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- (54) **Title:** LNK TRANSGENIC PLANTS

(57) **Abstract:** The present invention provides isolated LNK polypeptides and nucleic acids encoding the same. Also provided are methods of introducing a nucleic acid encoding LNK polypeptides and/or LNK antagonists into a plant cell, plant part or plant, e.g., to increase tolerance to abiotic stress, to delay development and/or prolong the life span of a plant, and/or to increase yield from the plant. Also provided are transformed plants, plant tissues, plant cells and plant seed comprising the nucleic acids, expression cassettes and vectors of the invention.



LNK TRANSGENIC PLANTS

FIELD OF THE INVENTION

[0001] The present invention relates to the field of plant molecular biology, more particularly to methods of expressing nucleic acids in plants.

BACKGROUND OF THE INVENTION

[0002] Light signaling pathways and the circadian clock interact to help organisms synchronize physiological and developmental processes with periodic environmental cycles. In plants, the interaction between light signaling pathways and the circadian clock plays a critical role in the process of day-length measurement, which contributes to adjust the floral transition to the most appropriate season of the year. The ability to genetically manipulate flowering time in order to optimize the timing of developmental processes in crop plants is critical to maximize crop yield under different growing conditions and in different geographic regions.

SUMMARY OF THE INVENTION

[0003] The inventors have found that LINK1 and LINK2, hereinafter referred to as LNK1 and LNK2, represent a new family of light and clock regulated morning genes that control both the pace of circadian rhythms and the photoperiodic regulation of flowering time. The invention relates in part to manipulating the expression of members of a novel plant specific gene family that plays a key role regulating floral transition and biomass production. In some embodiments, the invention relates to nucleic acids, expression cassettes, vectors and transgenic plants containing nucleic acids corresponding to LINK, hereinafter referred to as LNK genes (night-light inducible and clock regulated genes) such as, the homologous *LNK1* and *LNK2* genes of *A. thaliana* and orthologs of these genes, as well as fragments and variants of these nucleic acid sequences and functional RNA corresponding to, or polypeptides encoded by, these nucleic acids. The invention also relates to the introduction and/or manipulation of these nucleic acids in plants

to regulate plant growth and development and the transgenic plants generated thereby.

[0004] Accordingly, in one embodiment, the invention provides an isolated LNK1 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:1; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 1 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (b), (c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the nucleic acids are transcribed to express a functional RNA. The invention also encompasses expression cassettes and vectors comprising nucleic acids of the invention.

[0005] In an additional embodiment, the invention provides an isolated LNK2 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:3; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof;

(c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (b), (c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the nucleic acids are transcribed to express a functional RNA. The invention also encompasses expression cassettes and vectors comprising nucleic acids of the invention.

[0006] In additional embodiments, the invention provides a vector or expression cassette comprising a LNK promoter sequence operably associated with a nucleotide sequence of interest. In some embodiments the LNK1 promoter sequence is a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:7; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:7 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:7 or the complementary strand thereof; (d) a nucleotide sequence comprising nucleotides 1 to 50, 50 to 100, 100 to 150, 150 to 200, 200 to 250; 250 to 300, 300 to 350, 350 to 400, 400 to 450, 450 to 500, 500 to 550; 550 to 600, 600 to 650, 650 to 700, 700 to 750, 750 to 800, 800 to 850; 850 to

900, 900 to 950, or 950 to 1000 the nucleotide sequence of SEQ ID NO: 7 or the complementary strand thereof; (e) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a) - (c) or (d); (f) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a) - (c) or (d) under stringent hybridization conditions. In some embodiments, the nucleotide sequence of interest is in sense orientation relative to the promoter. In alternative embodiments, the nucleotide sequence of interest is in antisense orientation relative to the promoter. In further embodiments, the nucleotide sequence of interest is transcribed to form a functional RNA. In particular embodiments, the functional RNA is a LNK1 RNA antagonist.

[0007] In other embodiments the invention provides a vector or expression cassette comprising a LNK2 promoter sequence operably associated with a nucleotide sequence of interest wherein the LNK2 promoter sequence is a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO: 12; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 12 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 12 or the complementary strand thereof; (d) a nucleotide sequence comprising nucleotides 1 to 50, 50 to 100, 100 to 150, 150 to 200, 200 to 250; 250 to 300, 300 to 350, 350 to 400, 400 to 450, 450 to 500, 500 to 550; 550 to 600, 600 to 650, 650 to 700, 700 to 750, 750 to 800, 800 to 850; 850 to 900, 900 to 950, or 950 to 1000 the nucleotide sequence of SEQ ID NO: 12 or the complementary strand thereof; (e) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a) - (c) or (d); (f) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a) - (c) or (d) under stringent hybridization conditions. In some embodiments, the nucleotide sequence of interest is in sense orientation relative to the promoter. In alternative embodiments, the nucleotide sequence of interest is in antisense orientation relative to the promoter. In further embodiments, nucleotide sequence of interest is transcribed to form a functional RNA. In particular embodiments, the functional RNA is a LNK RNA antagonist (*e.g.*, a LNK1 and/or LNK2 antagonist).

[0008] The invention additionally encompasses transgenic plants containing LNK nucleic acids of the invention. In one embodiment, the invention provides a transgenic plant stably transformed with an isolated nucleic acid comprising a LNK1 nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:1; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 1 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 1 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the nucleic acids are transcribed to express a functional RNA. The invention also encompasses expression cassettes and vectors comprising nucleic acids of the invention.

[0009] In one embodiment, the invention provides a transgenic plant stably transformed with an isolated nucleic acid encoding a LNK1 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid

sequence of SEQ ID NO:2; and (c) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2.

[0010] In additional embodiments, the invention provides a transgenic plant stably transformed with an isolated LNK2 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:3; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter.

[0011] In one embodiment, the invention provides a transgenic plant stably transformed with an isolated nucleic acid encoding a LNK2 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; and (c) an amino acid sequence comprising at least 50,

100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4.

[0012] The invention also encompasses a transgenic plant stably transformed with both an isolated LNK1 and LNK2 nucleic acid of the invention.

[0013] In additional embodiments, the invention provides a transgenic plant stably transformed with a vector or expression cassette comprising a LNK promoter sequence operably associated with a nucleotide sequence of interest. In some embodiments the LNK1 promoter sequence is a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:7; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:7 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:7 or the complementary strand thereof; (d) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a), (b) or (c); (d) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (b) or (c) under stringent hybridization conditions. In some embodiments, the nucleotide sequence of interest is in sense orientation relative to the promoter. In alternative embodiments, the nucleotide sequence of interest is in antisense orientation relative to the promoter.

[0014] In other embodiments the LNK2 promoter sequence is a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO: 12; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 12 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 12 or the complementary strand thereof; (d) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a), (b) or (c); (d) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (b) or (c) under stringent hybridization conditions. In some embodiments, the nucleotide sequence of interest is in sense orientation relative to the promoter.

In alternative embodiments, the nucleotide sequence of interest is in antisense orientation relative to the promoter.

[0015] In representative embodiments, the transgenic plants of the invention display altered growth and/or development compared to a wild-type control plant grown under the same conditions. In some embodiments, the transgenic plants display increased vegetative growth and/or biomass compared to wild-type control plants. In additional embodiments, the transgenic plants produce biomass for a longer period of time than wild-type control plants. In the case of seed plants, in some embodiments, the transgenic plants display delayed flowering compared to wild-type plants. In additional embodiments, the transgenic plants of the invention have larger seed yield than wild-type plants. In further embodiments, the transgenic plants of the invention have an increased tolerance to abiotic stress (*e.g.*, high intensity light, low intensity light, drought (dehydration), high or low temperature and/or salinity (*e.g.*, salt) compared to a wild-type plant grown under the same conditions.

[0016] In alternative embodiments, transgenic plants of the invention display decreased vegetative growth and/or biomass compared to wild-type plants. In the case of seed plants, in some embodiments, the transgenic plants display shortened (*i. e.*, accelerated) flowering time compared to wild-type plants.

[0017] In some embodiments, the invention provides a method of modulating a circadian response of a plant, the method comprising stably transforming a plant cell with a LNK nucleic acid. One embodiment provides a method of modulating a circadian response of a plant comprising, stably transforming a plant cell with a LNK1 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:1; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%,

96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In additional embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant.

[0018] In some embodiments, the invention provides a method of modulating a circadian response of a plant, comprising stably transforming a plant cell with for example, a nucleic acid encoding a LNK1 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; and (c) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In additional embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant.

[0019] In another embodiment, the invention provides a method of modulating a circadian response of a plant, comprising stably transforming a plant cell with a

LNK2 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:3; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In additional embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant.

[0020] In an additional embodiment, the invention provides a method of modulating a circadian response of a plant, comprising stably transforming a plant cell with for example, a nucleic acid encoding a LNK2 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; and (c) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino

acid residues of the amino acid sequence of SEQ ID NO:4. In further embodiments, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In additional embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant.

[0021] In an additional embodiment, the invention provides a method of modulating a circadian response of a plant, comprising stably transforming a plant cell with both a LNK1 and LNK2 nucleic acid of the invention.

[0022] In additional embodiments, the invention provides a method of modulating the flowering time of a plant comprising, stably transforming a plant cell with a LNK nucleic acid. In additional embodiments, the invention provides a method of modulating the flowering time of a plant comprising, stably transforming a plant cell with a LNK1 and/or LNK2 nucleic acid of the of the invention such as, a nucleic acid described above or elsewhere herein. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In some embodiments, the method produces a transgenic plant in which the flowering time of the plant is accelerated. In other embodiments, the method produces a transgenic plant in which the flowering time of the plant is delayed.

[0023] In additional embodiments, the invention provides a method of modulating the biomass of a plant, the method comprising stably transforming a plant cell with a LNK nucleic acid. In some embodiments, the invention provides a method of modulating the biomass of a plant, the method comprising stably transforming a plant cell with a LNK1 and/or LNK2 nucleic acid of the of the invention such as, a nucleic acid described above or elsewhere herein. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the

nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In some embodiments, the method produces a transgenic plant in which the biomass of the plant is increased. In other embodiments, the method produces a transgenic plant in which the biomass of the plant is decreased.

[0024] In some embodiments, the invention provides a method of increasing yield of a plant comprising, stably transforming a plant cell with a LNK1 and/or LNK2 nucleic acid of the invention such as, a nucleic acid described above or elsewhere herein. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In some embodiments, the method produces a transgenic plant in which the biomass of the plant is increased. In other embodiments, the method produces a transgenic plant in which the biomass of the plant is decreased.

[0025] In additional embodiments, the invention provides a method of increasing yield of a plant comprising, stably transforming a plant cell with a LNK nucleic acid. In particular embodiments, the invention provides a method of increasing yield of a plant comprising, stably transforming a plant cell with a LNK1 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO: 1; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 1 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 1 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (e) a nucleotide sequence encoding an amino acid

sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In additional embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant.

[0026] In other embodiments, the invention provides a method of increasing yield of a plant comprising, stably transforming a plant cell with a LNK2 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:3; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a

nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In additional embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant.

[0027] In additional embodiments, the invention provides a method of increasing tolerance of a plant to an abiotic stress, the method comprising: stably transforming a plant cell with a LNK nucleic acid. In some embodiments, the invention provides a method of increasing tolerance of a plant to an abiotic stress, the method comprising: stably transforming a plant cell with a LNK1 and/or LNK2 nucleic acid of the invention such as, a nucleic acid described above or elsewhere herein. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In particular embodiments, the abiotic stress is a member selected from: high intensity light, low intensity light, drought (dehydration), high temperature, low temperature and high salinity (salt). In some embodiments, the method of the invention is applied to increase tolerance of a plant to a severe abiotic stress. In some embodiments, the method of the invention is applied to increase tolerance of a plant to a mild abiotic stress.

[0028] In additional embodiments, the invention encompasses a method of increasing the yield and/or tolerance of a plant to an abiotic stress comprising: stably transforming a plant cell with a LNK nucleic acid. In some embodiments, the invention provides a method of increasing the yield and/or tolerance of a plant

to an abiotic stress comprising: stably transforming a plant cell with a LNK1 and/or LNK2 nucleic acid of the invention such as, a nucleic acid described above or elsewhere herein. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant.

[0029] The invention also provided are expression cassettes, vectors, cells, plants and plant parts comprising the isolated nucleic acids of the invention operably associated with a LNK nucleotide sequence.

[0030] In some embodiments, the invention encompasses a vector or expression cassette comprising an isolated LNK polynucleotide. In some embodiments, the invention encompasses a vector or expression cassette comprising a LNK1 and/or LNK2 polynucleotide sequence of the invention operably associated with a promoter sequence. In some embodiments the expression cassette contains a sequence that encodes a selectable marker. Cells, plants and plant parts transformed with these nucleic acids, vectors, and expression cassettes are also encompassed by the invention. In particular embodiments, the transformed (*i.e.*, transgenic) plants are monocots. In other embodiments, the transformed plants are dicots. In particular embodiments, the cells, plants and/or plant parts correspond to sunflower, wheat, maize, soybean, rice, alfalfa or *Arabidopsis*.

[0031] In further embodiments, the invention encompasses a vector or expression cassette comprising an isolated LNK promoter sequence. In some embodiments, the invention encompasses a vector or expression cassette comprising a LNK1 and/or LNK2 promoter sequence operably associated with a nucleotide sequence of interest. In some embodiments, the nucleotide sequence of interest encodes a protein. In further embodiments, the protein encoded by the polynucleotides sequence of interest is a LNK polypeptide, such as LNK1 or LNK2 or a biologically active fragment or variant thereof. In other embodiments, the nucleotide sequence of interest encodes a protein. In some embodiments the expression cassette contains a polynucleotide sequence that is transcribed to form a functional RNA. In particular embodiments, the functional RNA is an antagonist of LNK, such as, an antagonist of LNK1 or LNK2 expression. In some embodiments the expression cassette contains a sequence that encodes a selectable

marker. Cells, plants and plant parts transformed with these nucleic acids, vectors, and expression cassettes are also encompassed by the invention. In particular embodiments, the transformed (*i.e.*, transgenic) plants are monocots. In other embodiments, the transformed plants are dicots. In particular embodiments, the cells, plants and/or plant parts correspond to sunflower, wheat, maize, soybean, rice, alfalfa or *Arabidopsis*.

[0032] As an additional aspect, the invention also encompasses products harvested from the transgenic plants of the invention and processed products produced therefrom.

[0033] The invention also provides seed produced from the plants of the invention and seed comprising the isolated nucleic acids, vectors and expression cassettes of the invention.

[0034] In further embodiments, the invention provides a method of introducing a nucleic acid into a plant, plant part or plant cell, the method comprising transforming the plant, plant part or plant cell with an isolated nucleic acid, expression cassette or vector of the invention.

[0035] In additional embodiments, the invention encompasses a method of expressing a nucleotide sequence of interest in a plant, the method comprising: (a) stably transforming a plant cell with an expression cassette or vector of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence of interest in the plant. In some embodiments, the nucleotide sequence is a LNK protein coding sequence. In further embodiments, the nucleotide sequence of interest encodes LNK1, LNK2, or a biologically active fragment thereof. In other embodiments, the nucleotide sequence of interest is transcribed to form a functional RNA. In further embodiments, the functional RNA is a LNK1 and/or LNK2 antagonist.

[0036] These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] Figure 1. Describes the genomic approach used to characterize light and clock interactions in the control of gene expression, which resulted in the

identification of *LNK* genes. (A) Experimental design. Plants were grown under 12h light/ 12h dark cycles for 14 days and then exposed or not to a 1h light pulse in the middle of the night or subjective day on the 15th day. (B) Comparative genome-wide expression analysis of the effect of a light pulse given during subjective day-time (x-axis) vs night-time (y-axis). (C) Overlap between 87 genes that are rhythmically expressed under multiple conditions, and 65 genes that showed a stronger induction by light during night-time compared to subjective day-time. (D) Microarray data corresponding to the relative response of *LNK* genes to a 1h light treatment given in the middle of the night or subjective day. (E) Relative expression levels of *LNK1* and *LNK2* measured by qRT-PCR. The analysis was conducted in wild-type, *phyAphyB* and *crycry2* plants grown under 12h light/12h dark cycles and exposed or not to a 1h light pulse in the middle of the night ($n=3$). (nd= not detectable). Data represent average \pm SEM. (F) Circadian expression of *LNK1* and *LNK2* genes. Expression was determined by qRT-PCR during the 2nd and 3rd day under free running conditions. $n=4$. Data are average \pm SEM.

[0038] Figure 2. *LNK1* and *LNK2* control hypocotyl elongation, photoperiodic dependent flowering and circadian rhythms. (A) Hypocotyl length of wild-type (WT), *link1*, *link2* and *linklink2* mutant seedlings grown under continuous white light (LL) ($n=6$ replicates of 10 seedlings each). (B) *Unkllink2* control the floral transition in plants grown under long day (LD, 16h light/8h dark) conditions. (C and D) Flowering time measured as the number of rosette leaves at bolting in LD (C) and short day (D) conditions (SD, 8h light/ 16h dark). ANOVA followed by a Tukey's multiple comparison test was used to evaluate the statistical significance of differences observed between genotypes. Error bars indicate +SEM (** $P<0.001$, * $P<0.05$). (E and FD) Circadian rhythms of leaf movement in continuous light ($n=7$). Plants were grown under LD cycles and then transferred to constant light and temperature conditions. Error bars indicate + SEM. Open and lined boxes indicate light and subjective night period, respectively.

[0039] Figure 3. *LNK1*, a nuclear protein, positively regulates expression of circadian genes with an afternoon phase. (A) *LNKLYFP* detection by confocal microscopy in *N. benthamiana* leaves transiently expressing 35S:*LNK1*:YFP. Arrowheads indicate nuclei expressing YFP. Scale bar= 50 μ m. (B) Phase

enrichment of circadian-regulated genes whose expression was down- or up-regulated in *link1;link2* mutant compared to WT plants, according to RNA-seq data of plants grown under continuous light conditions. The phase over-representation analysis was conducted with Phaser (<http://phaser.mocklerlab.org/>), and was based on the phases of gene expression estimated from data obtained using WT plants grown under LD conditions. Dashed line corresponds to $p=0.01$. (C) Average normalized expression of 36 genes from the cluster with the largest number of genes whose expression was altered in *link1;link2* mutants compared to WT plants grown under LD conditions. Normalized expression of *FKF1* (D), *PRR5* (E) and *ELF4* (F), three genes present in the cluster shown in (C). (C-F) Plants grown under LD cycles were sampled every four hours, starting 2h after lights on. n=3, Error bars indicate + SEM.

