain the dormancy of buds in the long term though. Gemma *et al*. (2007) reported that ABA concentration in stored onion bulbs decreased gradually and onset of sprouting occurred at minimal ABA concentration. This was followed by an increase in ABA concentration as sprout growth continued. No straightforward relationship between ABA and carbohydrate metabolism could be determined. ABA is an efficient inhibitor of germination and occurs in high concentrations in dormant seeds. Just as in sprouting buds, its concentration decreases also during seed germination, an indication that germination is controlled by equilibrium of auxin, gibberellin, and cytokinin on one hand and ABA on the other hand. The role of ABA during the abscission of leaves has been studied in seedling of Cleopatra mandarin (*Citrus resshni* Hort.) growing in water stress condition. Leaf abscission was induced by 1-aminocyclopropane-1-carboxylic acid (ACC) transported from roots to shoots. Water stress induced both ABA and ACC following which leaf abscission occurred (Gomez *et al*., 1996).

In addition, abscisic acid has a regulating effect of on the water balance. As soon as the water supply of cut wheat leaf blades is interrupted and the cell turgor decreases the concentration of ABA rises forty-fold within four hours. These effects were also observed in rooted shoots. A water loss of 5 – 10 percent (of the fresh weight) was sufficient to increase the ABA level. ABA induces the stomata to close thus minimizing further loss of water.

ABA reverses the effect of growth-stimulating hormones (auxin, gibberellins, cytokinin) in several tissues. Although the nature of the ABA receptor(s) remains unknown, substantial progress has been made recently in the characterization of some downstream elements of the ABA signaling pathways (Gosti *et al., 1999).*

Mutations in the ABA-insensitive loci ABI1to ABI5 reduce the sensitivity of seed germination to exogenous ABA. Conversely, mutations in the ERA1 (ENHANCED RESPONSE TO ABA) locus increase the sensitivity of seed germination to applied ABA. The ERA1 gene encodes the b subunit of a protein farnesyl transferase. Loss-of-function *era1*mutants display prolonged seed dormancy and improved ability to withstand drought, which suggests that farnesylation is essential for negative regulation of ABA action. The *abi3*, *abi4*, and *abi5* mutants exhibit additional defects in various aspects of seed maturation but do not seem to be altered in vegetative responses to ABA. The corresponding gene products thus may belong to a seed-specific branch of the ABA signaling network. In contrast, the *abi1*, *abi2*, and *era1*mutations are more pleiotropic in that they affect ABA sensitivity in both seeds and vegetative tissues.

ABI1 and ABI2 genes encode homologous proteins that belong to the 2C class of protein serine/threonine phosphatases. The mutant alleles *abi1-1* and *abi2-1* carry amino acid substitution at equivalent positions in the ABI1 and ABI2 PP2C domains. The *abi1-1*and *abi2-1*mutations are both dominant and lead to largely overlapping sets of phenotypic alterations including ABA-resistant seed germination and seedling growth, reduced seed dormancy, abnormal stomatal regulation, and defects in various responses to drought stress. The functions of the ABI1 and ABI2 phosphatases, however, could not be fully understood solely on the basis of the dominant mutant alleles identified thus far. First, it remained unclear whether these proteins actually are involved in ABA signaling. Indeed, it is possible that the wild-type ABI1 and ABI2 proteins do not normally contribute to ABA action and that only gain-of-function mutant forms can interfere with ABA responsiveness. Even if ABI1 and ABI2 are components of ABA signaling cascades, dominant mutant alleles do not permit us to conclude whether these proteins are positive or negative regulators of ABA responses. The study of Gosti *et al*. (1999) isolated seven distinct loss-of-function alleles of the *ABI1*gene as intragenic revertants of the *abi1-1*mutant. In marked contrast to the ABA-resistant *abi1-1*mutant, all of these intragenic revertants were more responsive to ABA than were wild-type plants, and this ABA supersensitivity phenotype was recessive. Moreover, each of these revertant alleles encodes an ABI1 protein that lacked any detectable phosphatase activity in an *in vitro* enzymatic assay. These results thus provide genetic evidence that a loss of ABI1 PP2C activity leads to an enhanced responsiveness to ABA, and hence that the wild-type ABI1 phosphatase is a negative regulator of ABA responses.

The ABI3 and ABI4 genes have been cloned and encode putative transcriptional regulators. The ABI3 gene product of *Arabidopsis* is essential for correct completion of seed maturation (Nambara *et al*., 1995) and seed development (Rohde *et al*., 2000). Developmental of embryo has three phases including early stage, mid stage and late stage. Normally, ABA is generally thought to play mostly growth inhibitory roles. McCarty *et al*. (1991) reported that phenotypic and molecular characterization of *abi3* mutant focused primarily on late embryo functions such as desiccation tolerance and dormancy. Similar to Koornneef *et al*. (1982) the mutations in *Arabidopsis* that reduce either the ability to synthesize or response to the plant hormone ABA, also reduce seed dormancy. A study of Nambara *et al*. (1995) for a regulatory role for the ABI3 gene in the establishment of embryo maturation in *Arabidopsis* found that ABI3 gene product can be most accurately described as one of the major regulators of the transition between embryo maturation and early seedling development, rather than simply a transducer of the abscisic acid signal.

ABI5 encodes a member of the basic leucine zipper transcription factor family, involved in ABA signaling during seed maturation and germination. The *Arabidopsis abi5* mutants have pleiotropic defects in ABA response, including decreased sensitivity to ABA inhibition of germination and altered expression of some ABA-regulated genes (Finkelstein and Lynch 2000). Comparison of seed and ABA-inducible vegetative gene expression in wild-type and abi5-1 plants indicates that ABI5 regulates subset of late embryogenesis-abundant genes during both developmental stages (Finkelstein and Lynch, 2000; Srinivas *et al*., 2001).

The jasmonate signal transduction pathway

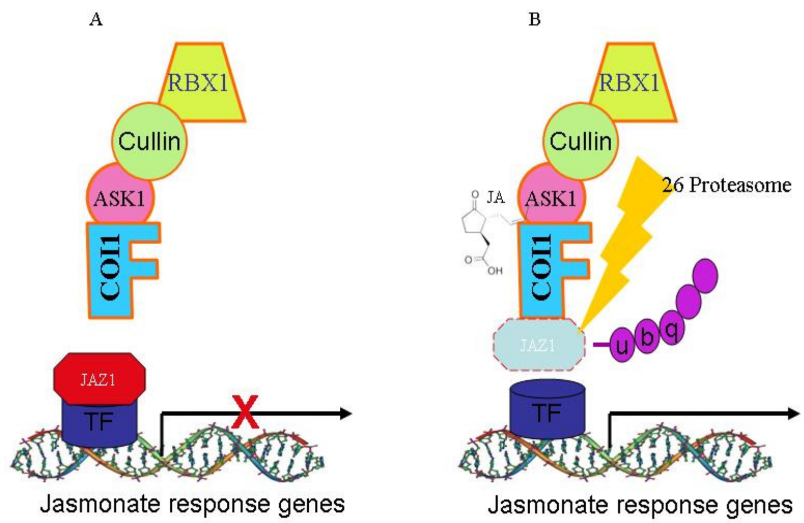

Figure 4 Jasmonate signaling pathway**.**

(A) In the absence of JA, the transcription of JA response genes is blocked by repressor proteins. (B) In the presence of JA concentrations, JA is bound by the F-box protein COI1 that is part of the SCF^{COI1} complex. The binding of JA stimulates the interaction of repressor proteins with SCF^{COI1} and promotes their ubiquitination, targeting them for destruction by the 26S proteasome. Subsequently the hormone transcription factors are released from their inhibition and JA response gene could be expressed.

JA signaling is also mediated by an SCF complex. The F-box gene COI1 (Coronatine Insensitive 1) was identified in a mutant screen for root elongation on medium containing coronatine (Benedetti *et al*., 1998; Xie *et al*., 1998). Coronatine is a toxin similar to methyl jasmonate in structure and is normally produced by *Pseudomonas syringae*. The coi1 mutants are male sterile and resistant to JA. Yeast two-hybrid and co-immunoprecipitation experiments showed that COI1 is part of an SCF complex that includes ASK1 or ASK2 and CUL1 (Xu *et al*., 2002). In another study, a yeast two-hybrid screen with COI1 as bait resulted in the recovery of a histone deacetylase called RPD3b. COI1 and RPD3b coimmunoprecipitate from plant extracts, suggesting that the histone deacetylase may be a COI1 substrate (Devoto *et al*., 2002). Thines *et al*. (2007) identified members of the jasmonate ZIM-domain (JAZ) protein family as key regulators of jasmonate signalling. JAZ1 protein acts to repress transcription of jasmonate-responsive genes. Jasmonate treatment causes JAZ1 degradation and this degradation is dependent on activities of the SCF^{COI1} ubiquitin ligase and the 26S proteasome. Furthermore, the jasmonoyl–isoleucine (JA–Ile) conjugate, but not other jasmonate-derivatives such as jasmonate, 12-oxo-phytodienoic acid, or methyl-jasmonate, promotes physical interaction between COI1 and JAZ1 proteins in the absence of other plant proteins. Our results suggest a model in which jasmonate ligands promote the binding of the SCF^{COI1} ubiquitin ligase to and subsequent degradation of the JAZ1 repressor protein, and implicate the SCF^{COI1}–JAZ1 protein complex as a site of perception of the plant hormone JA–Ile.

Nothing much is known about the specific downstream genes involved in the JA regulation of plant growth and development.

The ethylene signaling pathway

Ethylene is a gaseous plant hormone that plays a variety of roles in plant growth and development, such as biotic and abiotic stress responses, fruit ripening and senescence.

The essential components of ethylene signaling include a family of endoplasmic reticulum–localized receptors ETR1, ETR2 and EIN4, the Raf-like kinase CTR1 (for Constitutive Triple Response1), the enigmatic EIN2 (for Ethylene Insensitive2) protein, and the transcription factor EIN3 (Mineko and Shuichi, 2008; Guo and Ecker, 2003; Potuschak *et al*., 2003; Gagne *et al*., 2004). This pathway acts to promote transcription of a variety of ethylene-regulated genes through the action of the transcription factor EIN3 (Figure 5).

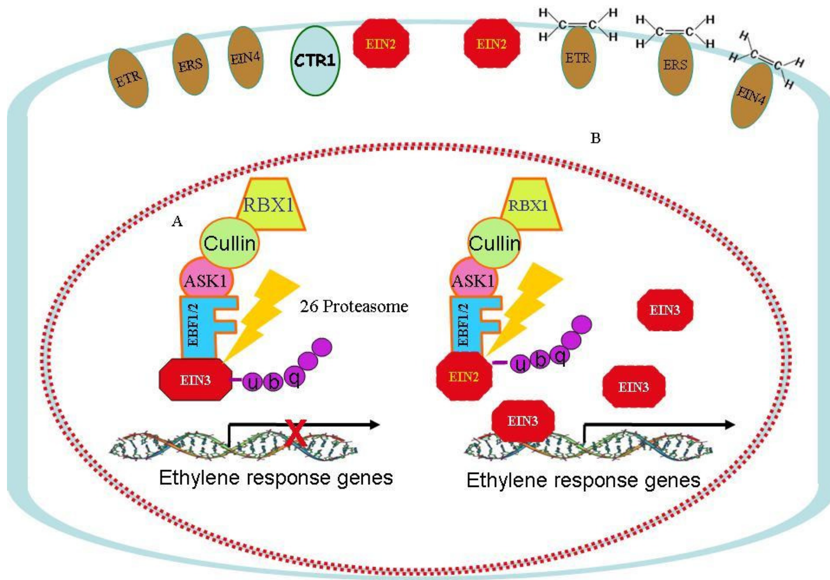

Figure 5 Ethylene signaling transduction pathway show two condition of ethylene concentration.

(A) In the absence of ethylene, EIN3 is ubiquitinated by the $SCF^{EBF1/2}$ complex, which targets it for degradation by 26S proteasome. (B) When ethylene is present, it binds to receptors (ETR, ERS and EIN4), which are integral membrane proteins of the endoplasmic reticulum membrane. EIN2 was degraded by $SCF^{EBF1/2}$, leading to EIN3 accumulation and to the activation of ethylene-response gene expression.

EIN3 is a key transcription factor in the signaling pathway. Several groups have shown that ethylene stabilizes EIN3 and that in the absence of ethylene, the protein is degraded by the proteasome. The E3 ubiquitin ligase responsible for the degradation is $SCF^{EBF1/2}$ (Guo and Ecker, 2003; Potuschak *et al*., 2003; Gagne *et al*., 2004). In the double mutant ebf1 ebf2, EIN3 is stabilized, resulting in a constitutive ethylene response including a constitutive triple response (Guo and Ecker, 2003). In summary, there is strong evidence that $SCF^{EBF1/2}$ degrades the transcriptional activator EIN3 in the absence of ethylene. Because a MAP kinase cascade has also been implicated in ethylene signaling (Novikova *et al*., 2000; Ouaked *et al*., 2003), it will be interesting to see if phosphorylation of EIN3 is required for its stabilization.

Ethylene, like auxin, gibberellin, and jasmonate controls transcription by regulatory protein degradation. However, unlike other plant hormones that stimulate the degradation of negative regulators, ethylene inhibits degradation of a positive regulator (EIN3).

The strigolactones signaling pathway

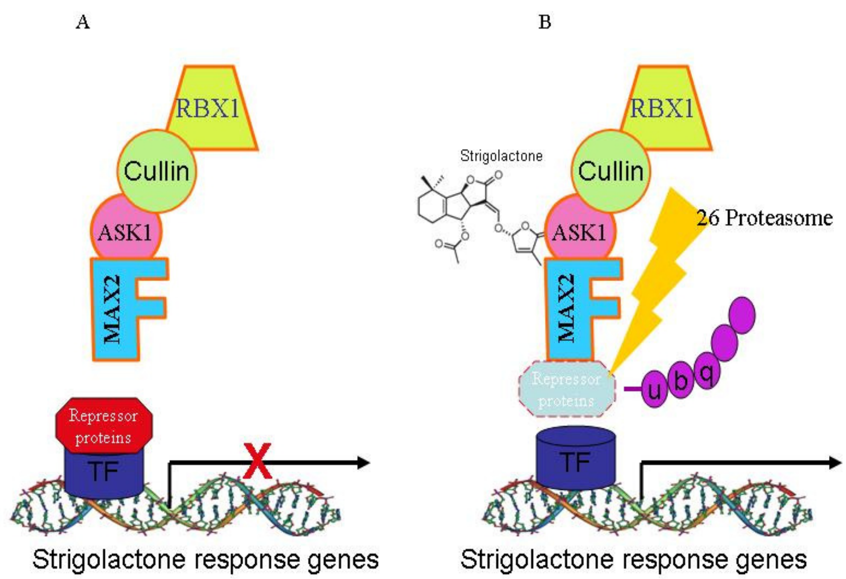

Figure 6 Strigolactone signaling transduction pathway.

(A) Under low strigolactone concentrations, the transcription factor gene is blocked by repressor proteins. (B) Under high hormone concentrations, strigolactone is bound by the F-box protein (MAX2) that in part of the SCF^{MAX2} complex. The binding stimulates the interaction of repressor proteins with SCF^{MAX2} and promotes the ubiquitination of repressor proteins, degrading them by the 26S proteasome and releasing the hormone transcription factor from their inhibition. Then strigolactone response gene could be expressed.

Strigolactones a group of terpenoid lactones have recently been confirmed as true plant growth regulators (Gomez *et al*., 2008; Umehara *et al*., 2008). They are compounds thought to be derived from carotenoids and are known to trigger the germination of parasitic plant seeds (*Striga* spp.) and but also to stimulate symbiotic fungi to form mycorrhiza that colonize roots and facilitate the uptake of soil nutrients by plants (Gomez-Roldan *et al*., 2008). Plants that have mutations in genes encoding carotenoid-cleaving dioxygenases (CCDs or MAX4) are highly branched, indicating that some substance normally suppresses the growth of lateral shoots. Mutant plants have a defect in the signaling pathway downstream of strigolactone. The defect is in a control component of the pathway, an F-box protein, which is postulated to transduce the hormone signal. These mutants are not deficient in strigolactone synthesis and do not respond to application of strigolactone. MAX2 is an F-box protein of strigolactone protein degradation pathway. The SCF^{MAX2} promotes the degradation of a protein that stimulates lateral branching (Ward and Leyser, 2004). In the case of senescence, MAX2 presumably degrades a protein that inhibits leaf senescence (Woo *et al*., 2001). Whether this is the same substrate as that which promotes lateral branching is unknown.

Brassinosteroids (BR) signaling pathway

Brassinosteriods are steroid hormones, first discovered in *Brassica napus*, rapeseed (Brassicaceae or mustard family). Physiological studies have demonstrated that BR can induce diverse cellular responses such as stem elongation, pollen tube growth, leaf bending or epinasty, root inhibition, induction of ethylene biosynthesis and fruit ripening, and xylem differentiation (Symons *et al*., 2006)

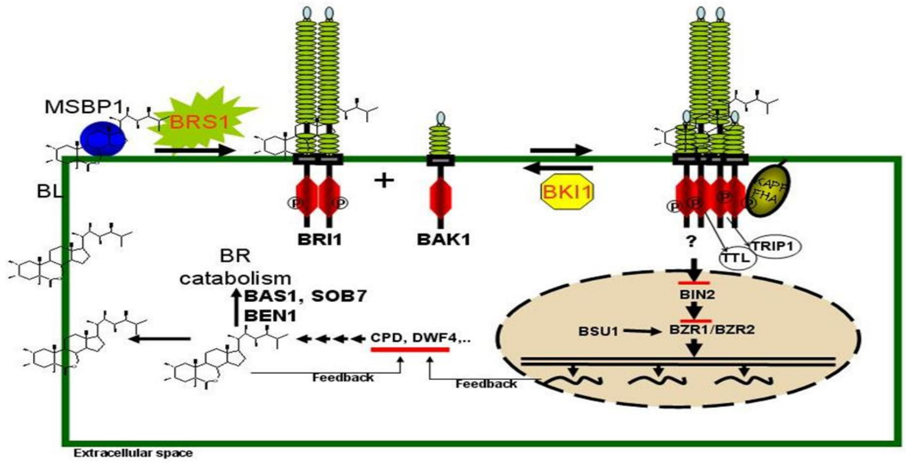

Figure 7 Brassinosteroid signaling transduction.

In low BR concentration, the BIN2 kinase rapidly phosphorylates the brassinosteroid-dependent transcriptional regulators, BES1 and BZR1 leading to their subsequent ubiquitination and degradation by 26S proteasome . When BR is perceived by the membrane-localised BRI1-BAK1 heterodimer, This lead to the accumulation of BES1 and BZR1 transcription factors then interact directly to promoter activated transcription and BR response genes were express.

Source: He *et al*. (2007)

Brassinosteroids (BR) are perceived by a cell surface receptor kinase, BRI1. Recent studies have demonstrated that BR binding to the extracellular domain of BRI1 induces a kinase activation and dimerization with another receptor kinase, BAK1. Activated BRI1 or BAK1 then regulate, possibly indirectly, the activities of BIN2 kinase and/or BSU1 phosphatase, which directly regulate the phosphorylation status and nuclear accumulation of two homologous transcription factors, BZR1 and BES1. BZR1 and BES1 directly bind to promoters of BR responsive genes to regulate their expression. The BR signaling pathway has become a paradigm for both receptor kinase signaling in plants and steroid signaling by cell surface receptors in general.

Other aspects of hormone signal transduction

Although each plant hormone has specific receptor(s) and responses genes in their signal transduction pathway then effected in different part of plant tissue or organ. Many aspects of plant development such as cell elongation, flowering and germination, are influenced by more than one hormone activity. The ubiquitin-proteasome pathway is not the only signal transduction pathway, but also other pathways are involved in signal transduction and result in plant responses. Some protein was not hormone receptor but act as a member of component that worked in pathway also important for expression of hormone response gene, such as SKP1, CULLIN, RBX1, HECT, G-protein, KNOTTED 1-LIKE HOMEBOX (KNOX), and MYB. First three are a member of SCF complexes. These proteins worked together so they should have some domain that supported their interaction as recognized domain for binding.

cAMP and RAS/RAF/MEK/ERK signaling pathway, are one of the oldest signaling molecules know (Dumaz and Marais, 2003). RAS proteins are small G-proteins that are embedded on the inner surface of the plasma membrane (Robinson and Cobb, 1997; Wellbrock *et al*., 2004). These pathways are also activated by growth factor and lead to the phosphorylation of many targets which then regulate cell fate.

MYB

MYB transcription factors represent a family of proteins that include the conserved MYB DNA-binding domain. The first MYB gene identified was the oncogene v-Myb derived from the avian myeloblastosis virus.

The proteins encoded by MYB genes are crucial to the control of proliferation and differentiation in a number of cell types. The MYB domain generally comprises up to three imperfect repeats, each forming a helix-turn-helix structure of about 53 amino acids. Three regularly spaced tryptophan residues, which form a tryptophan cluster in the three-dimensional helix-turn-helix structure, are characteristic of a MYB repeat. The three repeats in c-Myb are referred to as R1, R2 and R3; and repeats from other MYB proteins are categorised according to their similarity to R1, R2 or R3.

In contrast to animals, plants contain a large MYB-protein subfamily that is characterised by the presence of the R2 and R3-type of helix-turn-helix loops (R2R3MYB proteins). Plant MYB factors can act as transcriptional activators and some are associated closely with the activity of the circadian clock. Through analysis of whole genome sequencing data, a few genes encoding three Myb repeats have been detected in *A. thaliana* (Braun and Grotewold, 1999). These are potentially multifunctional MYB proteins that are involved in transcript splicing (Burns *et al*., 1999) and transcriptional regulation (Lei *et al*., 2000).

HECT (HECT ubiquitin protein ligase3)

The general function of the ubiquitination pathway is to conjugate ubiquitin to lys the residues within substrate proteins, thus targeting them for degradation by the proteasome (Smalle and Vierstra, 2004). The ubiquitin protein is attached to a protein substrate through the action of three enzymes: the ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin protein ligase (E3). HECT is one type of E3 ubiquitin protein ligase; the SCF complexes are another)

HECT E3s are large proteins. The HECT domain is a 350-amino acid motif and contains both an ubiquitin binding site and an Ub E2 binding site (Pickart, 2001) because the HECT E3 receives its ubiquitin from E2 and transfers it to the substrate. Typically, this process is then repeated several times to attach multiple ubiquitin molecules to the substrate, and polyubiquitination has been shown to be necessary for degradation of the substrate by the 26S proteasome (Wikinson, 2000: Smalle and Vierstra, 2004).

Table 1 List of Hormone Signal Transduction genes studied

Gene symbol	Gene name	Function
Auxin		
TIR1	Transport Inhibitor Response1	F-box protein ; involved in ubiquitin-mediated processes
PIN	Polar auxin transport (PIN-Form)	Auxin transport
ABP	Auxin-binding protein	Binds auxins ; putative auxin receptor
ARF1	Auxin response factors1	Transcription factor for auxin-dependent
ARF2	Auxin response factors2	gene expression that binds to Auxin
ARF6/8		Response Elements
AXR	Auxin resistant	Subunit of the RUB activating enzyme Similar to the ubiquitin-activating enzyme E1 Involved in auxin action
Gibberellin		
GID1	GA-insensitive dwarf1	Interact with the protein turnover complex (SCF).The SCF complex is able to degrade the DELLA repressor, thereby freeing GA-TRXN to stimulate gene transcription.
SLY1/GID2	SLEEPY1	F-box factor that targets DELLA proteins for proteasomal degradation ; a positive regulator of GA signaling orthologous to GID2
DELLA	DELLA	Repressor proteins, interfere with GA-
RGA	REPRESSOR OF GAI	dependent transcription factors (GA-
GAI	GA INSENSITIVE	TRXN) and degraded by GA-SCF
RGL1,2,3,5	RGA like 1	complex
PHOR1	photoperiod responsive	gibberellin response, encode photoperiod responsive protein

Table 1 (Continued)

Gene symbol	Gene name	Function
ABA		
ABI1	Abscisic acid insensitive1	Protein phosphatase ; dominant mutations confer ABA-insensitivity
ABI2	Abscisic acid insensitive2	encode for proteins serine/threonine phosphatases 2C (PP2C)
ABI3	Abscisic acid insensitive3	Promotes embryonic development ;B3 domain transcription factor
ABI5	ABA INSENSITIVE 5 (DNA binding , transcription factor, transcriptional activator)	Basic leucine zipper transcription factor, involved in ABA signaling during seed maturation and germination.
Ethylene		
EBF1/2	EIN3 Binding F-box protein	F-box protein
EIN2	Ethylene insensitive 2	channel-like transmembrane protein
EIN3	Ethylene insensitive 3	Ethylene response gene encoding a transcription factor
ETR	Ethylene receptor	Encode a receptor for ethylene located on the ER
ERS	Ethylene-response sensor	Functional same as Ethylene receptor
CTR	Constitutive triple response	Encode ETR protein in the absence of ethylene for plant seedling
Jasmonate		
COI1	Coronatine-insensitive1	F-box protein encode coronatine insensitive1 protein, jasmonate signal cascade
JAZ1	jasmonate ZIM-domain (JAZ)	Encode repressor protein that interfere with transcription factor and degraded by SCF^{COI1} complex

Table 1 (Continued)

Gene symbol	Gene name	Function
Brassinosteroid		
BRH1	BRASSINOSTEROID-RESPONSIVE RING-H2	Encodes a novel ring finger protein and forms an N-terminal hydrophobic domain and a C-terminal RING-H2 Signature. Expression is down regulated by brassinolide
BAK1	BRI1-associated receptor kinase1	The formation of the activated BRI1/BAK1 hetero-oligomer that lead to brassinosteroid-regulated gene transcription.
BRX	Brevis radix	Brassinosteroid-biosynthesis regulated
Strigolactone		
MAX2	More Axillary Branches2	F-box protein
MAX3	More Axillary Branches3	MAX3 and MAX4 are divergent members of the carotenoid cleavage dioxygenase family of enzyme
MAX4	More Axillary Branches4	
General		
HECT	HECT ubiquitin-protein ligase 3	Encodes HECT ubiquitin-protein ligase 3
MYB	MYB	Encode transcription factor that important for many hormone response gene
SIN1	Short integument1	Required for normal ovule development
CULLIN	CULLIN	Encode CULLIN a member of SCF complexes

Gene discovery

When candidate genes have been targeted for discovery in species for which DNA sequence data is not publicly available, information from other species can be used. Phylogenetic comparison of homologous genes (orthologs and paralogs) can identify conserved regions through phylogenetic shadowing. Primers for PCR amplification can be designed corresponding to the conserved regions. Fragments of the corresponding gene can then be amplified either from genomic DNA or from mRNA. The isolation of genes from mRNA could be difficult when the gene is expressed in low amounts, only in particular tissues and / or only at a particular developmental stage. Amplification of the fragments from genomic DNA circumvents the problem of unknown gene expression and unknown gene numbers, but has other limitations. The genome is much larger than the transcribed part and thus the possibility for non-specific priming is higher. If the target fragment includes an intron, the amplification could fail because of the large intron size. PCR amplification from genomic DNA can indicate multiple loci in the genome, but it is not known which of the loci is functionally expressed; which are may be pseudogenes or silent for other reasons.

The genetic code is the set of rules by which information encoded in genetic material (DNA or RNA sequences) is translated into proteins (amino acid sequences) by living cells. The code defines a mapping between tri-nucleotide sequences, called codons, and amino acids. A triplet codon in a nucleic acid sequence usually specifies a single amino acid (though insertion of two amino acids at one codon can occur unambiguously in different places in the same protein). Because the vast majority of genes are encoded with exactly the same code, this particular code is often referred to as the canonical or standard genetic code, or simply the genetic code, though in fact there are many variant codes.

The twenty amino acids found in proteins data bases. All 64 possible 3-letter combinations of the DNA coding units T, C, A and G are used either to encode one of these amino acids or as one of the three stop codons that signals the end of a sequence. While DNA can be decoded unambiguously, it is not possible to predict a DNA sequence from its protein sequence. Because most amino acids have multiple codons, a number of possible DNA sequences might represent the same protein sequence.

Degenerate Primer

A nucleotide sequence is called degenerate if one or more of its positions can be occupied by more than one of the four nucleotides (Kwok *et al*., 1994). The total number of different oligos in the resulting mixture is known as the degeneracy of the primer. Such primers are widely used in screening genomic DNA to identify homologues of already partially known genes. Degenerate primers are used to amplify conserved sequences of a gene or gene from the genome of an organism. Degenerate primer can generally be used when there is evidence of highly conserved regions or motifs of amino acids that can be designed into degenerate primers; these regions may be conserved between species.

DNA Cloning

Molecular cloning refers to the procedure of isolating a defined DNA sequence and obtaining multiple copies of it *in vivo*. Cloning is frequently employed to amplify DNA fragments containing genes, but it can be used to amplify any DNA sequence such as promoters, non-coding sequences and randomly fragmented DNA. It is utilised in a wide array of biological experiments and practical applications such as large scale protein production. In essence, in order to amplify any DNA sequence in a living organism that sequence must be linked to an origin of replication, a sequence element capable of directing the propagation of its self and any linked sequence. In practice, however, a number of other features are desired. A variety of specialized cloning vectors have been developed that allow protein expression, tagging, single stranded RNA and DNA production and a host of other manipulations. Cloning of any DNA fragment essentially involves four steps: fragmentation, ligation, transfection, and screening/selection. Although these steps are invariable among cloning procedures a number of alternative routes can be selected, these are summarised as a 'cloning strategy'. Initially, the DNA of interest needs to be isolated to provide a relevant DNA segment of suitable size. Subsequently, a ligation procedure is employed whereby the isolated fragment is inserted into a vector. The vector (which is frequently circular) is linearised by means of restriction enzymes, and incubated with the fragment of interest under appropriate conditions with an enzyme called DNA ligase. Following ligation the vector with the insert of interest is recircularized and can be transfected into cells. A number of alternative techniques are available, such as chemical sensitivation of cells, electroporation and biolistics. Finally, the transfected cells are cultured. As the aforementioned procedures are of particularly low efficiency, there is a need to identify the cells that have been successfully transfected with the vector construct containing the desired insertion sequence in the required orientation. Modern cloning vectors include selectable antibiotic resistance markers, which allow only cells in which the vector has been transfected, too grow. Additionally, the cloning vectors may contain colour selection markers which provide blue/white screening (α-factor complementation) on X-gal medium. Nevertheless, these selection steps do not absolutely guarantee that the DNA insert is present in the cells obtained. Further investigation of the resulting colonies is required to confirm that cloning was successful. This may be accomplished by means of PCR, restriction fragment analysis and/or DNA sequencing.

