

FLOWERING MODIFICATION IN THE PALM FAMILY

SEQUENCE SUBMISSION

[0001] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is entitled 2577217SequenceListing.txt, created on 12 November 2012 and is 35 kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] The present invention relates to the field of modifying flowering time, fruiting and seed setting in members of the palm family. More specifically, the present invention relates to a method for control and optimization of *Palmae* flowering time, fruiting and seed production by manipulating one gene from oil palm. The present invention can be used in various palm breeding efforts.

[0003] The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the Bibliography.

[0004] Palms are probably one of the most easily recognizable plant families, despite the relatively large size of the group – around 2400 species, according to a recent checklist (Govaerts and Dransfield, 2005). Palms are characterized by generally highly branched inflorescences with a basal prophyll, a tendency towards monoecy and dioecy and the association of flowers within small groups (usually condensed cincinni) which are often characteristic of specific clades.

[0005] At a certain point in their life cycle, annual plants undergo a major developmental transition and switch from vegetative to reproductive development. As this process is rarely reversible ensuring that the timing of this transition is optimal for pollination and seed development is a major factor in reproductive success. Physiological and genetic analysis of flowering has shown that multiple environmental and endogenous inputs influence the timing of the switch from vegetative to reproductive development. The molecular identity of these different inputs is being dissected using molecular genetic approaches in two model plants *Arabidopsis* (Srikanth and Schmid 2011) and rice. Changes of length of light/dark period, temperature, light quality and light fluences will affect plant flowering process. There are multiple pathways that quantitatively regulate an overlapping set of common targets, the floral pathway integrators, whose activities convert the shoot apical meristem to a reproductive fate.

An emerging theme is that changing the predominance of these input pathways could account for much of the plasticity and diversity of flowering time control within and between plant species (Blackman et al. 2010; Pin et al. 2010).

[0006] Oil palms are cultivated in the regions where they grow well and where there are oil mills. The following environmental conditions are needed for oil palm trees to produce enough flowers and fruits. (1) The year round temperature has to be: between 25° and 28° C. If the temperature drops below 25° C, the oil palm produces fewer leaves and is more susceptible to diseases, both of which compromise yields. Higher temperatures enable the oil palm to develop more leaves and to produce many fruit clusters. (2) Abundant sunshine. High light fluences drive efficient photosynthesis provided growth of the oil palm is not limited by water and mineral salts. The leaves grow large, the fruit ripens well, and the latter accumulates more oil. (3) Plentiful rain. Leave growth depends on water availability and is constrained by drought. Palm tress which produce few new leaves develop fewer flowers and fewer fruit clusters leading to lower yield.

[0007] Little research has been done on the genetic mechanism regulating flowering time or controlling the female/male flower ratio in oil palm. To precisely control the time of flowering, plants have evolved mechanisms to integrate seasonally predictable environmental cues (such as changes in photoperiod and prolonged periods of cold temperatures) and developmental cues (such as maturity). To allow this diversity of environmental cues to influence when flowering occurs in Arabidopsis, multiple pathways converge on a small number of genes, the floral integrator genes, including the floral promoters *FLOWERING LOCUS T (FT)* and *TWIN SISTER OF FT (TSF)* (Imaizumi 2010). FT and TSF are members of a family of proteins that contain a plant specific phosphatidylethanolamine-binding protein (PEBP) domain. In Arabidopsis, the PEBP family is divided into three subfamilies, FT-like, *TERMINAL FLOWER1 (TFL1)*-like, and Mother of FT and TFL1 (MFT)-like. FT and TFL1 are thought to be molecular switches for vegetative growth to reproductive development and MFT is phylogenetically ancestral to them (Kobayashi and Weigel 2007). In other plants, e.g. tomato, the balance between the activity of the tomato FT homolog *SINGLE FLOWER TRUSS* and that of the TFL1 homolog *SELF-FRUNING* affects a variety of development processes, such as flowering response, reiterative growth, termination cycles, leaf maturation, and stem growth (Carmel-Goren et al. 2003; Lifschitz et al. 2006). So far, there is no report on any flowering time regulator in the palm family.

[0008] Thus, it is desired to be able to control the flowering time and male/female flower ratio of plants in palms and particularly in oil palm.