[0040] Figure 4. *LNK1* and *LNK2* are necessary for the proper function of the circadian clock. *CCA1* (A), *LHY* (B), *PRR9* (C), *PRR7* (D), *PRR5* (E) and *TOC1* (F) mRNA expression measured by qRT-PCR in plants grown under 12h light/12h dark cycles and then transferred to continuous light. Values are expressed relative to *PP2A* and normalized to the maximum value of each gene. Data represent average + SEM (n=4). Open and lined boxes indicate subjective day and subjective night periods, respectively.

[0041] Figure 5. *LNK1* and *LNK2* are repressed by the *TOC1/PRR1* family of circadian clock components. (A-D) *LNK1* (A and C) and *LNK2* (B and D) expression measured by qRT-PCR in continuous light relative to *PP2A* (n=4), under 12h light/12h dark cycles and then transferred to continuous light. Error bars indicate + SEM. Open and lined boxes indicate subjective day and subjective night, respectively. (E) Model showing the proposed function of *LNK1* and *LNK2* in the circadian clock. Light regulates *LNK1* and *LNK2* expression in the morning, which then act to promote, directly or indirectly, the expression of a subset of afternoon genes, including the core clock gene *PRR5*. During the afternoon and early evening, *PRR9*, *PRR7*, *PRR5* and *TOC1* bind to the *LNK* promoters blocking their expression.

[0042] Figure 6. Sequence alignment of *LNK1* homologs in *Arabidopsis thaliana*. The length of the sequence aligned is shown, and degree of similarity between amino acids is highlighted (darker shading represents higher similarity).

[0043] Figure 7. Cladogram displaying *LNK1* homologs in a broad range of species from embryophyta group. *Arabidopsis thaliana* *LNK1* has three paralogs. Where multiple homologs were identified within a single species, the annotated gene model code is provided (if more than one transcript was identified, the one with higher score during the BLASTP was selected). Rc, *Ricinus communis*; Pp, *Physcomitrella patens*; Solyc, *Solanum lycopersicum*; Carubv, *Capsella rubella*; Os, *Oryza sativa*; Cs, *Cucumis sativus*; Medtr, *Medicago truncatula*; Sb, *Sorghum bicolor*. Percentage bootstrap values are presented for each node.

[0044] Figure 8. Temporal patterns of *LNK1* and *LNK2* expression under different conditions. (A) DD_DDHC, plants grown under temperature cycles (12h 22°C/12h 12°C) and continuous darkness, and then transferred to constant temperature conditions. Measurements correspond to the first 24h under constant temperature and dark conditions. (B) LL23_LDHH, plants grown under 12h light/12h dark cycles at constant temperature and then transferred to constant light and temperature conditions for three days. Measurements correspond to the 2nd and 3rd days under constant conditions. (C) COL_LDHH, plants grown and measured under 12h light/12h dark cycles at 22°C. (D) COL_SD, plants grown and measured under 8h light/16h dark cycles at 22°C. Data was obtained from Mockler, T. C, T. P. Michael, *et al.*, (2007). The Diurnal Project: Diurnal and Circadian Expression Profiling, Model-based Pattern Matching, and Promoter Analysis. Cold Spring Harbor Symposia on Quantitative Biology 72:353-363.

[0045] Figure 9. *LNK1* and *LNK2* expression in different mutant backgrounds. (A) Scheme of *LNK1* and *LNK2* showing the site of T-DNA insertions in the different mutant alleles. (B) All mutant alleles have null expression of the full length mRNA, evaluated using primers flanking the T-DNA insertion. Expression upstream the T-DNA insertion was detectable in some alleles (data not shown). Plants were grown in soil for 3 weeks in continuous light conditions. Samples harvested were processed until cDNA synthesis. Transcript presence was determined by PCR.

- [0046] Figure 10. Hypocotyl length phenotypes in different *linkl* and *Unk2* mutant lines. Plants were grown for 4 days under different light conditions. (A) DD: Continuous darkness. (B) LD: Long Day (C) LL: Continuous light. (D) SD: Short Day. (E) Be: Continuous Blue light. (F) Rc: Continuous Red light. Hypocotyl length under different light conditions is expressed relative to the hypocotyl length of each genotype under continuous darkness. ANOVA followed by a Tukey's multiple comparison test was used to evaluate statistical significance between genotypes. Error bars indicate +SEM (" >0.001 ", $**P<0.01$, $>>0.05$).
- [0047] Figure 11. Circadian rhythms of leaf movement in additional mutant alleles of *LNK1* and *LNK2*. (A and B) Circadian rhythm of leaf movement in continuous light (n=6). Plants were grown in 16h light/8h dark cycles. Error bars indicate +SEM. Open and lined boxes indicate light and subjective night period, respectively.
- [0048] Figure 12. Set of clusters obtained by high throughput sequencing. (A-I) Clusters of genes with similar expression patterns detected using a correlation based distance metric and a hierarchical clustering procedure followed by a hybrid adaptive dendrogram cut step. Data sets represent the average of normalized expression level for all genes within each cluster. Number of genes in each cluster is indicated between parentheses. Plants were grown and harvested in 16h light/8h dark cycles. Clusters obtained for genes down-regulated (A-E) or up-regulated (G-I) in *linkl ;link2* mutant. (F) Cluster formed by genes with a significant alteration in the temporal pattern of expression but without significant differences in expression levels between WT and *linkl ;link2*.
- [0049] Figure 13. Expression levels of flowering time genes in WT and *linkl ;link2* mutant plants: (A) *FT*, (B) *SOC1*. Plants were grown and harvested under 16h light/8h dark cycles and data was obtained by high throughput RNA-sequencing. CPM: Counts per Million.
- [0050] Figure 14. Expression of *CCA1* in the *Unk2* mutant. *CCA1* expression levels are 4h delayed in *Hnk2* mutant plants. mRNA expression measured by qRT-PCR in plants grown under 12h light/12h dark cycles and transferred to LL conditions. Values are expressed relative to *PP2A* and normalized to the maximum

value of each gene. Data represent average + SEM (n=4). Open and lined boxes indicate light and subjective night period, respectively.

[0051] Figure 15. Phylogenetic tree for the AtLNK genes, homolog genes in rice (*Oryza sativa*) and orthologs in sorghum (*Sorghum bicolor*) and soy (*Glycine max*) showing the evolutionary relationships for these taxa. The evolutionary history was inferred using the Neighbor-Joining method. In the bootstrap consensus tree inferred from 1000 replicates, representing the evolutionary history of the taxa analyzed, those branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.05). The analysis involved 23 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1028 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

[0052] Figure 16. Comparison of LNK gene regulation and function in rice and *Arabidopsis*. Expression of OsUBQ, OsLNK1, OsLNK2 and OsLNK3 was measured by qRT-PCR.

[0053] Figure 17. Molecular function of OsLNK2 in the control of the circadian clock and in the regulation of flowering time in rice.

DETAILED DESCRIPTION OF THE INVENTION

[0054] The present invention is based, in part, on the discovery that LNK1 and/or LNK2 represent of new family of light and clock regulated morning genes that control both the pace of circadian rhythms and the photoperiodic regulation of flowering time. The present invention will now be described in more detail with reference to the accompanying examples and drawings in which certain embodiments of the invention are shown. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[0055] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination.

[0056] Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0057] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0058] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

I. Definitions.

[0059] As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0060] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0061] The term "about," as used herein when referring to a measurable value such as a dosage or time period and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

[0062] The terms "comprise," "comprises" and "comprising" as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

- [0063] As used herein, the transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. *See, In re Herz*, 537 F.2d 549, 551-52, 190 U.S.P.Q. 461,463 (CCPA 1976); see also MPEP §2111.03. Thus, the term "consisting essentially of" when used in a claim or the description of this invention is not intended to be interpreted to be equivalent to "comprising."
- [0064] As used herein, "LINK" or "LNK", refers to a family of night-light inducible and clock regulated genes (LNK genes) that play a key role in the control of daily and seasonal rhythms in plants. Unless otherwise specified, it is understood that the term "LNK" may refer to LNK family members in *A. thaliana* (such as LNK1 *e.g.*, AT5G64170, represented by SEQ ID NOs:1-2 and 5-6), LNK2 *{e.g.*, AT3G54500, represented by SEQ ID NOs:3, 4, and 8-11), LNK3 (*e.g.*, AT3G12320, represented by SEQ ID NOs:13 and 14), LNK4 (*e.g.*, AT5G06980, represented by SEQ ID NOs:15 and 16), but also members from other night-light inducible and clock regulated genes in other plant species, including fragments and/or variants of these members, that display one or more biological activities of a LNK family member as described herein.
- [0065] As used herein "LNK agonist" refers to a nucleic acid or polypeptide that, increases, enhances or up regulates one or more LNK biological activities (including phenotypes), such as, a LNK biological activity described herein. For example, a LNK agonist may lead to plants having one or more accelerated circadian responses in agonist treated plants than in WT plants, shorter hypocotyls than WT plants under continuous white light or red light, earlier flowering (*i.e.*, accelerated flowering) in agonist treated plants than WT plants under long days (LD; 16 hr light/8 hr dark), shorter circadian rhythm leaf movement in agonist treated plants than in WT plants, increased *FKF1*, *FT* and/or *SOC1* expression in agonist treated plants or plant cells compared to WT plants or plant cells, and increased *PRR5* expression in agonist treated plants compared to WT plants or plant cells.
- [0066] As used herein "LNK antagonist" refers to a nucleic acid or polypeptide that, reduces, inhibits or down regulates one or more LNK biological activities

(including phenotypes), such as, a LNK biological activity described herein. For example, a LNK antagonist may lead to plants having an one or more delayed circadian responses in antagonist treated plants than in WT plants, longer hypocotyls than WT plants under continuous white light or red light; later flowering in antagonist treated plants than WT plants under long days (LD; 16 hr light/8 hr dark); longer circadian rhythm leaf movement than in WT plants; reduced *FKFI FT* and/or *SOCI* expression in antagonist treated plants or plant cells compared to WT plants or plant cells, and reduced *PRR5* expression in antagonist treated plants compared to WT plants or plant cells.

[0067] A "circadian response" is a physiological and/or developmental plant process influenced by circadian rhythms and/or photoperiodism in plants. In some embodiments, the circadian response is selected from the group consisting of: gene transcription, leaf movement, photosynthetic ability, stomatal opening, hypocotyl elongation, and a photoperiodic response such as, the control of flowering.

[0068] The term "biomass" refers to the amount of a plant body or a part thereof. The term encompasses substances, foods, materials, fuels, resources and the like derived from these plants or plant parts. Specifically, increased biomass may refer to hypertrophy of a subterranean stem (rhizom, corm, tuber, bulb), a terrestrial stem, a flowering stem or a vine, hypertrophy of a seed, acceleration of elongation of stem length, plant length, culm length or ear length, or enlargement of a source plant organ such as a leaf.

[0069] As used herein, a "long-day" plant is a plant that only flowers after having received more than 12 hours of light.

[0070] As used herein, a "short-day" plant is a plant that only flowers after having received less than 12 hours of light.

[0071] An "abiotic stress" is a stressor from one or more outside, non-living factors that adversely affects the productivity and/or the survival of the organism. Abiotic stressors include, but are not limited to: high light intensity, low light intensity, ozone, heat or high temperature, cold temperature, drought (dehydration), flooding stress (*e.g.*, due to waterlogging and/or submergence), stress following the removal of a flooding stressor (*e.g.*, dehydration in the period following removal of a flooding stress such as desubmergence stress), salt stress

(*e.g.*, high or excessive salt conditions), high winds, fire, poor pH (too alkaline and/or too acidic), soil compaction and/or high radiation. Those skilled in the art will appreciate that an abiotic stress will depend on the preferred conditions for that organism, and may well vary due to the presence of other biotic and/or abiotic stressors.

[0072] Parameters for abiotic stress factors are species specific and even variety specific and therefore vary widely according to the species/variety exposed to the abiotic stress. Thus, while one species may be severely impacted by a salinity level of 4.0 dS m^{-1} , another species may not be affected until at least a salinity level of 6.0 dS m^{-1} or even 10.0 dS m^{-1} . *See*, for example, Blaylock, **A.D.** ("Soil salinity, salt tolerance, and growth potential of horticultural and landscape plants," Univ. Wyoming, Cooperative Extension Service, Bulletin B-988, February 1994) in which different plants are categorized as sensitive, moderately sensitive, moderately tolerant and tolerant depending on the level of soil salinity required to affect plant growth. Thus, for example, the level of salinity that is excessive or high for a sensitive plant species or variety is not the same level of salinity that is excessive or high for a moderately sensitive plant or a tolerant plant. The same is true for other types of abiotic stress such as drought (dehydration), waterlogging and submergence stress. Thus, a level of drought that can be tolerated by a sensitive plant species/variety is different from the level of drought that can be tolerated by a plant species/variety that is more drought tolerant. Likewise, the level of flooding (*e.g.*, at the roots and/or the aerial parts of the plant) that can be tolerated by a sensitive plant species/variety is different from that for a plant that is more tolerant to excessive water (*e.g.*, wet roots and/or submergence).

[0073] "Severe" abiotic stress results in death in control plants (*e.g.*, plants not expressing an LNK polypeptide of the invention), for example, at least about 10%, 20%, 30%, 40%, 50% or even more control plants die, whereas the plants of the invention (*e.g.*, expressing a LNK polypeptide of the invention) exhibit an increased survival.

[0074] "Mild" abiotic stress is defined herein as conditions in which control plants do not die but their production is very low (*e.g.*, reduced by at least about 30%, 40%, 50% or more), whereas the plants of the invention exhibit increased

production as compared with and/or are less severely affected, as compared with control plants.

[0075] "Normal" growth conditions are conditions in which the plants are not exposed to significant biotic, abiotic, toxicological or nutritional stress, *e.g.*, conditions in which the plants are well irrigated and exposed to normal salt conditions. In particular embodiments, "normal" growth conditions are conditions in which plants are exposed to no detectable (*e.g.*, measurable) biotic, abiotic, toxicological or nutritional stress.

[0076] Thus, an "increased tolerance to abiotic stress" (and similar terms) as used herein, refers to the ability of a plant or part thereof exposed to abiotic stress and transformed with an isolated or recombinant nucleic acid of the invention (*e.g.*, encoding an LNK polypeptide of the invention) to withstand a given abiotic stress better than a control plant or part thereof (*e.g.*, a plant or part thereof that has been exposed to the same abiotic stress but has not been transformed with an isolated or recombinant nucleic acid molecule of the invention). Increased tolerance to abiotic stress can be measured using a variety of parameters including, but not limited to, the size and/or number of plants or parts thereof (*e.g.*, leaf number and/or size), productivity or yield (*e.g.*, of seed), relative water content, electrolyte leakage, stomata conductance, photosynthetic rate, internal CO₂ concentration, transpiration rate, chlorophyll fluorescence. Thus, in some embodiments of the invention, a transformed plant or part thereof comprising an isolated or recombinant nucleic acid molecule of the invention, thereby having increased tolerance to the abiotic stress, would have, for example, greater growth (*e.g.*, plant height) and/or survival and/or yield as compared with a plant or part thereof exposed to the same stress, but not having been transformed with an isolated or recombinant nucleic acid molecule of the invention.

[0077] An "increased yield" (and similar terms) as used herein refers to an enhanced or elevated production of a commercially and/or agriculturally important plant, plant biomass, plant part (*e.g.*, roots, tubers, seed, leaves, fruit), plant material (*e.g.*, an extract) and/or other product produced by the plant (*e.g.*, a recombinant polypeptide) by a plant or part thereof transformed with a nucleic acid of the invention (*e.g.*, encoding an LNK polypeptide of the invention) as compared

with a control plant or part thereof (*e.g.*, a plant or part thereof that has been exposed to the same growth conditions, but has not been transformed with a nucleic acid molecule of the invention).

[0078] The term "modulate" (and grammatical variations) refers to an increase or decrease.

[0079] As used herein, the terms "increase," "increases," "increased," "increasing" and similar terms indicate an elevation of at least about 15%, 20%, 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400%, 500% or more.

[0080] As used herein, the terms "reduce," "reduces," "reduced," "reduction" and similar terms such as, "decrease," "decreases," "decreased" and "decreasing" mean a decrease of at least about 15%, 20%, 25%, 35%, 50%, 75%, 80%, 85%, 90%, 95%, 97% or more. In particular embodiments, the reduction results in no or essentially no (*i.e.*, an insignificant amount, *e.g.*, less than about 10% or even 5%) detectable activity or amount.

[0081] "Wild-type" as used herein, refers to a plant cell, seed, plant component, plant tissue, plant organ or whole plant that has not been genetically modified or treated in an experimental sense. Wild-type cells, seed, components, tissue, organs or whole plants may be used as controls to compare levels of expression and the extent and nature of trait modification with cells, tissue or plants of the same species in which a transcription factor expression is altered, *e.g.*, in that it has been knocked out, overexpressed, or ectopically expressed.

[0082] As used herein, the term "heterologous" means foreign, exogenous, non-native and/or non-naturally occurring.

[0083] As used here, "homologous" means native. For example, a homologous nucleotide sequence or amino acid sequence is a nucleotide sequence or amino acid sequence naturally associated with a host cell into which it is introduced, a homologous promoter sequence is the promoter sequence that is naturally associated with a coding sequence, and the like.

[0084] As used herein a "chimeric nucleic acid," "chimeric nucleotide sequence" or "chimeric polynucleotide" comprises a promoter operably linked to a nucleotide sequence of interest that is heterologous to the promoter (or *vice versa*). In particular embodiments, the "chimeric nucleic acid," "chimeric nucleotide

sequence" or "chimeric polynucleotide" comprises a *LNK* coding sequence operably associated with a heterologous promoter. In other embodiments, the "chimeric nucleic acid," "chimeric nucleotide sequence" or "chimeric polynucleotide" comprises a *LNK* nucleic acid sequence that is transcribed to form a functional RNA and that is operably associated with a heterologous promoter. In further embodiments, the *LNK* nucleic acid sequence is operably associated with a homologous *LNK* promoter sequence.

[0085] A "promoter" is a nucleotide sequence that controls or regulates the transcription of a nucleotide sequence (*e.g.*, a coding sequence or functional RNA) that is operatively associated with the promoter. The coding sequence may encode a polypeptide and/or a functional RNA. Typically, a "promoter" refers to a nucleotide sequence that contains a binding site for RNA polymerase II and directs the initiation of transcription. In general, promoters are found 5', or upstream, relative to the start of the coding region of the corresponding coding sequence or functional RNA. The promoter region may comprise other elements that act as regulators of gene expression. These include a TATA box consensus sequence, and often a CAAT box consensus sequence (Breathnach *et al.*, *Annu. Rev. Biochem.* 50:349 (1981)). In plants, the CAAT box may be substituted by the AGGA box (Messing *et al.*, (1983) in *Genetic Engineering of Plants*, T. Kosuge, C. Meredith and A. Hollaender (eds.), Plenum Press, pp. 211-227). The promoter region, including all the ancillary regulatory elements, typically contain between about 100 and 1000 nucleotides, but can be as long as 2 kb, 3 kb, 4 kb or longer in length. Promoters according to the present invention can function as constitutive and/or inducible regulatory elements.