Detection of polymorphisms

Single-Strand Conformation Polymorphism (SSCP)

First announced in 1989 as a new means of detecting DNA polymorphisms, or sequence variations, SSCP analysis offers an inexpensive, convenient, and sensitive method for determining genetic variation (Sunnucks *et al.*, 2000). SSCP is a technique for detection of polymorphism of PCR products that have been amplified using specific primers and are then separated by electrophoresis on a nondenaturing polyacrylamide gel. The separation of different alleles is based on subtle differences in sequence (often a single base pair) which results in a different secondary structure and a measurable difference in mobility through the gel matrix (Orita *et al*., 1989). The mobility of double-stranded DNA in gel electrophoresis is dependent on strand size and length but is relatively independent of the particular nucleotide sequence. The mobility of single strands, however, is noticeably affected by very small changes in sequence, possibly one changed nucleotide out of several hundred. Small changes are noticeable because of the relatively unstable nature of single-stranded DNA. In the absence of a complementary strand, the single strand will undergo intrastrand base pairing to some extent, resulting in loops and folds that give the single strand a unique 3D structure. A single nucleotide change could dramatically affect the fragment's mobility through a gel by altering the intrastrand base pairing and its resulting 3D conformation. Like restriction fragment length polymorphisms (RFLPs), SSCPs are allelic variants of inherited, genetic traits that can be used as genetic markers. Unlike RFLP analysis, however, SSCP analysis can detect DNA polymorphisms and mutations at multiple places in DNA fragments (Orita *et al*., 1989). As a mutation scanning technique, though, SSCP is more often used to analyze the polymorphisms at single loci, especially when used for medical diagnoses (Sunnucks *et al*., 2000).

MATERIALS AND METHODS

Plant materials and DNA extraction

Oil palm (*E. guineensis*), vegetative tissues (leaves and root), vegetative meristem or floral meristem were excised from plants. Genomic DNA was be extracted from 100 mg tissue using the DNeasy Plant Mini Kit (Qiagen, Germany). Genomic DNA was stored at -20 ˚ C until required.

Plant materials were obtained from the Univanich breeding programme, seedlings from Univanich, seedlings from ASD-Costa Rica (bought from nursery Mongkol, Kanchanaburi) and seedlings from local producers in Topi x Yangumbi Tenera, Chumphon.

PCR amplification of candidate genes from oil palm

DNA sequences of each of the candidate genes were retrieved from public DNA sequence repositories using keyword searches. Using the obtained sequences, the non-redundant (NR) and EST databases of GENBANK/EMBL/DDBJ (http://www.ncbi.nlm.nih.gov) and the EST contig sequences from J. Craig Venter Institute (http://www.jcvi.org/cms/research/groups/plant-genomics/resources/) were searched using the BLAST algorithm. The corresponding genes from *Populus* were retrieved from the Joint Genomics Institute website (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html).

All obtained sequences were aligned using ClustalW or ClustalX program and adjusted using GeneDoc. The positions of introns in genomic sequences were indicated. Comparison of the sequences from many plant species revealed conservation of the exon and intron structure.

Because the sequences were retrieved from several sources and consisted of partial and complete cDNA, EST, or genomic DNA fragments from several plant species it was possible to broaden the usability of the primers designed. Because the exact sequence of the signals transduction genes in oil palm are unknown, degenerate primers were designed to match conserved codons flanking the intron locations, if available.

DNA fragments were amplified by a simple polymerase chain reaction (PCR) using the degenerate primers, *Taq* DNA polymerases (Fermentas, Qiagen or RBC) using standard PCR conditions. Temperatures for the annealing step during the PCR amplification were adjusted in case no suitable products were amplified. For some primer sets, Phusion DNA polymerase was used in an attempt to obtain specific PCR fragments. The amplification products were checked by agarose gel electrophoresis for presence of fragments corresponding to the expected sizes. Selected PCR products cloned for sequencing.

Table 2 Sequences of PCR primers used to amplify partial gene in oil palm.

Gene name	Primer sequence
Auxin	
Transport Inhibitor Response1	F) AAAGGCAAGCCTCACTTTGCNGAYTT
(TIR1)	R) GATGACATCCAAAGGGATCGCAT
Auxin response factor1(ARF1)	F) TACTTCCCTCAAGGTCAYATGGA
	R) GTTATCTGTGCATAAACYTCRTC
Auxin transport (PIN)	F) CTTCATATGTTCGTNTGGAGYTC
	R) CTGAACATTGCCATTCCNAGNCC
Auxin resistant (AXR)	F) GCACATGTTGTGGGTTGGCCNCC
	R) ACATCACCAACAAGCATCCARTC
Auxin binding protein (ABP)	F) GGTTTCTCTCAYATTACTGTNGCNGG
	R) ACTTCTTCACATGARTGYCTRTG
Giberellin	
GID1	F1) CCGTGTGCTTATGACGAYGGNTGG
	R1) AGATACGCTCTCCARTACCARTC
DELLA	F1)TCTTCCGACATGGCTGANGTNGC
	R1) TCTTGCTGCACGGCTTCTGCRCANGC
	F2) GACACTGTTCATTACAAYCCNTCNGA
	R2) TTGTGGTTCGCYTCYTGYTCNAC
	F3) GCNGCNTCTCAGGCCGGAGCTATG
	R3) CACGCCACCACGTTRCADATYTG
	F4) AAGCAAGGGATGCARTGGCCNGC
	R4) GCGATTAGTGGGCGGGTRTGCCANCC
SLY1/GID2	F) GCGCAGGACGAGCGGCTNTGGGA
	R) TGGAGCCGACGGAATCCNCCRAG
Abscisic acid	
ABI1/2	F) TTCGGTGTTTATGAYGGNCAYGG
	R) ACATCCCATAGTCCRTCRCTNGC
Abscisic acid	F) GGAAGGATCGTGCTACCCAAAG
insensitive3 (ABI3)	R) CGTTGGATCGAACAAATTCTCC
Abscisic acid	F) TTCGGTTCTATGAAYATGGAYGA
Insensitive5 (ABI5)	R) GCTGACTCTCKRTTYTTDATCAT

Table 2 (Continued)

Gene name	Primer sequence
Ethylene	
EIN3 Binding (EBF1/2) Factor	F1) CTTCCTGATGAATGCCTYTTYGARAT
	F2) AGACTTGCTGCTATTGCWGTNGG
	R) GCATTGCCCATGACCCARAANCC
Ethylene Receptor (ETR)	F1) TTTGGTGCTTTCATTGTTCTNTGYGG
	R1) GCAGCATGTGAAAGAGCAACNGCNAC
	F2) GAAGAATGTGCTTTGTGGATGCC
	R2) GGCGTCCTCATTTCRTGRTTCATNAC
	R3) GTTTGCATCAGACGTTTYTCRTCNCC
Jasmonate	
Coronatine insensitive (COI)	F) AAGGGTAAGCCCCGRGCNGCNATGTT
	R) ACTTCTAATCCTCTATCTCCDATNAC
Brassinosteroid	
BRH	F1) ATGGGTTTTCCAGTNGGNTA
	R1) GGCGTGCGGCAGAGCGGRCANGT
BRI1-associated receptor kinase1 (BAK1)	F1) GTTAATCCTTGYACTTGGTTYCAYGT
	R1) ATGTTGTTACTGTANAGYTCNCT
	F2) TACATGGCTAATGGAAGYGTNGC
	R2) CCAATCAAGYAACATRACRTCRTC
	R3) AGCATYCTNACNACYTCNGACATYTT
Brevis Radix (BRX)	F1) AAATGGCAAGCTCARAGNTGGTGG
	R1) CCTGGTTCATCCTCTTCNACCCAYTC
Strigolactone	
MORE AXILLARY BRANCHING2 (MAX2)	F1) GAGCTTGATTACTGGCCNCCNCA
	R1) ACTCTCATCTCTGTRCTCATRTC
MORE AXILLARY BRANCHING3 (MAX3)	F1) TGCAATGCCGAGGACATGCTNCTNCC
	R1) GTGAATGCCCARTCNGGRATCAT
MORE AXILLARY BRANCHING4 (MAX4)	F1) ACCGATAACGCCAACACNGGNGT
	R1) ACCGCGAACGAGTGGACCCANCCNGG
	F2) ATGGATATGTGCAGCATTHAAYCC
	R2) AGGGTGTTGGGGAARTTRCANGG

Table 2 (Continued)

Gene name	Primer sequence
General	
HECT	F1) CTTGGTTTATTTCCTCGNCCNTGG
	R1) TCAGGATAGCCTGGAAGNGTRAARTC
	F2) GATTTTACTCTTCCAGGYTAYCCNGA
	R2) GTATATCCATGATCRAAYTTDAT
	F3) GCTTTCTGCCAGTTTGTTACNGGNGC
	R3) TAATTAGCACATGTCATGACRCTNGG
Myb	F) TGTGGTAAAAGTTGYMGNYTNMGRTGG
	R) TTCTTTATTTCGTTATCNGTYCKNCCNGG
CULLIN	F1) AGAGAGAAGCATGATGARTTYATG
	R1) TCTGTAACCATTCCYTCCATYTT

F=Forward, R=Reverse, B=(C/G/T), D=(A/G/T), H=(A/C/T), M=(A/C), N=(A/G/C/T) , K=(G/T), R=(A/G), S=(G/C), W=(A/T), V=(A/G/C), Y=(C/T)

Cloning and sequencing of PCR fragments

PCR products that were larger than the length of the coding part of the targeted gene were purified using the MinElute PCR Purification Kit (Qiagen, Germany). The purified fragments were then legated in to pGem-T plasmid vector (Promega, USA) and transformed into competent cell (*Escherichia coli* ‘DH10B’ by electroporation using MicroPulser^{TM} (BIO-RAD, USA). The cloned cells were spread onto LB (Luria-Bertani) medium agar plates containing 100 µg/ml of antibiotic (ampicilin) 100 µl IPTG (100 mM) and 20 µl X-gal (50 mg/ml). The bacteria were then allowed to grow overnight at 37˚C. The blue-white colony selection was used to identify transformants. Individual colonies were picked for direct PCR amplification to check the presence of insert using M13 F/R or specific primers. The remainder of the same single colony was grown overnight in an incubator shaker at 37℃, 150 rpm in 5 ml of LB medium broth with 100 µg/ml of ampicilin. Plasmids were extracted from cultured cells using QIAprep Spin Miniprep kit (Qiagen, Germany) or GeneAid kit following manufacturers' procedures.

PCR-products were sent for sequencing by Macrogen Inc, Korea or 1stBase, Malaysia

Design of specific primers and detection of polymorphisms

The obtained sequences were then compared with sequences from other plant species to check whether the correct gene had been obtained. Specific primers were designed to amplify the candidate gene from oil palm. The specific primer sets targeted the introns of the genes because a higher level of polymorphism would be expected compared to exons.

Table 3 sequence of the specific primers

Primer name	Primer sequence
Transport Inhibitor Response1 (EgTIR1)	F) CGGCGATCGCCACCCATTGCAG
	R1) ATGGGAACACCCTCAACTCCTG
	R2) GAATCAGGGAAGCAGCTGAGCC
Auxin response factor1 (EgARF1)	F) TGGAACAGGTATCTTGAGTTTC
	R) TGTCTGGTTCAGCCTAAAAAG
Auxin resistant (EgAXR)	F) ATGTATGTGAAGGTGAGTATGGA
	R) TCCCCATCTTTATCTTCATACGT
Auxin transport (EgPIN1)	F1) GGAAGTGAGCATGGTGGAGCTGC
	R1) AGAGAGTCGATTTCCTGAGTACC
Auxin transport (EgPIN2)	F2) GGAGCAGCTCACCCAACCGATCA
	R2) AGTGAGTCCATTCCCTGGGTACC
Auxin transport (EgPIN3)	F3) TACGTCTTCCCACCGGCGCCCAC
	R3) TCCGACAGTATGGAGATGGAACG
Auxin transport (EgPIN4)	F4) TACGGTTTGCCGGCGACGGATCC
	R4) TCCGACAGTATGGAGATGGACTG
Abscisic acid insensitive3 (EgABI3)	F) GGAAGGATCGTGCTACCCAAAG
	R) CGTTGGATCGAACAAATTCTCC

To detect polymorphisms, SSCP assays were developed. PCR products were mixed with four volumes of loading dye (95% formamide, 10 mM NaOH, 0.025% xylene cyanol and 0.025% bromophenol blue) then denatured at 95°C for 10 min, and immediately placed on ice water to stabilize single strands. 2.5 µl aliquots were loaded on a 30 cm x 40 cm x 0.4 mm polyacrylamide (Sequagel MD, National Diagnostics, U.S.A.) gels attached to glass plate in 0.6x TBE buffer ran in 4°C refrigerator at constant 10 watt for 16 hr.

After SSCP, the DNA bands were revealed by silver staining. The gels on glass plates were covered with fix solution (10% acetic acid) and shaken gently on orbital shaker (ArmaLab, U.S.A.) for 30 min, then washed twice with reverse osmosis water for 10 min. Silver staining was carried out for 30 min on shaker using 1% silver nitrate (Fisher Scientific UK Limited) with 1.5 ml/l of 37% formaldehyde. Developing solution containing 50g/l sodium bicarbonate (Riedel-de Haën, GmbH), 1.5 ml/l of 37% formaldehyde, and 1 mg/l sodium thiosulphate, was used to develop the gels. Developing process were stopped by 10% acetic acid for 5 min and the gels were washed thoroughly with water 15 min, then left to air dry.

Phylogenetic analysis

Phylogenetic analysis was performed with MEGA4 program (Tamura *et al*., 2007) using the neighbor-joining method (Saitou and Nei, 1987). A bootstrap test was carried out with 1000 iterations (Felsenstein, 1985). Genetic distances were computed from amino acid sequence alignments using the Poisson correction method (Zuckerkandl and Pauling, 1965).

RESULTS AND DISCUSSION

Gene discovery in oil palm

Thirty fragments of genes involved in hormone signal transduction were successfully amplified from oil palm genomic DNA by PCR using degenerate primer sets corresponding to conserved regions of the target genes (Table 2). These genes are involved in several hormone signaling pathways including auxin, gibberellins, abscisic acid, jasmonate, brassinosteroids, ethylene and strigolactone. The obtained gene sequences were aligned together with sequences from other plant species. Comparison of the oil palm sequences with sequences from other plant species revealed a strict conservation of the exon and intron structure. Sizes of each of the gene fragments including exons and introns are show in table 4.

Table 4 Size of characterized gene fragments including exon and intron.

GENE	Name	Coding region	Intron length	Total fragment length
Auxin				
1 TIR1	Transport Inhibitor Response1	1325	488+86	1889
2 PIN	*Polar auxin transport (PIN-Form)*	1144	77	1221
3 PIN	*Polar auxin transport (PIN-Form)*	1139	76	1215
4 PIN	*Polar auxin transport (PIN-Form)*	1184	83	1267
5 PIN	*Polar auxin transport (PIN-Form)*	593	101	694
6 PIN	*Polar auxin transport (PIN-Form)*	1139	76	1215
7 PIN	*Polar auxin transport (PIN-Form)*	592	97	689
8 PIN	*Polar auxin transport (PIN-Form)*	582	127	709
9 ARF1	*Auxin response factor1*	159	350+111	620
10 AXR2/3	*Auxin resistant 2+3*	296	116	412
Gibberellin				
11 GID1	*GA-insensitive dwarf1*	280	0	280

Table 4 (Continued)

GENE	Name	Coding region	Intron length	Total fragment length
Abscisic acid				
12 ABI3	*Abscisic acid insensitive3*	251	139+244+111	745
13 ABI5	*Abscisic acid insensitive5*	922	0	922
Brassinosteroids				
14 BRX	*Brevis radix*	585	0	585
15 BAK	*BRI1-associated receptor kinase1*	429	245	674
Ethylene				
16 EBF	*EIN3 (Ethylene insensitive) Binding Factor*	862	0	862
17 ERS1	Ethylene response sensor	548	440	988
18 ERS2	Ethylene response sensor	548	431	979
19 ERS3	Ethylene response sensor	548	423	971
20 ETR	Ethylene receptor	863	0	863
Jasmonate				
21 COI1	Coronatine Insensitive1	764	0	764
Strigolactone				
22 MAX2	MORE AXILLARY BRANCHES2	229	0	229
23 MAX4	MORE AXILLARY BRANCHES4 (F1R1)	322	0	322
24 MAX4	MORE AXILLARY BRANCHES4 (F2R2)	98	0	98
25 MAX4	MORE AXILLARY BRANCHES4 (F2R2)	97	0	97
General				
26 HECT	*HECT ubiquitin-protein ligase3*	374	113	487
27 MYB	*Myb*	149	115	264
28 MYB	*Myb*	173	193	366
29 MYB	*Myb*	149	91	240

Discovery of auxin signal transduction genes

TIR1

Mutant screens in *Arabidopsis thaliana* identified TIR1 as a protein that appeared to be essential for auxin-dependent hypocotyl elongation and lateral root formation. TIR1 was found to be an F-box protein that binds auxin directly. Auxin binding to TIR1 promotes the association of the SCF^{TIR1} complex which then attaches chains of ubiquitin proteins to constitutively expressed transcriptional regulators of auxin-induced genes, thus targeting them for degradation by the proteasome and releasing the genes from their repression.

TIR1 belongs to a small family of 5 genes in *A. thaliana*: TIR1, AFB1, AFB2, AFB3, AFB5. At least four TIR homologs have been identified in the *Oryza* genome. *Populus* has three genes located on chromosomes, while a fourth homologous sequence was retrieved from an unassigned scaffold.

Figure 8 Structure model of TIR1 gene and primers used for amplify

Using degenerate primers (Table 2) developed from regions conserved in all plant species, a single fragment of size 1889 bp containing two introns, 488 and 86 bp long was amplified and cloned. The location of introns in the oil palm TIR1 homolog has been conserved. The exon shows a high degree of conservation with 20 other homologs from various plant species used for this alignment, including rice, *A. thaliana*, cotton, maize, grape, potato, poplar, citrus, and mustard. Appendix Figure 1 shows the alignment of the TIR1 coding region corresponding to 439 amino acids.

The phylogenetic relationship (Figure 8) of the oil palm TIR1 fragment and other sequences was obtained using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch. The TIR1 of oil palm groups with sequences from the grasses maize and rice. The five TIR1 homologs identified in the poplar genome showed some amino acids differences and they are separated in two groups. Similarly the four homologs of rice separated in two groups. As observed for the other plant species, there is most likely more than a single TIR1 homolog in the oil palm genome. However, no other clones were obtained.

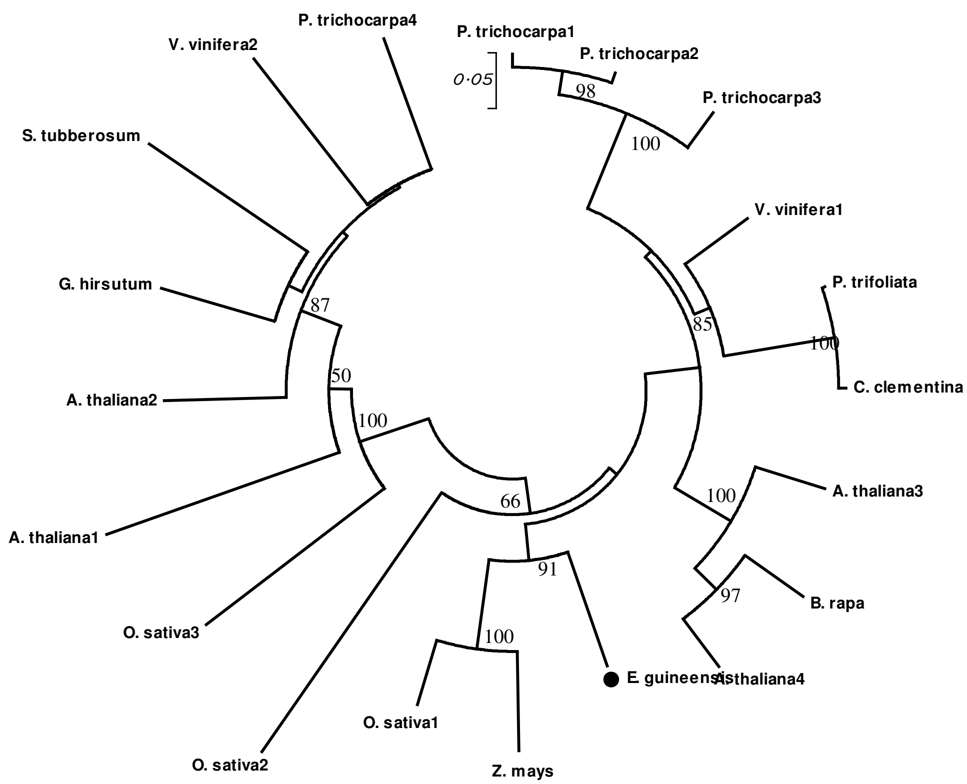

Figure 9 Phylogenetic relationship among TIR1 of oil palm and other plants.

Identification AXR (auxin resistant) genes of oil palm

AXR genes were identified in *Arabidopsis* because of their mutant phenotypes which include auxin resistance.

The AXR1 protein is related to the ubiquitin-activating enzyme (E1), which is required for signal transduction through the 26S proteasome degradation pathway. The proteasome-mediated degradation is common to many fundamental cellular processes in plants (Gray and Estelle, 2000). To attach the ubiquitin polypeptide to a protein, three steps are required: activation of the ubiquitin through an E1 enzyme, E2 and finally the transfer of the ubiquitin to the target protein catalysed by an E3 (SCF, HECT or other type).

Modification of CULLIN by RUB is very important for SCF^{TIR1} activity and normal auxin responsiveness (Pozo *et al*., 1998). Leyser *et al*. (1993) reported that mutations in the AXR1 gene decrease the number of RUB-CULLIN complexes results in and auxin-insensitive phenotype.

AXR2 and AXR3 are two related proteins for which homolog could be identified in several plant species. Mutant *axr3* plants displayed small, curled leaves, enhanced concentrations of anthocyanins, increased apical dominance, adventitious root formation, decreased root elongation and no root gravitropism.

A partial alignment of 31 AXR1 and AXR2 sequences showed sizes of introns were different each plants but exon quite similar.

A single clone and sequence of a partial AXR1/2, 3 fragment of oil palm was obtained. Its total length was 412 bp, consisting of 296 bp coding sequence and one intron of size 116 bp. Its sequence is rather short when compared to sequences from others plants though not unusual. The amino acid sequences translated from DNA fragment from 15 plant species varied from 92-129, though all had three highly conserved regions (Figure 11). Guilfoyle (1998) reported that AXR3 protein in

Arabidopsis thaliana has four conserved domains. Three mutant plants showed single amino acid changes in domainII and missing eleven amino acids in domain III. The oil palm AXR fragment included domainII and III (Figure 11).

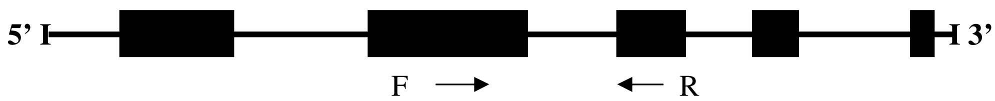

Figure 10 Structure model of AXR gene and primers used for amplify

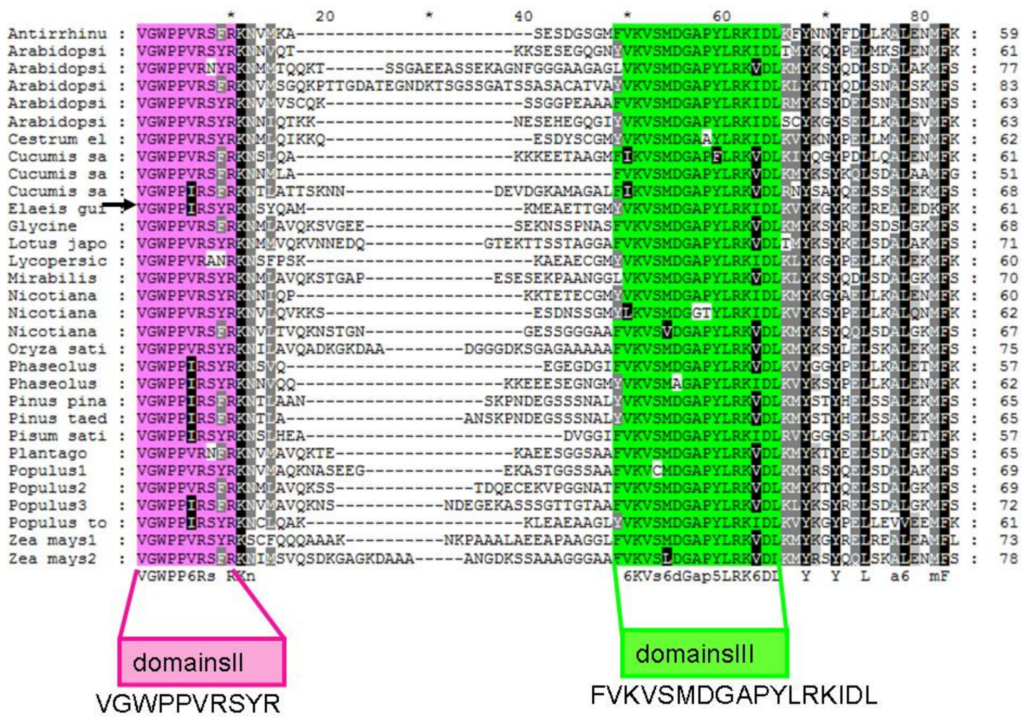

Figure 11 Alignment of AXR genes showing domainII and domainIII.

The phylogenetic analysis showed that the oil palm AXR gene is close to maize (*Zea mays*). This study got just only one clone of AXR but from data showed there should be more than one AXR gene homolog in the oil palm genome as *Arabidopsis*, tobacco, poplar and maize that show 4, 3, 3 and 2 AXR genes respectively.

Figure 12 Phylogenetic relationship of AXR proteins among oil palm and other plants. The unrooted tree was generated using the neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch amino acid sequence.

Identification of ARF1/ARF2 homologs in oil palm

Auxin response factors (ARFs) are transcription factors, reported to act as either repressors or activators that bind to auxin response elements in promoters of early auxin response genes. In the presence of low concentration of auxin, ARFs are repressed by AUX/IAA proteins. When the auxin concentration increases the degradation of AUX/IAA by SCF^{TIR1} is stimulated and the ARFs can initiate the transcription of auxin response genes.

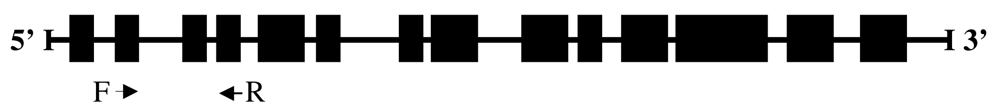

Figure 13 Structure model of ARF1, ARF2 gene and primers used for amplify

The ARF1 gene of *Arabidopsis thaliana* is homologous with the VIVIPAROUS1 (VP1) of maize (*Zea mays*). These proteins contain an amino – terminal DNA-binding domain that has some sequence similarity to a carboxyl-terminal B3 domain.

Wang *et al.* (2007) *Arabidopsis thaliana* and rice (*Oryza sativa* subs. Japonica), genome and identified 23 and 25 ARFs genes, named as AtARFs and OsARFs, respectively. The oil palm genome also should contain several ARF genes. Primers were designed and attempts were made to isolate homologs of ARF1/2/3 , and 6/ 8.

Searches of publicly accessible DNA databases revealed 27 sequences corresponding to ARF1/2 homologs from 20 species. A degenerate primer set (Table 2) was synthesized corresponding to conserved regions which spanned two introns.

A single clone and sequence was obtained corresponding to an ARF1 or ARF2 homolog. Its total length is 620 bp including 2 intron of 350 and 111 bp respectively. The position of both introns is conserved across all species. The coding sequence of this fragment is just 159 pb long (Appendix Figure 3). Translation of DNA to protein shows 53 amino acids that are conserved in all 12 plant species (Appendix Figure 3).

The phylogenetic tree showed that ARF1 of oil palm is close to *Acorus americanus* and rice (*Oryza sativa*), which are monocotyledonous herbs.

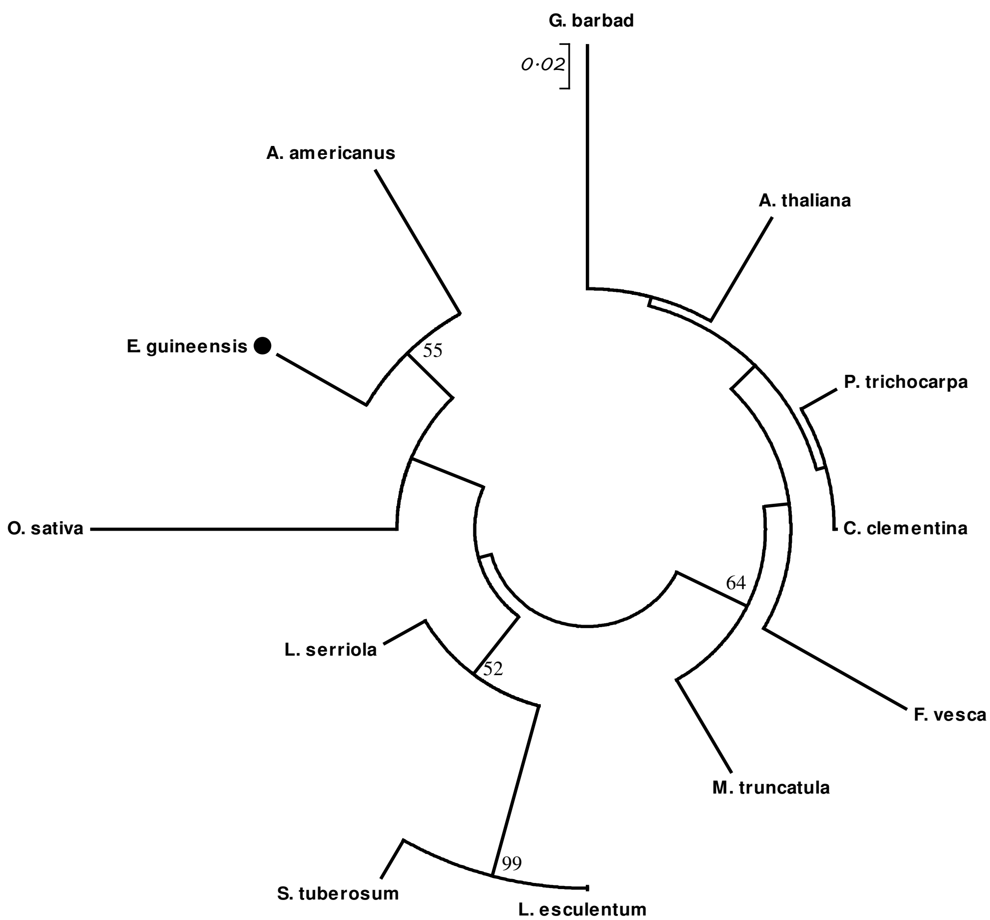

Figure 14 Phylogenetic relationships among ARF1 genes of oil palm and other plants. The unrooted tree was generated using genetic distances derived from amino acid sequence comparison by the neighbor-joining method in MEGA4. Bootstrap values from 1000 replicates are indicated at each branch. This tree shows ARF1 protein of oil palm close to sweet flag (*Acorus americanus*) and rice (*Oryza sativa*).