SUMMARY OF THE INVENTION

[0009] The present invention relates to the field of modifying flowering time, fruiting and seed setting in members of the palm family. More specifically, the present invention relates to a method for control and optimization of *Palmae* flowering time, fruiting and seed production by manipulating one gene from oil palm. The present invention can be used in various palm breeding efforts.

[0010] In a first aspect, the present invention provides an isolated nucleic acid encoding a *EgFT* protein comprising the amino acid sequence set forth in SEQ ID NO:2. In one embodiment, the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:1 or nucleotides 63-590 of SEQ ID NO:1. In another embodiment, the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:3. In a further embodiment, the nucleic acid encodes a variant *EgFT* protein. In one embodiment, the variant *EgFT* protein has at least 90% sequence identity with the *EgFT* protein while having the activity of the *EgFT* protein. In another embodiment, the variant *EgFT* protein has one or more amino acid changes in the amino acid sequence of the *EgFT* protein while having the activity of the *EgFT* protein. In one embodiment, the nucleic acid further comprises a plant operable promoter operably linked to the coding sequence.

[0011] In a second aspect, the present invention provides a construct or vector comprising an isolated nucleic acid as described herein. In one embodiment, the construct or vector is an expression construct or vector. In another embodiment, the construct or vector further comprises a selectable marker. In a further embodiment, the construct or vector comprises a Cre-lox recombination marker free system.

[0012] In a third aspect, the present invention provides a transgenic plant comprising a nucleic acid, construct or vector described herein. In one embodiment, the transgenic plant may be any plant species. In another embodiment, the transgenic plant may be a plant of the *Palmae* family. In a further embodiment, the transgenic plant may be an oil palm plant.

[0013] In a fourth aspect, the present invention provides a method of controlling, regulating or altering the flowering time in a plant using a nucleic acid, construct or vector described herein. In one embodiment, the transgenic plant may be any plant species. In another embodiment, the

transgenic plant may be a plant of the Palmae family. In a further embodiment, the transgenic plant may be an oil palm (*Elaeis guineensis*).

BRIEF DESCRIPTION OF THE FIGURES

[0014] Figures 1A and 1B show nucleotide sequence (SEQ ID NO:1) and encoded amino acid sequence (SEQ ID NO:2) of *FLOWERING LOCUS T (FT)* in oil palm and genomic organization of the *EgFT*. Boxes and lines represent exonic and intronic regions, respectively. Box and line individually indicate exon and intron regions. Numbers show the base pairs of nucleotides. Gray boxes indicate 3' or 5' untranslated regions associated with the expression of the gene product.

[0015] Figure 2 shows the identification of Euphorbiancious PEBP homologs. Amino acid sequence alignment of PEBP family members. Red stars on the upper row indicate the Tyr85(Y)/His88(H) and Gln140(Q)/Asp144(D) residues distinguishing all FT-like and TFL1/CEN-like members. The amino acid sequences for the family members from top to bottom are SEQ ID NO:4 to SEQ ID NO:20, respectively.

[0016] Figure 3 shows the Phylogenetic analysis of PEBP family proteins in oil palm. A Neighbor Joining phylogenetic analysis of multiple members of the PEBP family from several plant species including the sugar beet homologs. Bootstrap values for 1000 re-samplings are shown on each branch.

[0017] Figure 4 shows CaMV 35 promoter driven vector for plant transformation.

[0018] Figure 5 shows early flowering phenotypes in *EgFT*-overexpressing transgenic *Arabidopsis* plants.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention relates to the field of modifying flowering time, fruiting and seed setting in members of the palm family. More specifically, the present invention relates to a method for control and optimization of Palmae flowering time, fruiting and seed production by manipulating one gene from oil palm. The present invention can be used in various palm breeding efforts.

[0020] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention belongs.

[0021] As used herein, “allele” refers to any of one or more alternative forms of a gene locus, all of which alleles relate to a trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

[0022] As used herein, “EgFT” refers to the *Elaeis guineensis Flowering locus T* gene or its encoded protein. EgFT protein may also be referred to as *Elaeis guineensis* FT protein”.

[0023] As used herein, “gene” refers to a nucleic acid sequence that encompasses a 5' promoter region associated with the expression of the gene product, any intron and exon regions and 3' or 5' untranslated regions associated with the expression of the gene product.

[0024] As used herein, “genotype” refers to the genetic constitution of a cell or organism.

[0025] As used herein, “phenotype” refers to the detectable characteristics of a cell or organism, which characteristics are the manifestation of gene expression.