[0086] As used herein, the *LNK* promoter sequence is the region of nucleic acid sequence upstream (5') of a *LNK* (*e.g.*, *LNK1* and *LNK2*) coding sequence that is responsible for spatial and temporal regulation of *LNK* transcription. As disclosed herein, *LNK1* and *LNK2* are induced by light and expressed rhythmically with peak expression in the morning or at noon. Transcription is circadian regulated, but with an RNA maximum that is "later" in the 24-hour period than that of other known circadian genes, such as *CCA1* and *LHY* (Wang and Tobin, 1998; Schaffer *et al.*, 1998). *LNK*-like circadian rhythm or cyclic transcriptional regulation refers

to this type of a relatively delayed transcription maximum. Because LNK transcription reaches a maximal level at a different time point in the 24-hour period, the LNK promoter will allow for altering the setting of the circadian clock. For instance, by operable linking a LNK1 or LNK2 promoter sequence with a coding sequence corresponding to another circadian-regulated gene, the circadian set on this heterologously expressed protein will be altered toward that of the respective LNK1 or LNK2.

[0087] Sequences as short as 50 or 100 nucleotides from within the 5' regulatory region of LNK may also be employed as promoter sequences according to the invention. The degree to which such a sequence provides for LNK-like circadian rhythm or cyclic transcriptional regulation, when included in an expression vector, can be determined using techniques known in the art. Thus, the term "biologically active LNK1 promoter" or a "biologically active LNK2 promoter" refers to a 5' regulatory region of the respective LNK gene, or a part or a variant of such a region, that, when operably linked to the 5' end of a coding sequence and introduced into a plant, results in LNK-like circadian rhythm or cyclic transcript expression of the protein encoded by the coding sequence (*i.e.*, in the morning or at noon).

[0088] A "functional" RNA includes any untranslated RNA that has a biological function in a cell, *e.g.*, regulation of gene expression. Such functional RNAs include, but are not limited to, siRNA, shRNA, miRNA, antisense RNA, ribozymes, and the like. In some embodiments, functional RNAs are operably linked to a promoter in antisense orientation relative to the promoter.

[0089] By "operably linked" or "operably associated" as used herein, it is meant that the indicated elements are functionally related to each other, and are also generally physically related. For example, a promoter is operatively linked or operably associated to a coding sequence (*e.g.*, nucleotide sequence of interest) if it controls the transcription of the sequence. Thus, the term "operatively linked" or "operably associated" as used herein, refers to nucleotide sequences on a single nucleic acid molecule that are functionally associated. Those skilled in the art will appreciate that the control sequences (*e.g.*, promoter) need not be contiguous with the coding sequence, as long as they function to direct the expression thereof.

Thus, for example, intervening untranslated, yet transcribed, sequences can be present between a promoter and a coding sequence, and the promoter sequence can still be considered "operably linked" to the coding sequence.

[0090] A "heterologous nucleotide sequence" or "heterologous nucleotide sequence of interest" as used herein is a coding sequence that is heterologous to the *LNK* nucleotide sequence (*i.e.*, is not the native *LNK* sequence). The heterologous nucleotide sequence can encode a polypeptide or a functional RNA. A "heterologous promoter" is a promoter that is heterologous to the nucleotide sequence with which it is operatively associated. For example, according to the present invention, the *LNK* coding sequence can be operatively associated with a heterologous promoter (*e.g.*, a promoter that is not the native *LNK* promoter sequence with which the *LNK* coding sequence is associated in its naturally occurring state).

[0091] By the term "express," "expressing" or "expression" (or other grammatical variants) of a nucleic acid coding sequence, it is meant that the sequence is transcribed. In particular embodiments, the terms "express," "expressing" or "expression" (or other grammatical variants) can refer to both transcription and translation to produce an encoded polypeptide.

[0092] "Wild-type" nucleotide sequence or amino acid sequence refers to a naturally occurring ("native") or endogenous nucleotide sequence (including a cDNA corresponding thereto) or amino acid sequence.

[0093] The terms "nucleic acid," "polynucleotide" and "nucleotide sequence" can be used interchangeably herein unless the context indicates otherwise. These terms encompass both RNA and DNA, including cDNA, genomic DNA, partially or completely synthetic (*e.g.*, chemically synthesized) RNA and DNA, and chimeras of RNA and DNA. The nucleic acid, polynucleotide or nucleotide sequence may be double-stranded or single-stranded, and further may be synthesized using nucleotide analogs or derivatives (*e.g.*, inosine or phosphorothioate nucleotides). Such nucleotides can be used, for example, to prepare nucleic acids, polynucleotides and nucleotide sequences that have altered base-pairing abilities or increased resistance to nucleases. The present invention further provides a nucleic acid, polynucleotide or nucleotide sequence that is the complement (which can be

either a full complement or a partial complement) of a nucleic acid, polynucleotide or nucleotide sequence of the invention (*e.g.*, encodes a nucleic acid, polynucleotide or nucleotide sequence comprising, consisting essentially of, or consisting the complement of a *LNK* coding sequence of the invention). Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code, both in accordance with 37 C.F.R. §1.822 and established usage.

[0094] The nucleic acids and polynucleotides of the invention are optionally isolated. An "isolated" nucleic acid molecule or polynucleotide is a nucleic acid molecule or polynucleotide that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule or isolated polynucleotide may exist in a purified form or may exist in a non-native environment such as, for example, a recombinant host cell. Thus, for example, the term "isolated" means that it is separated from the chromosome and/or cell in which it naturally occurs. A nucleic acid or polynucleotide is also isolated if it is separated from the chromosome and/or cell in which it naturally occurs and is then inserted into a genetic context, a chromosome, a chromosome location, and/or a cell in which it does not naturally occur. The recombinant nucleic acid molecules and polynucleotides of the invention can be considered to be "isolated."

[0095] Further, an "isolated" nucleic acid or polynucleotide is a nucleotide sequence (*e.g.*, DNA or RNA) that is not immediately contiguous with nucleotide sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The "isolated" nucleic acid or polynucleotide can exist in a cell (*e.g.*, a plant cell), optionally stably incorporated into the genome. According to this embodiment, the "isolated" nucleic acid or polynucleotide can be foreign to the cell/organism into which it is introduced, or it can be native to the cell/organism (*e.g.*, *A. thaliana*), but exist in a recombinant form (*e.g.*, as a chimeric nucleic acid

or polynucleotide) and/or can be an additional copy of an endogenous nucleic acid or polynucleotide. Thus, an "isolated nucleic acid molecule" or "isolated polynucleotide" can also include a nucleotide sequence derived from and inserted into the same natural, original cell type, but which is present in a non-natural state, *e.g.*, present in a different copy number, in a different genetic context and/or under the control of different regulatory sequences than that found in the native state of the nucleic acid molecule or polynucleotide.

[0096] In representative embodiments, the "isolated" nucleic acid or polynucleotide is substantially free of cellular material (including naturally associated proteins such as histones, transcription factors, and the like), viral material, and/or culture medium (when produced by recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Optionally, in representative embodiments, the isolated nucleic acid or polynucleotide is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more pure.

[0097] As used herein, the term "recombinant" nucleic acid, polynucleotide or nucleotide sequence refers to a nucleic acid, polynucleotide or nucleotide sequence that has been constructed, altered, rearranged and/or modified by genetic engineering techniques. The term "recombinant" does not refer to alterations that result from naturally occurring events, such as spontaneous mutations, or from non-spontaneous mutagenesis.

[0098] A "vector" is any nucleic acid molecule for the cloning of and/or transfer of a nucleic acid into a cell. A vector may be a replicon to which another nucleotide sequence may be attached to allow for replication of the attached nucleotide sequence. A "replicon" can be any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, viral genome) that functions as an autonomous unit of nucleic acid replication in the cell, *i.e.*, capable of nucleic acid replication under its own control. The term "vector" includes both viral and nonviral (*e.g.*, plasmid) nucleic acid molecules for introducing a nucleic acid into a cell *in vitro*, *ex vivo*, and/or *in vivo*, and is optionally an expression vector. A large number of vectors known in the art may be used to manipulate, deliver and express LNK polynucleotides of the invention. Vectors may be engineered to contain sequences encoding selectable

markers that provide for the selection of cells that contain the vector and/or have integrated some or all of the nucleic acid of the vector into the cellular genome. Such markers allow identification and/or selection of host cells that incorporate and express the proteins encoded by the marker. A "recombinant" vector refers to a viral or non-viral vector that comprises one or more heterologous nucleotide sequences (*e.g.*, transgenes), *e.g.*, two, three, four, five or more heterologous nucleotide sequences.

[0099] Viral vectors have been used in a wide variety of gene delivery applications in cells, as well as living animal subjects. Plant viral vectors that can be used include, but are not limited to, *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes* and geminivirus vectors. Non-viral vectors include, but are not limited to, plasmids, liposomes, electrically charged lipids (cytofectins), nucleic acid-protein complexes, and biopolymers. In addition to a nucleic acid of interest, a vector may also comprise one or more regulatory regions, and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (*e.g.*, delivery to specific tissues, duration of expression, *etc.*).

[00100] The term "fragment," as applied to a nucleic acid or polynucleotide, will be understood to mean a nucleotide sequence of reduced length relative to the reference or full-length nucleotide sequence and comprising, consisting essentially of and/or consisting of contiguous nucleotides from the reference or full-length nucleotide sequence. Such a fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of oligonucleotides having a length of at least about 8, 10, 12, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 405, 410, 425, 450, 455, 460, 475, 500, 505, 510, 515 or 520 nucleotides or more from the reference or full-length nucleotide sequence, as long as the fragment is shorter than the reference or full-length nucleotide sequence. In representative embodiments, the fragment is a biologically active nucleotide sequence, as that term is described herein.

[00101] A "biologically active" nucleotide sequence is one that substantially retains at least one biological activity normally associated with the wild-type nucleotide

sequence, for example, the ability to drive transcription of an operatively associated coding sequence. In particular embodiments, the "biologically active" nucleotide sequence substantially retains all of the biological activities possessed by the unmodified sequence. By "substantially retains" biological activity, it is meant that the nucleotide sequence retains at least about 50%, 60%, 75%, 85%, 90%, 95%, 97%, 98%, 99%, or more, of the biological activity of the native nucleotide sequence (and can even have a higher level of activity than the native nucleotide sequence). For example, a biologically active promoter element is able to control, regulate and/or enhance the expression of a nucleotide sequence operably associated with the promoter. Methods of measuring expression of a nucleotide sequence are well known in the art and include Northern blots, RNA run-on assays and methods of measuring the presence of an encoded polypeptide (*e.g.*, antibody based methods or visual inspection in the case of a reporter polypeptide).

[00102] Two nucleotide sequences are said to be "substantially identical" to each other when they share at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or even 100% sequence identity.

[0100] Two amino acid sequences are said to be "substantially identical" or "substantially similar" to each other when they share at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or even 100% sequence identity or similarity, respectively.

[0101] As used herein "sequence identity" refers to the extent to which two optimally aligned polynucleotide or polypeptide sequences are invariant throughout a window of alignment of components, *e.g.*, nucleotides or amino acids.

[0102] As used herein "sequence similarity" is similar to sequence identity (as described herein), but permits the substitution of conserved amino acids (*e.g.*, amino acids whose side chains have similar structural and/or biochemical properties), which are well-known in the art.

[0103] As is known in the art, a number of different programs can be used to identify whether a nucleic acid has sequence identity or an amino acid sequence has sequence identity or similarity to a known sequence. Sequence identity or

similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the sequence identity alignment algorithm of Needleman *et al*, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *PNAS* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux *et al*, *Nucl. Acid Res.* 12:387-395 (1984), preferably using the default settings, or by inspection.

[0104] An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng *et al*, *J. Mol. Evol.* 35:351-360 (1987); the method is similar to that described by Higgins *etal*, *CABIOS* 5:151-153 (1989).

[0105] Another example of a useful algorithm is the BLAST algorithm, described in Altschul *et al*, *J. Mol. Biol.* 215:403-410, (1990) and Karlin *et al*, *PNAS* 90:5873-5787 (1993). A particularly useful BLAST program is the WU- BLAST-2 program which was obtained from Altschul *et al*, *Methods in Enzymology*, 266:460-480 (1996); http://blast.wustl.edu/blastl_READMEhtml. WU-BLAST-2 uses several search parameters, which are preferably set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

[0106] An additional useful algorithm is gapped BLAST as reported by Altschul *et al*, *Nucleic Acids Res.* 25:3389-3402 (1997).

[0107] The CLUSTAL program can also be used to determine sequence similarity. This algorithm is described by Higgins *et al*, *Gene* 73:237 (1988); Higgins *et al*, *CABIOS* 5:151-153 (1989); Corpet *et al*, *Nucleic Acids Res.* 16:10881-90 (1988);

Huang *et al*, CABIOS 8:155-65 (1992); and Pearson *et al*, Meth. Mol. Biol. 24:307-331 (1994).

[0108] The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer nucleotides than the nucleic acids disclosed herein, it is understood that in one embodiment, the percentage of sequence identity will be determined based on the number of identical nucleotides acids in relation to the total number of nucleotide bases. Thus, for example, sequence identity of sequences shorter than a sequence specifically disclosed herein, will be determined using the number of nucleotide bases in the shorter sequence, in one embodiment. In percent identity calculations relative weight is not assigned to various manifestations of sequence variation, such as, insertions, deletions, substitutions, *etc.*

[0109] Two nucleotide sequences can also be considered to be substantially identical when the two sequences hybridize to each other under stringent conditions. A non-limiting example of "stringent" hybridization conditions include conditions represented by a wash stringency of 50% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C. "Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" Elsevier, New York (1993). In some representative embodiments, two nucleotide sequences considered to be substantially identical hybridize to each other under highly stringent conditions. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH.

[0110] In some embodiments, LNK nucleotides of the invention hybridize with the LNK nucleotide sequences of SEQ ID NO:1 and/or SEQ ID NO:3 or the complement thereof, under moderate stringency conditions. Exemplary moderate

stringency conditions include hybridization in 40-45% formamide, 1M NaCl, 1% SDS at 37°C, and a wash in 0.5 to 1XSSC at 55-50°C.

[0111] In additional embodiments, LNK nucleotides of the invention hybridize with the LNK nucleotide sequences of SEQ ID NO: 1 and/or SEQ ID NO: 3 or the complement thereof, under low stringency conditions. A non-limiting example of "low stringency" hybridization conditions includes hybridization with a buffer solution of 30 to 35% formamide, 1M NaCl, 1% SDS at 37°C, and a wash in IX. to 2XSSC at 50-55°C.

[0112] Homologs of the disclosed *Arabidopsis* LNK proteins (e.g., LNK1, 2, 3 and 4) typically possess at least 60% sequence identity counted over full length alignment with the amino acid sequence of *Arabidopsis* LNK proteins using the NCBI Blast 2.0, gapped blastp set to default parameters. For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequence will show increasing percentage identities when assessed by this method, such as at least 70%, at least 75%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94% or at least 95% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs will typically possess at least 75% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% or more depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are described at the NCBI web-site, frequently asked questions page. One of ordinary skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided. The present invention provides not only the peptide homologs that are described above, but also nucleic acid molecules that encode such homologs. LNK proteins homologs

will typically also have one or more LNK protein biological activities described herein.

[0113] As used herein, the term "polypeptide" encompasses both peptides and proteins (including fusion proteins), unless indicated otherwise.

[0114] A "fusion protein" is a polypeptide produced when two heterologous nucleotide sequences or fragments thereof coding for two (or more) different polypeptides not found fused together in nature are fused together in the correct translational reading frame.

[0115] The polypeptides of the invention are optionally "isolated." An "isolated" polypeptide is a polypeptide that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated polypeptide may exist in a purified form or may exist in a non-native environment such as, for example, a recombinant host cell. The recombinant polypeptides of the invention can be considered to be "isolated."

[0116] In representative embodiments, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide. In particular embodiments, the "isolated" polypeptide is at least about 1%, 5%, 10%, 25%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more pure (w/w). In other embodiments, an "isolated" polypeptide indicates that at least about a 5-fold, 10-fold, 25-fold, 100-fold, 1000-fold, 10,000-fold, or more enrichment of the protein (w/w) is achieved as compared with the starting material. In representative embodiments, the isolated polypeptide is a recombinant polypeptide produced using recombinant nucleic acid techniques. In embodiments of the invention, the polypeptide is a fusion protein.

[0117] The term "fragment," as applied to a polypeptide, will be understood to mean an amino acid sequence of reduced length relative to a reference polypeptide or the full-length polypeptide (*e.g.*, LNK) and comprising, consisting essentially of, and/or consisting of a sequence of contiguous amino acids from the reference or full-length polypeptide. Such a fragment according to the invention may be, where appropriate, included as part of a fusion protein of which it is a constituent.

In some embodiments, such fragments can comprise, consist essentially of, and/or consist of polypeptides having a length of at least about 50, 75, 100, 125, 150, 160, 165, 170, 171, 172, 173 or 174 amino acids from the reference or full-length polypeptide, as long as the fragment is shorter than the reference or full-length polypeptide. In representative embodiments, the fragment is biologically active, as that term is defined herein.

[0118] A "biologically active" polypeptide is one that substantially retains at least one biological activity normally associated with the wild-type polypeptide or alternatively, acts to reduce at least one biological activity normally associated with the wild-type LNK polypeptide. For example, the ability to regulate the plant circadian clock functions and/or photoperiodic responses, to regulate flowering time and/or biomass production, to increase tolerance to abiotic stress and/or increase yield. In another embodiment, a biologically active polypeptide is capable of modulating *PRR5* and/or *ELF4* expression in a transgenic plant cell or plant that recombinantly expresses the polypeptide. In additional embodiments, a biologically active polypeptide is capable of modulating the expression of *FKFI*, *SOCI* and/or *FTI* in a transgenic plant cell or plant that recombinantly expresses the polypeptide. In some embodiments, the biologically active polypeptide is a LNK agonist and leads to an increased expression of *PRR5*, *ELF4* and/or *FKFI* compared to a wild-type plant or plant cell. In alternative embodiments, the biologically active polypeptide is a LNK antagonist and leads to a decreased expression of *PRR5*, *ELF4* and/or *FKFI* compared to a wild-type plant or plant cell. In particular embodiments, the "biologically active" polypeptide substantially retains all of the biological activities possessed by the unmodified (wild-type) LNK sequence. By "substantially retains" biological activity, it is meant that the polypeptide retains at least about 50%, 60%, 75%, 85%, 90%, 95%, 97%, 98%, 99%, or more, of the biological activity of the native LNK polypeptide (and can even have a higher level of activity than the native polypeptide). Methods of measuring circadian rhythms, flowering time, biomass, yield, alterations in gene expression profile, and tolerance to abiotic stress are known in the art, with non-limiting and exemplary methods described in the Examples herein.