Identification polar auxin transport proteins (PIN-Form) of oil palm

Active auxin transport requires energy for moving the polar IAA from the shoot apex to the root tip, and subsequent redistribution of auxin from the root tip to the basal portion of the root. The polar auxin transporters (PIN) belong to a family of genes that control multiple developmental processes in plants, including the formation of vascular tissue.

Members of the PIN protein family have considerable sequence similarity and several are functionally redundant, as indicated by the increasingly aberrant phenotypes of the multiple pin mutants. The PIN1 gene encodes a transmembrane protein with a vague similarity to a group of bacterial transporters. The *Arabidopsis pin1* mutants are characterized by bare, needle-like stems that lack flowers, a phenotype that can also be obtained in the wild type by chemical inhibition of auxin transport.

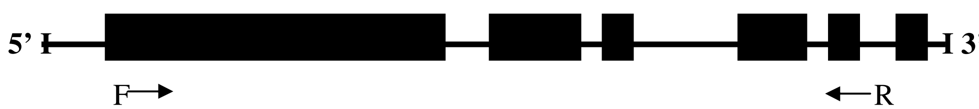

Figure 15 Structure model of PIN gene and primers used for amplify

PIN proteins are required for auxin efflux and different tissue require different members of the PIN family. Benkova *et al*. (2003) reported there are six genes in the *A. thaliana* PIN family of integral plasma membrane proteins which exert highly redundant functions.

PIN gene sequences were obtained from 6 plant species. *A. thaliana* had 5 PIN genes included, rice (*Oryza sativa*) 1 gene, maize (*Zea mays*) 1 gene, poplar 3 genes, *Brassica sp*. 3 genes and oil palm.

Seven clones were obtained by cloning PCR products amplified from oil palm genomic DNA using the PIN primer pair (Table 2). Comparison of exon size with other species appears too much different but intron quite small and not much different. From phylogeny tree show that *E. guineensis2*, *E. guineensis3* and *E. guineensis6* clones are very similar, clones *E. guineensis5* and *E. guineensis8* are same also another two clones, *E. guineensis4* and *E. guineensis9* are different from others. They correspond to four different genes.

This study shows that at least four different PIN genes occur in the oil palm genome.

Figure 16 Phylogenetic relationships of PIN among oil palm and other plants. Oil palm separated in two big groups. This study got four PIN genes.

Discovery of gibberellin signal transduction genes

Identification GA-insensitive dwarf1 (GID1) of oil palm

GID1 (GA-insensitive dwarf1) is a receptor of gibberellin. In the absence or low concentration of GA, GID1 is in the unbound state and DELLA proteins inhibit gene expression by interacting with transcription factors. Under higher GA concentration, the binding of GA to GID1 results in its association with an SCF E3 ubiquitin ligase complex containing the SLY1 F-box protein. The resulting ubiquitination and destruction of DELLA proteins by the 26S proteasome frees the transcription factors to activate gene expression.

Three GID1 homologs have been identified in the *A. thaliana* genome, but so far no mutant phenotypes of the *Arabidopsis* GID1 proteins have been described. GID1 was discovered in rice as a GA insensitive dwarf plant.

Vandenbussche *et al*. (2007) reported that homolog of GA signaling components can be identified in gymnosperms, monocotyledonous and dicotyledonous plants.

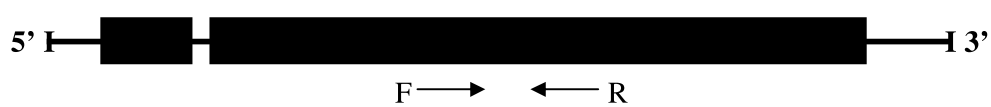

Figure 17 Structure model of GID1 gene and primers used for amplify

Two fragments corresponding to GID1 of oil palm were obtained, each 280 bp long, without intron. Both sequences are very similar, differing from each other at only 3 positions. It is possible that these correspond to two different loci, though without further study it cannot be excluded that they are derived from the same locus and represent two different alleles, or that the few differences are due to PCR errors.

Amino acid sequences were obtained by translating the coding region of 26 fragments from 21 plant species. The two nucleotide sequences corresponding to

GID1 of oil palm differ very little and result in identical amino acid sequences after translation. Phylogenetic analysis shows that the GID1 protein of oil palm grouped with grasses including wheat, barley, sugarcane, rice, maize, sorghum, and switchgrass (Figure 13). However the other monocot in the dataset, onion, did not group together with the palm and grasses.

GID2-SLY1 – could not be amplified. Should be a short fragment, but was not found so far.

Identification of DELLA

DELLA proteins are nuclear repressors of plant gibberellin (GA) responses. Arabidopsis has 5 DELLA proteins, namely GAI1, RGA1, RGL1, RGL2, and RGL3. Three of these have been shown to be involved in GA response (Fleck and Harberd, 2002). DELLA proteins interact with transcription factors and inhibit gene expression until destroyed by the 26S proteasome.

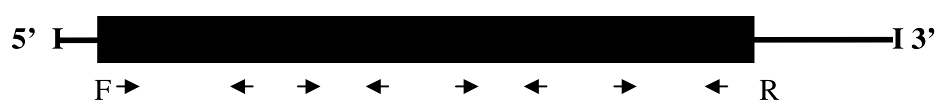

Figure 18 Structure model of DELLA gene and primers used for amplify

The comparison of nucleotide sequences of DELLA genes from several plant species indicates that they share several conserved domains. Consequently, several primer pairs were synthesized targeting different regions of the DELLA genes, but no sequences have been obtained so far. As the targeted fragments did not include introns, failure of the PCR amplification due to extreme length of the amplicon can be excluded. The reason for the PCR failure is thus unknown.

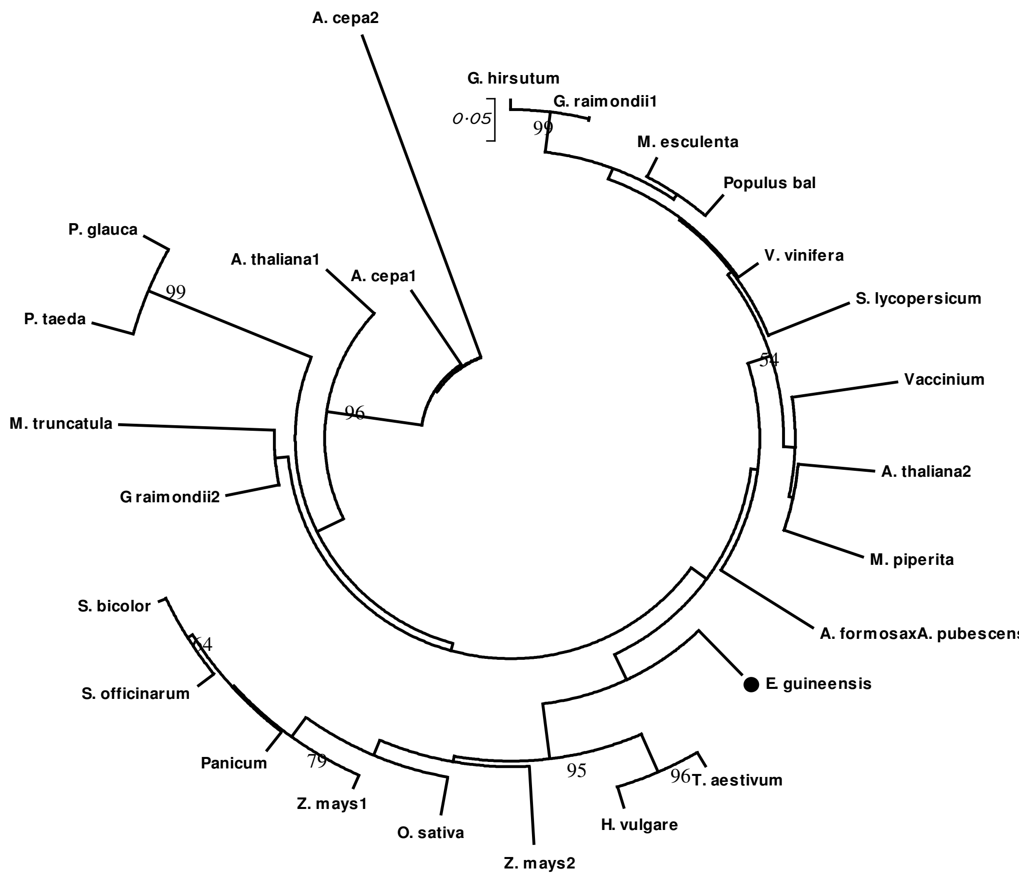

Figure 19 Phylogenetic relationship among GID1 sequences of oil palm and other plant species. The unrooted tree was generated from amino acid equences using neighbor-joining method implemented in MEGA4.Bootstrapvalues from 1000 replicates are indicated at each branch.

Discovery of abscisic acid signal transduction genes

ABA is involved in short-term physiological effects, as well as long-term developmental processes. Signal transduction pathways, which amplify the primary signal generated when the hormone binds to its receptor, are implicated for both the short-term and the long-term effects of ABA. Genetic studies have identified more than 50 loci involved in mediating ABA responses (Finkelstein *et al*., 2002), however, an ABI receptor protein has not been identified.

Identification of genes involved in ABA signaling usually involved screens based on the inhibition of seed germination or altered gene expression in response to exogenously applied ABA. Two genes that involved in abscisic acid signal transduction pathway were targeted. ABI3 and ABI5 are important transcription factors, their mutants reduce seed ABA responsiveness (Finkelstein and Lynch, 2000).

Identification of ABI3

Mechanism of ABA-regulated gene expression is up to ABA concentration. In the absence of ABA, the ABI3 transcription factor is degraded by 26S proteasome. In the other hand, ABA induced by development or environment signal such as embryo maturation, drought or nutrient stress. These conditions many transcription factor are produced for response to those signals. Transcription factor bound directly to ABA regulated gene promoter for activate transcription. ABI3 activate storage protein transcription to regulate multiple aspects of seed development.

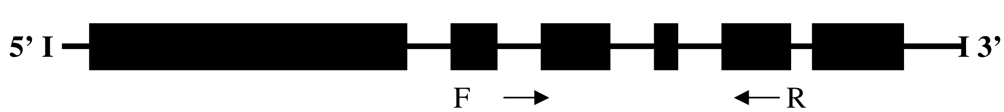

Figure 20 Structure model of ABI3 gene and primers used for amplify

A. thaliana has only a single ABI3 gene, though the LEC2 and FUS3 genes are closely related. ABI3 homologs were retrieved from 22 plant species. A single gene fragment corresponding to ABI3 was amplified and cloned using degenerate primer pair (Table 2). It is 745 bp long and includes 3 introns (139, 244, and 111 bp). Its coding sequence is just 251 bp. The positions of the introns are conserved among species. The phylogenetic tree (Figure 14) shows that ABI3 of oil palm is in the same group with monocotyledons plants including maize (*Zea mays*), rice (*Oryza sativa*), wild oat (*Avena fatua*) and wheat (*Triticum aestivum*).

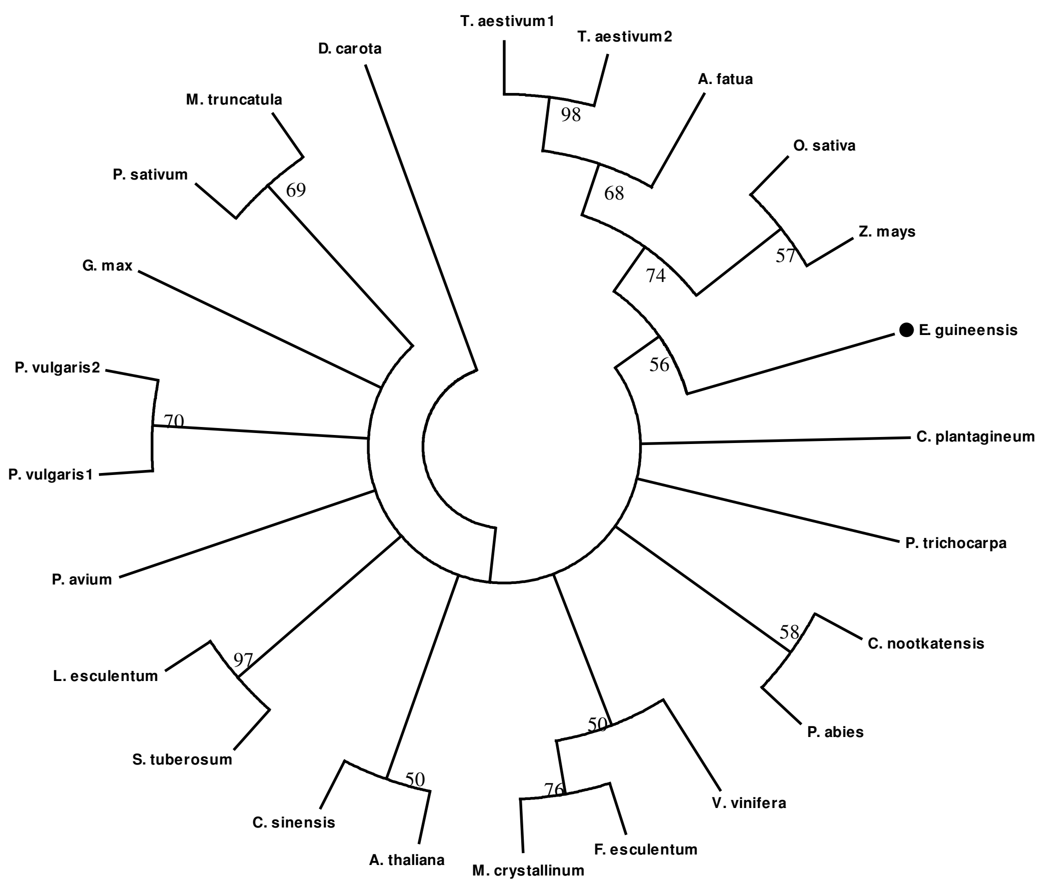

Figure 21 Phylogenetic relationship among ABI3 of oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch. It shows gene fragment of oil palm is close to others monocotyledons plant such as rice, wheat, wild oat and maize.

Identification ABI5

ABI5 (*Abscisic acid insensitive5)* encodes a member of the basic leucine zipper transcription factor family. It is involved in ABA signaling during late embryo development (Finkel and Lynch, 2000) seed maturation and germination. The *A. thaliana* abi5 mutants have pleiotropic defects in ABA response, including decreased sensitivity to ABA inhibition of germination and altered expression of some ABA-regulated genes. Comparison of seed and ABA-inducible vegetative gene expression in wild-type and abi5-1 plants indicates that ABI5 regulates subset of late embryogenesis-abundant genes during both developmental stages.

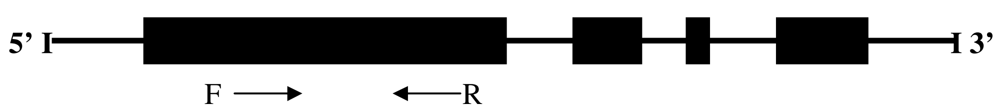

Figure 22 Structure model of ABI5 gene and primers used for amplify

ABI5 homologs were retrieved from 15 plant species. Degenerate PCR primers were synthesized corresponding to conserved region. The two identified conserved regions are separated by an intervening region that shows very little conservation among plant species.

The ABI5 gene fragment that was obtained is 922 bp in length without intron. A comparison of the oil palm ABI5 fragment shows that it has a conserved region separated an internal divergent region. The phylogenetic analysis of the oil palm ABI5 fragment amino acid sequence with those of 13 other plant species (Appendix Figure 7), indicates that the oil palm ABI5 most closely related to rice and maize.

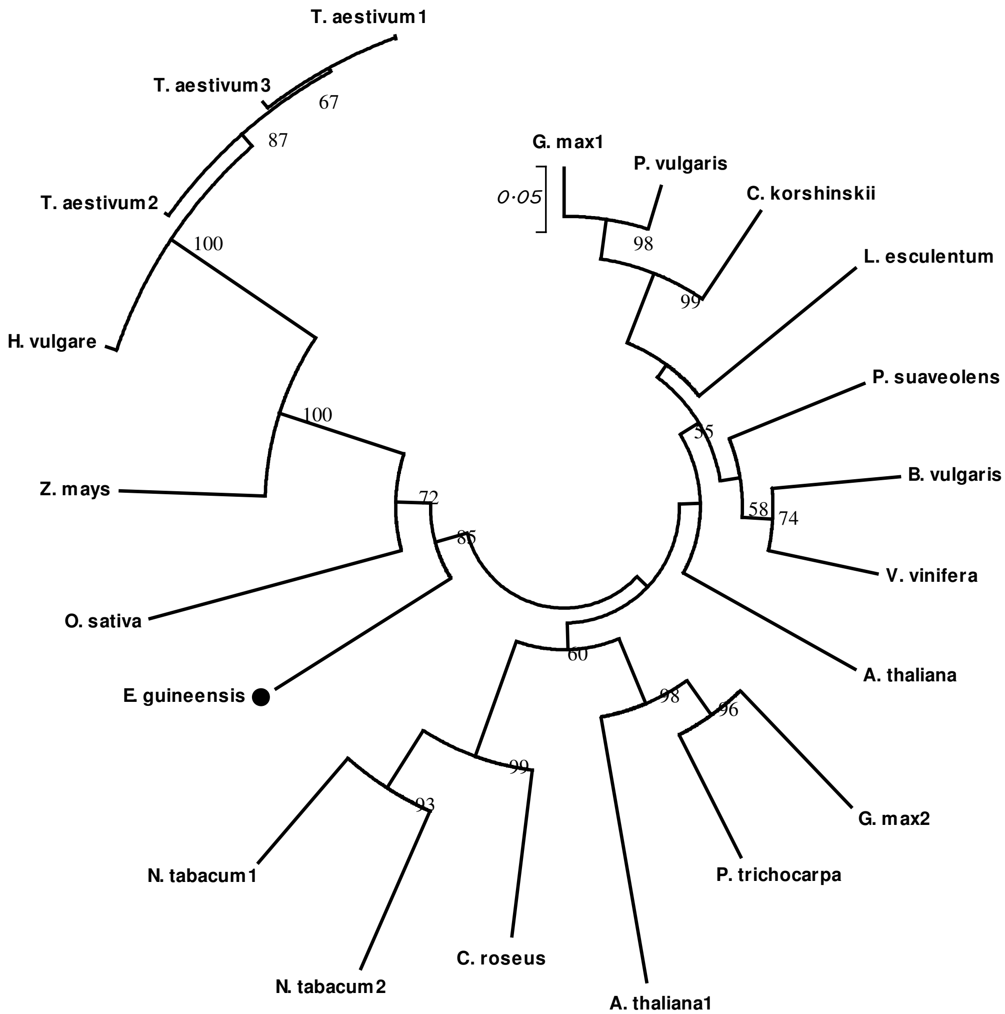

Figure 23 Phylogenetic relationship among ABI5 protein of oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

Other ABA genes:

ABI1 and ABI2, two closely related protein phosphatase 2C proteins could not be cloned, though a partial cDNA sequence could be obtained from another source.

Discovery of ethylene signal transduction genes

Nine PCR products corresponding to fragments of ethylene signal transduction genes have been cloned and sequenced. Three of them were EBF1/2 (EIN3 Binding F-box protein), others corresponded to ETR and ERS, the ethylene receptors.

Identification of EBF

EBF1 and EBF2 were identified as F-box proteins functioning in the ubiquitin-proteasome degradation pathway. When low concentrations of ethylene are present, these proteins interact with the EIN3 transcription factor and induce the ubiquitination of EIN3 leading to its destruction by the 26S proteasome. In higher ethylene conditions $SCF^{EBF1/2}$ interacts with EIN2 and marks it for degradation instead, leading to the accumulation of EIN3 transcription factor which then activates ethylene response gene expression.

Arabidopsis has 2/3 EBF homologs, while 1/2 sequences were obtained from rice. Designing of three primers were designed corresponded to conserved regions of the proteins.

Figure 24 Structure model of EBF gene and primers used for amplify

Three fragments of EBF homologs were amplified using 2 primer sets (Table2) sharing the same reverse primer but the two forward primers are about 300 bp separated. One fragment was amplified using first primer pair and two fragments were amplified using second primer pair resulting in a shorter fragment. Thus the three sequences largely overlap. They three sequences differ from each other at only 21 positions. Full length of first primer pair (F1R) is 861 bp and full length of another primer pair is 586 bp. None of them have an intron. Product of F1R primer pair was incomplete, lacking last 54 bp. Another two (F2R) were complete. A sequence of 179 amino acids was used for phylogenetic analysis. The phylogeny includes 2 *A. thaliana*, 2 soybean (*Glycine max*), 2 *Medicago spp*., 2 lettuce (*Lactuca sp*.), 4 poplar (*Populus trichocarpa*), 2 tomato (*Solanum lycopersicum*), grape (*Vitis vinifera*), maize (*Zea mays*), and 2 oil palm sequences. The phylogenetic tree showed oil palm was separate from other plant and close to maize (*Zea mays*) (Figure 16).

Figure 25 Phylogenetic relationship among EBF of oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

Identification of ETR

The ETR (Ethylene receptor) proteins are receptors localized in the membrane of the endoplasmic reticulum. Ethylene binds to these receptors in a transmembrane domain which results in EIN2 becoming active and turns on the EIN3 family of transcription factor bind to promoter directly then ethylene response gene express.

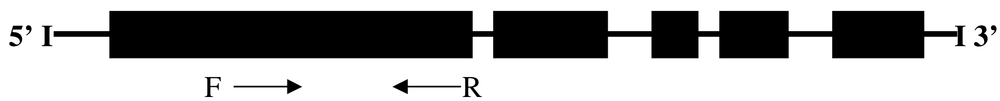

Figure 26 Structure model of ERS gene and primers used for amplify

Three ethylene receptor gene fragments were amplified using the F2R2 primer pair (Table 2) homolog ERS. They are three different genes. Full lengths were 988, 979, and 971 bp including one intron of sizes 440, 431, and 423 bp respectively (Table 4 and Appendix Figure 9). Their exon sizes are identical, 548 bp.

The analysis of phylogeny includes 1 *Arabidopsis thaliana*, 2 *Oryza sativa*, 1 *Actinidia deliciosa* , 1 *Brassica oleracea*, 1 *Carica papaya*, 1 *Chrysanthemum*, 1 *Citrus sinensis*, 1 *Cucumis melo*, 1 *Delphinium*, 1 *Dendrobium sp.*, 1 *Dimocarpus longan*, 1 *Durio zibethinus*, 2 *Fagus sp*, 1 *Gladiolus*, 2 *Glycine max*, 1 *Hibiscus rosa-sinensis*, 1 *Lactuca sativa*, 1 *Lilium formosanum*, 1 *Litchi chinensis*, 1 *Malus domesticus*, 1 *Musa acuminata*, 1 *Nicotiana tabacum*, 1 *Oncidium sp.*, 2 *Passiflora edulis*, 1 *Petunia sp.,* 1 *Phalaenopsis sp.,* 1 *Pisum sativum*, 1 *Pyrus communis*, 2 *Rosa hybrid*, 1 *Saccharum officinarum*, 1 *Vigna radiata*, 1 *Vitis vinifera*, 2 *Zea mays*, 1 *Ziziphus jujube*, and 3 oil palm (*Elaeis guineensis* sequences. They three sequences differ from each other at only 7 positions.

The phylogenetic analysis shows that the three of oil palm ERS partial genes were similar to *Citrus sinensis* (Figure 27).

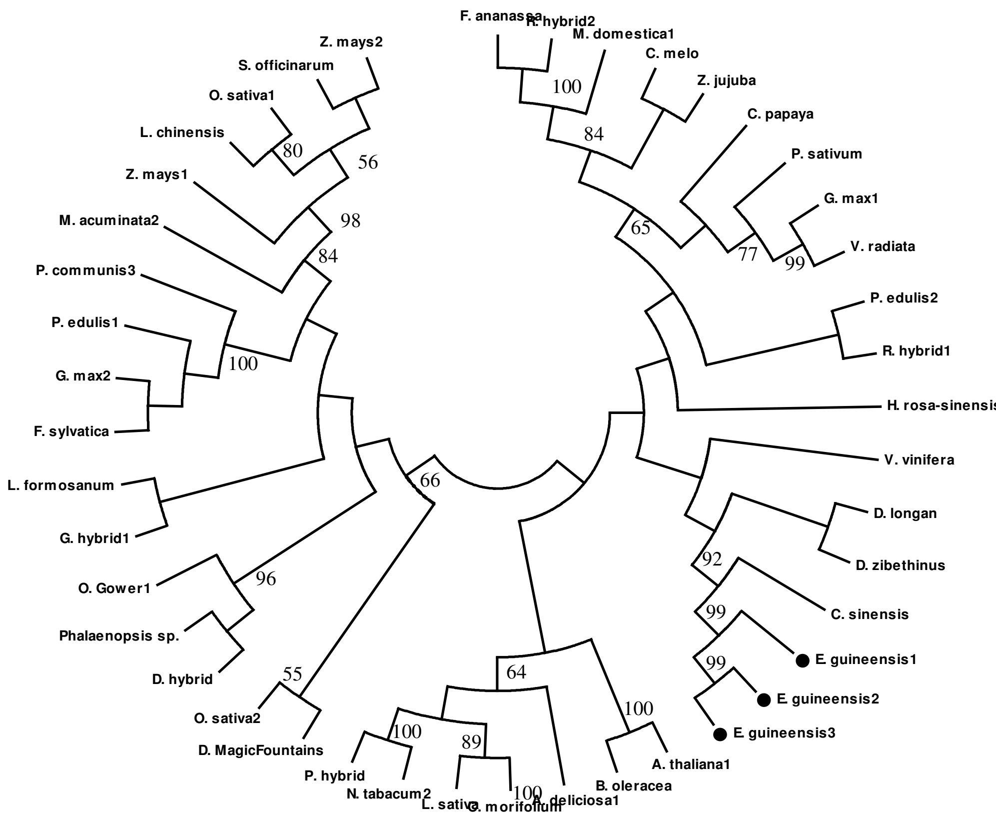

Figure 27 Phylogenetic relationship among ERS of oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

Figure 28 Structure model of ETR gene and primers used for amplify

Three fragments of Ethylene Receptor gene were amplified using the F2R3 primer pair (Table 2). This primer pair was designed to include 2 introns but results show that the oil palm genes have no any intron in this gene family. Three of them little bit different nucleotide but when translate to protein for phylogeny analysis they are still in the same group and close to rice and corn (Figure 29)

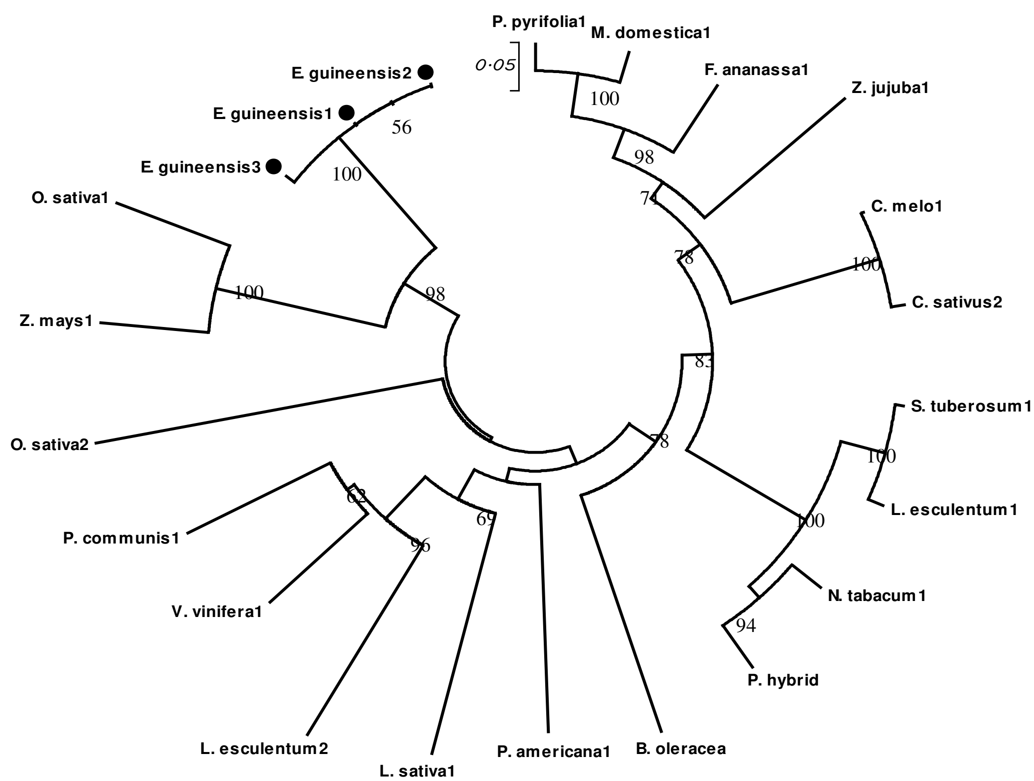

Figure 29 Phylogenetic relationship among ETR (F2R3) of oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

Discovery of some brassinostroid signal transduction genes

Steroid hormones have long been known in animals, but they have only recently been confirmed in plants. The brassinosteroids are a group of steroid hormones that play pivotal roles in a wide range of developmental phenomena in plants, including cell division and cell elongation in stems and roots, photomorphogenesis, reproductive development, leaf senescence, and stress responses (Clouse and Sasse 1998)

In this study three genes involved in Brassinosteroid signal transduction pathway were targeted.

Identification of BAK1 (BRI1-associated receptor kinase1)

The brassinosteroid signal transduction pathway is initiated by BR binding to an extra cellular domain of the receptor in the plasma membrane, The BRI protein contains 100 amino acids with island domain and LRR sequence. The binding between BR and BRI activates the phosporylation let them associated with another receptor at the LRR sequence. This second receptor is BRI associated receptor kinase1 (BAK1). This association of BRI and BAK then inhibits the BIN2 repressor protein resulting BR response gene expression.