[0026] The terms “polynucleotide,” “nucleic acid” and “nucleic acid molecule” are used interchangeably herein to refer to a polymer of nucleotides which may be a natural or synthetic linear and sequential array of nucleotides and/or nucleosides, including deoxyribonucleic acid, ribonucleic acid, and derivatives thereof. It includes chromosomal DNA, self-replicating plasmids, infectious polymers of DNA or RNA and DNA or RNA that performs a primarily structural role. Unless otherwise indicated, nucleic acids or polynucleotide are written left to right in 5' to 3' orientation. Nucleotides are referred to by their commonly accepted single-letter codes. Numeric ranges are inclusive of the numbers defining the range.

[0027] The terms “polypeptide,” “peptide,” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Amino acids may be referred to by their commonly known three-letter or one-letter symbols. Amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range.

[0028] No flowering time genes have yet been isolated from industrial plants, e.g. key industrial plants from the Arecaceae or Palmae (<http://en.wikipedia.org/wiki/Arecaceae>), including oil palm, date palm, coconut and other plants. Furthermore, the difficulties of transforming palm plants hinder their manipulation by genetic modification and improvements. Therefore, there has been no successful report on the control of flowering time of industrial plants using genes that promote flowering. It should be noted that given their long life cycle it is very time consuming to use traditional breeding methods to breed plants with optimal flowering

times and male/female flower ratio. Thus, it is an object of the present invention to control the flowering time and male/female flower ratio of plants with significant industrial importance; in particular, trees and shrubs in the palm family, such as oil palm, date palm, coconut and the like.

[0029] For model organisms such as *A. thaliana*, much work has been done to identify and characterize genes that flowering time. However, little has been done on similar genes in industrial trees. Moreover, the homology of gene sequences between the mustard family (Brassicaceae), of which *A. thaliana* is a member, and Arecaceae (or Palmae) is low because of the wide evolutionary distance between the two families. It is also not clear whether genes of limited sequence similarity may have similar biological function. Thus, the present invention relates to isolated genes that encode proteins with a phosphatidylethanolamine-binding domain and tested their effects on flowering time control in Arabidopsis and Palmae. Specifically, key flowering control genes from Palmae, such as FT, a member of a family of proteins that contain a phosphatidylethanolamine-binding protein (PEBP) domain, are the major subject of this invention. The present invention describes the process of cloning one gene controlling flowering time from oil palm and demonstrate its utility to accelerate flowering time in the model plant Arabidopsis.

[0030] Thus, in a first aspect, the present invention provides an isolated nucleic acid encoding a *EgFT* protein comprising the amino acid sequence set forth in SEQ ID NO:2. In one embodiment, the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:1 or nucleotides 63-590 of SEQ ID NO:1. In another embodiment, the nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:3. In a further embodiment, the nucleic acid encodes a variant *EgFT* protein. In one embodiment, the variant *EgFT* protein has at least 90% sequence identity with the *EgFT* protein while having the activity of the *EgFT* protein. In another embodiment, the variant *EgFT* protein has one or more amino acid changes in the amino acid sequence of the *EgFT* protein while having the activity of the *EgFT* protein. In one embodiment, the nucleic acid further comprises a plant operable promoter operably linked to the coding sequence.

[0031] In some embodiments, the polynucleotide may be one encoding a polypeptide of a variant of the amino acid sequences disclosed herein, which variant is an amino acid sequence disclosed herein having one or several amino acid residues substituted, deleted, inserted and/or added. The site at which one or several amino acid residues are substituted, deleted, inserted and/or added may be any site in the amino acid sequence, as long as the polypeptide with one or several amino acid residues substituted, deleted, inserted and/or added has the function of

regulating the flowering time of a plant. As used herein, the term "one or several amino acid residues" refers specifically to up to 10 amino acid residues in number, preferably to up to 6 amino acid residues, more preferably to up to 2 amino acid residues and even more preferably to one amino acid residue.