[0119] "Introducing" in the context of a plant cell, plant tissue, plant part and/or plant means contacting a nucleic acid molecule with the plant cell, plant tissue, plant part, and/or plant in such a manner that the nucleic acid molecule gains access to the interior of the plant cell or a cell of the plant tissue, plant part or plant. Where more than one nucleic acid molecule is to be introduced, these nucleic acid molecules can be assembled as part of a single polynucleotide or nucleic acid construct, or as separate polynucleotide or nucleic acid constructs, and can be located on the same or different nucleic acid constructs. Accordingly, these nucleic acid molecules can be introduced into plant cells in a single transformation event, in separate transformation events, or, *e.g.*, as part of a breeding protocol.

[0120] The term "transformation" as used herein refers to the introduction of a heterologous nucleic acid into a cell. Transformation of a cell may be stable or transient. Thus, a transgenic plant cell, plant tissue, plant part and/or plant of the invention can be stably transformed or transiently transformed. "Transient transformation" in the context of a polynucleotide means that a polynucleotide is introduced into the cell and does not integrate into the genome of the cell. As used herein, "stably introducing," "stably introduced," "stable transformation" or "stably transformed" (and similar terms) in the context of a polynucleotide introduced into a cell, means that the introduced polynucleotide is stably integrated into the genome of the cell (*e.g.*, into a chromosome or as a stable- extra-chromosomal element). As such, the integrated polynucleotide is capable of being inherited by progeny cells and plants. "Genome" as used herein includes the nuclear and/or plastid genome, and therefore includes integration of a polynucleotide into, for example, the chloroplast genome. Stable transformation as used herein can also refer to a polynucleotide that is maintained extrachromosomally, for example, as a minichromosome .

[0121] As used herein, the terms "transformed" and "transgenic" refer to any plant, plant cell, plant tissue (including callus), or plant part that contains all or part of at least one recombinant or isolated LNK nucleic acid, polynucleotide or nucleotide sequence of the invention. In representative embodiments, the recombinant or isolated nucleic acid, polynucleotide or nucleotide sequence is stably integrated into the genome of the plant (*e.g.*, into a chromosome or as a stable extra-

chromosomal element), so that it is passed on to subsequent generations of the cell or plant.

[0122] The term "plant part," as used herein, includes but is not limited to, reproductive tissues (*e.g.*, petals, sepals, stamens, pistils, receptacles, anthers, pollen, flowers, fruits, flower bud, ovules, seeds, embryos, nuts, kernels, ears, cobs and husks); vegetative tissues (*e.g.*, petioles, stems, roots, root hairs, root tips, pith, coleoptiles, stalks, shoots, branches, bark, apical meristem, axillary bud, cotyledon, hypocotyls, and leaves); vascular tissues (*e.g.*, phloem and xylem); specialized cells such as epidermal cells, parenchyma cells, collenchyma cells, sclerenchyma cells, stomates, guard cells, cuticle, mesophyll cells; callus tissue; and cuttings. The term "Plant part" also includes plant cells including plant cells that are intact in plants and/or parts of plants, plant protoplasts, plant tissues, plant organs plant cell tissue cultures, plant calli, plant clumps, and the like. As used herein, "shoot" refers to the above ground parts of a plant including the leaves and stems. The term "tissue culture" encompasses cultures of tissue, cells, protoplasts and callus.

[0123] As used herein, "plant cell" refers to a structural and physiological unit of the plant, which typically comprise a cell wall but also includes protoplasts. A plant cell of the present invention can be in the form of an isolated single cell, tissue culture cell or can be a part of a higher-organized unit such as, for example, a plant tissue (including callus) or a plant organ. Any plant (or groupings of plants, for example, into a genus or higher order classification) can be employed in practicing the present invention including angiosperms or gymnosperms, monocots or dicots. In some embodiments, the plant cell is from a long-day plant. In particular embodiments, the long-day plant is a member selected from the group consisting of *Arabidopsis thaliana*, wheat, barley, spinach and poplar. In some embodiments, the plant cell is from a short-day plant. In particular embodiments, the short-day plant is a member selected from: rice, maize, soybean, chrysanthemum, morning glory and cosmos.

[0124] Examples of transgenic plants and cells of the invention include, but are not limited to, plants and cells belonging to Brassicaceae (*e.g.*, *Arabidopsis thaliana*, cabbage, rapeseed), Gramineae (*e.g.*, rice, maize, barley, wheat, switchgrass, sugar cane, sorghum), Solanaceae (*e.g.*, tomato, eggplant, potato, tobacco), Leguminosae

(e.g., soybean, garden pea, bush bean), Convolvulaceae (e.g., sweet potato), Compositae (e.g., sunflower), Euphorbiaceae (e.g., cassava, Jatropha), Rosaceae (e.g., strawberry) and/or Salix (e.g., poplar), as well as, gymnosperms, ferns and mosses.

[0125] Exemplary transgenic plants and plant cells of the invention include, but are not limited to, corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa ssp.*), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rape (*Brassica napus*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annuus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tobacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Cofea spp.*), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus spp.*), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa spp.*), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), apple (*Malus pumila*), blackberry (*Rubus*), strawberry (*Fragaria*), walnut (*Juglans regia*), grape (*Vitis vinifera*), apricot (*Prunus armeniaca*), cherry (*Prunus*), peach (*Prunus persica*), plum (*Prunus domestica*), pear (*Pyrus communis*), watermelon (*Citrullus vulgaris*), duckweed (*Lemna*), oats, barley, vegetables, ornamentals, conifers, turfgrasses (e.g., for ornamental, recreational or forage purposes), and/or biomass grasses (e.g., switchgrass and miscanthus).

[0126] Turfgrass and cells which may be employed in practicing the present invention, include but are not limited to zoysiagrasses, bentgrasses, fescue grasses, bluegrasses, St. Augustinegrasses, bermudagrasses, bufallograsses, ryegrasses and/or orchardgrasses.

[0127] In particular embodiments, the transgenic plants of the invention are a member selected from wheat (*Triticum aestivum*), corn (*Zea mays*) and rice (*Oryza sativa*). In an additional embodiment, the transgenic plants are alfalfa or sunflower. In a particular embodiment, the transgenic plants are soybean (*Glycine max*).

[0128] Exemplary transgenic vegetables and cells of the invention include, but are not limited to, Solanaceous species (e.g., tomatoes; *Lycopersicon esculentum*), lettuce (e.g., *Lactuea sativa*), carrots (*Caucus carota*), cauliflower (*Brassica oleracea*), celery (*apium graveolens*), eggplant (*Solarium melongena*), asparagus (*Asparagus officinalis*), ochra (*Abelmoschus esculentus*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus spp.*), members of the genus *Cucurbita* such as Hubbard squash (*C. Hubbard*), Butternut squash (*C. moschata*), Zucchini (*C. pepo*), Crookneck squash (*C. crookneck*), *C. argyrosperma*, *C. argyrosperma ssp sororia*, *C. digitata*, *C. ecuadorensis*, *C. foetidissima*, *C. lundelliana*, and *C. martinezii*, and members of the genus *Cucumis* such as cucumber (*Cucumis sativus*), cantaloupe (*C. cantalupensis*) and/or musk melon (*C. melo*). Transgenic ornamentals and ornamental cells of the invention include, azalea (*Rhododendron spp.*), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa spp.*), tulips (*Tulipa spp.*), daffodils (*Narcissus spp.*), petunias (*Petunia hybrida*), carnation (*dianthus caryophyllus*), poinsettia (*Euphorbia pulcherima*), and/or chrysanthemum.

[0129] Transgenic conifers and conifer cells of the invention, include, for example pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and/or Alaska yellow-cedar (*Chamaecyparis nootkatensis*).

[0130] Also included as transgenic plants and cells of the invention are plants and cells that serve primarily as laboratory models, such as, *Arabidopsis*.

II. LNK nucleic acids promoters and functional RNA

Antisense and Co-Suppression

[0131] As demonstrated in the Examples, LNK genes such as, *LNK1* and *LNK2* mediate gene expression that leads to the inhibition of hypocotyl elongation and accelerated flowering. In some embodiments, the invention encompasses nucleic

acids that act as sense or anti-sense suppression of LNK (*e.g.*, LNK1 and/or LNK2) expression and that act as a LNK antagonist to for example, down-regulate the endogenous expression of a LNK polypeptide. Thus, in some embodiments, LNK (*e.g.*, LNK1 and/or LNK2) nucleic acids of the invention, including LNK sense or LNK anti-sense sequences, are used to interfere with the expression of endogenous homologous LNK nucleic acids in a transgenic plant or plant cell. A variety of sense and anti-sense technologies that may routinely be applied in preparing the compositions of the invention are known in the art. *See, e.g.*, Lichtenstein and Nellen (1997) *Antisense Technology: A Practical Approach* IRL Press at Oxford University Press, Oxford, U.K; Crowley *et al*, *Cell* 43:633-641 (1985); Rosenberg *et al*, *Nature* 313:703-706 (1985); Preiss *et al*, *Nature* 313:27-32 (1985); Melton *Proc. Natl. Acad. Sci.* 82:144-148 (1985); Izant and Weintraub *Science* 229:345-352 (1985); Kim and Wold, *Cell* 42:129-138 (1985); Smith *et al*, *Nature*, 334: 724-726 (1988); and Smith *et al*, *Plant Mol. Biol.* 14:369-379 (1990). (In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, *e.g.*, by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

[0132] A reduction or elimination of expression (*i.e.*, a "knock-down" or "knock-out") of a LNK protein or LNK homolog in a transgenic plant or plant cell, can be obtained by introducing a LNK antisense expression cassette or expression vector into a plant or plant cell as a cDNA. For antisense suppression, the regulatory protein or homolog cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full-length cDNA or gene, and need not be identical to the LNK message or gene found in the plant or plant cell type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target LNK gene or RNA. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous LNK coding sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably, the introduced antisense sequence in the vector/expression cassette will be at least 30 nucleotides in length, or at least 100 nucleotides. Transcription of an antisense construct as described results in the

production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous *LNK* gene in the plant cell.

[0133] Suppression of endogenous LNK expression can also be achieved using RNA interference (RNAi) or microRNA-based methods (Llave *et al*, *Science* 297: 2053-2056 (2002); Tang *et al*, *Genes Dev.* 17: 49-63 (2003)). RNAi is a post-transcriptional, targeted gene-silencing technique that uses double-stranded RNA (dsRNA) to incite degradation of messenger RNA (mRNA) containing the same sequence as the dsRNA (Constans, *The Scientist* 16:36 (2002)) Small interfering RNAs, or siRNAs are produced in at least two steps: an endogenous ribonuclease cleaves longer dsRNA into shorter, 21-23 nucleotide-long RNAs (Plasterk *Science* 296:1263-1265 (2002)). The siRNA segments then mediate the degradation of the target mRNA (Zamore, *Nature Struct. Biol.*, 8:746-50 (2001)). RNAi has been used for gene function determination in a manner similar to antisense oligonucleotides (Constans, *The Scientist* 16:36(2002)). Expression vectors and expression cassettes that express siRNAs in transiently and stably transfected cells have been engineered to express small hairpin RNAs (shRNAs), which get processed *in vivo* into siRNAs-like molecules capable of carrying out gene-specific silencing (Brummelkamp *et al.*, *Science* 296:550-553 (2002), and Paddison, *et al*, *Genes & Dev.* 16:948-958 (2002)). Post-transcriptional gene silencing by double-stranded RNA is discussed in further detail by Hammond *et al*, *Nature Rev Gen* 2: 110-119 (2001), Fire *et al*, *Nature* 391:806-811 (1998) and Timmons and Fire *Nature* 395:854 (1998). Vectors and expression cassettes in which RNA encoded by a transcription factor or transcription factor homolog cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Pat. No. 5,231,020. Such co-suppression (also termed sense suppression) does not require that the entire regulatory protein cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous *LNK* gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous regulatory protein gene is increased.

[0134] In some embodiments, the invention includes LNK antagonist nucleic acids, vectors and expression cassettes that express an untranslatable form of the LNK protein mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous LNK protein, thereby reducing or eliminating its activity and modulating one or more traits. Methods for producing such constructs are known in the art, *see, e.g.*, U.S. Pat. No. 5,583,021. In some embodiments, the constructs contain LNK nucleic acids comprising one or more premature stop codons introduced into LNK coding sequence. In other embodiments, the expression of an endogenous *LNK* gene is modified (*i.e.*, decreased) by gene silencing using double-stranded RNA (*see, e.g.*, Sharp Genes and Development 13:139-141 (1999)). Another method for abolishing or reducing the expression of a LNK gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in one or more *LNK* genes (*e.g.*, *LNK1*, *LNK2* and both *LNK1* and *LNK2*). Plants containing a single transgene insertion event at the desired gene can be crossed to generate homozygous plants for the mutation. Such methods are known in the art (see *e.g.*, Koncz *et al.*, (1992) Methods in Arabidopsis Research, World Scientific Publishing Co. Pte. Ltd., River Edge, N.J.).

LNK polypeptides and *LNK* coding sequences

[0135] In some embodiments, the invention provides LNK polypeptides. The term "LNK polypeptide" is intended to encompass the LNK polypeptides specifically described herein (*e.g.*, SEQ ID NO:2 and SEQ ID NO:4) as well as equivalents thereof, *e.g.*, that have substantially identical or similar amino acid sequences to the LNK polypeptides specifically described herein, and optionally biologically active equivalents that have one or more of the biological activities of the LNK polypeptides specifically described herein. The term "LNK polypeptide" also encompasses fragments of full-length LNK polypeptides (*e.g.*, SEQ ID NO:2 and SEQ ID NO:4), and optionally biologically active fragments, and biologically active equivalents thereof that have substantially identical or similar amino acid sequences to a fragment of a full-length LNK polypeptide. Further, the term "LNK

polypeptide" includes sequences from *A. thaliana* or can be a homolog from any other suitable plant species and also includes naturally occurring allelic variations, isoforms, splice variants and the like. The LNK polypeptide sequences can further be wholly or partially synthetic.

[0136] Biological activities associated with expression of LNK in a plant include, but are not limited to: modulating circadian rhythms, photoperiodic responses and plant phenotypes associated therewith; modulating the expression of key clock and clock output genes, such as *PRR5*, *ELF4* and/or *FKF1*; modulating flowering time and/or biomass production; modulating response to seasonal changes in photoperiod; and modulating tolerance to abiotic stress (*e.g.*, high light intensity or low light intensity). In some embodiments, the biologically active polypeptide is a LNK agonist and leads to an increased expression of *PRR5*, *ELF4* and/or *FKF1* compared to a wild-type plant or plant cell. In alternative embodiments, the biologically active polypeptide is a LNK antagonist and leads to a decreased expression of *PRR5*, *ELF4* and/or *FKF1* compared to a wild-type control plant or plant cell. In some embodiments, the biologically active polypeptide modulates circadian rhythms in a transgenic plant that recombinantly expresses the LNK polypeptide compared to a wild-type control plant. In additional embodiments, the biologically active polypeptide is a LNK agonist and leads to an accelerated flowering time and/or reduced biomass in a transgenic plant that recombinantly expresses the polypeptide compared to a wild-type control plant. In alternative embodiments, the biologically active polypeptide is a LNK antagonist and leads to a delayed flowering time and/or increased biomass in a transgenic plant that recombinantly expresses the polypeptide compared to a wild-type control plant.

[0137] In particular embodiments, the LNK polypeptide comprises, consists essentially of, or consists of an isolated LNK1 polypeptide of SEQ ID NO:2 or an equivalent thereof (including fragments and equivalents thereof).

[0138] In additional particular embodiments, the LNK polypeptide comprises, consists essentially of, or consists of an isolated LNK2 polypeptide of SEQ ID NO:4 or an equivalent thereof (including fragments and equivalents thereof).

[0139] Equivalents of the LNK polypeptides of the invention encompass those that have substantial amino acid sequence identity or similarity, for example, at least

about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more amino acid sequence identity or similarity with the amino acid sequences specifically disclosed herein (*e.g.*, SEQ ID NO:2 or SEQ ID NO:4) or a fragment thereof, optionally a biologically active fragment. Additional equivalents of the LNK polypeptides of the invention encompass those that have substantial amino acid sequence identity or similarity, for example, at least about 96%, 97%, 98%, 99% or more amino acid sequence identity or similarity with the amino acid sequence of SEQ ID NO: 10 or a fragment thereof, optionally a biologically active fragment.

[0140] Unless indicated otherwise, the LNK polypeptide can be a fusion protein. For example, it may be useful to express the LNK polypeptides as a fusion protein that can be recognized by a commercially available antibody (*e.g.*, FLAG motifs) or as a fusion protein that can otherwise be more easily purified (*e.g.*, by addition of a poly-His tail). Additionally, fusion proteins that enhance the stability of the protein may be produced, *e.g.*, fusion proteins comprising maltose binding protein (MBP) or glutathione-S-transferase. As another alternative, the fusion protein can comprise a reporter molecule. LNK is a transcription factor and LNK fusion proteins can also be generated for use in yeast two-hybrid systems (*e.g.*, GAL4-LNK fusions), as is known in the art.

[0141] It will further be understood that the LNK polypeptides specifically disclosed herein will typically tolerate substitutions in the amino acid sequence and substantially retain biological activity. To routinely identify biologically active LNK polypeptides of the invention other than those specifically disclosed herein, amino acid substitutions may be based on any characteristic known in the art, including the relative similarity or differences of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. In particular embodiments, conservative substitutions (*i.e.*, substitution with an amino acid residue having similar properties) are made in the amino acid sequence encoding the LNK polypeptide.

[0142] In making amino acid substitutions, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (*see*, Kyte *et al.*, J. Mol. Biol. 157:105 (1982)). It is accepted that the relative

hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

[0143] Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte *et al.*, *Id.*), and these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). It is understood that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in certain embodiments, the substitution of amino acids whose hydropathic indices are within +/- 2 is included. In certain embodiments, those which are within +/-1 are included, and in certain embodiments, those within +/- 0.5 are included.

[0144] It is also understood in the art that the substitution of amino acids can be made on the basis of hydrophilicity. U.S. Patent No. 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (± 3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in certain embodiments, the substitution of amino acids whose hydrophilicity values are within +/-2 is included, in certain embodiments, those which are within +/-1 are included, and in certain embodiments, those within +/- 0.5 are included.