Figure 30 Structure model of BAK1 gene and primers used for amplify

The cloned BAK1 gene fragment was 674 bp in length with one intron of 245 bp (Table 3). Comparison of the oil palm and 33 gene fragments from other plant species (2 Arabidopsis, 2 *Zea mays*, 1 Oryza, 1 *Solanum tuberosum*, 2 *Glycine max*, Sorghum, Coconut, grape, Pine, Aquilegia, *Daucus carota*, *P. vulgaris*, *Triticum* *aestivum*, *Hordeum vulgare*, and cotton. The phylogeny analysis showed that BAK1 of oil palm is close to coconut (Figure 32) but both of them quite different from rice.

Identification of BRX (Brevis radix)

The BRX gene fragment amplified from Genomic DNA of oil palm using degenerate primer (Table 2) gave product length 585 bp. It is close to rice and maize. It should have small intron when comparison with rice its show position of intron but too small. Shading orange color shows intron of rice and expected intron in oil palm (Figure 33).

Figure 31 Structure model of BRX gene and primers used for amplify

Other genes:

BRH could not be cloned in spite of several attempts.

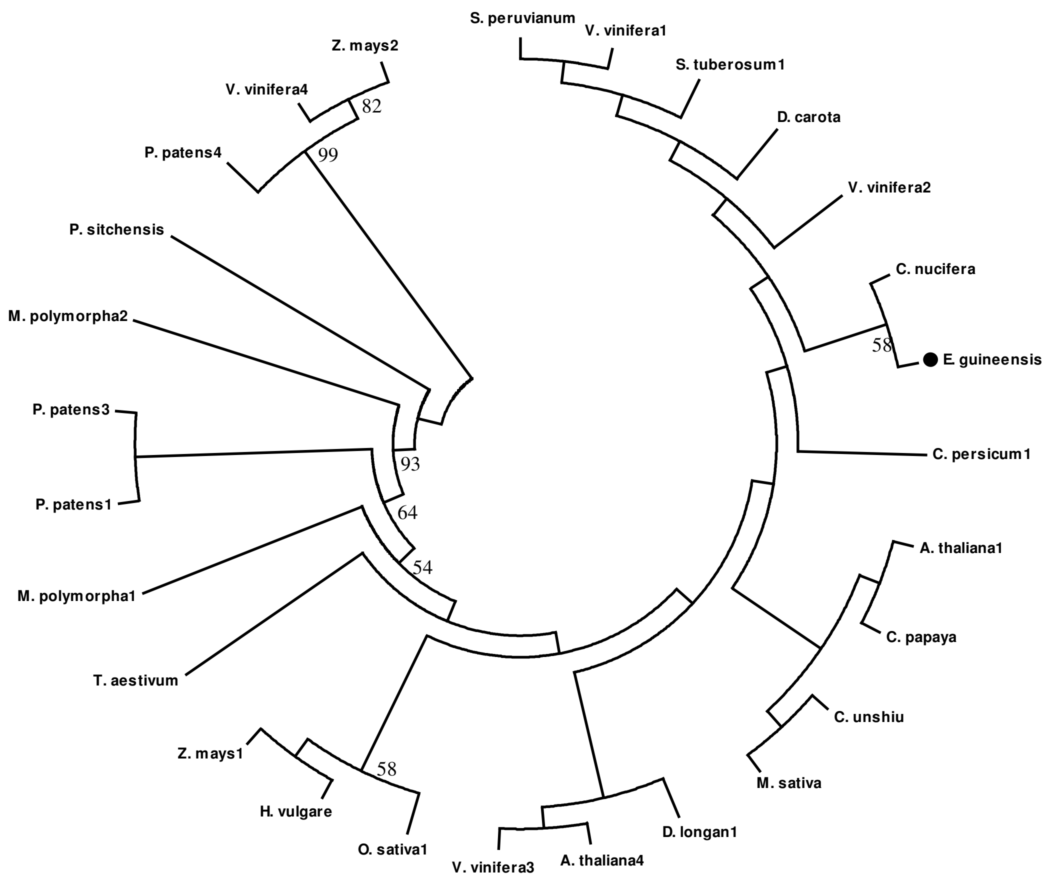

Figure 32 Phylogenetic relationship of BAK1 among oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

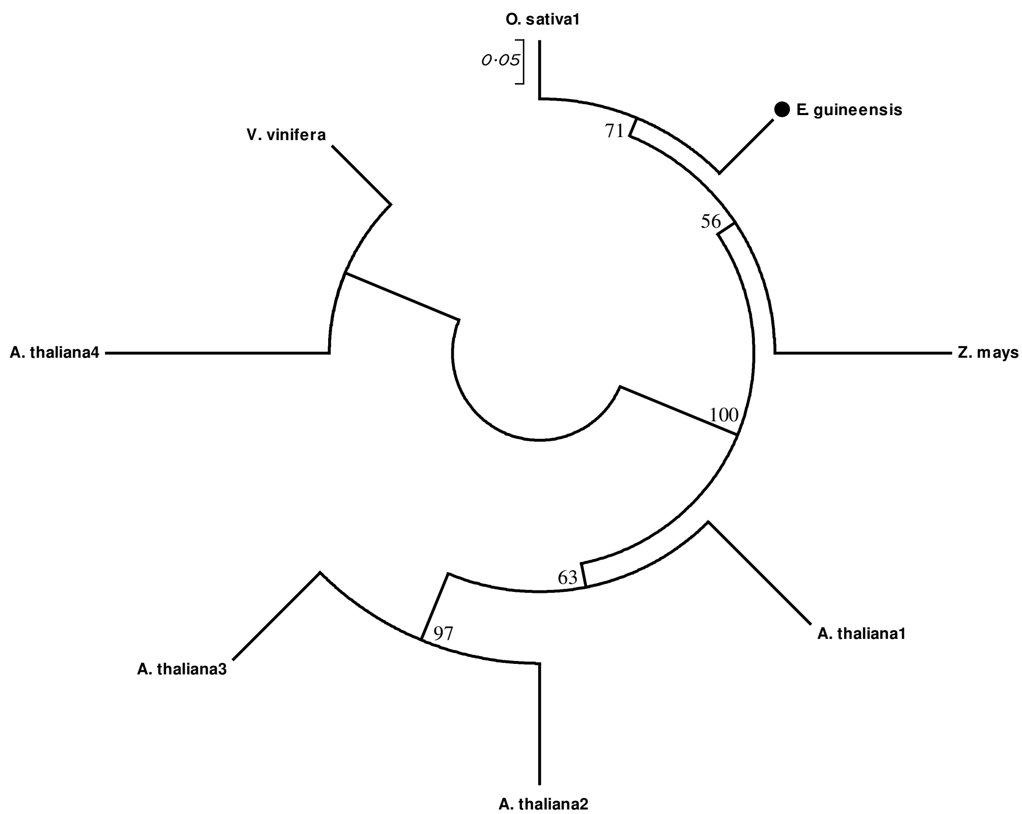

Figure 33 Phylogenetic relationship among BRX of oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

Discovery of jasmonate signal transduction genes

COI1 (Coronatine Insensitive) is an F-box of SCF complex of jasmonate signal transduction pathway. When is COI1 bound to SCF complex turn to SCF^{COI1} activated ubiquitination of repressor proteins and degraded this protein by 26S proteasome.

The coi1 mutant effect to male sterile, delayed anther development and incomplete anther dehiscence, or are impaired in filament elongation in *Arabidopsis*.

Figure 34 Structure model of COI1 gene and primers used for amplify

A fragment of the COI1 F-box gene was amplified and cloned. It was 764 bp long, without intron.

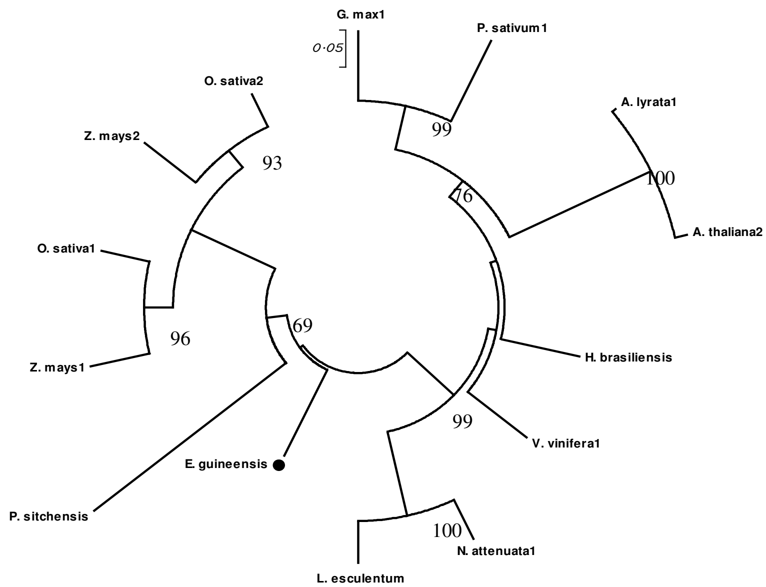

Figure 35 Phylogenetic relationship among oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch. COI1 of oil palm is in the same group of other monocotyledon plant such as rice, maize, wheat and sorghum.

Discovery of strigolactone signal transduction genes

Two families gene of MORE AXILLARY BRANCHING (MAX) were discovered in oil palm (MAX2 and MAX4). Recently four genes have been described in *Arabidopsis* of whose mutants all have more axillary branches (MAX1-MAX4). MAX1 encodes a cytochrome p450 family member. MAX2 encodes an F-box protein for Strigolactone signal transduction, suggesting MAX2-mediated target protein degradation operates in the MAX pathway to control bud out growth (Stirnberg *et al*., 2002). MAX3 and MAX4 are divergent members of the carotenoid cleavage dioxygenase family of enzyme, presumably involved in the synthesis of strigolactones.

Identification of MAX2

Figure 36 Structure model of MAX2 gene and primers used for amplify

A MAX2 fragment was amplified using degenerate primer pair (Table 2). Its size is 229 bp without intron. The phylogenetic tree showed that MAX2 from both grasses and dicotyledonous plants but oil palm did not group with grasses separated clearly (Figure 22)

Identification of MAX4

MORE AXILLARY BRANCHING4 (MAX4), RAMOSUS1 (RMS1) and DECREASED APICAL DOMINANCE1 (DAD1) genes of *Arabidopsis*, pea and petunia, respectively, are orthologous and function in a similar way (Bainbridge *et al*., 2005).

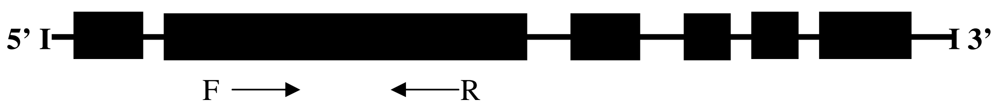

Figure 37 Structure model of MAX4 gene and primers used for amplify

In this study 2 pairs of degenerate primer (Table 2) were used to amplify oil palm genomic DNA. These primer pairs didn't overlap. The first primer pair (MAX4 F1R1) gave three fragments that little different (Figure 23) and their sizes were bigger, 322 bp without intron. Another primer pairs (MAX4 F2R2) gave three smaller fragments, 97-98 bp (Figure 23). They are different gene. Using MAX4 (F1R1) for phylogeny analysis, the phylogenetic tree showed that three of MAX4 oil palm were close together and separate in the same group of other monocotyledons plants (banana, rice and ginger).

MAX3 could not be cloned

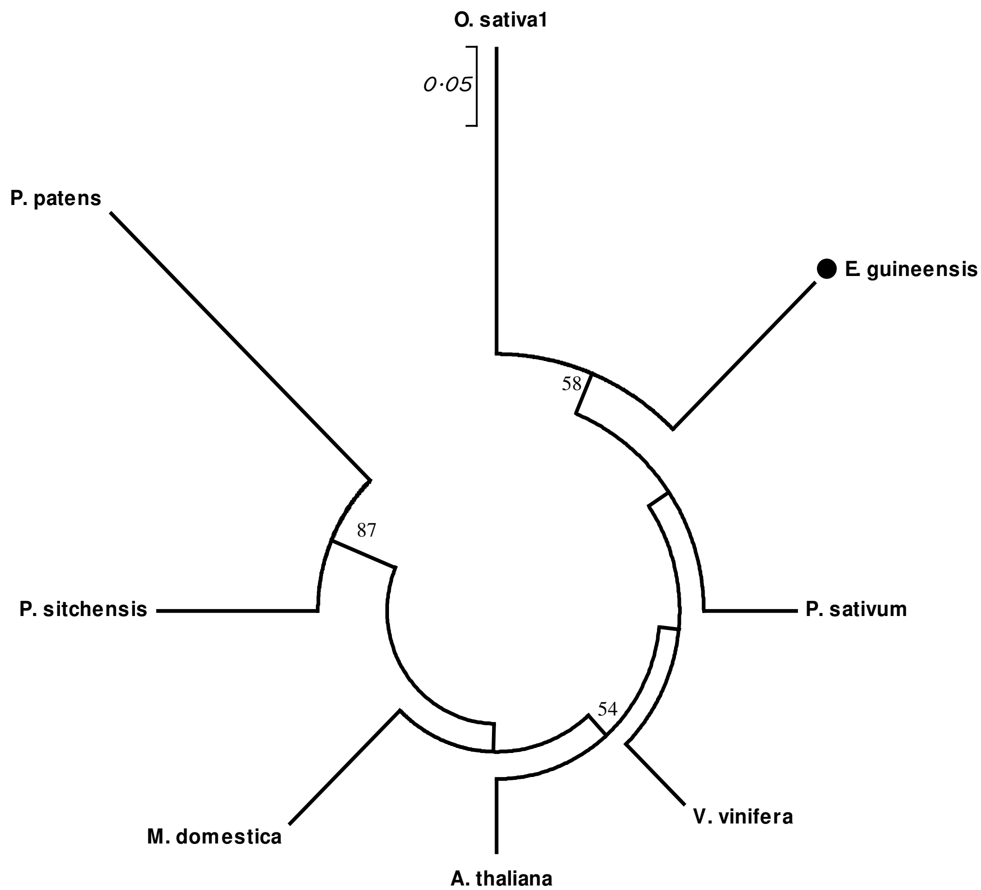

Figure 38 Phylogenetic relationship of MAX2 among oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

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CLUSTAL·W(1.60)·multiple·sequence·alignment·MAX4·F1R1

max4_Eg-1·······ACCGATAACGCCAACACAGGGGTCGTCAAGCTCGGCGACGGGCGGGTCGTCTGCCTCACC
max4_Eg-2·······ACCGATAACGCCAACACAGGAGTCGTCAAGCTCGGCGACGGGCGGGTCGTCTGCCTCACC
max4_Eg-3·······ACCGATAACGCCAACACAGGAGTCGTCAAGCTCGGCGACGGGCGGGTCGTCTGCCTCACC

max4_Eg-1·······GAGGCCATCAAGGGCTCCATCCAGATCGATCCCCACACGCTCGAGACCATCGGGAAGTTC
max4_Eg-2·······GAGACCATCAAGGGCTCCATCCAAATCGATCCCGACACGCTGGACACCATCGGGAGGTTC
max4_Eg-3·······GAGACCATCAAGGGCTCCATCCAAATCGATCCCGACACGCTGGACACCATCGGGAGGTTC

max4_Eg-1·······GAGTACACCGACGACTTGGGAGGTTTGATCCACTCGGCGCATCCCATCGTGACCGAGACG
max4_Eg-2·······GAGTACACCGACAATTTGGGCGGTCTGATCCACTCGGCGCATCCAATCGTGACCGACTCG
max4_Eg-3·······GAGTACACCGACAATTTGGGCGATCTGATCCACTCGGCGCATCCCATCGTGACCGACTCG

max4_Eg-1·······GAGTTCTTGACGCTGCTGCCCGACCTCGTGAGGCCTGGGTACACGGTGGCGAGGATGGAG
max4_Eg-2·······GAGTTCCTGACGCTGTTGCCGGACTTGGTGAGGCCGGGTTACACAGTGGTGAGAATGGCG
max4_Eg-3·······GAGTTCCTGACGCTGTTGCCGGACTTGGTGAGGCCGGGTTACACAGTGGTGAGAATGGCG

max4_Eg-1·······CCGGGGACCAACGAGAGGAAGGTCATCGGCAGGGTGAACTGCCGGGGAGGCCCGGCGCCG
max4_Eg-2·······CCGGGGAGCAACGAGCGGAAAGTGATCGGTAGGGCGAGTTGCCGGGGAGGCCCGGCGCCG
max4_Eg-3·······CCGGGGACCAACGAGAGGAAGGTCATCGGCAGGGTGAACTGCCGGGGAGGCCCGGCGCTG

max4_Eg-1·······GGTGGGTCCACTCGTTCGCGGT
max4_Eg-2·······GTTGGGTCCACTCGTTCGCGGT
max4_Eg-3·······GTTGGGTCCACTCGTTCGCGGT

Homology·of·MAX4·in·oil·palm
They·are·322·base·pair·in·length.
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CLUSTAL·W(1.60)·multiple·sequence·alignment·Max4·F2R2

max4_Eg-1·······ATGGATATGTGCAGCATAAACCCGGCTTATCTGGGCAAGAAGTATAGATACGCCTATGCC
MAX4_Eg-2·······ATGGATATGTGCAGCATAAACCCGGCTTATCTGGGCAAGAAGTATAGATACGCCTATGCC
MAX4_Eg-3·······ATT-ATATGTGCAGCATTAATCCTTCATATTTGGGAAGGAAATACAGATATGCCTATGCT

max4_Eg-1·······TGTGGTGCCCAAAGGCCTTGCAATTTCCCCAACACCCT
MAX4_Eg-2·······TGTGGTGCCCAAAGGCCGTGTAACTTCCC-AACACCCT
MAX4_Eg-3·······TGTGGAGCCCGACGGCCTTGTAACTTCCCCAACACCCT
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Figure 39 Partial alignments of MAX4 sequences. Above is MAX4 (F1R1), yellow shading show their base different. MAX4 (F2R2) gave just 97 and 98 bp in length.

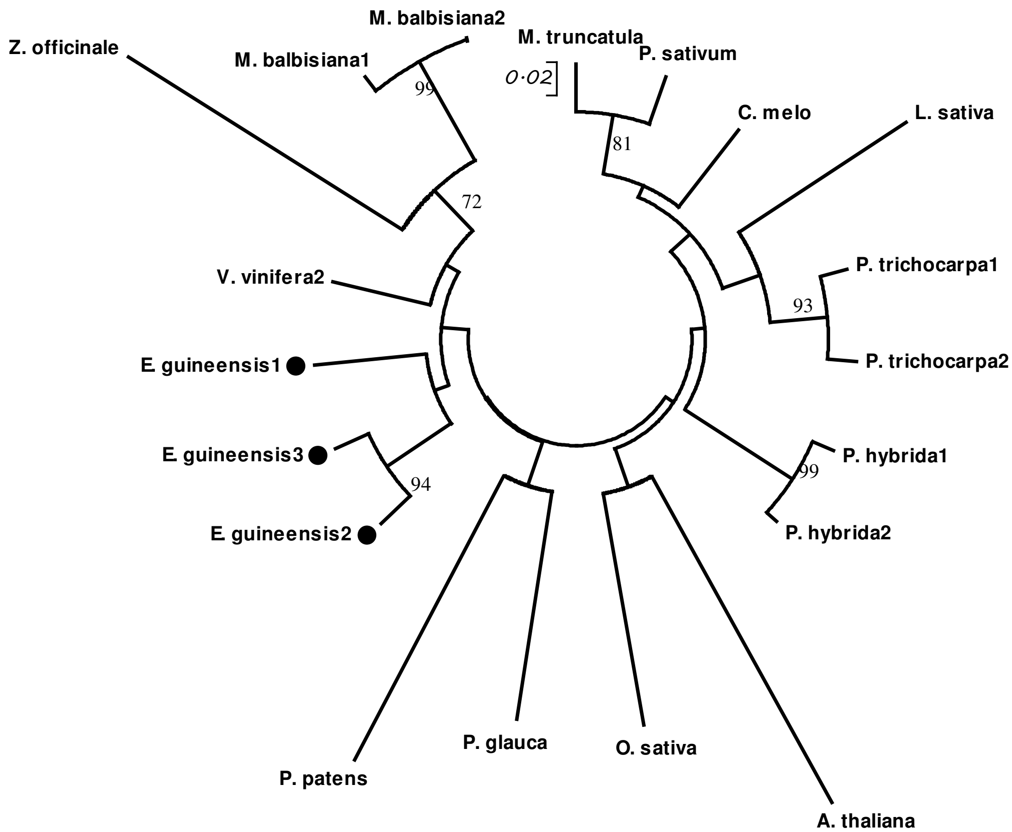

Figure 40 Phylogenetic relationship among MAX4 of oil palm and other plants, using MAX4 (F1R1) sequence for comparison. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

Discovery general genes involved in signal transduction pathway

Identification of a HECT homolog

Idividual ubiquitin (Ub)-protein ligase (E3s) cooperate with specific Ub-conjugating enzyme (E2s) to modify cognate substrates with polyubiquitin chains (Moon *et al*., 2004). Most known E3 enzymes, belong to one of three families. Members of the first family, whose founding member is E6-Associated Protein (E6AP), share a conserved about 350-residue region called the Homologous to E6AP C-Terminus (HECT) domain. Member of the second family are called Really Interesting New Gene (RING) (Wang and Pickart 2005).

HECT E3 genes were identified from the *Arabidopsis* genomic sequence, seven HECT E3 in Arabidopsis genome were called UPL3 through UPL7 (Downes *et al*., 2003). RT-PCR data show that each of these genes is expressed in seedlings. The upl3 mutant show highly branched trichomes (up to seven branches versus two to three in the wild type similar to gibberellic acid pathway regulates trichome development, and the constitutive GA response mutant spy-5, like upl3, has supernumerary trichome branches (Perazza *et al*., 1998) UPL3 is allelic to the KAKTUS gene, which had previously been identified as exhibiting a defect in trichome development (Downes *et al*., 2003). Indeed, upl3 mutants display increased elongation of hypocotyls on GA, consistene with a hypersensitive response to GA (Wang and Pickart 2005). HECT is play role in ubiquitination because conjugation of SCF complex need HECT domain and this process lead to gene expression and plant response to hormone.

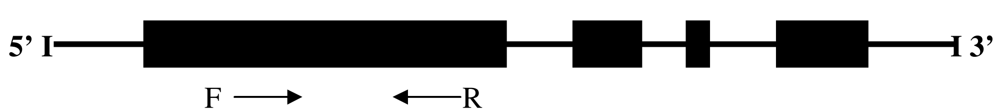

Figure 41 Structure model of HECT gene and primers used for amplify

Two fragment of HECT discovered from oil palm genome using degenerate primer pair (Table 2). Its size is 487 bp obtain one intron size 113 bp (Appendix Figure 16). Its exon is normal and highly conserve. They are 124 amino acids using for analyzed phylogeny comparison with other 17 plants. Figure 42 shows two HECT of are similar and close to pine and quite different with other monocotyledon plant such as rice, barley, sugarcane, and maize.

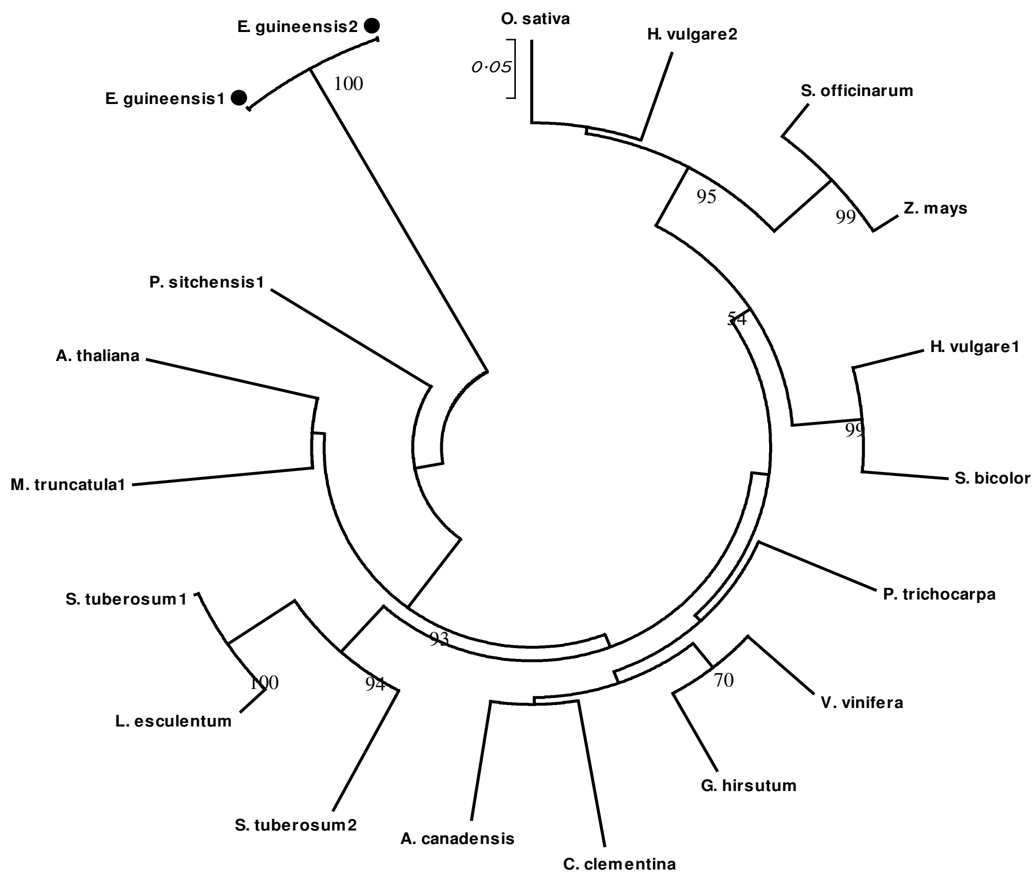

Figure 42 Phylogenetic relationship among HECT of oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch.

Identification of some MYB transcription factors

GAMYB found three fragments and they are too different. These fragments not big include one intron 115, 193, and 91 respectively. Their full fragment lengths are 264, 366, and 240 bp but their coding region same size (149 bp). Phylogenetic relationship show that the first one (oil palm1) close to Arabidopsis, apple (*Malus domesticus*) and tomatoes (*Solanum lycopersicum*). Another oil palm is close to strawberry (*Fragaria ananassa*) and legume (*Lotus japonicus*).

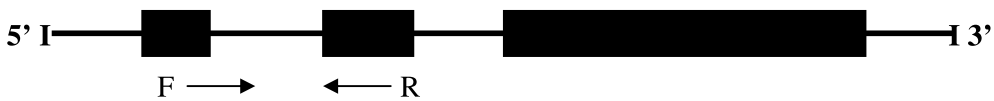

Figure 43 Structure model of MYB gene and primers used for amplify

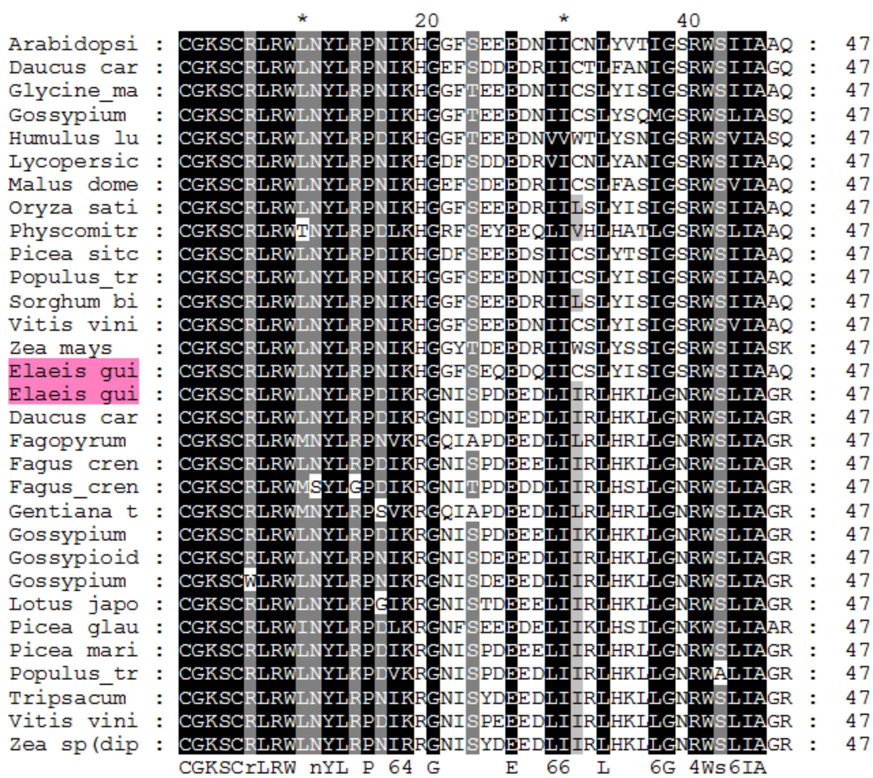

Figure 44 Partial alignment of MYB homologues using DNA fragments.

They are two different MYB genes in oil palm that different both coding region and intron length.

Figure 45 Phylogenetic relationship of MYB among oil palm and other plants. The unrooted tree was generated using MEGA4 program by neighbor-joining method. Bootstrap values from 1000 replicates are indicated at each branch. It shows two gene fragments of oil palm are different gene. First one (*E. guineensis1*) is close to grape, soybean and poplar (*V. vinifera*, *G. max* and *P. trichocarpar* respectively). *E. guineensis2* close to strawberry and the third one close to another grape (*V. vinifera2*)

Development and Analysis of Specific Primers

Locus Specific primers

Specific primers were designed flanking the introns or extending into the introns in such a way different loci could be amplified individualy in each gene family. All of the target sequences have introns. Eighteen of the cloned gene fragments included at least one intron. Eight of them were chosen for design specific primer pair. Twelve different oil palms seedling were screened for polymorphism. Table 4 showed the specific primers that were designed from sequences that for PCR-SSCP. In later experiment 48 oil palm accessions of the UV breeding programs were screened for polymorphism.

Polymorphism analysis

Primer pairs for the specific amplification of oil palm TIR1, AXR1, ARF1, PIN and ABI3 genes were designed. The amplified fragments include at least one intron. The PCR-SSCP assay was used to detect the presence of any polymorphism at these genes. The specific primer pairs amplified a simgle fragment with greater efficiency than the degenerate primers.