[0032] When the amino acids are altered, for example, by substitution, it is preferable to be conservatively substituted. This means that a particular amino acid residue is substituted with a different amino acid in which the properties of the amino acid side-chain are conserved. Non-limited examples of such the conservative substitution include substitution between hydrophobic amino acids such as alanine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine, substitution between hydrophilic amino acids such as arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine and threonine, substitution between amino acids having an aliphatic side chain such as glycine, alanine, valine, leucine, isoleucine and proline, substitution between amino acids having a hydroxy-containing side chain such as serine, threonine and tyrosine, substitution between amino acids having a sulfur atom-containing side chain such as cysteine and methionine, substitution between amino acids having a carboxylic acid- and amide-containing side chain such as aspartic acid, asparagine, glutamic acid and glutamine, substitution between amino acids having a base-containing side chain such as arginine, lysine and histidine, and substitution between amino acids having an aromatic-containing side chain such as histidine, phenylalanine, tyrosine and tryptophan. The substitutions between amino acids having the same amino acid side-chain properties may retain the biological activity of the polypeptide.

[0033] In some embodiments, the polynucleotide may be a variant of a polynucleotide selected from the group consisting of the polynucleotides described herein, which variant has one to 30 nucleotides substituted, deleted, inserted and/or added. The site at which nucleotides are substituted, deleted, inserted and/or added may be any site, as long as the polynucleotide with substituted, deleted, inserted and/or added nucleotides has the function of regulating the flowering time of a plant.

[0034] Examples of methods for preparing a nucleic acid encoding a protein comprising altered amino acids are well known to those skilled in the art, including site-directed mutagenesis (Kramer and Fritz, 1987). Examples of specific methods for altering nucleotides also include methods using a commercially available kit (e.g. Transformer Site-Directed Mutagenesis Kit: Clontech; QuickChange Site Directed Mutagenesis Kit: Stratagene) and methods using polymerase chain reaction (PCR). These methods are well known to those

skilled in the art. The amino acid sequence of a protein may also be mutated in nature due to the mutation of a nucleotide sequence. A nucleic acid encoding proteins having the amino acid sequence of a natural *EgFT* protein wherein one or more amino acids are substituted, deleted, and/or added are also included in the polynucleotide of the present invention, so long as they encode a protein functionally equivalent to a natural *EgFT* protein. Also natural *EgFT* protein homologs in related *Palmae* plants which show high identities to the sequences of the *EgFT* protein are also included in the polynucleotide of the present invention, so long as they encode a protein functionally equivalent to a natural *EgFT* protein. Additionally, nucleotide sequence variants that do not give rise to amino acid sequence changes in the protein (degeneracy variants) are also included in the polynucleotide of the present invention.

[0035] In a second aspect, the present invention provides a construct or vector comprising an isolated nucleic acid as described herein. In one embodiment, the construct or vector is an expression construct or vector. In another embodiment, the construct or vector further comprises a selectable marker. In a further embodiment, the construct or vector comprises a Cre-lox recombination marker free system, such as well known in the art.

[0036] The construct typically includes regulatory regions operatively linked to the 5' side of the nucleic acid described herein (such as a nucleic acid encoding a *EgFT* protein) and/or to the 3' side of the nucleic acid. A cassette containing all of these elements is also referred to herein as an expression cassette. The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. The regulatory regions (i.e., promoters, transcriptional regulatory regions, and translational termination regions) and/or the polynucleotide encoding a signal anchor may be native/analogous to the host cell or to each other. The promoters and tissue-specific promoters are particularly useful for preparing constructions for the transformation of oil palm, as well as for the transformation of other oil crops. Alternatively, the regulatory regions and/or the polynucleotide encoding a signal anchor may be heterologous to the host cell or to each other. See, U.S. Patent No. 7,205,453 and U.S. Patent Application Publication Nos. 2006/0218670, 2006/0248616 and 20090100536, and the references cited therein. The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include those described in International Publication No. WO 2008/094127 and the references cited therein.

[0037] A number of promoters can be used in the practice of the invention. The promoters can be selected based on the desired outcome. That is, the nucleic acids can be combined with

constitutive, tissue-preferred, or other promoters for expression in the host cell of interest. Such constitutive promoters include, for example, the core promoter of the Rsyn7 (WO 99/48338 and U.S. Pat. No. 6,072,050); the core CaMV 35S promoter (Odell et al., 1985); rice actin (McElroy et al., 1990); ubiquitin (Christensen and Quail, 1989; Christensen et al., 1992); pEMU (Last et al., 1991); MAS (Velten et al., 1984); ALS promoter (U.S. Pat. No. 5,659,026), and the like. Other constitutive promoters include, for example, those disclosed in U.S. Pat. Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142.