[0145] The LNK polypeptides of the present invention also encompass LNK polypeptide fragments (optionally, biologically active LNK fragments), and equivalents thereof (optionally, biologically active equivalents). The length of the LNK fragment is not critical. Illustrative functional LNK protein fragments comprise at least about 50, 75, 100, 125, 150, 160, 165, 170, 171, 172, 173 or 174 amino acids of a LNK polypeptide.

[0146] In representative embodiments, the invention provides an isolated LNK polypeptide comprising, consisting essentially of, or consisting of a LNK1 amino acid sequence selected from the group consisting of: (a) the amino acid sequence of SEQ ID NO:2; (b) an amino acid sequence having at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more amino acid sequence identity or similarity with the amino acid sequence of SEQ ID NO:2, optionally wherein the LNK polypeptide is biologically active; and (c) a fragment of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 155, 160, 171, 172, 173 or 174 amino acid residues of the amino acid sequence of (a) or (b) above, optionally wherein the fragment is biologically active.

[0147] In representative embodiments, the invention provides an isolated LNK polypeptide comprising, consisting essentially of, or consisting of a LNK2 amino acid sequence selected from the group consisting of: (a) the amino acid sequence of SEQ ID NO:4; (b) an amino acid sequence having at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more amino acid sequence identity or similarity with the amino acid sequence of SEQ ID NO:4, optionally wherein the LNK polypeptide is biologically active; and (c) a fragment of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 155, 160, 171, 172, 173 or 174 amino acid residues of the amino acid sequence of (a) or (b) above, optionally wherein the fragment is biologically active.

[0148] The invention further provides antibodies and antibody fragments that specifically bind to the LNK polypeptides of the invention. Anti-LNK antibodies may be produced using standard procedures described in a number of texts, including Harlow and Lane (1988). The determination that a particular agent binds substantially only to a LNK protein (*e.g.*, LNK1 and/or LNK2) may readily be made by using or adapting techniques known in the art. One suitable *in vitro* assay

makes use of the Western blotting procedure (described in many standard texts, including Harlow and Lane (1988)). Western blotting may be used to determine that a given LNK protein binding agent, such as an anti-LNK1 or LNK2 monoclonal antibody, binds substantially only to LNK1 and/or LNK2. The term "antibody" or "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibody can be monoclonal or polyclonal and can be of any species of origin, including for example mouse, rat, rabbit, horse, goat, sheep or human, or can be a chimeric, humanized or human antibody. *See, e.g., Walker et al., Molec. Immunol. 26:403-411 (1989).* The antibodies can be recombinant monoclonal antibodies produced according to the methods disclosed in U.S. Patent No. 4,474,893 or 4,816,567. The antibodies can also be chemically constructed according to the method disclosed in U.S. Patent No. 4,676,980.

[0149] Antibody fragments included within the scope of the present invention include, for example, Fab, F(ab')₂, and Fc fragments, and the corresponding fragments obtained from antibodies other than IgG. Such fragments can be produced by known techniques. For example, F(ab')₂ fragments can be produced by pepsin digestion of the antibody molecule, and Fab fragments can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse *et al.*, *Science* 254:1275-1281 (1989)).

[0150] Monoclonal antibodies according to the present invention can be produced in a hybridoma cell line according to the technique of Kohler *et al.*, *Nature* 265:495-97 (1975). For example, a solution containing the appropriate antigen can be injected into a mouse and, after a sufficient time, the mouse sacrificed and spleen cells obtained. The spleen cells are then immortalized by fusing them with myeloma cells or with lymphoma cells, typically in the presence of polyethylene glycol, to produce hybridoma cells. The hybridoma cells are then grown in a suitable medium and the supernatant screened for monoclonal antibodies having the desired specificity. Monoclonal Fab fragments can be produced in *E. coli* by

recombinant techniques known to those skilled in the art. *See, e.g.*, Huse, Science 246:1275-1281 (1989).

[0151] Antibodies specific to a LNK polypeptide can also be obtained by phage display techniques known in the art.

[0152] The invention also provides nucleic acids encoding LNK polypeptides, optionally biologically active LNK polypeptides. The nucleic acid can be from any plant species of origin (*e.g.*, *A. thaliana*) or can be partially or completely synthetic. In representative embodiments, the nucleic acid encoding the LNK polypeptide is an isolated nucleic acid.

[0153] Two nucleotide or amino acid sequences are orthologs of each other if they share a common ancestral sequence and diverged when a species carrying that ancestral sequence split into two species. Orthologous sequences are also homologous sequences. LNK orthologs from other organisms, in particular other plants, can be routinely identified using methods known in the art (*e.g.*, orthologs from species belonging to the Asteraceae family [also known as the *Compositae* family], such as a species of lettuce). For example, PCR and other amplification techniques and hybridization techniques can be used to identify such orthologs based on their sequence similarity to the sequences set forth herein.

[0154] In representative embodiments, the invention encompasses polynucleotides encoding the LNK polypeptides of the invention having substantial nucleotide sequence identity with the polynucleotides specifically disclosed herein encoding LNK (*e.g.*, the LNK1 coding sequence in SEQ ID NO:1 (*i.e.*, nucleotides 302-2152), and the LNK2 coding sequence in SEQ ID NO:3 (*i.e.*, nucleotides 324-2333)), or fragments thereof, and which encode a LNK polypeptide (including fragments), optionally a biologically active LNK polypeptide. In some embodiments, the polynucleotides encoding the LNK polypeptides have substantial nucleotide sequence identity with the coding sequence in SEQ ID NO: 1 or the coding sequence in SEQ ID NO: 3, or fragments thereof, and which encode a LNK polypeptide (including fragments), optionally a biologically active LNK polypeptide.

[0155] The invention also provides polynucleotides encoding the LNK polypeptides of the invention, wherein the polynucleotide hybridizes to the

complete complement of the LNK nucleic acid sequences specifically disclosed herein (*e.g.*, SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO: 13 and SEQ ID NO: 15), or fragments thereof, under stringent hybridization conditions as known by those skilled in the art and encode a LNK polypeptide (including fragments), optionally a biologically active LNK polypeptide. In some embodiments, the polynucleotide hybridizes to nucleotides 300 to 500, 500 to 1000, 1000 to 1500, or 1500-2000 of SEQ ID NO:1 or SEQ ID NO:3, or fragments thereof, under stringent hybridization conditions and encode a LNK polypeptide (including fragments), optionally a biologically active LNK polypeptide. Further, it will be appreciated by those skilled in the art that there can be variability in the polynucleotides that encode the LNK polypeptides of the present invention due to the degeneracy of the genetic code. The degeneracy of the genetic code, which allows different nucleotide sequences to code for the same protein, is well known in the art. Moreover, plant or species-preferred codons can be used in the polynucleotides encoding the LNK polypeptides of the invention, as is also known in the art.

[0156] The invention also provides polynucleotides encoding fragments of a full-length LNK polypeptide, optionally biologically active fragments. Exemplary polynucleotides encoding LNK fragments comprise at least about 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 505, 510, 515 or 520 or more nucleotide bases of the coding polynucleotide sequence of SEQ ID NO:1 (*i.e.*, nucleotides 302-2152), or SEQ ID NO:3 (*i.e.*, nucleotides 324-2333).

[0157] In exemplary, but non-limiting, embodiments, the invention provides a nucleic acid (*e.g.*, recombinant or isolated) comprising, consisting essentially of, or consisting of a nucleotide sequence encoding a LNK polypeptide, the nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence comprising the coding nucleotide sequence of SEQ ID NO:1 (*i.e.*, nucleotides 302-2152), or SEQ ID NO:3 (*i.e.*, nucleotides 324-2333); (b) a nucleotide sequence comprising at least about 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 505, 510, 515 or 520 or more consecutive nucleotides of the coding nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3 (*e.g.*, encoding a fragment, optionally a functional fragment of SEQ

ID NO:3); (c) a nucleotide sequence having at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more sequence identity to the nucleotide sequence of (a) or (b); (d) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a) or (b) under stringent hybridization conditions; and (e) a nucleotide sequence that differs from the nucleotide sequence of any of (a) to (d) due to the degeneracy of the genetic code. In representative embodiments, the nucleotide sequence encodes a biologically active LNK polypeptide (including biologically active fragments of a full-length LNK polypeptide).

[0158] In representative embodiments, the nucleotide sequence encodes the polypeptide of SEQ ID NO:2, or an equivalent polypeptide having substantial amino acid sequence identity or similarity with SEQ ID NO:2 (optionally, a biologically active equivalent). In representative embodiments, the nucleotide sequence encodes an equivalent (optionally, a biologically active equivalent) of the polypeptide of SEQ ID NO:2 and hybridizes to the complete complement of coding sequence of SEQ ID NO: 1 under stringent hybridization conditions.

[0159] In additional representative embodiments, the nucleotide sequence encodes the polypeptide of SEQ ID NO:4, or an equivalent polypeptide having substantial amino acid sequence identity or similarity with SEQ ID NO:4 (optionally, a biologically active equivalent). In representative embodiments, the nucleotide sequence encodes an equivalent (optionally, a biologically active equivalent) of the polypeptide of SEQ ID NO:4 and hybridizes to the complete complement of coding sequence of SEQ ID NO: 3 under stringent hybridization conditions.

[0160] In further embodiments, the nucleotide sequence is a nucleic acid selected from the group consisting of: (a) a nucleic acid encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (b) a nucleic acid encoding a polypeptide having an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; (c) a nucleic acid encoding a polypeptide having an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2; (d) a nucleic acid encoding a polypeptide comprising the LNK2 amino

acid sequence of SEQ ID NO:4; (e) a nucleic acid encoding a polypeptide having an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; (f) a nucleic acid encoding a polypeptide comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4; (g) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (h) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (i) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (j) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (k) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)- (i) or (j); and (l) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(i) or (j) under stringent hybridization conditions.

[0161] Accordingly, in one embodiment, the invention provides an isolated LNK3 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO: 13; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 13 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 13 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK3 amino acid sequence of SEQ ID NO: 14; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 14; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO: 14; (g) a nucleotide sequence having at

least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (b), (c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the nucleic acids are transcribed to express a functional RNA. The invention also encompasses expression cassettes and vectors comprising LNK3 nucleic acids of the invention.

[0162] In an additional embodiment, the invention provides an isolated LNK4 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO: 15; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 15 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 15 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK4 amino acid sequence of SEQ ID NO: 16; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 16; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO: 16; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (b), (c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative

embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the nucleic acids are transcribed to express a functional RNA. The invention also encompasses expression cassettes and vectors comprising LNK4 nucleic acids of the invention.

[0163] The invention further provides expression cassettes comprising a LNK nucleic acid. In some embodiments the expression cassettes comprise a LNK promoter sequence. In some embodiments the expression cassettes comprise a nucleic acid encoding a LNK polypeptide or a nucleic acid that expresses a LNK functional RNA. Thus, in some embodiments, the invention provides expression cassettes comprising a LNK nucleic acid encoding a LNK polypeptide operably associated with a promoter. In some embodiments, the LNK nucleic acid is operably associated with a heterologous promoter. In alternative embodiments, the LNK nucleic acid is operably associated with endogenous LNK promoter sequence.

[0164] In further embodiments, the expression cassettes comprise a LNK1 nucleic acid operably associated with a promoter. In some embodiments, the LNK1 nucleic acid is in sense orientation relative to the promoter. In further embodiments, the LNK1 nucleic acid encodes a polypeptide. In some embodiments, the expression cassettes comprise a LNK1 nucleic acid in antisense orientation relative to the promoter. In further embodiments, the LNK1 nucleic acid expresses a functional RNA.

[0165] In further embodiments, the expression cassettes comprise a LNK2 nucleic acid operably associated with a promoter. In some embodiments, the LNK2 nucleic acid is in sense orientation relative to the promoter. In further embodiments, the LNK2 nucleic acid encodes a polypeptide. In some embodiments, the expression cassettes comprise a LNK2 nucleic acid in antisense orientation relative to the promoter. In further embodiments, the LNK2 nucleic acid expresses a functional RNA.

[0166] In some embodiments, the expression cassettes comprise a LNK promoter sequence operably associated with a nucleic acid of interest. In some embodiments, the expression cassette comprises a LNK1 promoter. In other embodiments, the expression cassette comprises a LNK2 promoter. In some

embodiments, the LNK1 nucleic acid is in nucleic acid encoding the LNK polypeptide is operably associated with a *LNK* promoter sequence of the invention. In embodiments, the nucleic acid encoding the LNK polypeptide is operably associated with a heterologous promoter.

[0167] The heterologous promoter can be any suitable promoter known in the art (including bacterial, yeast, fungal, insect, mammalian, and plant promoters). In particular embodiments, the promoter is a promoter for expression in plants. The selection of promoters useable with the present invention can be made among many different types of promoters. Thus, the choice of promoter depends upon several factors, including, but not limited to, cell- or tissue-specific expression, desired expression level, efficiency, inducibility and/or selectability. For example, where expression in a specific tissue or organ is desired in addition to inducibility, a tissue- specific promoter can be used (*e.g.*, a root specific promoter). In contrast, where expression in response to a stimulus is desired a promoter inducible by other stimuli or chemicals can be used. Where continuous expression is desired throughout the cells of a plant, a constitutive promoter can be chosen.

[0168] Non-limiting examples of constitutive promoters include cestrum virus promoter (cmp) (U.S. Patent No. 7,166,770), an actin promoter (*e.g.*, the rice actin 1 promoter; Wang *et al*, Mol. Cell. Biol. 12:3399-3406 (1992); as well as U.S. Patent No. 5,641,876), Cauliflower Mosaic Virus (CaMV) 35S promoter (Odell *et al*, Nature 313:810-812 (1985)), CaMV 19S promoter (Lawton *et al*, Plant Mol. Biol. 9:315-324 (1987)), an opine synthetase promoter (*e.g.*, nos, mas, ocs, *etc.*; (Ebert *et al*, PNAS 84:5745-5749 (1987)), Adh promoter (Walker *et al*, PNAS 84:6624-6629 (1987)), sucrose synthase promoter (Yang & Russell, PNAS 87:4144-4148 (1990)), and a ubiquitin promoter.

[0169] In some embodiments, the expression cassettes of the invention can further comprise enhancer elements and/or tissue preferred elements in combination with the promoter. In some embodiments, the expression cassette comprises a constitutive S35 promoter operably associated with a polynucleotide sequence encoding LNK having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. In some embodiments, the expression cassette comprises a constitutive S35 promoter operably associated with a polynucleotide sequence encoding LNK.

- [0170] In some embodiments, the heterologous promoter is a promoter for expression in a monocot plant. In further embodiments the heterologous promoter is selected from: ZmUbil (Ubiquitin), Act1 (Actin), OsTubA1, (Tubulin), OsCcl (Cytochrome c), rubi3 (polyubiquitin), APX (ascorbate peroxidase), SCP1, PGD1 (phosphogluconate dehydrogenase), R1G1B (early drought induced protein) and EIF5 (translation initiation factor).
- [0171] In some embodiments, the heterologous promoter is a promoter for expression in a dicot plant. In further embodiments the heterologous promoter is a CsVMV (cassava vein mosaic virus) or ScBV (sugarcane *bacilliform badnavirus*) promoter. In other embodiments, the heterologous promoter is an CaMV 35S promoter.
- [0172] Some non-limiting examples of tissue-specific promoters useable with the present invention include those driving the expression of seed storage proteins (*e.g.*, 13-conglycinin, cruciferin, napin phaseolin, *etc.*), zein or oil body proteins (such as oleosin), or proteins involved in fatty acid biosynthesis (including acyl carrier protein, stearoyl- ACP desaturase and fatty acid desaturases (fad 2-1)), and other nucleic acids expressed during embryo development (such as Bce4, *see, e.g.*, Kridl *et al*, Seed Sci. Res. 1:209-219 (1991); as well as EP Patent No. 255378). Thus, the promoters associated with these tissue-specific nucleic acids can be used in the present invention.
- [0173] Additional examples of tissue-specific promoters usable with the present invention include, but are not limited to, the root-specific promoters RCc3 (Jeong *et al*, Plant Physiol. 153:185-197 (2010)) and RB7 (U.S. Patent No. 5,459,252), the lectin promoter (Lindstrom *et al*, Der. Genet. 11:160-167 (1990); and Vodkin *et al*, Prog. Clin. Biol. Res. 138:87-98 (1983)), corn alcohol dehydrogenase 1 promoter (Dennis *et al*, Nucleic Acids Res. 12:3983-4000 (1984)), S-adenosyl-L-methionine synthetase (SAMS) (Vander Mijnsbrugge *et al*, Plant and Cell Physiology, 37(8):1108-1115 (1996)), corn light harvesting complex promoter (Bansal *et al*, PNAS 89:3654-3658 (1992)), corn heat shock protein promoter (O'Dell *et al*, EMBO J. 5:451-458 (1985); and Rochester *et al*, EMBO J. 5:451-458 (1986)), pea small subunit RuBP carboxylase promoter (Cashmore, "Nuclear genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase" pp.

29-39 In: Genetic Engineering of Plants, Hollaender ed., Plenum Press 1983; and Poulsen *et al*, Mol. Gen. Genet. 205:193-200 (1986)), Ti plasmid mannopine synthase promoter (Langridge *et al*, PNAS 86:3219-3223 (1989)), Ti plasmid nopaline synthase promoter (Langridge *et al*, (1989), *supra*), petunia chalcone isomerase promoter (van Tunen *et al*, EMBO J. 7:1257-1263 (1988)), bean glycine rich protein 1 promoter (Keller *et al*, Genes Dev. 3:1639-1646 (1989)), truncated CaMV 35S promoter (O'Dell *et al*, Nature 313:810-812 (1985)), potato patatin promoter (Wenzler *et al*, Plant Mol. Biol. 13:347-354 (1989)), root cell promoter (Yamamoto *et al*, Nucleic Acids Res. 18:7449 (1990)), maize zein promoter (Kriz *et al*, Mol. Gen. Genet. 207:90-98 (1987); Langridge *et al*, Cell 34:1015-1022 (1983); Reina *et al*, Nucleic Acids Res. 18:6425 (1990); Reina *et al*, Nucleic Acids Res. 18:7449 (1990); and Wandelt *et al*, Nucleic Acids Res. 17:2354 (1989)), globulin-1 promoter (Belanger *et al*, Genetics 129:863-872 (1991)), α -tubulin cab promoter (Sullivan *et al*, Mol. Gen. Genet. 215:431-440 (1989)), PEPCase promoter (Hudspeth *et al*, Plant Mol. Biol. 12:579-589 (1989)), R gene complex-associated promoters (Chandler *et al*, Plant Cell 1:1175-1183 (1989)), and chalcone synthase promoters (Franken *et al*, EMBO J. 10:2605-2612 (1991)). Particularly useful for seed-specific expression is the pea vicilin promoter (Czako *et al*, Mol. Gen. Genet. 235:33-40 (1992); as well as U.S. Patent No. 5,625,136). Other useful promoters for expression in mature leaves are those that are switched on at the onset of senescence, such as the SAG promoter from *Arabidopsis* (Gan *et al*, Science 270:1986-1988 (1995)).