The PCR-SSCP analysis of TIR1 (1173 bp), AXR1 (306 bp) and ARF1 (583 bp) could not detect any polymorphism in a sample of 12 palm trees from different origins (Figure 46). Four different PIN gene specific primer pairs were designed (Table 4 and Appendix Figure 4). The fragment for the PIN1 and PIN2 loci contained 2 introns, while the PIN3 and PIN4 fragments were designed including a single intron. Their expected sizes were 830, 790, 597, and 600 bp respectively. These primer pairs were used with 12 oil palms from different sources to check polymorphism.

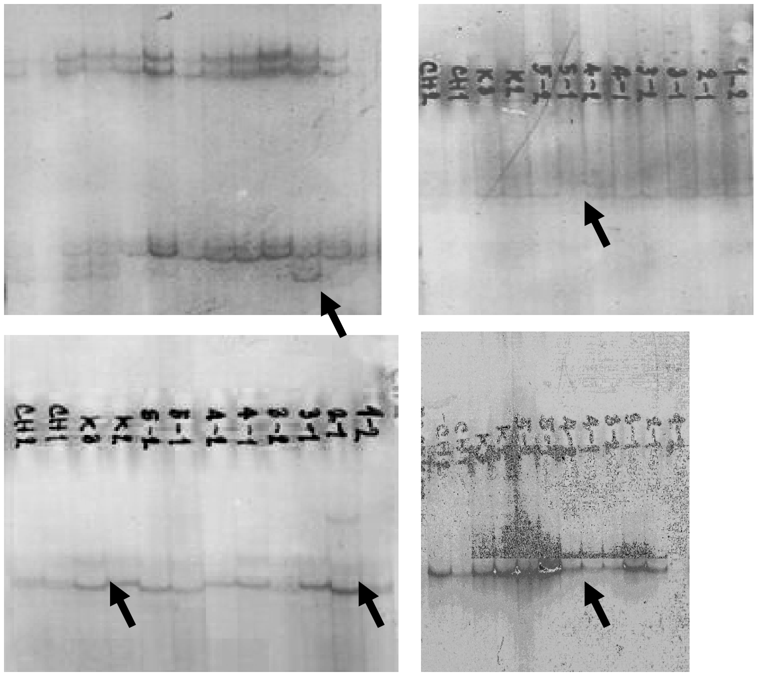

Figure 46 SSCP pattern of PIN1 using EgPIN1 specific primer tested 12 oil palms from different sources, seedlings from Univanich (1-2, 2-1, 3-1, 3-2, 4-1, 4-2, 5-1, 5-2), seedlings from ASD-Costa Rica (K2 and K3) and seedlings from local producers in Chumphon (CH1 and CH2). Arrows show polymorphism.

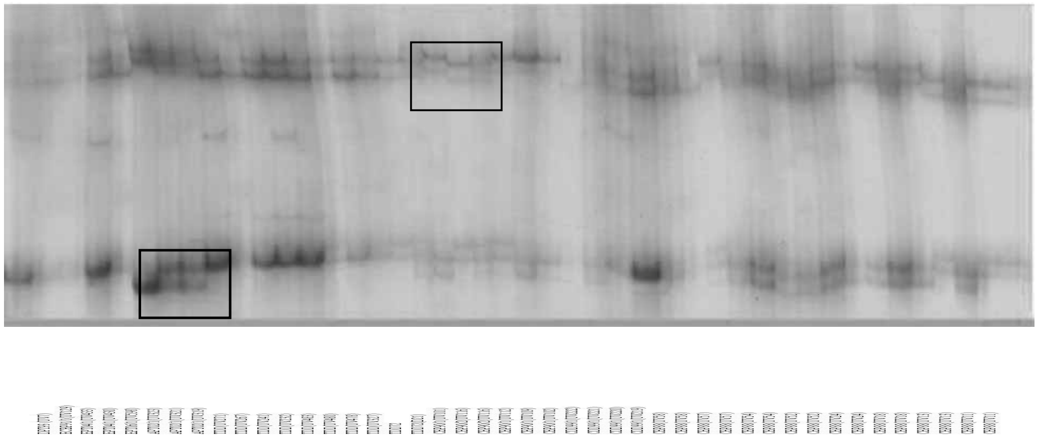

Figure 47 SSCP pattern of PIN1 gene using EgPIN1 specific primer tested with oil palm from Univanich breeding programme. It shows some polymorphism.

SSCP patterns show that bands from different loci (primer pairs) give very different banding patterns but within each locus the patterns are quite similar. Genomic DNA was amplified from oil palm trees corresponding to the different SSCP patterns and the PCR product was then sent for direct sequencing using specific internal primers. Appendix Figure 17 shows the PIN sequences obtained using PIN1 specific primer pair. All five samples sent for sequencing show some polymorphism. The alignments for the different sequences at the PIN2, PIN3 and PIN4 loci are shown in Appendix Figure 17, 18, 19, and 20 respectively.

A primer pair specific for the oil palm ABI3 locus was designed (Table 4). The amplified fragment was 684 bp long and contained 3 introns so should be suitable as a tool to identify polymorphism in an oil palm population. This specific primer pair was used to screen 12 oil palms from different sources. The SSCP result shows that the ABI3 gene fragments all give same band pattern. So this gene also can not be used for the identification of oil palm from different source.

DISSCUSSION

Forty-nine degenerate primer pairs were designed to screen oil palm genomic DNA and RNA. Thirty DNA fragments were successfully amplified and characterized using these primers. These fragments correspond to genes involved in the signal transduction pathways of auxin, gibberellins, abscisic acid, brassinosteroids, jasmonate, ethylene, and strigolactones. They are seven genes fragments of auxin, one of gibberellin, two of abscisic acid, two of brassinosteroid, one of jasmonate, five of ethylene and three of strigolactone. In addition four gene fragments included in hormone signaling pathway HECT, and MYB.

Some degenerate primer pairs gave more than one fragment product. These genes are members of small gene families so should have more than one involved in oil palm genome.

Eighteen of the gene fragments included some non-coding sequence from one or more introns. For eight of them a specific primer pair was designed and tested for polymorphism in twelve different oil palms. The TIR1, ARF1, AXR2, and ABI3 specific primer pairs amplified a unique DNA fragment but results from their SSCP (single strand conformation polymorphism) pattern showed that they didn't harbour any polymorphism. The four PIN specific primer pairs each corresponded to a unique locus. The SSCP band patterns showed some polymorphism for each of them. After sequencing the corresponding alleles, the individual mutations causing the differences in electrophoresis during SSCP could be identified.

CONCLUSTION

The machinery of hormone signal transduction and hormone response is complex. In addition to the large gene families of transcriptional regulators, there are likely to be multiple SCFs that function in hormone response. Many genes of hormone signal transduction have been identified and characterized in *Arabidopsis* and rice genome.

Since transcription factors and signaling genes are known to regulate important developmental and physiological processes in plants, these genes would be good candidates that may cause some of the variation observed in oil palm. This study shows that most likely all of the genes are also present in the oil palm genome.

A major challenge for the future studies is to convert the obtained DNA sequences into markers that can be applied in oil palm population that selected only strong and produce higher amount of oil plants. From this study eighteen gene members that including intron, eight of them use as model for design eight specific primer pairs and test with twelve different sources oil palm. Four of them gave polymorphism.

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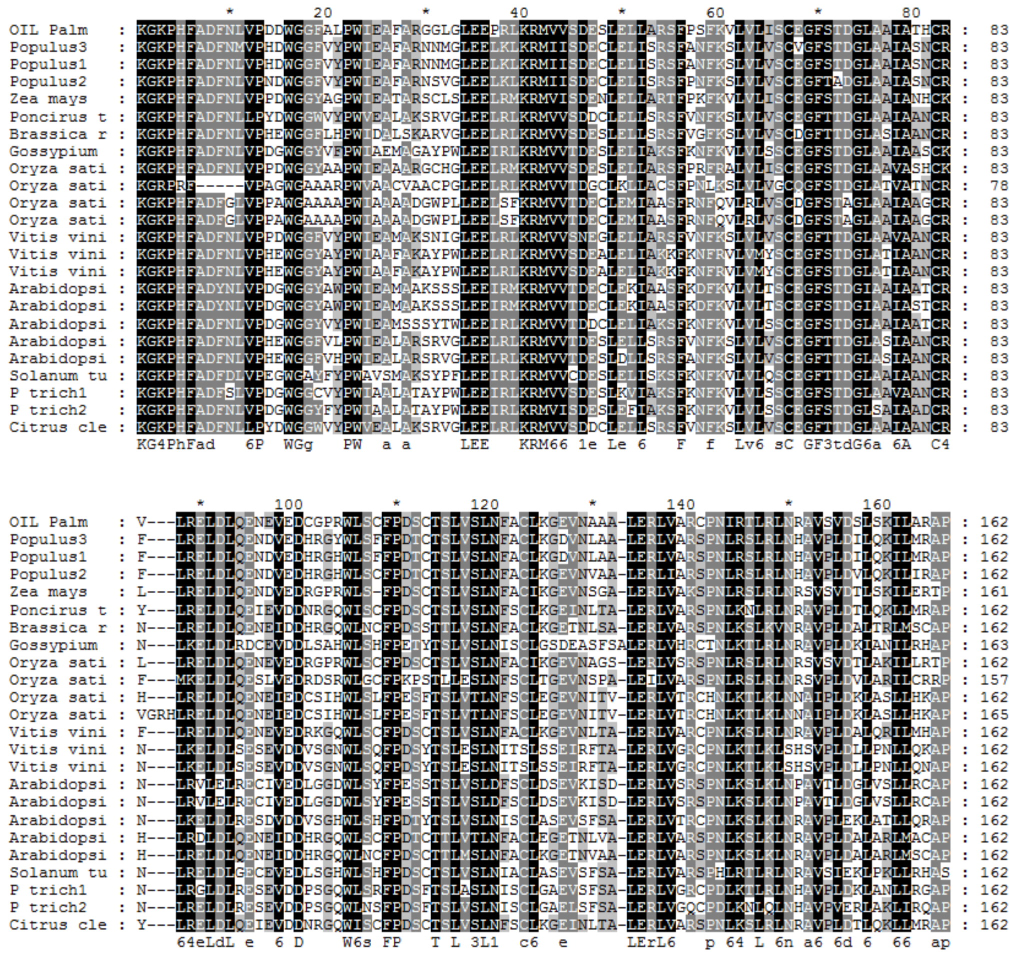
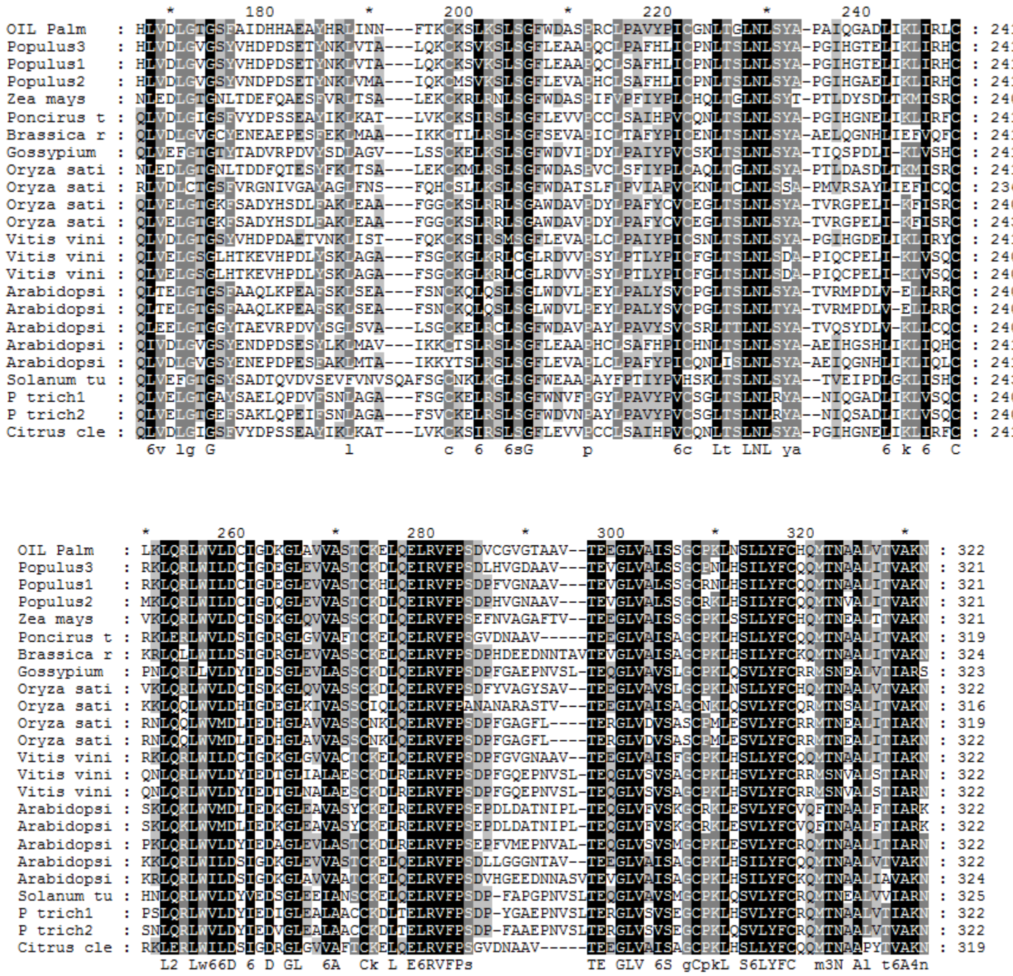
APPENDICES

Appendix A

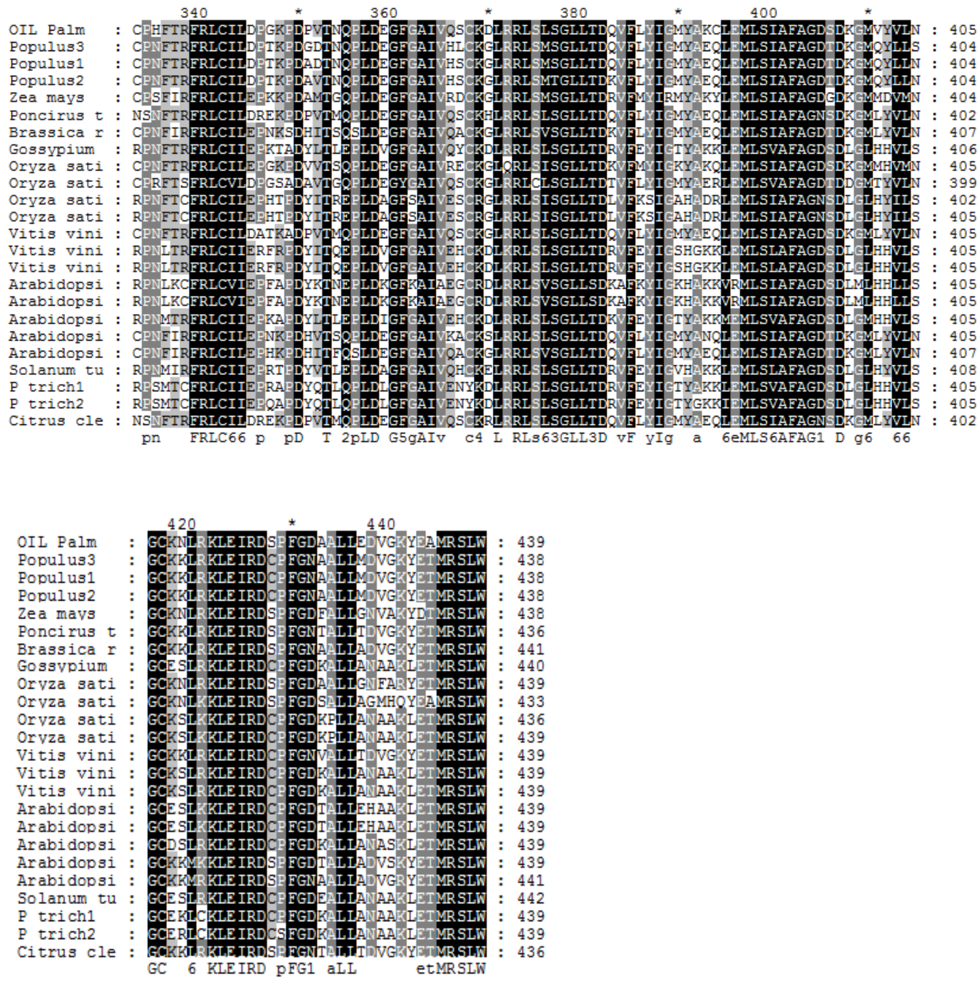
Alignment sequences



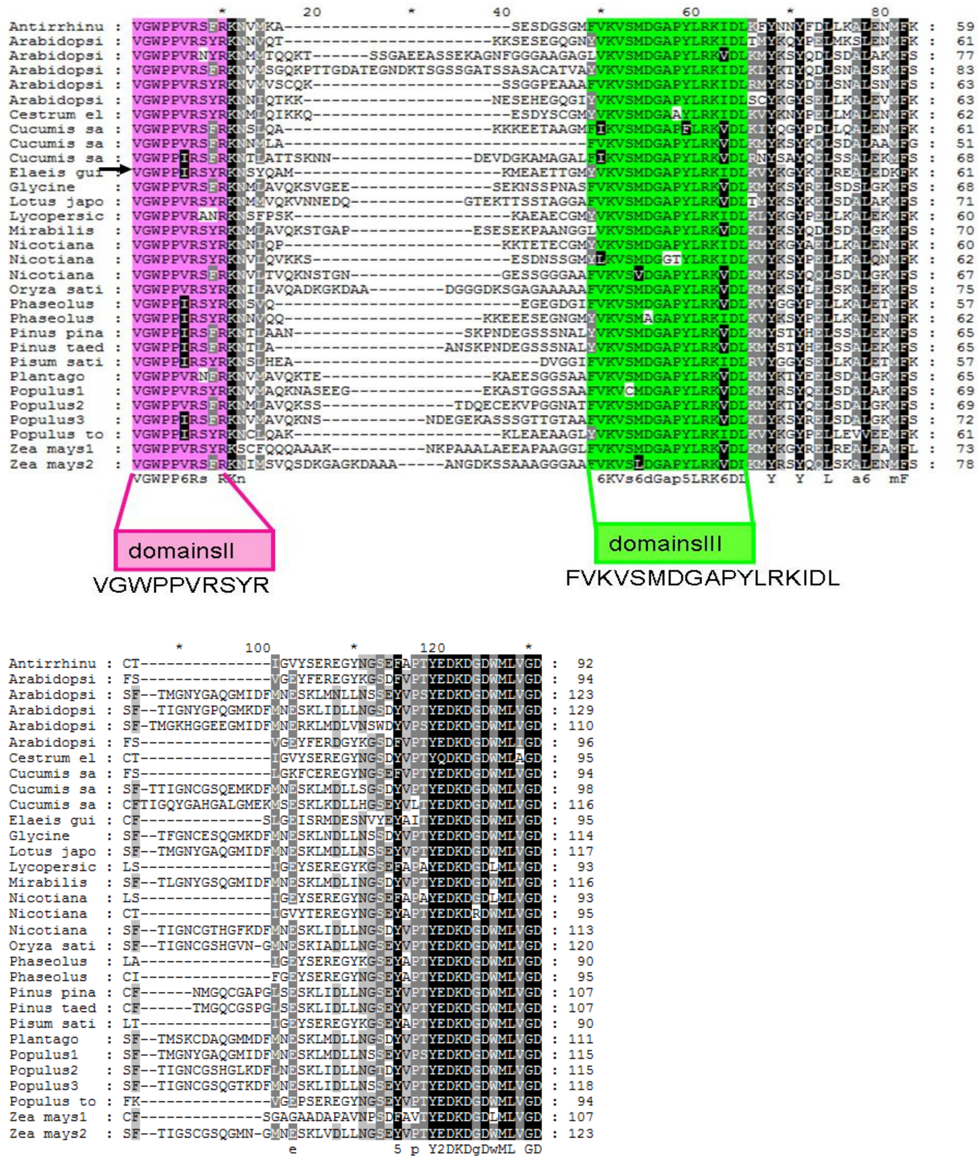
Appendix Figure 1 Alignment of TIR1 sequence fragments.



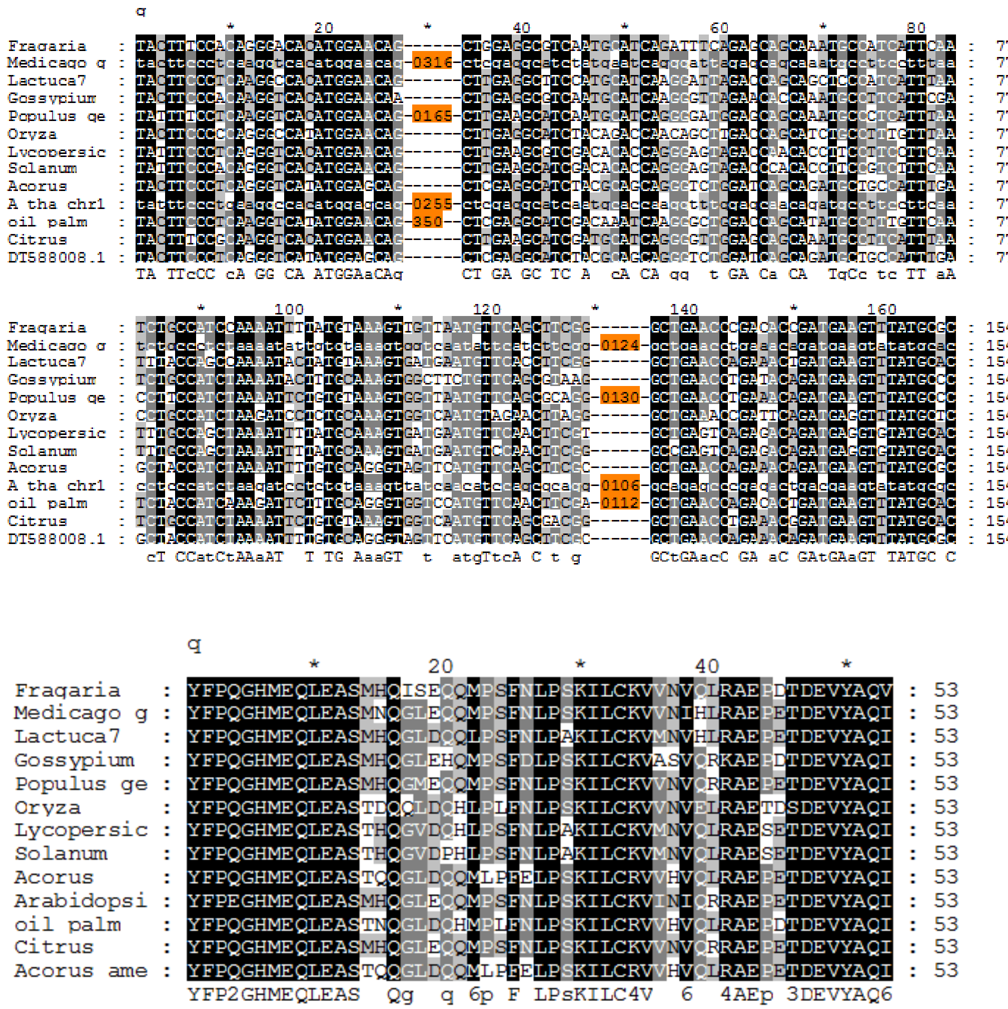
Appendix Figure 1 (Continued)



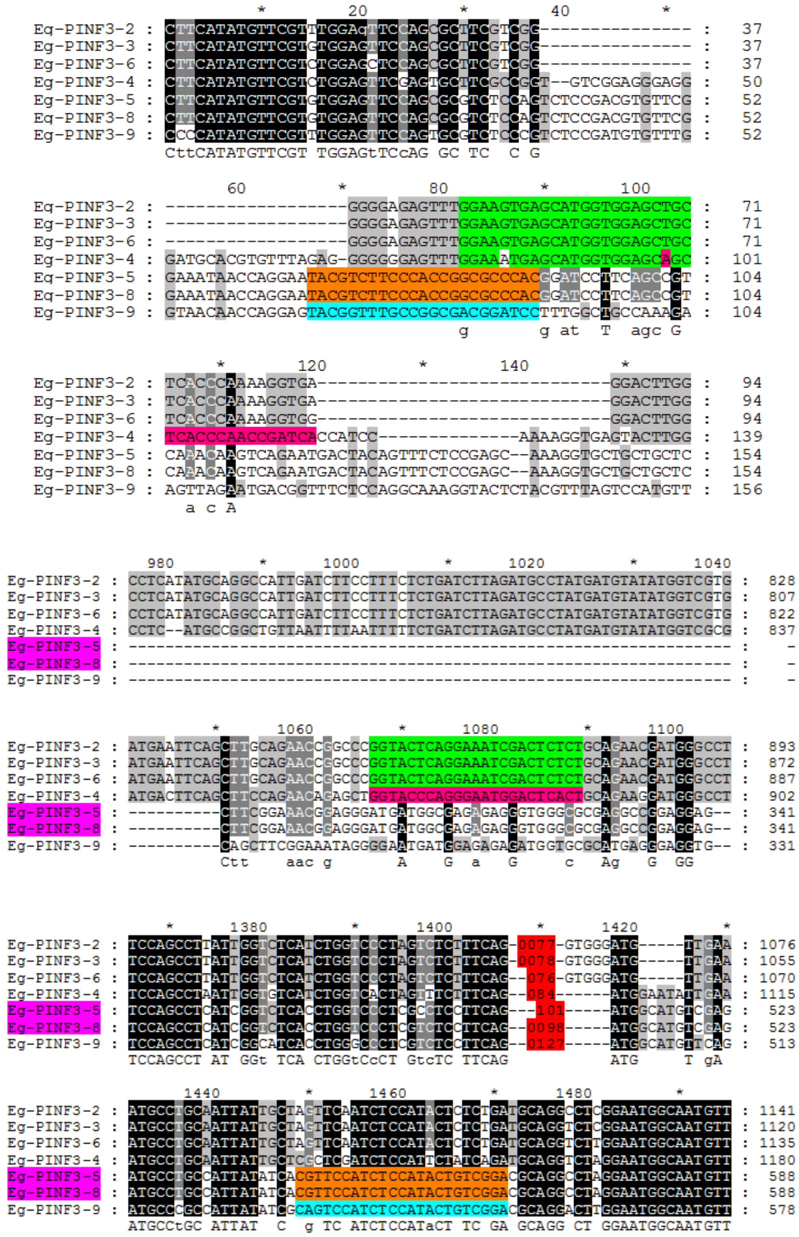
Appendix Figure 1 (Continued)



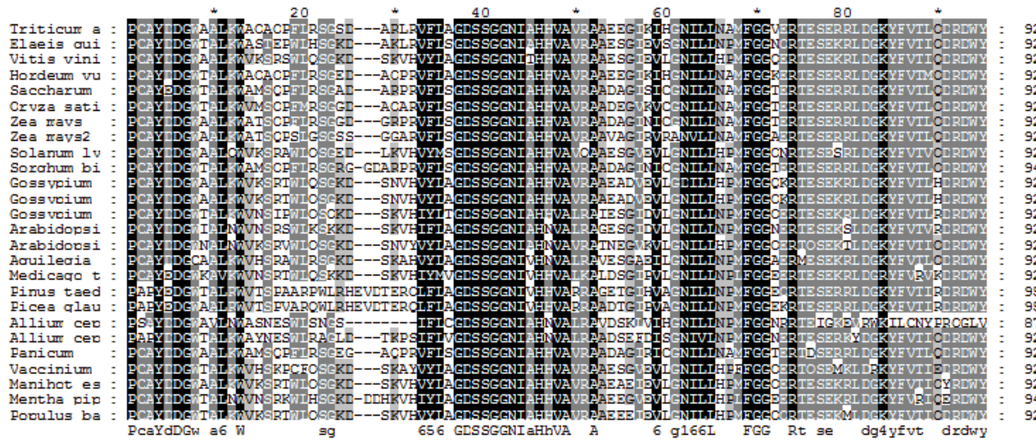
Appendix Figure 2 Alignment of AXR3 protein fragments. DomainII and DomainIII of AXR3 protein are shaded. These conserved domains are important because mutations in them relate to different phenotypes.



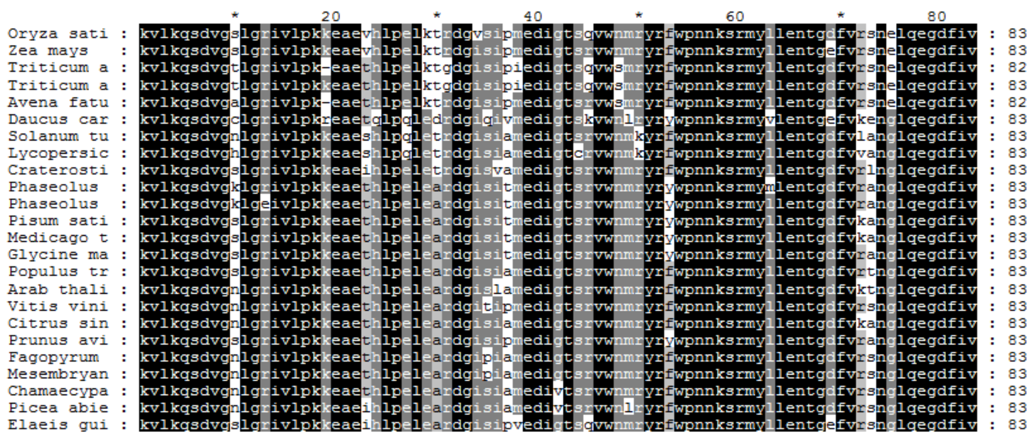
Appendix Figure 3 Partial alignment of ARF1 sequences using DNA fragments. Shadings are positions and sizes of intron that show oil palm Has two intron 350 bp and 112 bp in length. Partial alignment of ARF1 amino acid sequences show that ARF1 of oil palm obtain 53 amino acids.