[0038] Other promoters include inducible promoters, particularly from a pathogen-inducible promoter. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. Other promoters include those that are expressed locally at or near the site of pathogen infection. In further embodiments, the promoter may be a wound-inducible promoter. In other embodiments, chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. The promoter may be a chemical-inducible promoter, where application of the chemical induces gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression. In addition, tissue-preferred promoters can be utilized to target enhanced expression of a polynucleotide of interest within a particular plant tissue. Each of these promoters are described in U.S. Pat. Nos. 6,506,962, 6,575,814, 6,972,349 and 7,301,069 and in U.S. Patent Application Publication Nos. 2007/0061917 and 2007/0143880.

[0039] Generally, the expression cassette may additionally comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Usually, the plant selectable marker gene will encode antibiotic resistance, with suitable genes including at least one set of genes coding for resistance to the antibiotic spectinomycin, the streptomycin phosphotransferase (spt) gene coding for streptomycin resistance, the neomycin phosphotransferase (nptII) gene encoding kanamycin or geneticin resistance, the hygromycin phosphotransferase (hpt or aphiv) gene encoding resistance to hygromycin, acetolactate synthase (als) genes. Alternatively, the plant selectable marker gene will encode herbicide resistance such as resistance to the sulfonylurea-type herbicides, glufosinate, glyphosate, ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D), including genes coding for resistance to herbicides which act to inhibit the action of glutamine synthase such as phosphinothricin or basta (e.g., the bar gene). See generally, International Publication No. WO 02/36782, U.S. Patent No. 7,205,453 and U.S.

Patent Application Publication Nos. 2006/0218670, 2006/0248616, 2007/0143880 and 2009/0100536, and the references cited therein. See also, Jefferson et al. (1991); De Wet et al. (1987); Goff et al. (1990); Kain et al. (1995) and Chiu et al. (1996). This list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used. The selectable marker gene is also under control of a promoter operable in the plant species to be transformed. Such promoters include those described in International Publication No. WO 2008/094127 and the references cited therein.

[0040] Alternatively, the expression cassette may additionally comprise a Cre-lox recombination marker free system, such as described herein. Such a system is useful for producing selection marker free transgenic oil palm plants or other plants.

[0041] In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g. transitions and transversions may be involved.

[0042] In a third aspect, the present invention provides a transgenic plant comprising a nucleic acid, construct or vector described herein. In one embodiment, the transgenic plant may be any plant species. In another embodiment, the transgenic plant may be a plant of the Palmae family. In a further embodiment, the transgenic plant may be an oil palm (*Elaeis guineensis*).

[0043] Once a nucleic acid has been cloned into an expression vector, it may be introduced into a plant cell using conventional transformation procedures. The term "plant cell" is intended to encompass any cell derived from a plant including undifferentiated tissues such as callus and suspension cultures, as well as plant seeds, pollen or plant embryos. Plant tissues suitable for transformation include leaf tissues, root tissues, meristems, protoplasts, hypocotyls, cotyledons, scutellum, shoot apex, root, immature embryo, pollen, and anther. "Transformation" means the directed modification of the genome of a cell by the external application of recombinant DNA from another cell of different genotype, leading to its uptake and integration into the subject cell's genome. In this manner, genetically modified plants, plant cells, plant tissue, seed, and the like can be obtained.

[0044] DNA constructs containing the nucleic acids of the present invention can be used to transform any monocot or dicot plant and particularly oil palm plants or plants of the Palmae

family. The constructs may be introduced into the genome of the desired plant host by a variety of conventional techniques. Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. Transformation protocols may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation, as is well known to the skilled artisan. For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the DNA constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria. Thus, any method, which provides for effective transformation/transfection may be employed. See, for example, U.S. Patent Nos. 7,241,937, 7,273,966 and 7,291,765 and U.S. Patent Application Publication Nos. 2007/0231905 and 2008/0010704 and references cited therein. See also, International Published Application Nos. WO 2005/103271 and WO 2008/094127 and references cited therein. Techniques which have been used to transform oil palm include biolistic-mediated transformation and *Agrobacterium*-mediated transformation. See, for example, Masli et al. (2009); Omidvar et al. (2008); Parveez et al. (2008); Abdullah et al. (2005); Parveez et al. (2000); Chowdhury, et al. (1997); and U.S. Patent Application Publication Nos. 2009/0038032 and 2010/071608.