[0174] In addition, promoters functional in plastids can be used. Non-limiting examples of such promoters include the bacteriophage T3 gene 9 5' UTR and other promoters disclosed in U.S. Patent No. 7,579,516. Other promoters useful with the present invention include but are not limited to the S-E9 small subunit RuBP carboxylase promoter and the Kunitz trypsin inhibitor gene promoter (Kti3).

[0175] In some embodiments, inducible promoters can be used with the present invention. Examples of inducible promoters useable with the present invention include, but are not limited to, tetracycline repressor system promoters, Lac repressor system promoters, copper-inducible system promoters, salicylate-inducible system promoters (*e.g.*, the PRLa system), glucocorticoid-inducible

promoters (Aoyama *et al*, Plant J. 11:605-612 (1997)), and ecdysone-inducible system promoters. Other non-limiting examples of inducible promoters include ABA- and turgor-inducible promoters, the auxin-binding protein gene promoter (Schwab *et al*, Plant J. 4:423-432 (1993)), the UDP glucose flavonoid glycosyltransferase promoter (Ralston *et al*, Genetics 119:185-197 (1988)), the IVIPI proteinase inhibitor promoter (Cordero *et al*, Plant J. 6:141-150 (1994)), the glyceraldehyde-3-phosphate dehydrogenase promoter (Kohler *et al*, Plant Mol. Biol. 29:1293-1298 (1995); Martinez *et al*, J. Mol. Biol. 208:551-565 (1989); and Quigley *et al*, J. Mol. Evol. 29:412-421 (1989)) the benzene sulphonamide-inducible promoters (U.S. Patent No. 5,364,780) and the glutathione S-transferase promoters. Likewise, one can use any appropriate inducible promoter described in Gatz *et al*, Current Opinion Biotechnol. 7:168-172 (1996) and Gatz *et al*, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108 (1997).

[0176] Other suitable promoters include promoters from viruses that infect the host plant including, but not limited to, promoters isolated from Dasheen mosaic virus, Chlorella virus (*e.g.*, the Chlorella virus adenine methyltransferase promoter; Mitra *et al*, Plant Molecular Biology 26:85 (1994)), tomato spotted wilt virus, tobacco rattle virus, tobacco necrosis virus, tobacco ring spot virus, tomato ring spot virus, cucumber mosaic virus, peanut stump virus, alfalfa mosaic virus, and the like.

[0177] The expression cassettes of the invention may further comprise a transcriptional termination sequence. Any suitable termination sequence known in the art may be used in accordance with the present invention. The termination region may be native with the transcriptional initiation region, may be native with the nucleotide sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthetase and nopaline synthetase termination regions. *See also*, Guerineau *et al*, Mol. Gen. Genet. 262:141 (1991); Proudfoot, Cell 64:671 (1991); Sanfacon *et al*, Genes Dev. 5:141 (1991); Mogen *et al*, Plant Cell 2:1261 (1990); Munroe *et al*, Gene 91:151 (1990); Ballas *et al*, Nucleic Acids Res. 17:7891 (1989); and Joshi *et al*, Nucleic Acids Res. 15:9627 (1987). Additional exemplary termination sequences are the pea RubP carboxylase small

subunit termination sequence and the Cauliflower Mosaic Virus 35S termination sequence. Other suitable termination sequences will be apparent to those skilled in the art.

[0178] Further, in particular embodiments, the nucleotide sequence of interest (*e.g.*, heterologous nucleotide sequence of interest) is operably associated with a translational start site. The translational start site can be derived from the *LNK* coding sequence or, alternatively, can be the native translational start site associated with a heterologous nucleotide sequence of interest, or any other suitable translational start codon.

[0179] In illustrative embodiments, the expression cassette includes in the 5' to 3' direction of transcription, a promoter, a nucleotide sequence of interest (*e.g.*, a heterologous nucleotide sequence of interest), and a transcriptional and translational termination region functional in plants.

[0180] Those skilled in the art will understand that the expression cassettes of the invention can further comprise enhancer elements and/or tissue preferred elements in combination with the promoter. In some embodiments, the expression cassette comprises a promoter sequence operably associated with the first intron of *Arabidopsis Cox5c2*.

[0181] Further, in some embodiments, it is advantageous for the expression cassette to comprise a selectable marker gene for the selection of transformed cells.

[0182] Selectable marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (*HPT*), as well as genes conferring resistance to herbicidal compounds. Herbicide resistance genes generally code for a modified target protein insensitive to the herbicide or for an enzyme that degrades or detoxifies the herbicide in the plant before it can act. *See, DeBlock et al, EMBO J. 6:2513 (1987); DeBlock et al, Plant Physiol. 91:691 (1989); Fromm et al, BioTechnology 8:833 (1990); Gordon-Kamm et al, Plant Cell 2:603 (1990).* For example, resistance to glyphosphate or sulfonylurea herbicides has been obtained using genes coding for the mutant target enzymes, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and acetolactate synthase (ALS). Resistance to glufosinate ammonium, boromoxynil, and 2,4-dichlorophenoxyacetate (2,4-D)

have been obtained by using bacterial genes encoding phosphinothricin acetyltransferase, a nitrilase, or a 2,4- dichlorophenoxyacetate monooxygenase, which detoxify the respective herbicides.

[0183] Selectable marker genes that can be used according to the present invention further include, but are not limited to, genes encoding: neomycin phosphotransferase II (Fraley *et al*, CRC Critical Reviews in Plant Science 4:1 (1986)); cyanamide hydratase (Maier-Greiner *et al*, PNAS 88:4250 (1991)); aspartate kinase; dihydrodipicolinate synthase (Peri *et al*., BioTechnology 11:715 (1993)); the bar gene (Toki *et al*, Plant Physiol. 100: 1503 (1992); Meagher *et al*, Crop Sci. 36:1367 (1996)); tryptophane decarboxylase (Goddijn *et al*, Plant Mol. Biol. 22:907 (1993)); neomycin phosphotransferase (NEO; Southern *et al*, J. Mol. Appl. Gen. 1:327 (1982)); hygromycin phosphotransferase (HPT orHYG; Shimizu *et al*, Mol. Cell. Biol. 6:1074 (1986)); dihydrofolate reductase (DHFR; Kwok *et al*, PNAS 83:4552 (1986)); phosphinothricin acetyltransferase (DeBlock *et al*, EMBO J. 6:2513 (1987)); 2,2- dichloropropionic acid dehalogenase (Buchanan-Wollatron *et al*, J. Cell. Biochem. 13D, 330 (1989)); acetohydroxyacid synthase (United States Patent No. 4,761,373 to Anderson *et al*; Haughn *et al*, Mol. Gen. Genet. 221:266 (1988)); 5-enolpyruvyl-shikimate-phosphate synthase (*aroA*; Comai *et al*, Nature 317:741 (1985)); haloarylnitrilase (WO 87/04181 to Stalker *et al*); acetyl-coenzyme A carboxylase (Parker *et al*, Plant Physiol. 92: 1220 (1990)); dihydropteroate synthase (*sull*; Guerineau *et al*, Plant Mol. Biol. 15:127 (1990)); and 32 kDa photosystem II polypeptide (*psbA*; Hirschberg *et al*, Science 222: 1346 (1983)).

[0184] Also included are genes encoding resistance to: chloramphenicol (Herrera-Estrella *et al*, EMBO J. 2:987 (1983)); methotrexate (Herrera-Estrella *et al*, Nature 303:209 (1983); Meijer *etal*, Plant Mol. Biol. 16:807 (1991)); hygromycin (Waldron *et al*, Plant Mol. Biol. 5:103 (1985); Zhijian *et al*, Plant Science 108:219 (1995); Meijer *etal*, Plant Mol. Bio. 16:807 (1991)); streptomycin (Jones *et al*, Mol. Gen. Genet. 210:86 (1987)); and spectinomycin (Bretagne- Sagnard *et al*, Transgenic Res. 5:131 (1996)); bleomycin (Hille *et al*, Plant Mol. Biol. 7:171 (1986)); sulfonamide (Guerineau *et al*, Plant Mol. Bio. 15:127 (1990); bromoxynil (Stalker *et al*, Science 242:419 (1988)); 2,4-D (Streber *et al*, Bio/Technology

7:8 11 (1989)); phosphinothricin (DeBlock *et al*, EMBO J. 6:25 13 (1987)); spectinomycin (Bretagne-Sagnard *et al.*, Transgenic Research 5:13 1 (1996)).

[0185] Other selectable marker genes include the *pat* gene (for bialaphos and phosphinothricin resistance), the *ALS* gene for imidazolinone resistance, the *HPH* or *HYG* gene for hygromycin resistance, the *Hml* gene for resistance to the He-toxin, and other selective agents used routinely and known to one of ordinary skill in the art. *See generally*, Yarranton, Curr. Opin. Biotech. 3:506 (1992); Christopherson *et al*, PNAS 89: 63 14 (1992); Yao *et al*, Cell 71:63 (1992); Reznikoff, Mol. Microbial. 6:24 19 (1992); Barkley *et al*, THE OPERON 177-220 (1980); Hu *et al*, Cell 48:555 (1987); Brown *et al*, Cell 49:603 (1987); Figge *et al*, Cell 52:7 13 (1988); Deuschle *et al*, PNAS 86:5400 (1989); Fuerst *et al*, PNAS 86:2549 (1989); Deuschle *et al*, Science 248:480 (1990); Labow *et al*, Mol. Cell. Biol. 10:3343 (1990); Zambretti *et al*, PNAS 89:3952 (1992); Baim *et al*, PNAS 88:5072 (1991); Wyborski *et al*, Nuc. Acids Res. 19:4647 (1991); Hillenand-Wissman, Topics in Mol. and Struct. Biol. 10: 143 (1989); Degenkolb *et al*, Antimicrob. Agents Chemother. 35: 159 1 (1991); Kleinschmidt *et al*, Biochemistry 27: 1094 (1988); Gatz *et al*, Plant J. 2:397 (1992); Gossen *et al*, PNAS 89:5547 (1992); Oliva *et al*, Antimicrob. Agents Chemother. 36:9 13 (1992); Hlavka., HANDBOOK OF EXPERIMENTAL PHARMACOLOGY 78 (1985); and Gill *et al*, Nature 334:72 1 (1988).

[0186] The nucleotide sequence of interest can additionally be operably linked to a sequence that encodes a transit peptide that directs expression of an encoded polypeptide of interest to a particular cellular compartment. Transit peptides that target protein accumulation in higher plant cells to the chloroplast, mitochondrion, vacuole, nucleus, and the endoplasmic reticulum (for secretion outside of the cell) are known in the art. Transit peptides that target proteins to the endoplasmic reticulum are desirable for correct processing of secreted proteins. Targeting protein expression to the chloroplast (for example, using the transit peptide from the RubP carboxylase small subunit gene) has been shown to result in the accumulation of very high concentrations of recombinant protein in this organelle. The pea RubP carboxylase small subunit transit peptide sequence has been used to express and target mammalian genes in plants (U.S. Patent Nos. 5,717,084 and

5,728,925). Alternatively, mammalian transit peptides can be used to target recombinant protein expression, for example, to the mitochondrion and endoplasmic reticulum. It has been demonstrated that plant cells recognize mammalian transit peptides that target endoplasmic reticulum (U.S. Patent Nos. 5,202,422 and 5,639,947).

[0187] Further, the expression cassette can comprise a 5' leader sequence that acts to enhance expression (transcription, post-transcriptional processing and/or translation) of an operably associated nucleotide sequence of interest. Leader sequences are known in the art and include sequences from: picornavirus leaders, *e.g.*, EMCV leader (Encephalomyocarditis 5' noncoding region; Elroy-Stein *et al.*, PNAS USA, 86:6126 (1989)); potyvirus leaders, *e.g.*, TEV leader (Tobacco Etch Virus; Allison *et al.*, Virology, 154:9 (1986)); human immunoglobulin heavy-chain binding protein (BiP; Macajak and Sarnow, Nature 353:90 (1991)); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4; Jobling and Gehrke, Nature 325: 622 (1987)); tobacco mosaic virus leader (TMV; Gallie, MOLECULAR BIOLOGY OF RNA, 237-56 (1989)); and maize chlorotic mottle virus leader (MCMV; Lommel *et al.*, Virology 81:382 (1991)). See also, Della-Cioppa *etal*, Plant Physiology 84:965 (1987).

[0188] The heterologous nucleotide sequence(s) of interest in the expression cassette can be any nucleotide sequence(s) of interest and can be obtained from prokaryotes or eukaryotes (*e.g.*, bacteria, fungi, yeast, viruses, plants, mammals) or the heterologous nucleotide sequence can be synthesized in whole or in part. Further, the heterologous nucleotide sequence can encode a polypeptide or can be transcribed to produce a functional RNA. In particular embodiments, the functional RNA can be expressed to improve an agronomic trait in the plant (*e.g.*, drought resistance, heat resistance, salt resistance, disease resistance, insect and other pest resistance [*e.g.*, a *Bacillus thuringiensis* endotoxin], herbicide resistance, and the like), to confer male sterility, to improve fertility and/or enhance nutritional quality (*e.g.*, enzymes that enhance nutritional quality). The nucleotide sequence may be used in the sense orientation or antisense orientation relative to the promoter to achieve suppression of endogenous plant genes, as is known by those skilled in the art (*see, e.g.*, U.S. Patent Nos. 5,283,184; and 5,034,323).

[0189] The heterologous nucleotide sequence can encode a polypeptide that imparts a desirable agronomic trait to the plant (as described above), confers male sterility, improves fertility and/or improves nutritional quality. Other suitable polypeptides include enzymes that can degrade organic pollutants or remove heavy metals. Such plants, and the enzymes that can be isolated therefrom, are useful in methods of environmental protection and remediation. Alternatively, the heterologous nucleotide sequence can encode a therapeutically or pharmaceutically useful polypeptide or an industrial polypeptide (*e.g.*, an industrial enzyme). Such polypeptides include, but are not limited to antibodies and antibody fragments, cytokines, hormones, growth factors, receptors, enzymes and the like.

[0190] Heterologous nucleotide sequences of interest suitable to confer tolerance to the herbicide glyphosate include, but are not limited to the *Agrobacterium* strain CP4 glyphosate resistant EPSPS gene (*aroA:CP4*) as described in U.S. Patent No. 5,633,435 or the glyphosate oxidoreductase gene (GOX) as described in U.S. Patent No. 5,463,175. Other heterologous nucleotide sequences include genes conferring resistance to herbicides that act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonyleurea-type herbicides (*e.g.*, mutant forms of the acetolactate synthase (ALS) gene that lead to such resistance, in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides that act to inhibit the action of glutamine synthase, such as phosphinothricin or basta (*e.g.*, the bar gene). The bar gene encodes resistance to the herbicide basta, the *nptII* gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS gene encodes resistance to the herbicide chlorsulfuron.

[0191] Suitable heterologous nucleotide sequences that confer insect tolerance include those which provide resistance to pests such as rootworm, cutworm, European Corn Borer, and the like. Exemplary nucleotide sequences include, but are not limited to, a *Bacillus* insect control protein gene (*see, e.g.*, WO 99/31248; U.S. Patent Nos. 5,689,052; 5,500,365; 5,880,275); *Bacillus thuringiensis* toxic protein genes (*see, e.g.*, U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; 6,555,655; 6,541,448; and 6,538,109; Geiser, *et al.*, Gene 48: 109 (1986)); and lectins (Van Damme *et al.*, Plant Mol. Biol. 24:825 (1994)).

- [0192] Alternatively, the heterologous nucleotide sequence can encode a reporter polypeptide (*e.g.*, an enzyme), including but not limited to Green Fluorescent Protein, beta-galactosidase, luciferase, alkaline phosphatase, the *GUS* gene encoding beta- glucuronidase, and chloramphenicol acetyltransferase.
- [0193] Where appropriate, the heterologous nucleic acids may be optimized for increased expression in a transformed plant, *e.g.*, by using plant preferred codons. Methods for synthetic optimization of nucleic acid sequences are available in the art. The nucleotide sequence can be optimized for expression in a particular host plant or alternatively can be modified for optimal expression in monocots or dicots. *See, e.g.*, EP 0 359 472, EP 0 385 962, WO 91/16432; Perlak *et al*, PNAS 88, 3324 (1991), and Murray *et al*, Nucl. Acids Res. 17:477 (1989), and the like. Plant preferred codons can be determined from the codons of highest frequency in the proteins expressed in that plant. Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences which may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.
- [0194] The invention further provides vectors comprising the LNK nucleic acids of the invention and expression cassettes of the invention, including expression vectors, transformation vectors and vectors for replicating and/or manipulating the nucleotide sequences in the laboratory. The vector can be a plant vector, animal (*e.g.*, insect or mammalian) vector, bacterial vector, yeast vector or fungal vector. Generally, according to the present invention, the vector is a plant vector, a bacterial vector, or a shuttle vector that can replicate in either host under appropriate conditions. Bacterial and plant vectors are well-known in the art. Exemplary plant vectors include plasmids (*e.g.*, pUC or the Ti plasmid), cosmids, phage, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) and plant viruses.

III. Transgenic plants, plant parts and plant cells.

[0195] The invention also provides transgenic plants, plant parts and plant cells comprising the nucleic acids, expression cassettes and vectors of the invention.

[0196] Accordingly, as one aspect, the invention provides a cell comprising a LNK nucleic acid, expression cassette, or vector of the invention. The cell can be transiently or stably transformed with the nucleic acid, expression cassette or vector. Further, the cell can be a cultured cell, a cell obtained from a plant, plant part, or plant tissue, or a cell *in situ* in a plant, plant part or plant tissue. Cells can be from any suitable species, including plant (*e.g.*, *Arabidopsis thaliana* and *Helianthus annuus*), bacterial, yeast, insect and/or mammalian cells. In representative embodiments, the cell is a plant cell or bacterial cell.

[0197] The invention also provides a plant part (including a plant tissue culture) comprising a nucleic acid, expression cassette, or vector of the invention. The plant part can be transiently or stably transformed with the nucleic acid, expression cassette or vector. Further, the plant part can be in culture, can be a plant part obtained from a plant, or a plant part *in situ*. In representative embodiments, the plant part comprises a transgenic cell of the invention, as described in the preceding paragraph.

[0198] Seed comprising the LNK nucleic acid, expression cassette, or vector of the invention are also provided. Optionally, the nucleic acid, expression cassette or vector is stably incorporated into the genome of the seed.