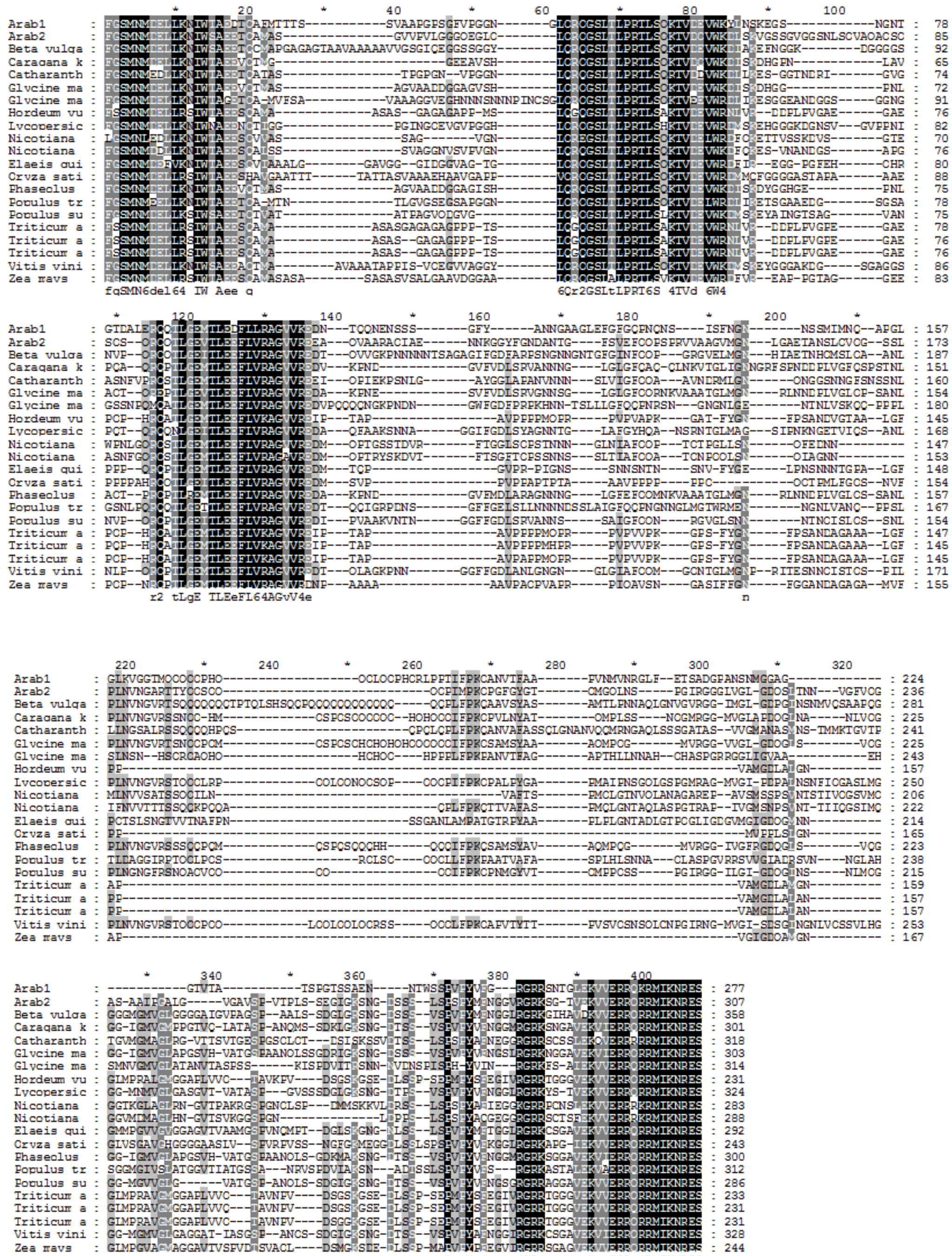
Appendix Figure 4 Forward primers that design for specific form of four PIN genes. F1 is green, F2 is pink, F3 is an orange, and F4 is blue. Reverse primers that design for specific form of four PIN genes. R1 is green, R2 is pink, R3 is an orange, and R4 is blue.



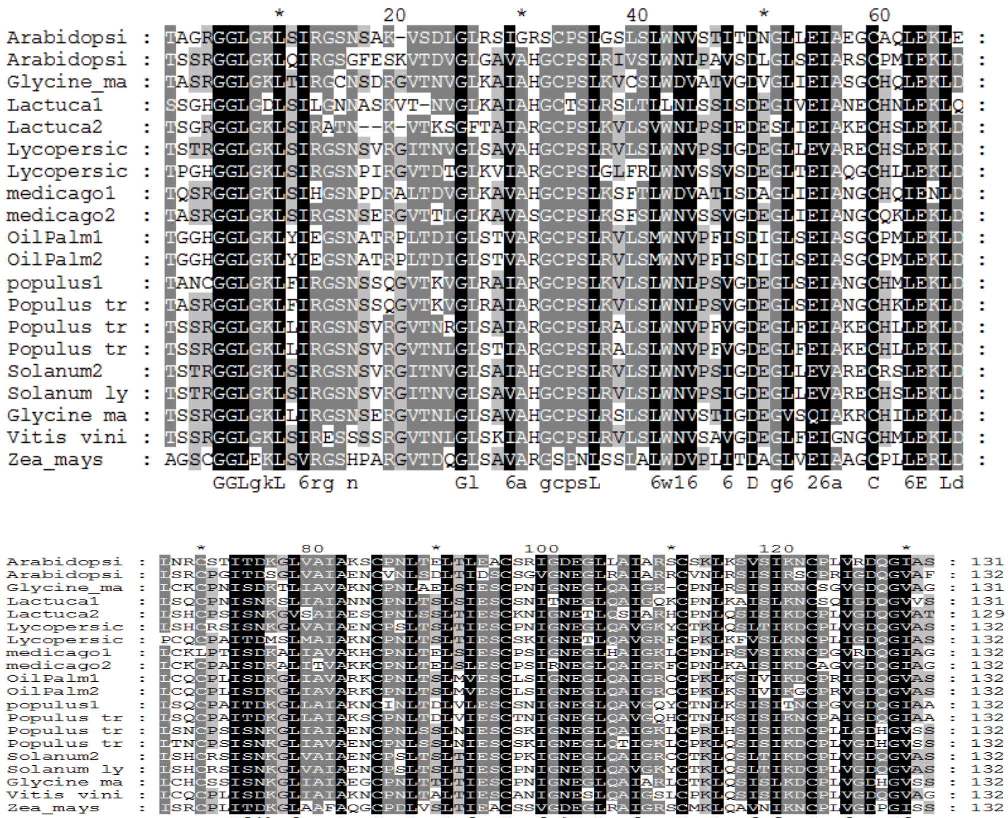
Appendix Figure 5 Partial alignment of GA-insensitive dwarf1 (GID1) homologues using protein fragments. These fragments obtain 87-94 amino acids.



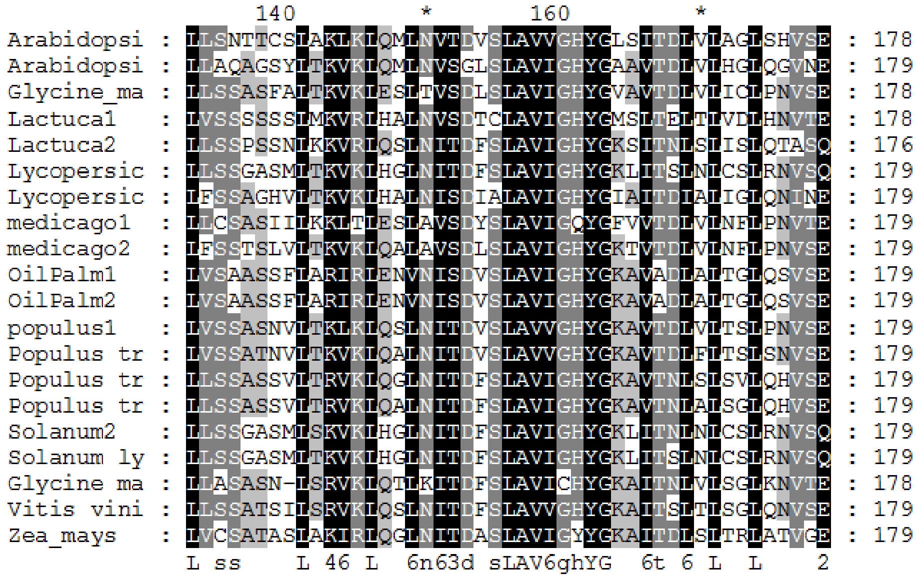
Appendix Figure 6 Partial alignment of ABI3 homologues using protein fragments.



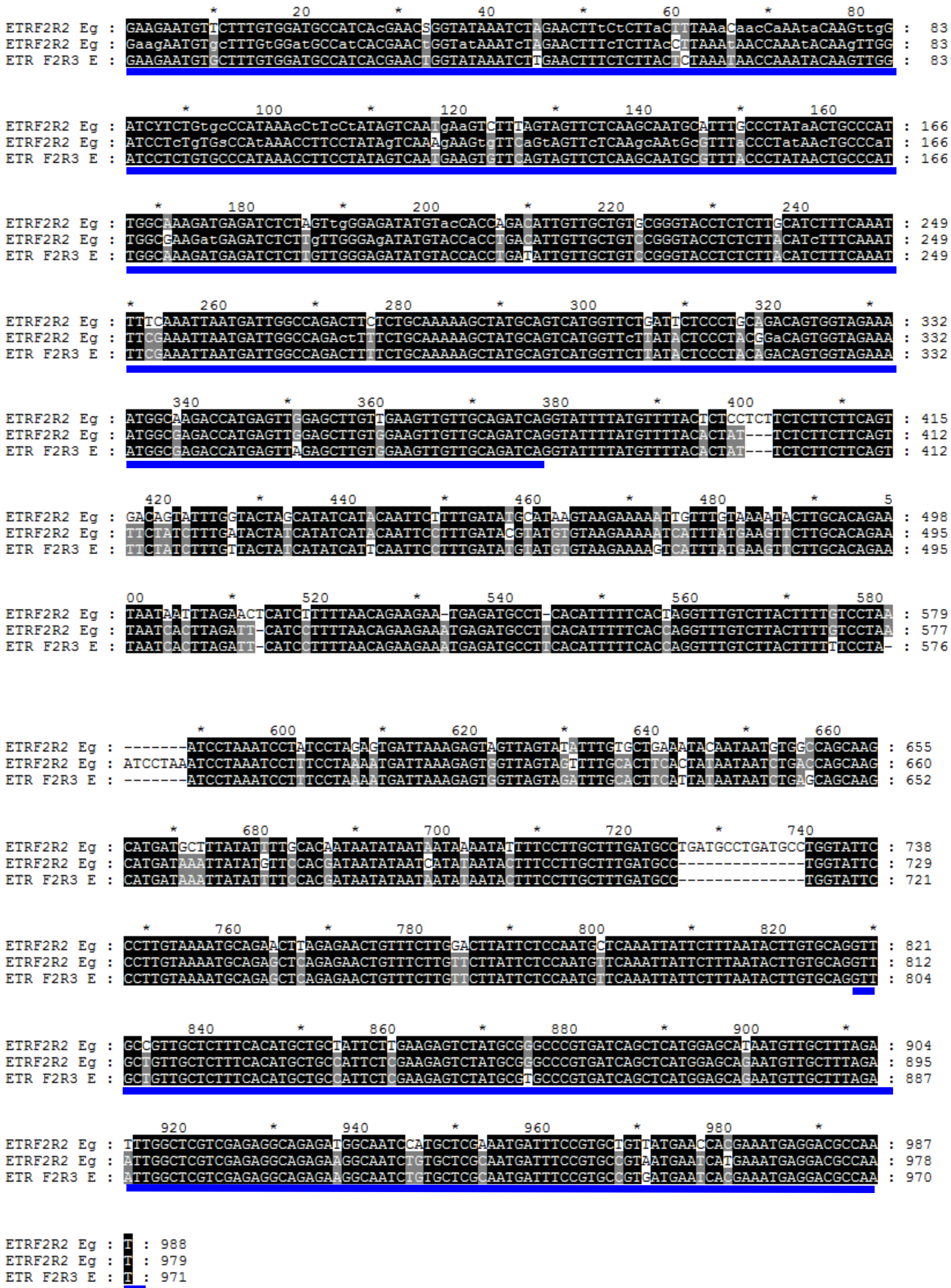
Appendix Figure 7 Partial alignment of ABI5 sequences using protein fragments. Alignment's problematic because of the variable insertion deletions.



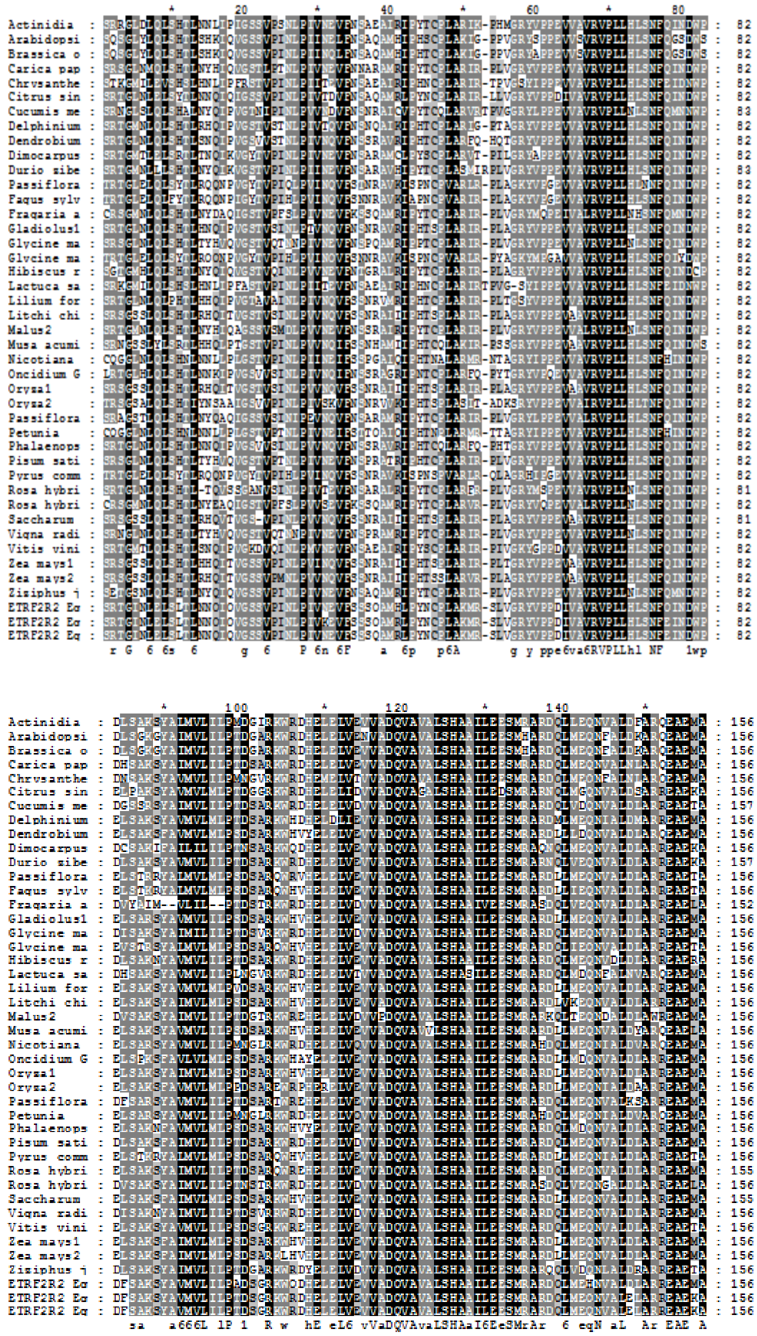
Appendix Figure 8 Partial amino acid sequence EBF1 including 2 oil palm sequences.



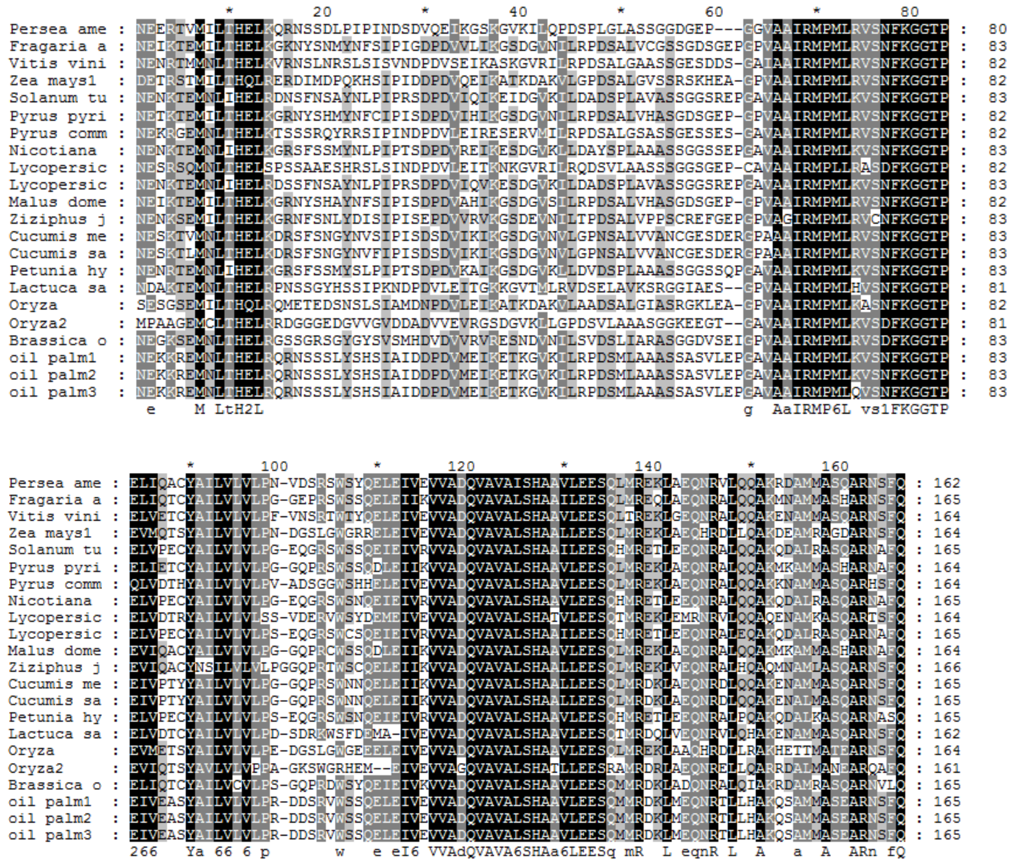
Appendix Figure 8 (Continued)



Appendix Figure 9 Partial alignment of ETR (ERS) DNA sequences. Each fragment contains one intron of different length 439, 432 and 421. Exons are underlined.



Appendix Figure 10 Partial alignment of ETR (ERS) sequences using protein fragments obtain 156 amino acids.



Appendix Figure 11 Partial alignment of ETR (F2R3) sequences using amino acids fragments.

```
                 *       180         *         200         *         220         *         240
Persea ame : KVMSHGMRRPMHSVSGLLSVMQLENLGPDQRLIVDAVAKTSSVVSTLINDVMEISTVDNGTLSLQM--KSFRLHPMIKEAACL : 243
Fragaria a : KVMSDGMRRPMHSVLGLLSMMQDESLNNDQRVIVDAMVRTSNVLSTLINDAMDNPAKDSGRFPLEM--RPFRLQPMIKEAACL : 246
Vitis vini : KVMSHGLRRPMHSILGLLSMFQDETMSFKQKIVIDTIMKTSNVLSTLINDVMEISAKDNGRFPLEM--RPFRLHSMIKEASCL : 245
Zea mays1  : TAMYDGMRRPMHSILGLVSMMQQESMNPEQRLVMDAIAKTSSVASTIMNDVMQTSTMNCEHLSLVR--RPFNLHSFIKEVVGV : 245
Solanum tu : MVMSHGLRRPMHSILGLLSLLQDEKLGNEQRLLVDSMVKTSNVVSTLIDDVMDTSTKDNGRFPLEM--RHFQLHSMIKEAACL : 246
Pyrus pyri : KVMSDGMRRPMHSILGLLSLMQDDTLDRDQRVIVDAMVRTSNVLSTLINDVMDNSAKESGRFPLEV--RSFGLHGMIKEAACL : 245
Pyrus comm : KAMSHGMRRPMHMILGLLSMFQENLSFK-QSIIVDTMEKTSYVICTLINDVMEMSAKDNERFPLEM--RPFELHSMIKEAACL : 244
Nicotiana  : MVMSHGLRRPMHSILGLLSLLQDDNMGIEQRLLVDAMAKTSSVVSTLINDVMDTSTKDNSRFPLEM--RHFQLHSMIKEAACL : 246
Lycopersic : KVMNNGMRRPMHSILGLLSIFQDEKASSDQRMIVDTMVKTSTVLSTLINDAMEISAKDDGRFPVEM--KPFQLHLLVREASCL : 245
Lycopersic : MVMSHGLRRPMHSILGLLSLLQDEKLGNEQRLLVDSMVKTSNVVSTLIDDVMDTSTKDNGRFPLEM--RYFQLHSMIKEAACL : 246
Malus dome : KVMSDGMRRPMHSILGLLSLMQDNTLDNDQRVIVDAMVRTSNVLSTLINDVMDNSAKESGRFPLEM--RSFGLHATIKEAACL : 245
Ziziphus j : KVMSNGMRRPMHSILGLLSMMQDENLSNEQQVLVDTMVRTSSVVTTLVDDMMDNSTKDNGRFPLEM--RSFHLHSMIKEAACL : 247
Cucumis me : KVMSDGMRRPMHSIMGLLSMLQNENMNDDQRIILDAMVRTGNVVSTLIDDVMEDPIKDSARFPLELEMRSFRLHSMIKEAACL : 248
Cucumis sa : KVMSDGMRRPMHSIMGLLSMLQNENMNDDQRIILDAMVRTGNVVSTQIDDVMEHPIKDSARFPLELEMRSFRLHSMIKEAACL : 248
Petunia hy : MVMSHGLRRPMHSILGLLSLLQDDKLGNEQQLLVDAMAKTSNVVSTLINDVIDTSTKDNGRFPLEM--KHFQLHSMIKEAACL : 246
Lactuca sa : KVMSHGMRRPMHSIMGLLSILQDDQKNTNQTNIIDTISKTSSVLSTLINDVMEISAKDTGRLPLEI--RPFQLHSMVKEACCL : 243
Oryza      : TAMYDGMRRPMHSILGLVSMMQQENMNPEQRLVMDAIVKTSSVASTIMNDVMQTSTVNREYLSLVR--RAFNLHSLVKEAISV : 245
Oryza2     : GVMSQGMRRPIHSILGLVSMVQEEALAPEQRLVVDTMARTATVVSTLVNDVMEMSADSRERFPLET--RPFHLHAMIRDAACV : 242
Brassica o : KAMSEGMRRPMHSILGLLSMIQDEKLSNEQKMIVDTMVKTGNVMSNLVGDAMDVS---DGRFVTEM--KPFSLHRTVREAACL : 243
oil palm1  : RAMSQGMRRPIHSILGILSMMQQEKLSQEQRLVVDTMAITGSVISTLINDVMDTSTIDSERLSLIM--RPFQLHSMIKEAASV : 246
oil palm2  : RAMSQGMRRPIHSILGILSMMQQEKLSQEQRLVVDTMAITGSVISTLINDVMDTSTIDSERLSLIM--RPFQLHSMIKEAASV : 246
oil palm3  : RAMSQGMRRPIHSILGILSMMQQEKLSQEQRLVVDTMAITGSVISTLINDVMDTSTIDSERLSLIM--KPFQLHSMIKEAASV : 246
               Ms G6RRP6Hs6 G66S6 Q       Q  66D 6  T  V  t16 D 6  s     r       4 F Lh  64ea  6
```