[0045] Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant which possesses the transformed genotype and thus the desired phenotype, e.g., a transgenic plant. A "transgenic plant" is a plant into which foreign DNA has been introduced. A "transgenic plant" encompasses all descendants, hybrids, and crosses thereof, whether reproduced sexually or asexually, and which continue to harbor the foreign DNA. Regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the desired nucleotide sequences. See for example, International Published Application No. WO 2008/094127 and references cited therein.

[0046] The foregoing methods for transformation are typically used for producing a transgenic variety in which the expression cassette is stably incorporated. After the expression

cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. In one embodiment, the transgenic variety could then be crossed, with another (non-transformed or transformed) variety, in order to produce a new transgenic variety. Alternatively, a genetic trait which has been engineered into a particular cotton line using the foregoing transformation techniques could be moved into another line using traditional backcrossing techniques that are well known in the plant breeding arts. For example, a backcrossing approach could be used to move an engineered trait from a public, non-elite variety into an elite variety, or from a variety containing a foreign gene in its genome into a variety or varieties which do not contain that gene. As used herein, "crossing" can refer to a simple X by Y cross, or the process of backcrossing, depending on the context. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

[0047] Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Transgenic seeds can, of course, be recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The cultivated transgenic plants will express the DNA of interest in a tissue-preferred or tissue-specific manner as described herein.

[0048] In a sixth aspect, the present invention provides a method of controlling, regulating or altering the flowering time in a plant using a nucleic acid, construct or vector described herein. In one embodiment, the transgenic plant may be any plant species. In another embodiment, the transgenic plant may be a plant of the Palmae family. In an additional embodiment, the transgenic plant may be an oil palm (*Elaeis guineensis*). In some embodiments, the method for controlling the flowering time of plants includes the steps of introducing the polynucleotide, recombinant vector or polypeptide of the present invention into a plant.

[0049] In one embodiment, an expression vector described herein is introduced into plant cells to obtain a transgenic plant, in which the flowering is promoted. Even a transgenic plant is not necessary to be obtained. Depending on the host plant, the nucleic acids of the present invention may be introduced into plant cells such that the nucleic acid encoding a flowering protein described herein can be expressed in the plant cells.

[0050] In another embodiment, a nucleotide sequence is integrated into the genomic DNA of a plant to enhance the expression of the endogenous flowering gene. Preferably, examples of such a nucleotide sequence include an expression control sequence. More specifically, examples of such an expression control sequence include promoter sequences and enhancer sequences.

Such an expression control sequence is operably integrated into the genomic DNA of a plant to enhance the expression of the endogenous flowering gene in the plant, thereby promoting flowering of the plant.

[0051] In a further embodiment, the protein encoded by the flowering gene is introduced into plant cells by injecting the protein into, for example, phloem sap. The protein encoded by the flowering gene migrates to shoot apices and axillary buds to promote flowering of the plant. The protein encoded by the flowering gene may be produced using a recombinant microorganism or extracted from a wild-type plant or transgenic plant. Alternatively, grafting may be performed using, as a rootstock, a plant (for example, a transgenic plant) accumulating the protein encoded by the flowering gene.

[0052] In another embodiment, in order to control the flowering time of oil palm, it is preferable that any of various inducible promoters, for example, a copper ion inducible promoter (e.g. see WO 08/111,661) is ligated to a polynucleotide of the present invention and then introduced into a plant of the order Palmae family, in particular, into *Elaeis guineensis*, and that the flowering time of the plant is controlled by exposing the transgenic plant to copper ions. According to this construction, the flowering time of plants can be controlled more effectively as compared with the construction in which, for example, a DNA fragment of the *A. thaliana* FT gene, which has an amino acid sequence homology of less than 90% with the EgFT protein, is ligated to the above promoter and introduced into a plant. Further, this construct may prevent emergence of undesirable phenotypes, such as morphological aberration.

[0053] The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are within the skill of the art. See, e.g., Maniatis *et al.*, 1982, *Molecular Cloning* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York); Sambrook *et al.*, 1989, *Molecular Cloning*, 2nd Ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York); Sambrook and Russell, 2001, *Molecular Cloning*, 3rd Ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York); Ausubel *et al.*, 1992), *Current Protocols in Molecular Biology* (John Wiley & Sons, including periodic updates); Glover, 1985, *DNA Cloning* (IRL Press, Oxford); Russell, 1984, *Molecular biology of plants: a laboratory course manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.); Anand, *Techniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); Guthrie and Fink, *Guide to Yeast Genetics and Molecular Biology* (Academic Press, New York, 1991); Harlow and Lane, 1988, *Antibodies*, (Cold Spring

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EXAMPLES

[0054] The present invention is described by reference to the following Examples, which is offered by way of illustration and is not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized.