[0199] The invention also contemplates a transgenic plant comprising a LNK nucleic acid, expression cassette, or vector of the invention. The plant can be transiently or stably transformed with the LNK nucleic acid, expression cassette or vector. In representative embodiments, the plant comprises a cell or plant part of the invention, as described in the preceding paragraphs. In some embodiments, the transgenic plant has an altered circadian rhythm compared to a non-transgenic control wild-type plant. In additional embodiments, the transgenic plant has an altered *PRR5*, *ELF4* and/or *FKF1* expression profile compared to a non-transgenic control wild-type plant. In some embodiments, the transgenic plant has an accelerated flowering time and/or decreased biomass compared to a non-transgenic control wild-type plant. In alternative embodiments, the transgenic plant has a

delayed flowering time and/or increased biomass compared to a non-transgenic control wild-type plant. In additional embodiments, the transgenic plant has increased yield compared to a non-transgenic control wild-type plant. In a further embodiment, the transgenic plant has an increased tolerance to an abiotic stress (*e.g.*, high intensity light, low intensity light, drought, high temperature, low temperature and/or high salinity (*e.g.*, salt)) compared to a non-transgenic control wild-type plant.

[0200] In some embodiments, the invention encompasses a transgenic plant stably transformed with an isolated nucleic acid encoding a LNK polypeptide. In additional embodiments, the invention encompasses a transgenic plant stably transformed with an isolated nucleic acid comprising a LNK1 nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:1; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter.

[0201] In additional embodiments, the invention provides a transgenic plant stably transformed with an isolated nucleic acid encoding a LNK1 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; and (c) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2.

[0202] The invention also encompasses a transgenic plant stably transformed with an isolated LNK2 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:3; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:4; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter.

[0203] In additional embodiments, the invention provides a transgenic plant stably transformed with an isolated nucleic acid encoding a LNK2 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; and (c) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4.

[0204] The invention also encompasses a transgenic plant stably transformed with both an isolated LNK1 and LNK2 nucleic acid of the invention. In particular embodiments, the nucleic acid sequence encodes the LNK1 polypeptide sequence of SEQ ID NO:2 and/or the LNK2 polypeptide sequence of SEQ ID NO:4.

[0205] In additional embodiments, the invention encompasses transgenic plant stably transformed with an isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:1; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof; (d) the nucleotide sequence of SEQ ID NO:3; (e) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (f) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a), (c), (d) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (c), (d) or (f) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (f)-(h) due to the degeneracy of the genetic code.

[0206] In particular embodiments, the transgenic plant comprises at least 10, 15, 20, 25 or 30 consecutive nucleotides of the LNK1 nucleotide sequence of SEQ ID

NO:1, or the complementary strand thereof. In additional particular embodiments, the transgenic plants comprises at least 10, 15, 20, 25 or 30 consecutive nucleotides of the LNK2 nucleotide sequence of SEQ ID NO:3, or the complementary strand thereof.

[0207] In some embodiments, the transgenic plants are transformed with a vector comprising a LNK nucleic acid. In some embodiments, the vector comprises a LNK1 nucleic acid such as described herein. In some embodiments, the vector comprises a LNK2 nucleic acid such as described herein.

[0208] In one embodiment, the invention provides a transgenic plant stably transformed with an isolated nucleic acid comprising a LNK3 nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO: 13; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 13 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 13 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK3 amino acid sequence of SEQ ID NO: 14; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 14; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200 or 250 contiguous amino acid residues of the amino acid sequence of SEQ ID NO: 14; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter. In further embodiments, the nucleic acids are transcribed to express a functional RNA. The

invention also encompasses expression cassettes and vectors comprising nucleic acids of the invention.

[0209] In one embodiment, the invention provides a transgenic plant stably transformed with an isolated nucleic acid encoding a LNK3 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK3 amino acid sequence of SEQ ID NO: 14; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 14; and (c) an amino acid sequence comprising at least 50, 100, 150, 200 or 250 contiguous amino acid residues of the amino acid sequence of SEQ ID NO: 14.

[0210] In additional embodiments, the invention provides a transgenic plant stably transformed with an isolated LNK4 nucleic acid comprising a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO: 15; (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 75 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 15 or the complementary strand thereof; (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 15 or the complementary strand thereof; (d) a nucleotide sequence encoding a polypeptide comprising the LNK4 amino acid sequence of SEQ ID NO: 16; (e) a nucleotide sequence encoding an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 16; (f) a nucleotide sequence encoding an amino acid sequence comprising at least 50, 100, 150, 200 or 250 contiguous amino acid residues of the amino acid sequence of SEQ ID NO: 16; (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)-(e) or (f); (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(c) or (d) under stringent hybridization conditions; and (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (e) due to the degeneracy of the genetic code. In a further embodiment, the nucleic acids are operably associated with a promoter. In some embodiments, the nucleic acids are in sense orientation relative to the promoter. In alternative embodiments, the nucleic acids are in antisense orientation relative to the promoter.

[0211] In one embodiment, the invention provides a transgenic plant stably transformed with an isolated nucleic acid encoding a LNK4 polypeptide selected from the group consisting of: (a) a polypeptide comprising the LNK4 amino acid sequence of SEQ ID NO: 16; (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 16; and (c) an amino acid sequence comprising at least 50, 100, 150, 200 or 250 contiguous amino acid residues of the amino acid sequence of SEQ ID NO: 16.

[0212] The invention also encompasses a transgenic plant stably transformed with both an isolated LNK3 and LNK4 nucleic acid of the invention. In particular embodiments, the nucleic acid sequence encodes the LNK3 polypeptide sequence of SEQ ID NO: 14 and/or the LNK4 polypeptide sequence of SEQ ID NO: 16.

[0213] In additional embodiments, the transgenic plants are transformed with an expression cassette comprising a LNK nucleic acid. In some embodiments, the expression cassette comprises a LNK promoter sequence operably associated with a nucleic acid sequence of interest. In a further embodiment the expression cassette comprises a LNK coding sequence or is transcribed to form a functional LNK RNA. In further embodiments, the expression cassette comprises a LNK nucleic acid coding sequence or a LNK nucleic acid that is transcribed to form a functional RNA, operably associated with a promoter. In some embodiments, the promoter is an endogenous LNK promoter sequence. In alternative embodiments, the promoter is a heterologous promoter sequence. In further embodiments, the expression cassette comprises a selectable marker.

[0214] In further embodiments, the transgenic plants are transformed with an expression cassette comprising a LNK1 nucleic acid. In some embodiments, the expression cassette comprises a LNK1 promoter sequence operably associated with a nucleic acid sequence of interest. In a further embodiment the expression cassette comprises a LNK1 coding sequence or is transcribed to a functional LNK1 RNA. In further embodiments, the expression cassette comprises a LNK1 nucleic acid coding sequence or a LNK1 nucleic acid that is transcribed to a functional RNA, operably associated with a promoter. In some embodiments, the promoter is an endogenous LNK1 promoter sequence. In alternative embodiments, the

promoter is a heterologous promoter. In further embodiments, the expression cassette comprises a selectable marker.

[0215] In additional embodiments, the transgenic plants are transformed with an expression cassette comprising a LNK2 nucleic acid. In some embodiments, the expression cassette comprises a LNK2 promoter sequence operably associated with a nucleic acid sequence of interest. In a further embodiment the expression cassette comprises a LNK2 coding sequence or is transcribed to a functional LNK2 RNA. In further embodiments, the expression cassette comprises a LNK2 nucleic acid coding sequence or a LNK nucleic acid that is transcribed to a functional RNA, operably associated with a promoter. In some embodiments, the promoter is an endogenous LNK2 promoter sequence. In alternative embodiments, the promoter is a heterologous promoter. In further embodiments, the expression cassette comprises a selectable marker.

[0216] The invention also encompasses a crop comprising a plurality of the transgenic plants of the invention, as described herein. Non-limiting examples of the types of crops comprising a plurality of transgenic plants of the invention include an agricultural field, a golf course, a residential lawn or garden, a public lawn or garden, a road side planting, an orchard, and/or a recreational field (*e.g.*, a cultivated area comprising a plurality of the transgenic plants of the invention).

[0217] Products harvested from the plants of the invention are also provided. Non-limiting examples of a harvested product include a seed (*e.g.*, sunflower seeds and grain), a leaf, a stem, a shoot, a fruit, flower, root, biomass (*e.g.*, for biofuel production) and/or extract.

[0218] In some embodiments, a processed product produced from the harvested product is provided. Non-limiting examples of a processed product include a protein (*e.g.*, a recombinant protein), an extract, a medicinal product (*e.g.*, artemisin as an antimalarial agent), a fiber or woven textile, a fragrance, dried fruit, a biofuel (*e.g.*, ethanol), a tobacco product (*e.g.*, cured tobacco, cigarettes, chewing tobacco, cigars, and the like), an oil (*e.g.*, sunflower oil, corn oil, canola oil, and the like), a nut butter, a seed butter (*e.g.*, sunflower butter), a flour or meal (*e.g.*, wheat flour, corn meal) and/or any other animal feed (*e.g.*, soy, maize, barley, rice, alfalfa) and/or human food product (*e.g.*, a processed wheat, maize,

rice or soy food product). Further, processed product can be cut, dried, cooked, canned, frozen, dehydrated, powdered, ground and/or mixed with other ingredients.

III. Methods of transforming plants and plant cells

[0219] The invention also provides methods of delivering (*i.e.*, introducing) a nucleic acid, expression cassette or vector of the invention to a target plant or plant cell (including callus cells or protoplasts), plant parts, seed, plant tissue (including callus), and the like. The invention further comprises host plants, cells, plant parts, seeds, tissue culture (including callus) transiently or stably transformed with the nucleic acids, expression cassettes or vectors of the invention. In representative embodiments, the methods of the invention comprise transforming the plant, plant part or plant cell with a nucleic acid, expression cassette, or vector comprising an *LNK* coding sequence operably associated with a promoter. In other embodiments, the methods of the invention comprise transforming the plant, plant part or plant cell with a nucleic acid, expression cassette, or vector comprising an *LNK* nucleotide sequence transcribed to form a functional RNA in operable association with a promoter.

[0220] Also provided by the invention are seed produced from the inventive transgenic plants. Optionally, the seed comprise a nucleic acid, expression cassette or vector of the invention stably incorporated into the genome.

[0221] Methods of introducing nucleic acids, transiently or stably, into plants, plant tissues, cells, protoplasts, seed, callus and the like are known in the art. Stably transformed nucleic acids can be incorporated into the genome. Exemplary transformation methods include biological methods using viruses and *Agrobacterium*, physicochemical methods such as electroporation, floral dip methods, polyethylene glycol, ballistic bombardment, microinjection, and the like. Other transformation technology includes the whiskers technology that is based on mineral fibers (see '*e.g.*, U.S. Patent No. 5,302,523 and 5,464,765) and pollen tube transformation. In one form of direct transformation, the vector is microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA (Crossway, Mol. Gen. Genetics 202: 179 (1985)).

- [0222] In another protocol, the genetic material is transferred into the plant cell using polyethylene glycol (Krens *et al.*, Nature 296:72 (1982)).
- [0223] In still another method, protoplasts are fused with minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the nucleotide sequence to be transferred to the plant (Fraley *et al.*, PNAS 79: 1859 (1982)).
- [0224] Nucleic acids may also be introduced into the plant cells by electroporation (Fromm *et al.*, PNAS 82:5824 (1985)). In this technique, plant protoplasts are electroporated in the presence of nucleic acids comprising the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the nucleic acid. Electroporated plant protoplasts reform the cell wall, divide and regenerate. One advantage of electroporation is that large pieces of DNA, including artificial chromosomes, can be transformed by this method.
- [0225] Ballistic transformation typically comprises the steps of: (a) providing a plant material as a target; (b) propelling a microprojectile carrying the heterologous nucleotide sequence at the plant target at a velocity sufficient to pierce the walls of the cells within the target and to deposit the nucleotide sequence within a cell of the target to thereby provide a transformed target. The method can further include the step of culturing the transformed target with a selection agent and, optionally, regeneration of a transformed plant. As noted below, the technique may be carried out with the nucleotide sequence as a precipitate (wet or freeze-dried) alone, in place of the aqueous solution containing the nucleotide sequence.
- [0226] Any ballistic cell transformation apparatus can be used in practicing the present invention. Exemplary apparatus are disclosed by Sandford *et al.*, Particulate Science and Technology 5:27 (1988), Klein *et al.*, Nature 327:70 (1987), and in EP 0 270 356. Such apparatus have been used to transform maize cells (Klein *et al.*, PNAS 85:4305 (1988)), soybean callus (Christou *et al.*, Plant Physiol. 87:671 (1988)), McCabe *et al.*, BioTechnology 6:923 (1988), yeast mitochondria (Johnston *et al.*, Science 240:1538 (1988)), and Chlamydomonas chloroplasts (Boynton *et al.*, Science 240:1534 (1988)).

[0227] Alternately, an apparatus configured as described by Klein *et al.*, (Nature 70:327 (1987)) may be utilized. This apparatus comprises a bombardment chamber, which is divided into two separate compartments by an adjustable-height stopping plate. An acceleration tube is mounted on top of the bombardment chamber. A macroprojectile is propelled down the acceleration tube at the stopping plate by a gunpowder charge. The stopping plate has a borehole formed therein, which is smaller in diameter than the microprojectile. The macroprojectile carries the microprojectile(s), and the macroprojectile is aimed and fired at the borehole. When the macroprojectile is stopped by the stopping plate, the microprojectile(s) is propelled through the borehole. The target is positioned in the bombardment chamber so that a microprojectile(s) propelled through the bore hole penetrates the cell walls of the cells in the target and deposit the nucleotide sequence of interest carried thereon in the cells of the target. The bombardment chamber is partially evacuated prior to use to prevent atmospheric drag from unduly slowing the microprojectiles. The chamber is only partially evacuated so that the target tissue is not desiccated during bombardment. A vacuum of between about 400 to about 800 millimeters of mercury is suitable.

[0228] In alternate embodiments, ballistic transformation is achieved without use of microprojectiles. For example, an aqueous solution containing the nucleotide sequence of interest as a precipitate may be carried by the macroprojectile (*e.g.*, by placing the aqueous solution directly on the plate-contact end of the macroprojectile without a microprojectile, where it is held by surface tension), and the solution alone propelled at the plant tissue target (*e.g.*, by propelling the macroprojectile down the acceleration tube in the same manner as described above). Other approaches include placing the nucleic acid precipitate itself ("wet" precipitate) or a freeze-dried nucleotide precipitate directly on the plate-contact end of the macroprojectile without a microprojectile. In the absence of a microprojectile, it is believed that the nucleotide sequence must either be propelled at the tissue target at a greater velocity than that needed if carried by a microprojectile, or the nucleotide sequenced caused to travel a shorter distance to the target (or both).

[0229] In particular embodiments, the nucleotide sequence is delivered by a microprojectile. The microprojectile can be formed from any material having sufficient density and cohesiveness to be propelled through the cell wall, given the particle's velocity and the distance the particle must travel. Non-limiting examples of materials for making microprojectiles include metal, glass, silica, ice, polyethylene, polypropylene, polycarbonate, and carbon compounds (*e.g.*, graphite, diamond). Non-limiting examples of suitable metals include tungsten, gold, and iridium. The particles should be of a size sufficiently small to avoid excessive disruption of the cells they contact in the target tissue, and sufficiently large to provide the inertia required to penetrate to the cell of interest in the target tissue. Particles ranging in diameter from about one-half micrometer to about three micrometers are suitable. Particles need not be spherical, as surface irregularities on the particles may enhance their carrying capacity.

[0230] The nucleotide sequence may be immobilized on the particle by precipitation. The precise precipitation parameters employed will vary depending upon factors such as the particle acceleration procedure employed, as is known in the art. The carrier particles may optionally be coated with an encapsulating agent such as polylysine to improve the stability of nucleotide sequences immobilized thereon, as discussed in EP 270356 (column 8).

[0231] Alternatively, plants may be transformed using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. *Agrobacterium*-mediated nucleic acid transfer exploits the natural ability of *A. tumefaciens* and *A. rhizogenes* to transfer DNA into plant chromosomes. *Agrobacterium* is a plant pathogen that transfers a set of genes encoded in a region called T-DNA of the Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, into plant cells. The typical result of transfer of the Ti plasmid is a tumorous growth called a crown gall in which the T-DNA is stably integrated into a host chromosome. Integration of the Ri plasmid into the host chromosomal DNA results in a condition known as "hairy root disease". The ability to cause disease in the host plant can be removed by deletion of the genes in the T-DNA without loss of DNA transfer and integration. The DNA to be transferred is attached to border sequences that define the end points of an integrated T-DNA.

- [0232] Transfer by means of engineered *Agrobacterium* strains has become routine for many dicotyledonous plants. Some difficulty has been experienced, however, in using *Agrobacterium* to transform monocotyledonous plants, in particular, cereal plants. However, *Agrobacterium* mediated transformation has been achieved in several monocot species, including cereal species such as rye, maize (Rhodes *et al*, Science 240:204 (1988)), and rice (Hiei *etal*, Plant J. 6:271 (1994)).
- [0233] While the following discussion will focus on using *A. tumefaciens* to achieve gene transfer in plants, those skilled in the art will appreciate that this discussion also applies to *A. rhizogenes*. Transformation using *A. rhizogenes* has developed analogously to that of *A. tumefaciens* and has been successfully utilized to transform, for example, alfalfa, *Solarium nigrum L.*, and poplar (U.S. Patent No. 5,777,200). As described by U.S. Patent No. 5, 773,693, it is preferable to use a disarmed *A. tumefaciens* strain, however, the wild-type *A. rhizogenes* may be employed. An illustrative strain of *A. rhizogenes* is strain 15834.
- [0234] In particular protocols, the *Agrobacterium* strain is modified to contain the nucleotide sequences to be transferred to the plant. The nucleotide sequence to be transferred is incorporated into the T-region and is typically flanked by at least one T-DNA border sequence, optionally two T-DNA border sequences. A variety of *Agrobacterium* strains are known in the art particularly, and can be used in the methods of the invention. See, e.g., Hooykaas, Plant Mol. Biol. 13:327 (1989); Smith *et al*, Crop Science 35:301 (1995); Chilton, PNAS 90, 3119 (1993); Mollony *et al*, Monograph Theor. Appl. Genet NY 19, 148 (1993); Ishida *et al*, Nature Biotechnol. 14:745 (1996); and Komari *etal*, The Plant J. 10:165 (1996).
- [0235] In addition to the T-region, the Ti (or Ri) plasmid contains a vir region. The vir region is important for efficient transformation, and appears to be species-specific.
- [0236] Two exemplary classes of recombinant Ti and Ri plasmid vector systems are commonly used in the art. In one class, called "cointegrate," the shuttle vector containing the gene of interest is inserted by genetic recombination into a non-oncogenic Ti plasmid that contains both the cis-acting and trans-acting elements required for plant transformation as, for example, in the PMLJ1 shuttle vector of DeBlock *et al*, EMBO J. 3:1681 (1984), and the non-oncogenic Ti

plasmid pGV2850 described by Zambryski *et al*, EMBOJ. 2:2 143 (1983). In the second class or "binary" system, the gene of interest is inserted into a shuttle vector containing the cis-acting elements required for plant transformation. The other necessary functions are provided in trans by the non-oncogenic Ti plasmid as exemplified by the pBIN19 shuttle vector described by Bevan, Nucleic Acids Research 12:8711 (1984), and the non-oncogenic Ti plasmid PAL4404 described by Hoekma, *et al*, Nature 303:179 (1983).