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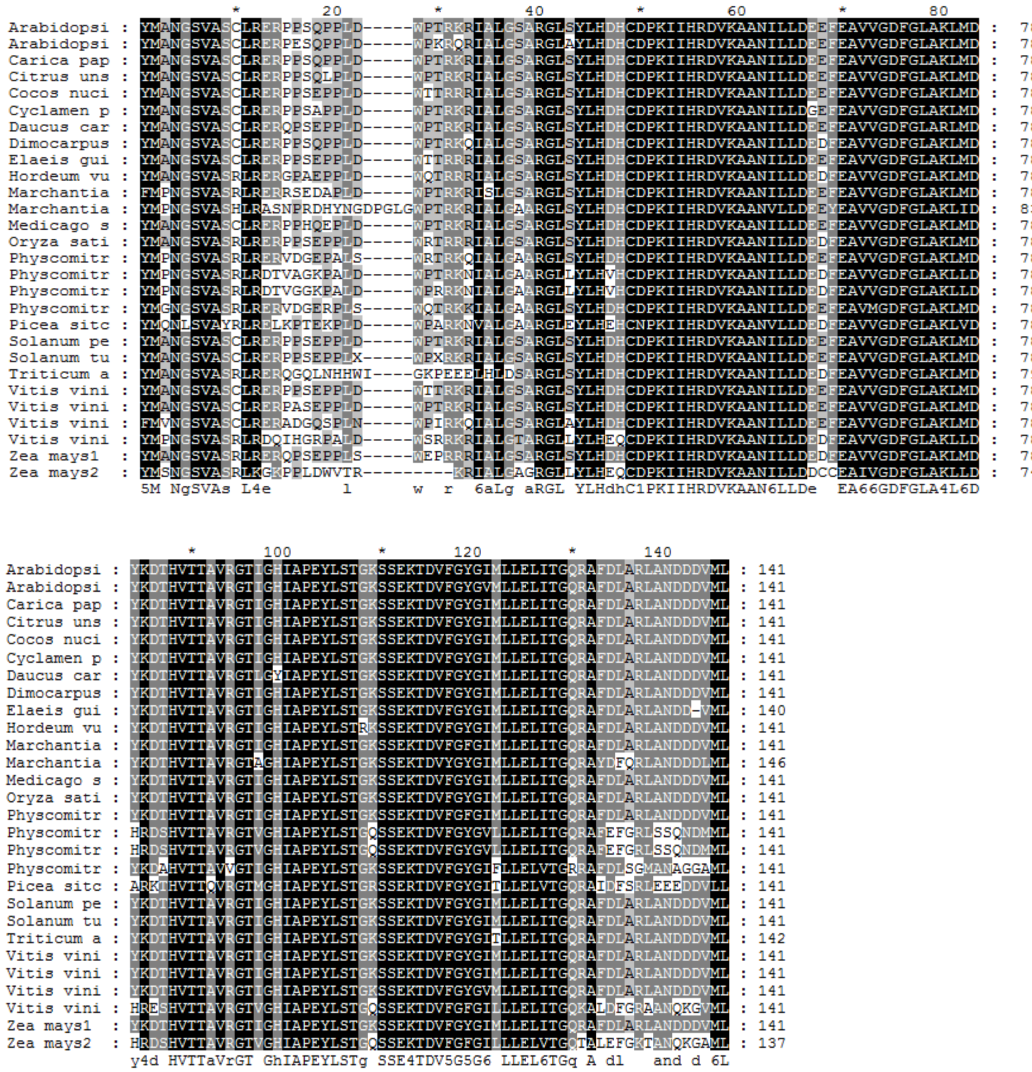
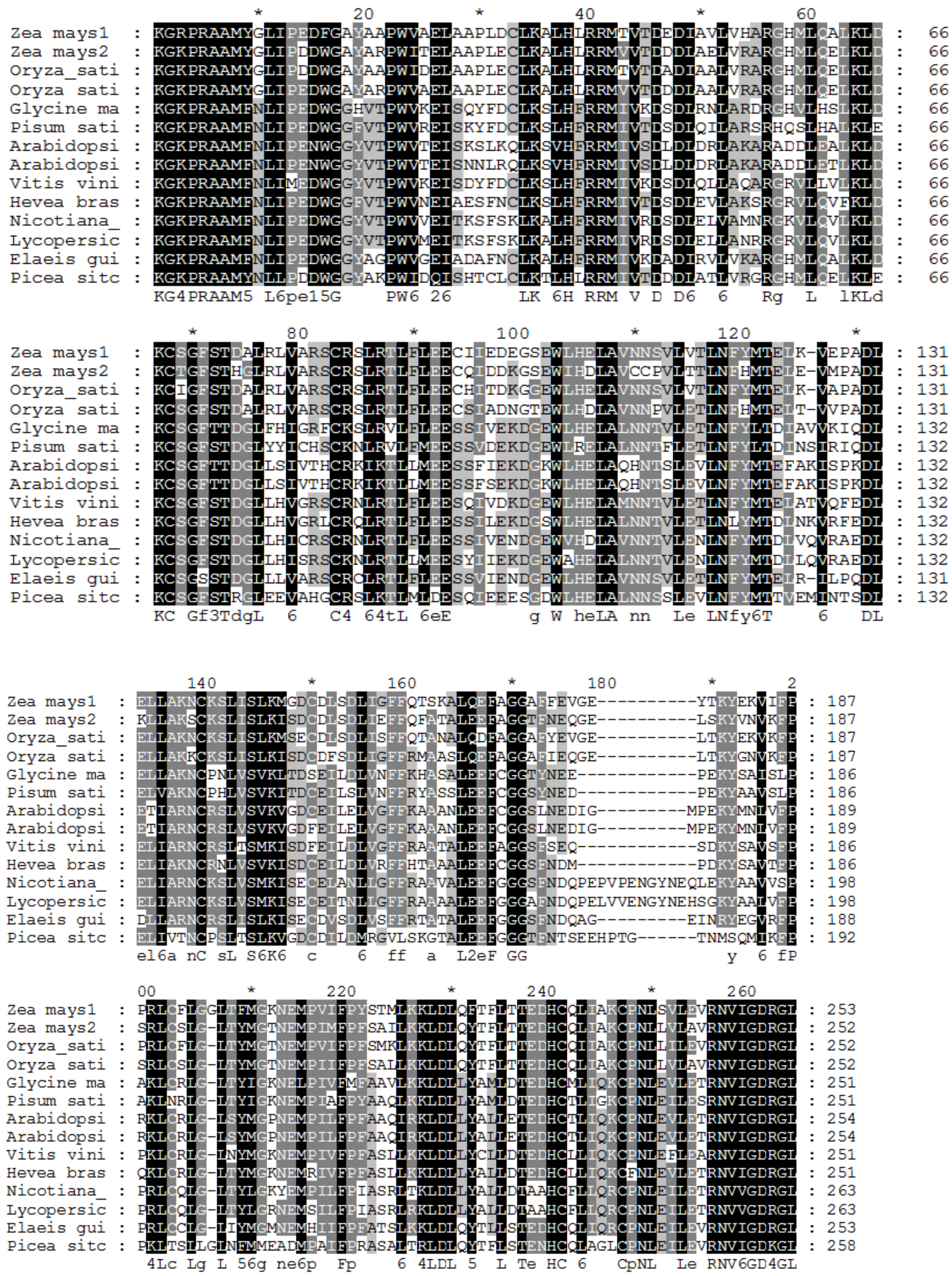
               *       260         *
Persea ame : AKCICVFKGFDFGVQVEKTVPDWVI : 268
Fragaria a : AKCLCVYRGFGFAIEVDKSIADHVI : 271
Vitis vini : AKCLCVYKGFGFAVDIRNPLPDQVI : 270
Zea mays1  : VRCLTGCKGVEFEFQVENSLPERII : 270
Solanum tu : AKCLCAYRGYNISIEVDKSLPNHVL : 271
Pyrus pyri : AKCLCVFRGFGFAIDVNKSLPDHVM : 270
Pyrus comm : AKCLCMYKGFSFEIDVQSSLPNQVI : 269
Nicotiana  : AKCLCAHRGYNISIEVDKSLPNYVM : 271
Lycopersic : VKCLCVYKGFGFSTDVPTSLPNQVM : 270
Lycopersic : AKCLCAYRGYNISIEVDKSLPNHVL : 271
Malus dome : AKCLCVFRGFDFAIDVDKSLPDHVM : 270
Ziziphus j : AKCLCLYRGFDFAVEVDKSLPDNVM : 272
Cucumis me : AKCLCAYKGFGFAFEVQRSLPDHVM : 273
Cucumis sa : AKCLCAYKGFGFAFEVQRSLPDHVM : 273
Petunia hy : AKSLCAYRGYKISIEVDKSLPNHVM : 271
Lactuca sa : VKCLCIYQGFGFSMEVPSSIPNLVM : 268
Oryza      : VRCLTGCKGIDFEFEVDNSLPERVV : 270
Oryza2     : ARCLCDFRGFGFAVHVENALPDLVV : 267
Brassica o : ARCLCLYNGFRFTVDAEMSLPDNVV : 268
oil palm1  : ARCLCDCRGFGFEFQVDNAVPDRVV : 271
oil palm2  : ARCLCDCRGFGFEFQVDNAVPDRVV : 271
oil palm3  : ARCLWDCRGFGFEFQVDNAVPDRVV : 271
             a4c6c   G  f       6p  66
```

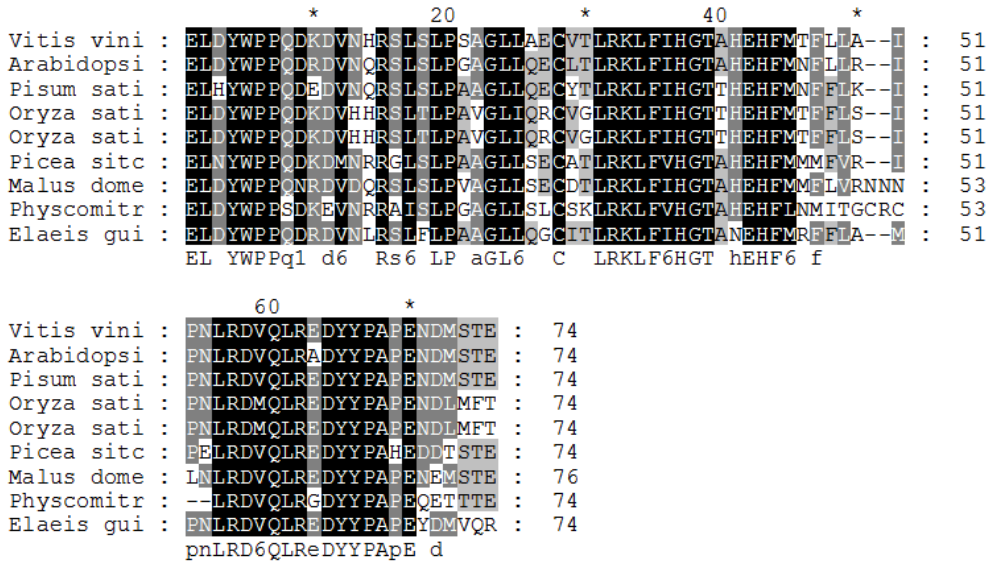
Appendix Figure 11 (Continued)



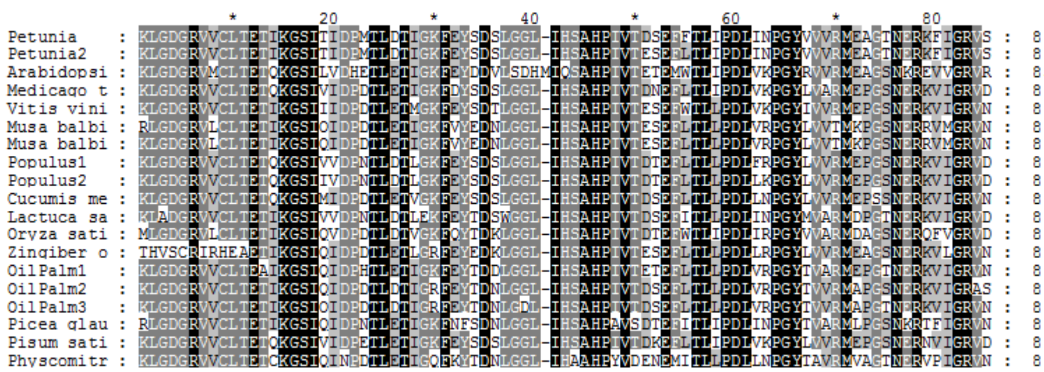
Appendix Figure 12 Partial alignment of BAK1 sequences using amino acid fragments.



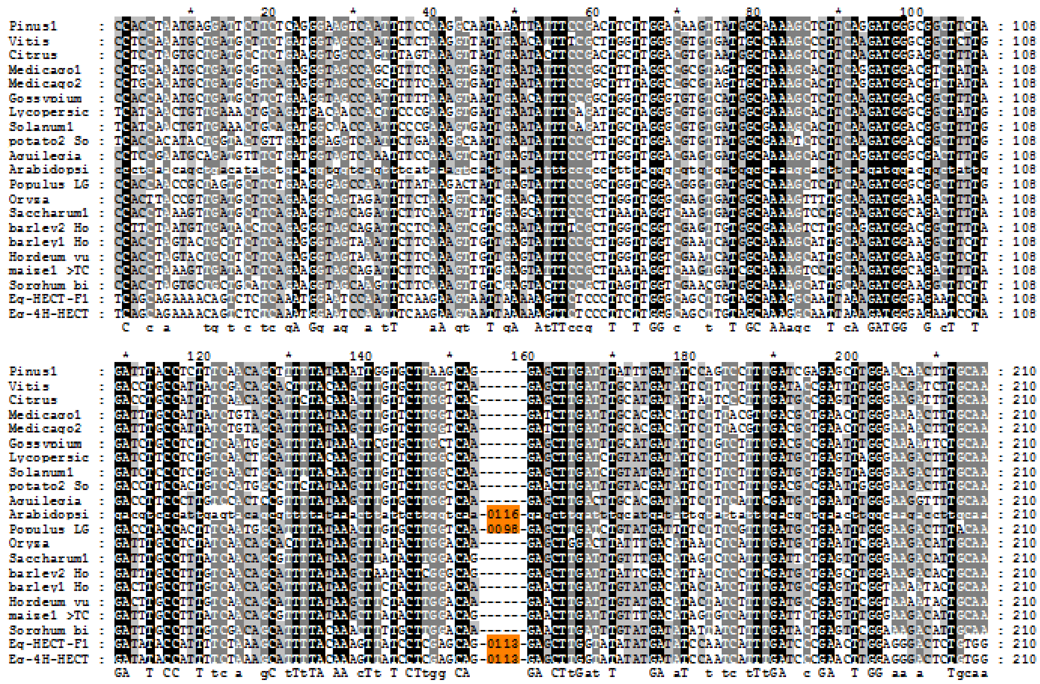
Appendix Figure 13 Partial alignments of COI1 sequences using amino acids sequences.

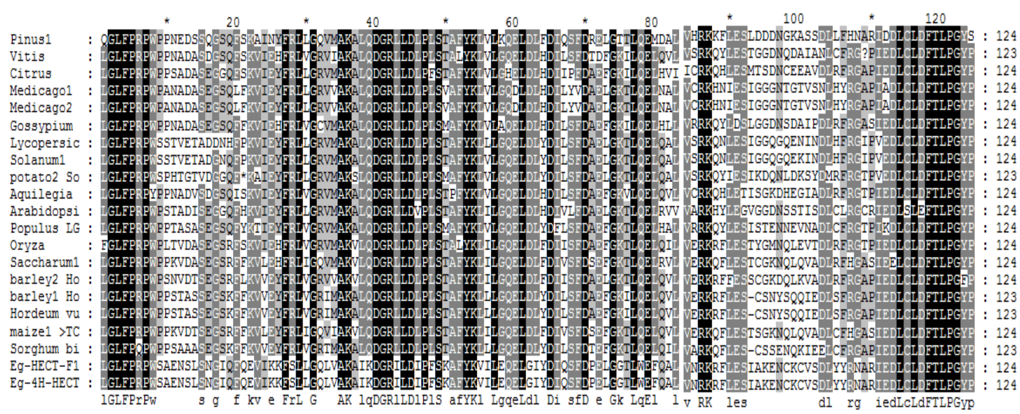


Appendix Figure 14 Partial alignment of MAX2 sequences using protein fragments.

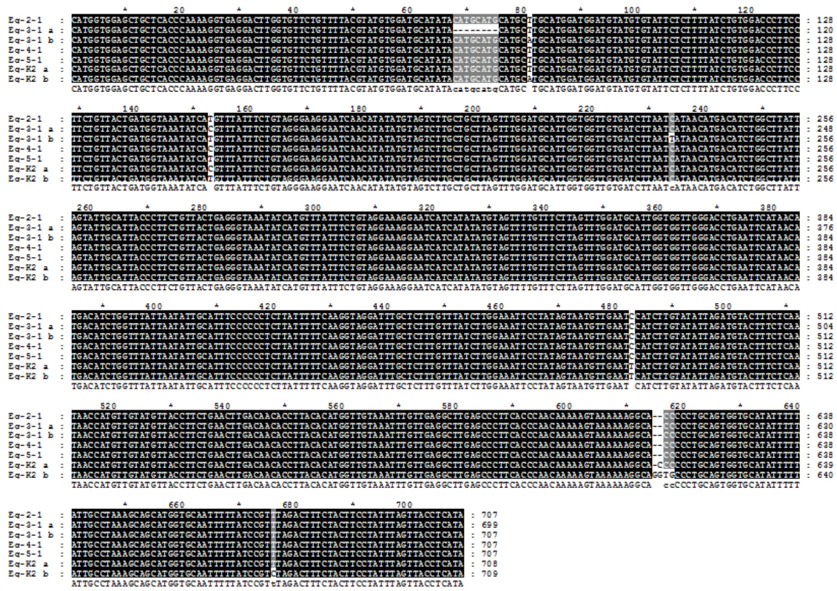


Appendix Figure 15 Partial alignment of MAX4 sequences using protein fragments.

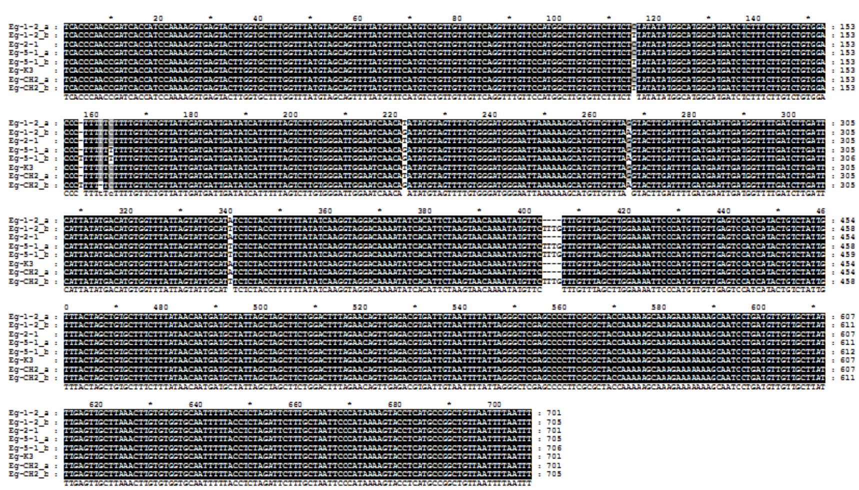




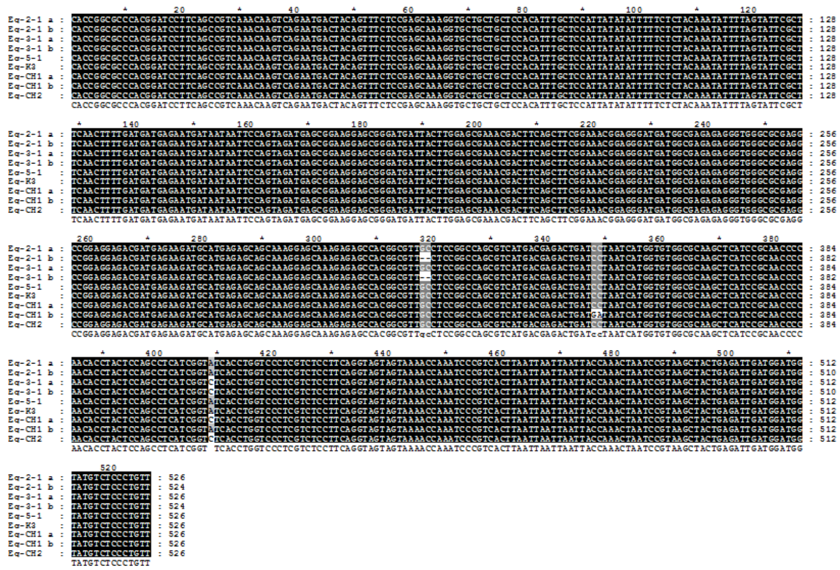
Appendix Figure 16 Partial alignment of HECT sequences using DNA fragments. Partial alignment of HECT sequences using protein fragments.



Appendix Figure 17 Sequences of PCR product using PIN1 specific primer. They are quite similar and only two position some sample deletion and made them different.



Appendix Figure 18 Sequences of PCR product using PIN2 specific primer. They are quite similar and only seven position that different.



Appendix Figure 19 Sequences of PCR product using PIN3. They are 526 bp in length.

Appendix Figure 20 Sequences of PCR product using PIN4 specific primer. Their products are different many positions and primer designed including intron but their sequences incompletes.

Appendix B

Original sequences

AAAGGCAAGCCTCACTTTGCCGACTTCAACTTGGTTCCCGATGACTGGGGTGGCTTCGCCCTCCCCT
GGATCGAGGCCTTCGCACGAGGCGGCCTTGGCCTCGAGGAGCCCCGGCTGAAGAGGATGGTCGTCT
CCGACGAGAGCCTCGAGCTCCTCGCGCGCTCCTTCCCCAGTTTCAAGGTCCTGGTTCTCATCAGCTG
CGAGGGGTTCAGCACCGACGGGCTTGCGGCGATCGCCACCCATTGCAGGTGATCCACCTCACACCT
TTCTGTTCATTCTCTAGTGGCTAGGTGGGTTCAAAATTGGATCTTGATCATGTTTGGCTATCATACTT
TCGGTAGCTTTTGTGTCATTGATTGATGCAAAAAATCGAATTTTTATCATGTTGCCTTATGTTTGTTT
CTGCTAATTATTTAGTGTTCTTTGTTATTTGGATGTGCACCAGTTGAATTGAATCCCATTCATGTCTC
TCCAGCTGGATTCTGTTTCCCTCATCTCTCTCTCTCTCTCTCTCTGTTGAAATAATTTTCATGTCCT
AATTTGGTATCCAATTATTTATGGATCGACGCAGATGGGTGTTTTGTATGAAGGTATTTTTTGAAAA
ACCCTTTTATTATTATTGTTAAAAATTAATATGGTTATATAGGCATGGGACAAACTTATGCAAACTG
GGACACTTGTTGAACATTAAATAATGTTCTAAGGGTATTTAACCTTTTTTTTGGGTTTTTAAGGGTCC
TTAGGGAGCTTGATTTACAGGAAAATGAGGTGGAAGATTGTGGGCCTCGGTGGCTCAGCTGCTTCC
CTGATTCCTGTACTTCGTTAGTGTCCCTGAATTTCGCCTGTTTGAAAGGGGAGGTGAATGCTGCTGC
ATTGGAGAGGCTTGTTGCGAGGTGTCCCAACATTAGGACCTTGCGTCTCAATCGTGCCGTGTCTGTT
GATTCACTGTCCAAGATTCTTGCTCGCGCACCCCACTTGGTCGACTTGGGCACTGGTTCATTCGCCAT
AGACCACCATGCTGAAGCTTACCACAGGCTGATCAATAACTTCACTAAATGCAAGTCGCTGAAAAG
CTTGTCTGGGTTTTGGGATGCTTCACCCCGCTGCCTTCCGGCTGTGTATCCCATTTGTGGAAACCTGA
CCGGTCTCAACTTGAGCTATGCCCCAGCAATTCAAGGTGCTGACCTTATCAAGCTAATACGTCTGTG
TTTGAAACTTCAACGGCTTTGGGTGTATCCCATTTGTGGAAACCTGACCGGTCTCAACTTGAGCTAT
GCCCCAGCAATTCAAGGTGCTGACCTTATCAAGCTAGTGAGTTCTTGTTGGCTCATTTTCCTGGAATT
GACTGGGTTGCTATGCGTCAAAATGACTTAGTATTGGCTCGAATCCTTCTGCAGGTATTGGATTGTA
TTGGAGACAAAGGATTGGCGGTTGTGGCCAGCACTTGTAAAGAGTTGCAGGAGTTGAGGGTGTTCC
CATCTGATGTCTGTGGTGTGGGAACTGCTGCTGTGACAGAGGAAGGGCTTGTGGCCATATCCTCAGG
ATGCCCAAAGCTGAACTCCTTGCTTTACTTTTGCCATCAGATGACGAATGCTGCGCTCGTCACTGTT
GCGAAGAACTGTCCACATTTCACACGCTTCAGATTATGTATCCTTGATCCAGGGAAGCCCGACCCAG
TCACTAATCAGCCATTGGATGAAGGTTTTGGGGCAATTGTGCAGTCATGTAAAGATCTAAGGCGGTT
ATCATTATCAGGCCTTCTCACAGACCAGGTCTTCTTGTACATTGGCATGTATGCTAAATGCCTAGAG
ATGCTCTCAATTGCATTTGCTGGTGACAGTGATAAGGGAATGGTGTATGTGCTCAATGGATGCAAAA
ATCTGAGGAAATTGGAAATAAGGGATAGCCCATTTGGGGATGCTGCACTTTTGGAGGATGTGGGGA
AGTATGAAGCGATGCGATCCCTTTGGATGTCATC

Appendix Figure 21 EgTIR1 Oil palm (*Elaeis guineensis*) homolog of the *A. thaliana* TIR1 gene, exons1-3, partial sequence. Including exon1 1-248, intron1 249-736, exon2 737-1232, intron2 1233-1399 , and exon3 1400-1980

GATTTACTTCCCTCAAGGTCATATGGAACAGGTATCTTGAGTTTCCACATATTAGATCTATATTATGA
CTCCGAGACGCATTTTTCGTGGTTTTACTATCAAATGTCAGATCTCTTTTGATTAGATACTGGACTTA
TCTTGGGGTTGTCTTAGATAAGTGCCTATTATGAGTGCCATGATCCTTGATACTGCTTTAAGAATCCA
AGACATTCATTATAAGTCTAATATTACTGTTTTGTCACACTGCGACATGCTAGTTTTGCATCATGAAT
CATAAGCTAATCATATTAGCATGACTTATGTATGGCTAAGATGAGCTGATCTCTATATTGATCTCTG
TTTCTGTTTTCTTTTCTTTTTTTCTTGCGCGTGTGTTTTGCAGCTCGAGGCATCGACAAATCAAGGGCT
GGACCAGCATATGCCTTTGTTCAATCTACCATCAAAGATTCTTTGCAGGGTGGTCCATGTTCAACTT
CGAGTAAGCTCTTGTTGGTCACTGTATAATATGTTGTTCATCATGTTAGATGTGGTATGTGGAGGCG
TTAGCTTTATTTCTCACTACCTGTGTTATTCATCACCTTTCTTTTTAGGCTGAACCAGACACTGATGA
AGTTTATGCACAGATAACAATCACTAGT

Appendix Figure 22 EgARF1 Oil palm (*Elaeis guineensis*) homolog of the *Oryza sativa* ARF1 gene, exons1-3, partial sequence. Including exon1 1-27, intron1 28-378, exon2 379-474, intron2 475-586, and exon3 587-624

GATTGCACATGTTGTGGGTTGGCCTCCAATCCGATCATATAGGAAGAATAGCTACCAAGCAATGAA
GATGGAGGCAGAGACCACTGGAATGTATGTGAAGGTGAGTATGGATGGGGCTCCTTATCTGAGGAA
AATTGATCTCAAGGTCTACAAGGGGTACAAGGAGCTCAGAGAGGCCTTGGAGGATAAGTTCAAATG
CTTTTCCTTAGGTGAGTAAGATGATGATAAACAGTTCTCTTAAAAACAAAAAAAAAAAAAAAAAGT
CAATGATAAAAAAGAGAAGAAGATGATTTAATTCTTCTTGATCTTTCCTTTTTCTATTCAGGTGAGA
TTTCAAGGATGGATGAAAGCAATGTGTATGAATATGCTATCACGTATGAAGATAAAGATGGGGATT
GGATGCTTGTTGGTGATGTAATCACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGA
GCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCA
TGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAA
GCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACT
GCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAG
AGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGC
TGCGGCGAGCGGT

Appendix Figure 23 EgAXR Oil palm (*Elaeis guineensis*) homolog of the *Zea mays* ARF1 gene, exons1-2, partial sequence. Including exon1 1-205, intron1 206-321, and exon2 322-412

AAGGTgTTGAAGCAGAGTGACGTGGGTAGCCtGGGAAGGATCGTGCTACCCAAAGTGAGCGAAATC
CGACACGGTTTGAATTAATATACAGTTTGTTTCCATCCTTTTTCTGCTCCCAAATTTGGCACCATGGA
AGGGTTTTCTTCTTTGTCTGAACAAAGGTTTGAAGTATTTGATGTtGCTGTATCACCAGAAGGAAGCG
GAGATCCATCTACCGGAGCTCGAAGCAAGGGACGGGATATCAATTCCAGTGGAGGACATAGGCACC
TCCCAAGTATGGAACATGCGGTACAGGTACTTGACGTTGTAATAGCGCTCTTTTGATTTGAGTAACG
CCTCCCAAAAGAAGGAAAAAGAATTTTTTTTTAGCGTAAAGTAGTTTCATTAAATCAAGTATAAAG
AATATATGGAAGTTTGATTGATGCTATCTACTTCTTGCATGCATCACAAGTTCTTTTTTTTGTTTGGA
AAAGAGAAACGTTTGCTATAATTTTCTTTTAAAAAAAGAGAGCAAATAATCATCTGGTGATCGATG
CAGATTTTGGCCCAACAACAAGAGCCGCATGTATCTCCTGGAGAACACAGGTACAGTTTGGAAGGA
AATCTTTCATCTTGATAGCTTCCTCTTGTTCAATGTCAAATTCTTTTGATACTTATTGTTGCTTTTGGT
CTCATGTATGTGTTGTGGGGATTCAGGAGAATTTGTTCGATCCAACGGCCTGCAGGAGGGAGACTTC
ATAGTGAT

Appendix Figure 24 EgABI3 Oil palm (*Elaeis guineensis*) homolog of the *Oryza sativa* ABI3 gene, exons1-4, partial sequence. Including exon1 1-54, intron1 55-193, exon2 194-294, intron2 295-538, exon3 539-585, intron3 586-696, and exon4 697-745

TTCGGTTCTATGAATATGGATGAGTTCGTCAAGAACATCTGGACCGCGGAGGAGAGCCAGGTCATA
GCCGCCGCACTTGGCGGAGCCGTGGGCGGGGGCATCGACGGTGGCGTGGCCGGAACGGGTCTCCAG
CGCCAGGGCTCGCTGACGCTGCCGCGGACTCTCAGCCAGAAGACGGTCGACGAGGTGTGGAGGGAC
TTCATCCGGGAGGGCGGGCCAGGGTTCGAGCATCAGCACCGGCCTCCACCAGCAGCGGCAACCGAC
CCTCGGGGAAATGACCCTCGAGGAATTTCTAGTGAGAGCTGGAGTTGTTAGAGAGGACATGACTCA
GCCAGGGGTGCCGAGGCCGATTGGTAATAGCAGTAACAACAGCAATACCAACAGCAATGTGTTTTA
TGGAGAGTTGCCGAATTCAAACAATAATACCGGGCCTGCTCTCGGGTTCCCTCAGACGAGTCTGAG
CAATGGGACCGTGGTGACAAACGCATTTCCCAACAGCTCCGGTGCCAATTTAGCGATGCCGGCTAC
CGGGACGAGGCCATATGCAGCTCCTCTGCCTCTGGGAAATACTGCTGATTTGGGGACACCACAGGG
GTTGATAGGGGATGGAGTCATGGGGATTGGAGATCAGGGTATGAACAATGGAATGATGCCTGGCGT
GGTTGGTGTGGGGGGAGCTGGGGTTACAGTTGCAGCCATGGGTTCCCCAGTGAACCAGATGCCGAC
GGATGGGCTTAGCAAGGGCAATGGGAACCTGTCATCCCTGTCCCCAGTTCCATATATGTTCACCGGG
GGCCTTAGGGGGAGGAAATGCAGCGGGGCAGTGGAGAAGGTAGTGGAGAGGAGACAGAGGAGAA
TGATTAAAAACAGAGAGTCAGC

Appendix Figure 25 EgABI5 Oil palm (*Elaeis guineensis*) homolog of the *Oryza sativa* ABI5 gene

TACATGGCTAATGGAAGTGTTGCATCATGCTTAAGAGGTACTCATCATTCTTTTATTATCTGATACA
ACAGAATGCATTTTCCTGATATCAGTATAGCTAATATTATTTTAATAAAATTATGTGATTGCGGCTAT
TTCACTTATGTTTTGAAAATAGTCCGAGACAGTATTGTTGTGCTTATGTTAAAAGTTCTCTATTCAAA
AAAAGAAAAAAAAAAAAAACAAAGAAAAGATTTGATTGTTAAGAATGCTGTAAACTGTAAAGAGCC
TCCTACTTCTTGTAGAGCGGCCACCATCTGAACCTCCGCTTGATTGGACAACTCGGCGAAGGATTGC
ATTGGGATCTGCAAGGGGGCTGTCATATTTGCATGATCATTGTGATCCAAAAATTATTCATCGTGAT
GTCAAAGCTGCAAATATTTTATTGGATGAAGAGTTTGAGGCAGTTGTTGGAGACTTTGGGTTGGCCA
AGCTCATGGACTACAAGGATACCCATGTAACAACTGCTGTTCGTGGAACAATTGGACACATTGCTCC
AGAGTATCTATCTACTGGAAAGTCCTCAGAGAAGACTGATGTTTTTGGATACGGAATCATGCTTTTG
GAACTTATTACAGGCCAGAGGGCATTCGATCTTGCCAGGCTTGCAAATGATGACGTCATGTTGCTTG
ATTGG

Appendix Figure 26 EgBAKI Oil palm (*Elaeis guineensis*) homolog of the Cocos nucifera BAK1 gene, exons 1-2, partial gene. Exon1 1-37, intron1 38-283 and exon2 284-675

AAATGGCAAGCTCAGAGGTGGTGGGCCGAGAACTACGACAAGGTCATGGAGCTCTACAACGTCCA
GAGGTTCAATCGCCAGGATGCCCCCCTCCCCATCCCTCCTGGCTCCGAAGATGAGGTGAGACTTGTA
TAAAGGCCTTCTCAGCAAACCATCCTTCTTAGTTTTTGTCCAAACTCTTTCGGGCCTTGGTTAACTCG
TGGCTTGGTATTTCTTTCTTCAGAGCTCGAAGGAGGACAGCCCAGTGACCCCGCCGTTAAACAAAGA
ACGGCTTCCCCGTAACTTTCACAGGCCATTGATGGGTGGTGGCGGTGGTGGAGCGATGGGTTACAC
ATCATCAGACTCACTTGAGCACCGTTCCAGCCACCACTGCCACCACCATCATGGGCGCTATTATCAT
GATTCAGGTGGTCTGACATCAACACTGAAGCTGTCAAGCATCAGTGGAGCAAAGATGGAGACATCA
TCGATGGATGCATCGGTGCAAACAAGCTCATCTCCGGCGGACCAATCGGGGGAGCCATCAGTGGCA
GTGAGCAATGCCAGTGACCAGGAGAGGGAATGGGTTGAAGAGGATGAACCAGG

Appendix Figure 27 EgBRX Oil palm (*Elaeis guineensis*) homolog of the *Oryza sativa* BRX gene

AGACTTGCTGCTATTGCAGTGGGTACTGGTGGCCATGGTGGGCTGGGTAAGCTTTACATTGAAGGA
AGTAATGCAACTCGTCCGCTCACTGATATTGGACTGTCTACTGTGGCCCGTGGTTGCCCTTCCCTAC
GGGTTCTGTCAATGTGGAATGTTCCCTTCATCAGTGACATTGGCCTATCCGAGATTGCAAGTGGATG
TCCTATGCTGGAAAAGCTTGACCTGTGCCAGTGTCCTTTAATCTCAGATAAGGGTTTGATAGCTGTT
GCTCGGAAGTGTCCCAACTTGACATCTCTGATGGTAGAATCTTGCTTGAGCATAGGCAATGAAGGCC
TGCAGGCTATCGGTCGCTGCTGCCCAAAGTTGAAGTCTATTGTTATTAAGGACTGCCCACGTATTGG
TGACCAAGGAGTTGCAAGCCTGGTCTCTGCAGCTTCATCTTTTCTAGCCAGGATTAGGCTTGAGAAC
GTGAATATCAGTGATGTGTCTCTTGCTGTCATTGGGCACTATGGGAAGGCTGTTGCTGATCTAGCAC
TTACTGGCCTCCAGTCAGTAAGTGAGAGGGTTTCTGGGTCATGGGCAATGC

Appendix Figure 28 EgEBF Oil palm (*Elaeis guineensis*) homolog of the *Zea may* EBF gene

AAGGGTAAGCCCCGAGCTGCGATGTTCAACCTCATACCCGAAGACTGGGGCGGCTACGCGGGACCC
TGGGTCGGCGAGATCGCCGACGCCTTCAACTGCCTCAAGGCCCTCCATTTCCGCCGGATGATCGTGA
AAGATGCCGATATCCGTGTCCTGGTCAAGGCCAGGGGCCACATGCTCCAGGCGCTCAAGCTCGATA
AGTGTTCGGGGTCCTCCACCGATGGCCTCTTGCTCGTTGCCCGCTCTTGCAGATGCCTTAGAACCTTA
TTTTTGGAAGAAAGCTCTGTTATTGAGAATGATGGTGAATGGCTTCATGAGCTTGCTGTCAATAATT
CTGTGCTTGAGACTTTGAATTTCTATATGACGGAGCTTAGAATCTTGCCACAAGACCTTGATTTATTA
GCTAGGAACTGCCGGTCATTGATTTCTCTGAAGATCAGTGAGTGTGATGTCTCTGATCTAGTCAGCT
TTTTCCGCACAGCAACAGCACTTGAAGAATTTGGTGGCGGCTCATTTAATGATCAAGCAGGAGAAA
TCAACAGGTACGAGGGCGTTCGCTTTCCTCCCAGGCTATGCTGCTTGGGACTCATTTATATGGGGAT
GAATGAAATGCACATAATATTTCCATTTGCCACTTCACTCAAGAAGCTAGACCTGCAGTATACTTTG
CTCAGCACTGAAGACCATTGTCAGCTGATTCAGCGGTGTCCCAATCTTGAAATTCTTGAGGTAAGAA
ACGTGATAGGAGATAGAGGATTAGAAGT

Appendix Figure 29 EgCOI1 Oil palm (*Elaeis guineensis*) homolog of the *Oryza sativa* COI1 gene

CTTCATATGTTCGTTTGGAgTTCCAGCGCTTCGTCGGGGGGAGAGTTTGGAAGTGAGCATGGTGGAG
CTGCTCACCCAAAAGGTGAGGACTTGGTGTTCTGTTTTACGTATGTGGATGCATATACATGCATGCA
TGCTTGCATGGATGGATGTATGTGTATTCTCTTTTATCTGTGGACCCTTCCTTCTGTTACTGATGGTA
AATATCATGTTTATTTCTGTAGGGAAGGAATCAACATATATGTAGTCTTGCTGCTTAGTTTGGATGC
ATTGGTGGTTGTGATCTTAATCATAACATGACATCTGGCTTATTAGTATTGCATTACCCTTCTGTTAC
TGAGGGTAAATATCATGTTTATTTCTGTAGGAAAGGAATCATCATATATGTAGTTTTGTTTCTTAGTT
TGGATGCATTGGTGGTTGGGACCTGAATTCATAACATGAACATCTGGTTTTATTAATATTGGCATTT
CCCCCCTCTTATTTTTCAAGGTGGGATTTTGCTTCTTTGTTTATCTTGGAAATTTCTATAGTAATGTtG
AATCCATCTTGTATATTAGATGTACTTTCTCCAATAACCATGTTGTATGTTACCTTCTGAACTTGACA
ACACCTTACTCATGGTTGTAAATTTGTTGAGGCTTGAGCCCTTCACCCAACAAAAAGTAAAAAAGGC
ACCCCCTGCAGTGGTGCATATTTTTATTGCCTAAAGCAGCATGGTGCAATTTTTATCCGTTTAGACTT
TCTACTTCCTATTTAGTTACCTCATATGCAGGCCATTGATCTTCCTTTCTCTGATCTTAGATGCCTATG
ATGTATATGGTCGTGATGAATTCAGCTTGCAGAACCGGCCCGGTACTCAGGAAATCGACTCTCTGCA
GAACGATGGGCCTACCGAGCTCCTCCCAAAATCTGGGGCTGCTGCGGAAATCAAGCAGACTTCCAT
GCCTCCTGCCGGTGTGATGACCAGGCTTATTTTGATCATGGTCTGGCGAAAGCTAATTAGAAATCCG
AATACCTACTCCAGCCTTATTGGTCTCATCTGGTCCCTAGTCTCTTTCAGGTTATGGTCATTCCAAAA
CAATGTACAGAGAGAGAGAGAGAGAGCATGAATCTTAGAATCTAATTGTTACATTTTGCAGGTGGGAT
GTTGAAATGCCTGCAATTATTGCTAGTTCAATCTCCATACTCTCTGATGCAGGCCTCGGAATGGCAA
TGTTCAG

Appendix Figure 30 EgPINF3-2 oil palm (*Elaeis guineensis*) homolog of the *Zea mays* gene, exons 1-2, partial gene. Exon1 1-1063, intron1 1064-1140 and exon2 1141-1221.

CTTCATATGTTCGTGTGGAGTTCCAGCGCTTCGTCGGGGGGAGAGTTTGGAAGTGAGCATGGTGGA
GCTGCTCACCCAAAAGGTGAGGACTTGGTGTTCTGTTTTACGTATGTGGATGCATATACATGCATGC
ATGCTTGCATGGATGGATGTATGTGTATTCTCTTTTATCTGTGGACCCTTCCTTCTGTTACTGATGGT