EXAMPLE 1

Materials and Methods

[0055] *Transgenic plasmids construction and materials: Elaeis guineensis Flowering locus T gene* was firstly identified from a database of sequenced cDNA library prepared from *Elaeis guineensis* leaf. This cDNA fragment was PCR-amplified with forward primer 5'-ATAAGTCGACATGCCAAGAGAAAGGGATCCTTT-3' (SEQ ID NO:21) and reverse primer 5'-TTATTTCTAGATTAAAGGTTGCATCCTTCTCCCGCCGGA-3' (SEQ ID NO:2). The PCR fragment was inserted in the sense orientation into the *SalI/XbaI* sites of pCABMIA1300-3HA vector.

[0056] *RNA extraction, EgFT cloning and analysis:* 100 mg leaf tissues were ground to fine powder in liquid N₂ and extracted with plant RNA purification reagent (Invitrogen, CA USA). RNA concentration was measured by Nanodrop (Thermo, DE, USA). M-MLV reverse

transcriptase (Promega, WI, USA) was used for reverse transcription reactions and cDNAs production. The cDNAs were used to amplify *FT* coding region. Real-time PCR was performed with Power SYBR® Green PCR Master mix (Applied Biosystems, CA, USA) and run in ABI7900HT. All samples were run in triplicates and the data was analyzed with RQ manager at a pre-set Ct value (Applied Biosystems, CA, USA). Ct values included in the analyses were based on three biological replicates, with three technical replicates for each biological sample. Standard deviation was calculated based on the three biological replicates.

EXAMPLE 2

Identified *EgFT* Coding Region and Genomic Sequence

[0057] *Elaeis guineensis* Flowering locus *T* gene was firstly identified from a database of sequenced cDNA library prepared from *Elaeis guineensis* leaf (Fig. 1A). The genomic sequence was identified from the deep sequencing database of oil palm genomic sequencing (Dr. Yue Genghua, unpublished data). Box and line indicate exon and intron regions, respectively (Fig. 1B). Numbers showed the base pairs of nucleotides. Gray boxes indicate 3' or 5' untranslated regions associated with the expression of the gene product.

[0058] Putative *EgFT* protein show high similarity with rice Hd3a protein and Arabidopsis FT, which is known to be a flowering promoter (Fig. 2). The phylogenetic analysis also suggests the *EgFT* is clustered in a FT protein group (Fig. 3).

EXAMPLE 3

Transgenic Arabidopsis Overexpressing *EgFT* Showed Early Flowering Phenotypes

[0059] To assess the potential roles of putative *EgFT* genes in flowering time control, the putative *EgFT* gene (1300-3HA-*EgFT*, Fig. 4) was overexpressed in Arabidopsis using a cauliflower mosaic virus 35S promoter. The 35S:*EgFT* construct was introduced into the WT Arabidopsis Col-0 plants. Flowering dipping method was used to obtain transgenic Arabidopsis lines which overexpressed *EgFT* (1300-3HA-*EgFT*, Fig. 4) in WT Col-0 background. Early flowering phenotypes were easily observed in T3 plants either under long day or short day light condition compared with WT Col-0 control plants (Table 1, Table 2 and Fig. 5). Another obvious advantage of *EgFT* overexpression is that it can generate early flower but doesn't adversely affect other plants traits such as weak vegetative growth and small plants size, found

in *Jatropha curcas* FT overexpression in Arabidopsis and Jatropha (Dr. Nam-Hai Chua, personal communication).

TABLE 1
Flowering Traits for 35S:EgFT
Under Long Day Light Condition

Line	Bolting Time (days)	Total Leaf No. formed at Bolting	Time of Anthesis (days)	Rosette Leaves	Cauline Leaves
Col-0	16.00±2.00	8.88±0.83	21.05±1.60	9.63±0.74	1.63±0.74
35S:EgFT					
#1	7.75±1.04	6.38±0.74	13.75±1.75	8.00±1.07	1.75±0.71
#2	7.25±0.71	6.50±0.76	13.00±0.93	7.75±1.28	1.63±0.52
#3	8.25±1.91	6.75±0.89	14.38±1.92	8.00±1.20	1.50±0.53

TABLE 2
Flowering Traits for 35S:EgFT
Under Short Day Light Condition

Line	Bolting Time (days)	Total Leaf No. formed at Bolting	Time of Anthesis (days)	Rosette Leaves	Cauline Leaves
Col-0	42.50±8.90	23.50±8.60	52.50±8.47	25.00±7.33	5.63±0.74
35S:EgFT					
#1	13.50±4.07	7.38±0.74	24.63±3.25	12.00±2.83	2.75±1.03
#2	9.13±0.35	6.50±0.53	21.00±0.00	8.50±1.41	3.78±0.74
#3	12.75±2.71	7.25±1.04	24.88±1.89	10.63±1.50	1.88±0.99

[0060] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise

noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if the range 10-15 is disclosed, then 11, 12, 13, and 14 are also disclosed. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0061] It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. Embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a protein selected from the group consisting of
a *EgFT* protein having the amino acid sequence set forth in SEQ ID NO:2, and,
a protein having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2 and having the flower promoting activity of the *EgFT* protein.
2. The isolated nucleic acid of claim 1, wherein the protein having at least 90% identity is one in which one or more of the amino acids of SEQ ID NO:2 are substituted, deleted, added, and/or inserted.
3. The isolated nucleic acid of claim 1, wherein the nucleic acid has the nucleotide sequence set forth in SEQ ID NO:1 or the nucleotide sequence set forth in nucleotides 63-590 of SEQ ID NO:1.
4. The isolated nucleic acid of any one of claims 1-3 which further comprises a plant operable promoter operably linked to the nucleic acid.
5. An expression vector comprising the isolated nucleic acid of any one of claims 1-4.
6. A transgenic plant cell, plant or plant seed comprising the isolated nucleic acid of any one of claims 1-4 stably integrated into its genome.
7. The transgenic plant cell, plant or plant seed of claim 6, wherein the plant is a member of the Palmae family.
8. The transgenic plant cell, plant or plant seed of claim 5, wherein the plant is an oil palm plant (*Elaeis guineensis*).
9. A method for producing a transgenic plant which comprises introducing the isolated nucleic acid of any one of claims 1-4 or an expression vector comprising the isolated nucleic acid of any one of claims 1-4 into a plant, wherein the transgenic plant has the nucleic acid stably integrated in its genome.

10. A method for producing a transgenic plant which comprises transfecting the isolated nucleic acid of any one of claims 1-4 or an expression vector comprising the isolated nucleic acid of any one of claims 1-4 into a plant cell or plant cells and regenerating a transgenic plant from the transfected plant cell or transfected plant cells, wherein the transgenic plant has the nucleic acid stably integrated in its genome.
11. The method of claim 9 or 10, wherein the plant is a member of the Palmae family.
12. The method of claim 9 or 10, wherein the transgenic plant is an oil palm plant (*Elaeis guineensis*).
13. A method of promoting the flowering time of a plant which comprises introducing the nucleic acid of any one of claims 1-4 or an expression vector comprising the isolated nucleic acid of any one of claims 1-4 into a plant, wherein the isolated nucleic acid is expressed in the plant thereby promoting the flowering time of the plant.
14. A method of promoting the flowering time of a plant comprising transfecting the nucleic acid of any one of claims 1-4 or an expression vector comprising the isolated nucleic acid of any one of claims 1-4 into a plant cell or plant cells and growing a plant from the transfected plant cell or transfected plant cells, wherein the isolated nucleic acid is expressed in the plant thereby promoting the flowering time of the plant.
15. The method of claim 13 or 14, wherein the plant is a member of the Palmae family.
16. The method of claim 13 or 14, wherein the transgenic plant is an oil palm plant (*Elaeis guineensis*).
17. The method of claim 13 or 14, wherein the nucleic acid is overexpressed.
18. An isolated protein selected from the group consisting of
a EgFT protein having the amino acid sequence set forth in SEQ ID NO:2, and.

a protein having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2 and having the flower promoting activity of the *EgFT* protein.

19. An isolated nucleic acid encoding an antisense RNA complementary to the transcription product of the nucleic acid of any one of claims 1-3.