AAATATCATGTTTATTTCTGTAGGGAAGGAATCAACATATATGTAGTCTTGCTGCTTAGTTTGGATG
CATTGGTGGTTGTGATCTTAATCATAACATGACATCTGGCTTATTAGTATTGCATTACCCTTCTGTTA
CTGAGGGTAAATATCATGTTTATTTCTGTAGGAAAGGAATCATCATATATGTAGTTTTGTTTCTTAGT
TTGGATGCATTGGTGGTTGGGACCTGAATTCATAACATGACATCTGGTTTATTAATATTGCATTTCCC
CCCTCTTATTTTTCAAGGTAGGATTTGCTCTTTGTTTATCTTGGAAATTCCTATAGTAATGTTGAATC
CATCTTGTATATTAGATGTACTTTCTCAATAACCATGTTGTATGTTACCTTCTGAACTTGACAACACC
TTACACATGGTTGTAAATTTGTTGAGGCTTGAGCCCTTCACCCAACAAAAAGTAAAAAAGGCACCC
CCTGCAGTGGTGCATATTTTTATTGCCTAAAGCAGCATGGTGCAATTTTTATCCGTTTAGACTTTCTA
CTTCCTATTTAGTTACCTCATATGCAGGCCATTGATCTTCCTTTCTCTGATCTTAGATGCCTATGATGT
ATATGGTCGTGATGAATTCAGCTTGCAGAACCGGCCCGGTACTCAGGAAATCGACTCTCTGCAGAA
CGATGGGCCTACCGAGCTCCTCCCAAAATCTGGGGCTGCTGCGGAAATCAAGCAGACTTCCATGCC
TCCTGCCGGTGTGATGACCAGGCTTATTTTGATCATGGTCTGGCGAAAGCTAATTAGAAATCCGAAT
ACCTACTCCAGCCTTATTGGTCTCATCTGGTCCCTAGTCTCTTTCAGGTTATGGTCATTCCAAAACAA
TGTACAGAGAGAGAGAGAGAGCATGAATCTTAGAATCTAATTGTTACATTTTGCAGGTGGGATGTT
GAAATGCCTGCAATTATTGCTAGTTCAATCTCCATACTCTCTGATGCAGGTCTCGGAATGGCAATGT
TCAG

Appendix Figure 31 Eg-PINF3-3 oil palm (*Elaeis guineensis*) homolog of the *Zea mays* gene, exons 1-2, partial gene. Exon1 1-1057, intron1 1058-1134 and exon2 1135-1215.

CTTCATATGTTCGTGTGGAGTTCCAGCGCGTCTCCAGTCTCCGACGTGTTCGGAAATAACCAGGAAT
ACGTCTTCCCACCGGCGCCCACGGATCCTTCAGCCGTCAAACAAGTCAGAATGACTACAGTTTCTCC
GAGCAAAGGTGCTGCTGCTCCACATTTGCTCCATTATATATTTTTCTCTACAAATATTTTAGTATTCG
CTTCAACTTTTGATGATGAGAATGATAATAATTCCAGTAGATGAGCGGAAGGAGCGGGATGATTAC
TTGGAGCGAAACGACTTCAGCTTCGGAAACGGAGGGATGATGGCGAGAGAGGGTGGGCGCGAGGC
CGGAGGAGACGATGAGAAGATGCATGAGAGCAGCAAAGGAGCAAAGAGAGCCACGGCGTTGCCTC
CGGCCAGCGTCATGACGAGACTGATCCTAATCATGGTGTGGCGCAAGCTCATCCGCAACCCCAACA
CCTACTCCAGCCTCATCGGTCTCACCTGGTCCCTCGCCTCCTTCAGGTAGTAGTAAAACCAAATCCC
GTCACTTAATTAATTAATTACCAAACTAATCCGTAAGCTACTGAGATTGATGGATGGTATGTCTCCC
TGTTGGTATTCCAGATGGCATGTCGAGATGCCTGCCATTATATCACGTTCCATCTCCATACTGTCGG
ACGCAGGCCTAGGAATGGCAATGTTCAG

Appendix Figure 32 EgPINF3-5 oil palm (*Elaeis guineensis*) homolog of the *Zea mays* gene, exons 1-2, partial gene. Exon1 1-511, intron1 512-613 and exon2 614-694

CTTCATATGTTCGTCTGGAGTTCGAGTGCTTCGCCGGTGTCGGAGGGAGGGATGCACGTGTTTAGAG
GGGGGGAGTTTGGAAATGAGCATGGTGGAGCAGCTCACCCAACCGATCACCATCCAAAAGGTGAGT
ACTTGGTGCTTTGGTTTATGTAGCAGTTTTATGTTTCATGTCTGTTGTTGTTCAGGTTTGTTCCATGGC
TTGTGTTCTTTCTCTATATATGGCATGGCATGATCTCTTTCTTGTCTGTGGACCCTTTTTCTTTTGTTCT
GTTATTGATGATTGATATCATTTTTAGTCTTGTGGGATTGGAATCAACATATATGTAGTTTTGTGGGA
TGGGAATTAAAAAAGCATGTTGTTTAGGTACTTGATTTTGATGAATTGATGGTTTTGATCTTGATTCA
TTATATGACATGTGGTTTATTAGTATTGCATATCTCTACCTTTTTTATATCAAGGTAGGACAAAATAT
CACATTCTAAGTAACAAAATATGTTCTTTGTTTAGCTTGGAAAATTCCCATGTTGGAGTCCATCATA
CTGTCTATTGTTTACTAGCTGTGCTTTCTTTATAACAATGATGCTATTAGCTAGCTTCTGGACTTTAG
AACAGTTGAGGCGTGATTGTAATTTTATTAGGGCTCGAGCCCCTTCGCGCTACCAAAAAGCAAAGA
AAAAAAGCAATCCTGATGTTGTTGCTTATTTGAGTTGCTTAAACTTGTGTGGTGCAATTTTTACCTCT
AGATTCTTTGCTAATTCCCATAAAAGTACCTCATGCCGGCTGTTAATTTTAATTTTTCTGATCTTAGA
TGCCTATGATGTATATGGTCGCGATGACTTCAGCTTCCAGAACAGAGCTGGTACCCAGGGAATGGA
CTCACTGCAGAAGGATGGGCCTAGTCTCTCGAAGCCCGGTTCCAATTCCACAGCTGAACTCCACCCG
AAATCTGGGGCTGATGGAGAAATCAAGCCGACTTCGATGCCACCTGCTAGTGTGATGACCAGGCTT
ATTTTGATCATGGTCTGGCGAAAGCTAATTAGAAATCCAAACACCTACTCCAGCCTAATTGGTGTCA
TCTGGTCACTAGTTTCTTTCAGGTTATGGTCATCCCAAAACAACCTTCCCTTATTTCACAATTTGCTT
ACGAATGAATCCTAGGATCTCATCATTTTATTTTGCAGATGGAATATTGAAATGCCTGCAATTATTG
CTCGCTCGATCTCCATTCTATCAGATGCAGGTCTAGGAATGGCAATGTTCAG

Appendix Figure 33 Eg-PINF3-4 oil palm (*Elaeis guineensis*) homolog of the *Zea mays* gene, exons 1-2, partial gene. Exon1 1-1102, intron1 1103-1186 and exon2 1187-1267.

CCGTGTGCTTATGACGATGGTTGGACCGCCCTCAAATGGGCCTCCACCGAACCTTGGCTCCATAGCG
GTAAGGATGCCAAGCTCCGGGTTTTCCTCTCAGGGGATAGCTCTGGTGGGAACATTGCACACCATGT
TGCTGTCAGGGCTGCTGAGTCGGGAATTGAGGTCTCTGGGAACATTCTCCTTAATCCCATGTTTGGT
GGGAACCAACGAACCGAGTCGGAGAAGAGATTGGATGGAAAGTACTTTGTCACGATTCAGGACAG
GGACTGGTATTGGAGAGCGCATCT

Appendix Figure 34 EgGID1 Oil palm (*Elaeis guineensis*) homolog *Triticum aestivum* GID1 gene

CTTCATATGTTCGTCTGGAGCTCCAGCGCTTCGTCGGGGGGAGAGTTTGGAAGTGAGCATGGTGGA
GCTGCTCACCCAAAAGGTGGGGACTTGGTGTTCTGTTTTACGTATGTGGATGCATATACATGCATGC
ATGCTTGCATGGATGGATGTATGTGTATTCTCTTTTATCTGTGGACCCTTCCTTCTGTTACTGATGGT
AAATATCATGTTTATTTCTGTAGGGAAGGAATCAACATATATGTAGTCTTGCTGCTTAGTTTGGATG
CATTGGTGGTAGTGATCTTAATCATAACATGACATCTGGCTTATTAGTATTGCATTACCCTTCTGTTA
CTGAGGGTAAATATCATGTTTATTTCTGTAGGAAAGGAATCATCATATATGTAGTTTTGTTTCTTAGT
TTGGATGCATTGGTGGTTGGGACCTGAATTCATAACATGACATCTGGTTTATTAATATTGCATTTCCC
CCCTCTTATTTTTCAAGGTAGGATTTGCTCTTTGTTTATCTTGGAAATTCCTATAGTAATGTTGAATC
CATCTTGTATATTAGATGTACTTTCTCAATAACCATGTTGTATGTTACCTTCTGAACTTGACAACACC
TTACACATGGTTGTAAATTTGTTGAGGCTTGAGCCCTTCACCCAACAAAAAGTAAAAAAGGCACCC
CCTGCAGTGGTGCATATTTTTATTGCCTAAAGCAGCATGGTGCAATTTTTATCCGTTTAGACTTTCTA
CTTCCTATTTAATTACCTCATATGCAGGCCATTGATCTTCCTTTCTCTGATCTTAGATGCCTATGATGT
ATATGGTCGTGATGAATTCAGCTTGCAGAACCGGCCCGGTACTCAGGAAATCGACTCTCTGCAGAA
CGATGGGCCTACCGAGCTCCTCCCAAAATCTGGGGCTGCTGCGGAAATCAAGCAGACTTCCATGCC
TCCTGCCGGTGTGATGACCAGGCTTATTTTGATCATGGTCTGGCGAAAGCTAATTAGAAATCCGAAT
ACCTACTCCAGCCTTATTGGTCTCATCTGGTCCCTAGTCTCTTTCAGGTTATGGTCATTCCAAAACGA
TGTACAGAGAGAGAGAGAGAGCATGAATCTTAGAATCTAATTGTTACATTTTGCAGGTGGGATGTT
GAAATGCCTGCAATTATTGCTAGTTCAATCTCCATACTCTCTGATGCAGGTCTTGGAATGGCAATGT
TCAG

Appendix Figure 35 Eg-PINF3-6 oil palm (*Elaeis guineensis*) homolog of the *Zea mays* gene, exons 1-2, partial gene. Exon1 1-1057, intron1 1058-1134 and exon2 1135-1215

CTTCATATGTTCGTGTGGAGTTCCAGCGCGTCTCCAGTCTCCGACGTGTTCGGAAATAACCAGGAAT
ACGTCTTCCCACCGGCGCCCACGGATCCTTCAGCCGTCAAACAAGTCAGAATGACTACAGTTTCTCC
GAGCAAAGGTGCTGCTGCTCCACATTTGCTCCATTATATATTTTTCTCTACAAATATTTTAGTATTCG
CTTCAACTTTTGATGATGAGAATGATAATAATTCCAGTAGATGAGCGGAAGGAGCGGGATGATTAC
TTGGAGCGAAACGACTTCAGCTTCGGAAACGGAGGGATGATGGCGAGAGAGGGTGGGCGCGAGGC
CGGAGGAGACGATGAGAAGATGCATGAGAGCAGCAAAGGAGCAAAGAGAGCCACGGCGTTGCCTC
CGGCCAGCGTCATGACGAGACTGATCCTAATCATGGTGTGGCGCAAGCTCATCCGCAACCCCAACA
CCTACTCCAGCCTCATCGGTCTCACCTGGTCCCTCGTCTCCTTCAGGTAGTAGTAAAACCAAATCCC
GTCACTTAATTAATTACCAAACTAATCCGTAAGCTACTGAGATTGATGGATGGTATGTCTCCCTGTT
GGTATTCCAGATGGCATGTCGAGATGCCTGCCATTATATCACGTTCCATCTCCATACTGTCGGACGC
AGGCCTAGGAATGGCAATGTTCAG

Appendix Figure 36 EgPINF3-8 oil palm (*Elaeis guineensis*) homolog of the *Zea mays* gene, exons 1-2, partial gene. Exon1 1-510, intron1 511-608 and exon2 609-689.

CCCCATATGTTCGTTTGGAGTTCCAGTGCGTCTCCCGTCTCCGATGTGTTTGGTAACAACCAGGAGT
ACGGTTTGCCGGCGACGGATCCTTTGGCTGCCAAAGAAGTTAGAATGACGGTTTCTCCAGGCAAAG
GTACTCTACGTTTAGTCCATGTTCTTTCTATGCATATAGTTCAGCTTTCTTAGCTTGATAAAAATATT
GAGCTTCTTGGATCGAAGTTATTCCCAGTGGTGGATGGGCGCAAGGAGAGGGATGATTACATGGAG
CGAGAGGACTTCAGCTTCGGAAATAGGGGAATGATGGAGAGAGATGGTGCGCATGAGGGAGGTGG
CGACGAGAAGATGCATGAGAGCACCAAAGGAGGAGGGAGAGGCACGGTGTTGCCCCCGGCCAGCG
TCATGACGAGGCTGATCCTCATCATGGTGTGGCGGAAGCTCATCCGCAACCCCAACACCTACTCCAG
CCTCATCGGCATCACCTGGGCCCTCGTCTCCTTCAGGTAAACCAAATCCTAACTGCCAAACTAGTTA
TATTCATCAAATTGGTGCATCTTTTTTCCTTTTTTTTTTGTTTCTGAAAAAATTCCATCCATAGTATGT
CTCACTTTTGCTCTTGCTGGTATTGCAGATGGCATGTTCAGATGCCCGCCATTATATCGCAGTCCATC
TCCATACTGTCGGACGCAGGACTTGGAATGGCAATGTTCAG

Appendix Figure 37 EgPINF3-9 oil palm (*Elaeis guineensis*) homolog of the *Zea mays* gene, exons 1-2, partial gene. Exon1 1-500, intron1 501-628 and exon2 629-709.

AAAACATGCGATCCAGCTCCGGCCGCATGGCGGCCGCGGGAATTCGATTCTTGGTTTATTTCCTCGT
CCGTGGTCAGCAGAAAACAGTCTCTCAAATGGAATCCAATTTCAAGAAGTAATTAAAAAGTTCTCC
CTTCTTGGGCAGCTTGTAGCAAAGGCAATTAAAGATGGGAGAATCCTAGATATACCATTTTCTAAAG
CATTTTACAAAGTTATCCTCGAGCAGGTTTTCTCTTAAAACTATATCTAAACTATTTTACTCTCAATT
TTTTTTTTTTTTTGTTTTTTTTTGTTCAATCATACAGCCTGATAATAAAATTTTGCATTTATATTTCTTT
AGGAGCTTGGTATATATGATATCCAATCATTTGATCCCGAACTTGGAGGGACTCTGTGGGAGTTTCA
AGCTCTTGTCAATAGGAAAAGATTTTTAGAGTCCATTGCTAAGGAAAATTGCAAGTGTGTATCAGAT
TTGTATTACCGCAATGCTAGAATTGAGGATCTTTGTCTTGATTTCACTCTTCCAGGCTATCCTGAATC
ACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCATA
GCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGA
AATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGT
GCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACC
TGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGG

Appendix Figure 38 EgHECT Oil palm (*Elaeis guineensis*) homolog *Pinus sp.* HECT gene, exons 1-2, partial gene. Including exon1 1-177, intron1 178-291, and exon2 292-488

TAATGAGAAGAAAAGGGAGATGAACCTGACTCATGAGTTGAGACAGAGAAACTCATCCAGTTTGTA
CAGTCACTCGATTGCAATTGATGATCCAGATGTAATGGAGATTAAAGAAACCAAAGGTGTGAAGAT
ACTGAGGCCAGACTCCATGCTTGCTGCTGCAAGTAGTGCAAGTGTGCTCGAACCGGGAGCTGTTGCT
GCTATACGAATGCCGATGTTAAAGGTTTCCAATTTCAAAGGTGGGACACCAGAGATTGTTGAAGCA
AGCTATGCGATACTGGTTTTGGTCCTCCCTAGAGATGATTCGAGGGTTTGGAGCTCCCAGGAACTAG
AGATTGTTGAGGTAGTGGCGGATCAGGTTGCTGTTGCCCTCTCTCATGCAGCAGTTTTGGAGGAATC
GCAGATGATGAGAGACAAACTGATGGAGCAGAACAGGACTTTGCTGCATGCGAAGCAGAGTGCCA
TGATGGCAAGTGAAGCAAGGAACTCATTTCAAAGAGCCATGAGCCAGGGAATGAGGAGACCCATC
CACTCCATCTTGGGTATATTGTCGATGATGCAACAGGAAAAATTGAGCCAAGAACAAAGGCTTGTA
GTTGATACGATGGCAATAACTGGTAGTGTCATTTCAACATTGATTAATGATGTTATGGACACATCTA
CTATCGACAGTGAGCGCTTATCTTTGATTATGAGACCCTTCCAGCTGCATTCTATGATTAAGGAAGC
TGCTAGTGTTGCAAGATGTCTTTGTGATTGTAGAGGTTTTGGTTTTGAATTTCAGGTTGACAATGCAG
TGCCTGATCGGGTTGTT

Appendix Figure 39 EgETR (F2R3) oil palm (*Elaeis guineensis*) homolog *Oryza sativa* ETR gene

GAAGAATGTTCTTTGTGGATGCCATCAcGAACSGGTATAAATCTAGAACTTtCtCTTaCTTTAAaCaacCa
AAtaCAAGttgGATCYTCTGtgcCCATAAAcCtTcCtATAGTCAATgAaGTCTTTAGTAGTTCTCAAGCAATG
CATTTGCCCTATaACTGCCCATTGGCAAAGATGAGATCTCTAGTtgGGAGATATGTacCACCAGACATT
GTTGCTGTGCGGGTACCTCTCTTGCATCTTTCAAATTTTCAAATTAATGATTGGCCAGACTTCTCTGC
AAAAAGCTATGCAGTCATGGTTCTGATTCTCCCTGCAGACAGTGGTAGAAAATGGCAAGACCATGA
GTTGGAGCTTGTTGAAGTTGTTGCAGATCAGGTATTTTATGTTTTACTCTCCTCTTCTCTTCTTCAGTG
ACAGTATTTGGTACTAGCATATCATACAATTCTTTTGATATGCATAAGTAAGAAAAATTGTTTGTAA
AATACTTGCACAGAATAATAATTTAGAACTCATCTTTTTAACAGAAGAATGAGATGCCTCACATTTT
TCACTAGGTTTGTCTTACTTTTGTCCTAAATCCTAAATCCTATCCTAGAGTGATTAAAGAGTAGTTAG
TATATTTGTGCTGAAATACAATAATGTGGCCAGCAAGCATGATGCTTTATATTTTGCACAATAATAT
AATAATAAAATATTTTCCTTGCTTTGATGCCTGATGCCTGATGCCTGGTATTCCCTTGTAAAATGCAG
AACTTAGAGAACTGTTCTTGGACTTATTCTCCAATGCTCAAATTATTCTTTAATACTTGTGCAGGTT
GCCGTTGCTCTTTCACATGCTGCTATTCTTGAAGAGTCTATGCGGGCCCGTGATCAGCTCATGGAGC
ATAATGTTGCTTTAGATTTGGCTCGTCGAGAGGCAGAGATGGCAATCCATGCTCGAAATGATTTCCG
TGCTGTTATGAACCACGAAATGAGGACGCCAAT

Appendix Figure 40 Eg ETRF2R2 (1) oil palm homolog *Citrus sinensis* ERS gene, exons 1-2, partial gene. Including exon1 1-378, intron1 379-818, and exon2 819-988

GAGCTTGATTACTGGCCCCCACAGGACAGGGATGTGAACCTGAGGAGCCTCTTCCTTCCAGCAGCC
GGCCTGCTACAGGGGTGCATCACCCTGCGCAAGTTGTTCATCCATGGTACCGCCAACGAACACTTCA
TGAGGTTTTTCCTGGCGATGCCCAACCTTAGGGACGTGCAGCTGCGAGAAGACTACTATCCAGCGCC
GGAGTATGATATGGTACAGAGATGAGAGT

Appendix Figure 41 EgMAX2 oil palm (*Elaeis guineensis*) homolog *Picea sitchensis* MAX2 gene

ACCGATAACGCCAACACAGGGGTCGTCAAGCTCGGCGACGGGCGGGTCGTCTGCCTCACCGAGGCC
ATCAAGGGCTCCATCCAGATCGATCCCCACACGCTCGAGACCATCGGGAAGTTCGAGTACACCGAC
GACTTGGGAGGTTTGATCCACTCGGCGCATCCCATCGTGACCGAGACGGAGTTCTTGACGCTGCTGC
CCGACCTCGTGAGGCCTGGGTACACGGTGGCGAGGATGGAGCCGGGGACCAACGAGAGGAAGGTC
ATCGGCAGGGTGAACTGCCGGGGAGGCCCGGCGCCGGGTGGGTCCACTCGTTCGCGGT

Appendix Figure 42 EgMAX4 (F1R1) oil palm (*Elaeis guineensis*)

GAAGAATGTGCTTTGTGGATGCCATCACGAACTGGTATAAATCTAGAACTTTCTCTTACCTTAAATA
ACCAAATACAAGTTGGATCCTCTGTGSCCATAAACCTTCCTATAGTCAAAGAAGTGTTCAGTAGTTC
TCAAGCAATGCGTTTACCCTATAACTGCCCATTGGCGAAGATGAGATCTCTTGTTGGGAGATATGTA
CCACCTGACATTGTTGCTGTCCGGGTACCTCTCTTACATcTTTCAAATTTCGAAATTAATGATTGGCC
AGActTTTCTGCAAAAAGCTATGCAGTCATGGTTcTTATACTCCCTACGGaCAGTGGTAGAAAATGGC
GAGACCATGAGTTGGAGCTTGTGGAAGTTGTTGCAGATCAGGTATTTTATGTTTTACACTATTCTCTT
CTTCAGTTTCTATCTTTGATACTATCATATCATACAATTCCTTTGATACGTATGTGTAAGAAAAATCA
TTTATGAAGTTCTTGCACAGAATAATCACTTAGATTCATCCTTTTAACAGAAGAAATGAGATGCCTT
CACATTTTTCACCAGGTTTGTCTTACTTTTGTCCTAAATCCTAAATCCTAAATCCTTTCCTAAAATGA
TTAAAGAGTGGTTAGTAGTTTTGCACTTCACTATAATAATCTGACCAGCAAGCATGATAAATTATAT
GTTCCACGATAATATAATCATATAATACTTTCCTTGCTTTGATGCCTGGTATTCCCTTGTAAAATGCA
GAGCTCAGAGAACTGTTTCTTGTTCTTATTCTCCAATGTTCAAATTATTCTTTAATACTTGTGCAGGT
TGCTGTTGCTCTTTCACATGCTGCCATTCTCGAAGAGTCTATGCGGGCCCGTGATCAGCTCATGGAG
CAGAATGTTGCTTTAGAATTGGCTCGTCGAGAGGCAGAGAAGGCAATCTGTGCTCGCAATGATTTCC
GTGCCGTAATGAATCATGAAATGAGGACGCCAAT

Appendix Figure 43 Eg ETRF2R2 (2) oil palm homolog *Citrus sinensis* ERS gene, exons 1-2, partial gene. Including exon1 1-378, intron1 379-809, and exon2 810-979

GATTAGGGTGTTGGGGAAATTGCAAGGCCTTTGGGCACCACAGGCATAGGCGTATCTATACTTCTTG
CCCAGATAAGCCGGGTTTATGCTGCACATATCCATAATCACTAGT

Appendix Figure 44 EgMAX4 (F2R2) oil palm (*Elaeis guineensis*)

GAAGAATGTGCTTTGTGGATGCCATCACGAACTGGTATAAATCTTGAACTTTCTCTTACTCTAAATA
ACCAAATACAAGTTGGATCCTCTGTGCCCATAAACCTTCCTATAGTCAATGAAGTGTTCAGTAGTTC
TCAAGCAATGCGTTTACCCTATAACTGCCCATTGGCAAAGATGAGATCTCTTGTTGGGAGATATGTA
CCACCTGATATTGTTGCTGTCCGGGTACCTCTCTTACATCTTTCAAATTTCGAAATTAATGATTGGCC
AGACTTTTCTGCAAAAAGCTATGCAGTCATGGTTCTTATACTCCCTACAGACAGTGGTAGAAAATGG
CGAGACCATGAGTTAGAGCTTGTGGAAGTTGTTGCAGATCAGGTATTTTATGTTTTACACTATTCTCT
TCTTCAGTTTCTATCTTTGTTACTATCATATCATTCAATTCCTTTGATATGTATGTGTAAGAAAAGTC
ATTTATGAAGTTCTTGCACAGAATAATCACTTAGATTCATCCTTTTAACAGAAGAAATGAGATGCCT
TCACATTTTTCACCAGGTTTGTCTTACTTTTTTCCTAATCCTAAATCCTTTCCTAAAATGATTAAAGA
GTGGTTAGTAGATTTGCACTTCATTATAATAATCTGAGCAGCAAGCATGATAAATTATATTTTCCAC
GATAATATAATAATATAATACTTTCCTTGCTTTGATGCCTGGTATTCCCTTGTAAAATGCAGAGCTCA
GAGAACTGTTTCTTGTTCTTATTCTCCAATGTTCAAATTATTCTTTAATACTTGTGCAGGTTGCTGTTG
CTCTTTCACATGCTGCCATTCTCGAAGAGTCTATGCGTGCCCGTGATCAGCTCATGGAGCAGAATGT
TGCTTTAGAATTGGCTCGTCGAGAGGCAGAGAAGGCAATCTGTGCTCGCAATGATTTCCGTGCCGTG
ATGAATCACGAAATGAGGACGCCAAT

Appendix Figure 45 Eg ETRF2R2 (3) Oil palm homolog *Citrus sinensis* ERS gene, exons 1-2, partial gene. Including exon1 1-378, intron1 379-801, and exon2 802-971