[0237] Binary vector systems have been developed where the manipulated disarmed T-DNA carrying the heterologous nucleotide sequence of interest and the vir functions are present on separate plasmids. In this manner, a modified T-DNA region comprising foreign DNA (the nucleic acid to be transferred) is constructed in a small plasmid that replicates in *E. coli*. This plasmid is transferred conjugatively in a tri-parental mating or via electroporation into *A. tumefaciens* that contains a compatible plasmid with virulence gene sequences. The vir functions are supplied in *trans* to transfer the T-DNA into the plant genome. Such binary vectors are useful in the practice of the present invention.

[0238] In particular embodiments of the invention, super-binary vectors are employed. See, e.g., U.S. Patent No. 5,591,615 and EP 604662. Such a super-binary vector has been constructed containing a DNA region originating from the hypervirulence region of the Ti plasmid pTiBo542 (Jin *et al*, J. Bacteriol. 169:4417 (1987)) contained in a super-virulent *A. tumefaciens* A281 exhibiting extremely high transformation efficiency (Hood *et al*, Biotechnol. 2:702 (1984); Hood *et al*, J. Bacteriol. 168:1283 (1986); Komari *et al*, J. Bacteriol. 166:88 (1986); Jin *et al*, J. Bacteriol. 169:4417 (1987); Komari, Plant Science 60:223 (1987); ATCC Accession No. 37394.

[0239] Exemplary super-binary vectors known to those skilled in the art include pTOK162 (Japanese patent Appl. (Kokai) No. 4-222527, EP 504,869, EP 604,662, and United States Patent No. 5,591,616) and pTOK233 (Komari, Plant Cell Reports 9:303 (1990); Ishida *et al*, Nature Biotechnology 14:745 (1996)). Other super-binary vectors may be constructed by the methods set forth in the above references. Super-binary vector pTOK162 is capable of replication in both *E. coli* and in *A. tumefaciens*. Additionally, the vector contains the *virB*, *virC* and *virG*

genes from the virulence region of pTiBo542. The plasmid also contains an antibiotic resistance gene, a selectable marker gene, and the nucleic acid of interest to be transformed into the plant. The nucleic acid to be inserted into the plant genome is typically located between the two border sequences of the T region. Super-binary vectors of the invention can be constructed having the features described above for pTOK162.

[0240] The T-region of the super-binary vectors and other vectors for use in the invention are constructed to have restriction sites for the insertion of the genes to be delivered. Alternatively, the DNA to be transformed can be inserted in the T-DNA region of the vector by utilizing *in vivo* homologous recombination. *See*, Herrera-Esterella *et al*, EMBO J. 2:987 (1983); Horch *et al*, Science 223:496 (1984). Such homologous recombination relies on the fact that the super-binary vector has a region homologous with a region of bpR322 or other similar plasmids. Thus, when the two plasmids are brought together, a desired gene is inserted into the super-binary vector by genetic recombination via the homologous regions.

[0241] In plants stably transformed by *Agrobacteria-mediated* transformation, the nucleotide sequence of interest is incorporated into the plant nuclear genome, typically flanked by at least one T-DNA border sequence and generally two T-DNA border sequences.

[0242] Plant cells may be transformed with *Agrobacterici* by any means known in the art, *e.g.*, by co-cultivation with cultured isolated protoplasts, or transformation of intact cells or tissues. The first uses an established culture system that allows for culturing protoplasts and subsequent plant regeneration from cultured protoplasts. Identification of transformed cells or plants is generally accomplished by including a selectable marker in the transforming vector, or by obtaining evidence of successful bacterial infection.

[0243] Protoplasts, which have been transformed by any method known in the art, can also be regenerated to produce intact plants using known techniques.

[0244] Plant regeneration from cultured protoplasts is described in Evans *et al*, Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co. New York, 1983); and Vasil I. R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. II, 1986). Essentially all plant species

can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugar-cane, sugar beet, cotton, fruit trees, and legumes.

[0245] Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently root. Alternatively, somatic embryo formation can be induced in the callus tissue. These somatic embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and plant hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

[0246] The regenerated plants are transferred to standard soil conditions and cultivated in a conventional manner. The plants are grown and harvested using conventional procedures.

[0247] Alternatively, transgenic plants may be produced using the floral dip method (*See, e.g., Clough et al, Plant J. 16:735-743 (1998)*, which avoids the need for plant tissue culture or regeneration. In one representative protocol, plants are grown in soil until the primary inflorescence is about 10 cm tall. The primary inflorescence is cut to induce the emergence of multiple secondary inflorescences. The inflorescences of these plants are typically dipped in a suspension of *Agrobacterium* containing the vector of interest, a simple sugar (*e.g., sucrose*) and surfactant. After the dipping process, the plants are grown to maturity and the seeds are harvested. Transgenic seeds from these treated plants can be selected by germination under selective pressure (*e.g., using the chemical bialaphos*). Transgenic plants containing the selectable marker survive treatment and can be transplanted to individual pots for subsequent analysis. *See, Bechtold, et al, Methods Mol Biol. 82:259-266 (1998); Chung et al, Transgenic Res 9:471-476 (2000); Clough et al, A. Plant J. 16:735-743 (1998); Mysore et al, Plant J. 21:9-16 (2000); Tague et al, Transgenic Res. 10:259-267 (2001); Wang et al, Plant Cell Rep. 22:274-281 (2003); Ye et al, Plant J. 19:249-257 (1999).*

[0248] The particular conditions for transformation, selection and regeneration can be optimized by those of skill in the art. Factors that affect the efficiency of transformation include the species of plant, the target tissue or cell, composition of the culture media, selectable marker genes, kinds of vectors, and light/dark conditions. Therefore, these and other factors may be varied to determine what an optimal transformation protocol is for any particular plant species. It is recognized that not every species will react in the same manner to the transformation conditions and may require a slightly different modification of the protocols disclosed herein. However, by altering each of the variables, an optimum protocol can be derived for any plant species.

[0249] In some embodiments, the invention encompasses a method of expressing a LNK polypeptide in a plant, plant part or plant cell. In representative embodiments, the method comprises transforming the plant, plant part or plant cell with a nucleic acid, expression cassette, or vector of the invention comprising a nucleotide sequence encoding the LNK polypeptide. The plant can be transiently or stably transformed. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In some embodiments, the expressed polypeptide is a LNK1 polypeptide as described herein. In additional embodiments, the expressed polypeptide is a LNK2 polypeptide as described herein. In further embodiments, the nucleic acid, expression cassette, or vector expresses both a LNK1 and LNK2 polypeptide as described herein.

[0250] The invention additionally provides a method of expressing a LNK nucleic acid in a plant, plant part or plant cell. In representative embodiments, the method comprises transforming the plant, plant part or plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK polynucleotide sequence of the invention. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In particular embodiments, the transcription of the LNK polynucleotide produces a functional RNA. In further embodiments, the functional RNA is a member selected from:

siRNA, shRNA, miRNA, antisense RNA and a ribozyme. In particular embodiments, the functional RNA is a LNK antagonist. In some embodiments, the polynucleotide encodes a LNK polypeptide of the invention. In additional embodiments, the LNK polypeptide is a LNK agonist. In alternative embodiments, the LNK polypeptide is a LNK antagonist.

[0251] The invention additionally provides a method of expressing a LNK1 nucleic acid in a plant, plant part or plant cell. In representative embodiments, the method comprises transforming the plant, plant part or plant cell with a LNK1 nucleic acid, expression cassette, or vector operably associated with a LNK polynucleotide sequence of the invention. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In representative embodiments, the method comprises transforming the plant, plant part or plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK1 polynucleotide sequence of the invention. In particular embodiments, the transcription of the LNK1 polynucleotide produces a functional RNA. In further embodiments, the functional RNA is a member selected from: siRNA, shRNA, miRNA, antisense RNA and a ribozyme. In particular embodiments, the functional RNA is a LNK1 antagonist. In some embodiments, the polynucleotide encodes a LNK1 polypeptide of the invention. In additional embodiments, the LNK1 polypeptide is a LNK1 agonist. In alternative embodiments, the LNK1 polypeptide is a LNK1 antagonist.

[0252] The invention additionally provides a method of expressing a LNK2 nucleic acid in a plant, plant part or plant cell. In representative embodiments, the method comprises transforming the plant, plant part or plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK2 polynucleotide sequence of the invention. In further embodiments, the method further comprises the steps of (i) regenerating a stably transformed plant from the stably transformed plant cell; and (ii) expressing the nucleotide sequence in the plant. In representative embodiments, the method comprises transforming the plant, plant part or plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK2 polynucleotide sequence of the invention. In

particular embodiments, the transcription of the LNK2 polynucleotide produces a functional RNA. In further embodiments, the functional RNA is a member selected from: siRNA, shRNA, miRNA, antisense RNA and a ribozyme. In particular embodiments, the functional RNA is a LNK2 antagonist. In some embodiments, the polynucleotide encodes a LNK2 polypeptide of the invention. In additional embodiments, the LNK2 polypeptide is a LNK2 agonist. In alternative embodiments, the LNK2 polypeptide is a LNK2 antagonist.

[0253] In some embodiments, the invention provides a method of modulating a circadian response in a plant, comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK polynucleotide sequence of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the LNK polynucleotide sequence in the plant (*e.g.*, in an amount effective to modulate a circadian response of the plant). In some embodiments the resulting transgenic plant displays a modulated (*i.e.*, altered) expression of *PRR5*, *ELF4* and/or *FKF1*. In some embodiments, the resulting transgenic plant displays and increased expression of LNK protein. In some embodiments, the resulting transgenic plant displays and increased expression of *PRR5*, *ELF4* and/or *FKF1*. In alternative embodiments, the resulting transgenic plant displays a decreased (*i.e.*, reduced) expression of LNK protein. In other embodiments, the resulting transgenic plant displays a decreased expression of *PRR5*, *ELF4* and/or *FKF1*. In some embodiments, the LNK polynucleotide is a LNK agonist. In alternative embodiments, the LNK polynucleotide is a LNK antagonist.

[0254] In some embodiments, the invention provides a method of modulating a circadian response in a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence of the nucleic acid in the plant (*e.g.*, in an amount effective to modulate a circadian response of the plant). In some embodiments the resulting transgenic plant displays a modulated expression of *PRR5*, *ELF4* and/or *FKF1*. In some embodiments, the resulting transgenic plant displays and increased expression of LNK protein. In

some embodiments, the resulting transgenic plant displays an increased expression of *PRR5*, *ELF4* and/or *FKFl*. In alternative embodiments, the resulting transgenic plant displays a decreased (*i.e.*, reduced) expression of LNK protein. In other embodiments, the resulting transgenic plant displays a decreased expression of *PRR5*, *ELF4* and/or *FKFl*. In some embodiments, the LNK polypeptide is a LNK agonist. In alternative embodiments, the LNK polypeptide is a LNK antagonist.

[0255] In some embodiments, the invention provides a method of modulating the adjustment of daily and/or seasonal rhythms in a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence of the nucleic acid in the plant (*e.g.*, in an amount effective to alter daily and/or seasonal rhythms in a plant). In some embodiments the resulting transgenic plant displays a modulated (*i.e.*, altered) expression of *PRR5*, *ELF4* and/or *FKFl*. In some embodiments, the resulting transgenic plant displays an increased expression of LNK protein. In some embodiments, the resulting transgenic plant displays an increased expression of *PRR5*, *ELF4* and/or *FKFl*. In alternative embodiments, the resulting transgenic plant displays a decreased (*i.e.*, reduced) expression of LNK protein. In other embodiments, the resulting transgenic plant displays a decreased expression of *PRR5*, *ELF4* and/or *FKFl*. In some embodiments, the LNK polypeptide is a LNK agonist. In alternative embodiments, the LNK polypeptide is a LNK antagonist.

[0256] In additional embodiments, the invention provides a method of modulating the adjustment of daily and/or seasonal rhythms in a plant, wherein the method comprises: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK polynucleotide sequence of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective in an amount effective to alter daily and/or seasonal rhythms in a plant). In some embodiments the resulting transgenic plant displays a modulated (*i.e.*, altered) expression of *PRR5*, *ELF4* and/or *FKFl*. In some embodiments, the

resulting transgenic plant displays and increased expression of LNK protein. In some embodiments, the resulting transgenic plant displays and increased expression of *PRR5*, *ELF4* and/or *FKF1*. In alternative embodiments, the resulting transgenic plant displays a decreased (*i.e.*, reduced) expression of LNK protein. In other embodiments, the resulting transgenic plant displays a decreased expression of *PRR5*, *ELF4* and/or *FKF1*. In some embodiments, the LNK polynucleotide is a LNK agonist. In alternative embodiments, the LNK polynucleotide is a LNK antagonist. In particular embodiments, transcription of the LNK polynucleotide produces a functional RNA. In further embodiments, the functional RNA is a member selected from: siRNA, shRNA, miRNA, antisense RNA, and a ribozyme

[0257] In additional embodiments, the invention provides a method of increasing the yield from a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK polynucleotide sequence of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to increase the yield from the plant). Still further, the invention provides a method of the prolonging (*e.g.*, increasing) the life span and/or delaying development of a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK polynucleotide sequence of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to prolong the life span and/or delay the development of a plant). In particular embodiments, transcription of the LNK polynucleotide produces a functional RNA. In further embodiments, the functional RNA is a member selected from: siRNA, shRNA, miRNA, antisense RNA, and a ribozyme. In particular embodiments, the functional RNA is a LNK antagonist.

[0258] In some embodiments, the invention provides a method of increasing the yield from a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an

amount effective to increase the yield from the plant). Still further, the invention provides a method of the prolonging (*e.g.*, increasing) the life span and/or delaying development of a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to prolong the life span and/or delay the development of a plant). In some embodiments, the resulting transgenic plant displays a decreased (*i.e.*, reduced) expression of LNK protein. In other embodiments, the resulting transgenic plant displays a decreased expression of *PRR5*, *ELF4* and/or *FKF1*. In particular embodiments, the LNK polypeptide is a LNK antagonist.

[0259] In additional embodiments, the invention provides a method of delaying the flowering time of a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector operably associated with a LNK polynucleotide sequence of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to delay the flowering time of a plant). In particular embodiments, transcription of the LNK polynucleotide produces a functional RNA. In further embodiments, the functional RNA is a member selected from: siRNA, shRNA, miRNA, antisense RNA, and a ribozyme. In particular embodiments, the functional RNA is a LNK antagonist.

[0260] In some embodiments, the invention provides a method of delaying the flowering time of a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to delay the flowering time of the plant). In particular embodiments, the LNK polypeptide is a LNK antagonist.

[0261] In some embodiments, the invention provides a method of increasing the biomass of a plant, the method comprising: (a) stably transforming a plant cell

with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to increase the biomass of the plant). In particular embodiments, the LNK polypeptide is a LNK antagonist.

[0262] In some embodiments, the invention provides a method of accelerating the flowering time of a plant, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to accelerate the flowering time of the plant). In particular embodiments, the LNK polypeptide is a LNK agonist.

[0263] The invention also encompasses a method of increasing tolerance of a plant to abiotic stress, the method comprising: (a) stably transforming a plant cell with a nucleic acid, expression cassette, or vector encoding an LNK polypeptide of the invention; (b) regenerating a stably transformed plant from the stably transformed plant cell of (a); and (c) expressing the nucleotide sequence in the plant (*e.g.*, in an amount effective to increase the tolerance of the plant to an abiotic stress). The method optionally includes the further step of exposing the plant to the abiotic stress (*e.g.*, growing the plant under the abiotic stress conditions). Abiotic stress is as described elsewhere herein. In representative embodiments of the foregoing methods, the abiotic stress comprises drought, salt stress, submergence stress and/or waterlogging stress, and/or stress after removal of a submergence stressor (*e.g.*, desubmergence stress).

[0264] In some embodiments, the methods of the invention can be used to increase the yield of a plant and/or increase tolerance of a plant to abiotic stress. In further embodiments, the abiotic stress comprises drought, salt stress, waterlogging stress, submergence stress, and/or desubmergence stress. Thus, in some embodiments, the transgenic plants of the invention are grown under the abiotic stress conditions. Alternatively, in additional embodiments, the transgenic plants are grown under normal cultivation conditions.

[0265] In one embodiment, the invention provides a method of increasing yield and/or increasing tolerance of a plant to abiotic stress, the method comprising: (a) stably transforming a plant cell with an isolated nucleic acid encoding a polypeptide selected from the group consisting of (i) a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2; (ii) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2; and (iii) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2. In a further embodiment, the invention further includes regenerating a stably transformed plant from the stably transformed plant cell; and expressing the nucleotide sequence in the plant.

[0266] In one embodiment, the invention provides a method of increasing yield and/or increasing tolerance of a plant to abiotic stress, the method comprising: (a) stably transforming a plant cell with an isolated nucleic acid encoding a polypeptide selected from the group consisting of (i) a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4; (ii) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; and (iii) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4. In a further embodiment, the invention further includes regenerating a stably transformed plant from the stably transformed plant cell; and expressing the nucleotide sequence in the plant.

[0267] Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

EXAMPLE 1

EXPERIMENTAL PROCEDURES

Plant Material

[0268] All the *Arabidopsis* lines used in Examples 1 and 2 were Columbia ecotype. *linkl-1* (SALK_024353), *linkl-2* (SALK_063322), *linkl-3* (GABI_044A09), *Unk2-1* (GABI_484F07), *Unk2-2* (SALK_116103) and *Unk2-3* (SALK_141609) mutants were obtained from the *Arabidopsis* Biological Research Center (ABRC) and the Gabi Kat T-DNA insertion collections, respectively. The *linkl;link2* double mutant was obtained by crossing the simple mutants *linkl-1* (At5g64170) and *Unk2-1* (At3g54500). The clock and photoreceptor mutants used in this study were *prr7-3;prr9-1* (31), *tocl-101*(42), *phyA-211;phyB-9*(43) and *cryl-bl04;cry2-l*(44).

Growth Conditions and physiological measurements

[0269] For flowering time experiments, seeds were stratified for four days in the dark at 4°C and then transferred to soil. The plants were grown at 22 °C under long days (LD, 16h light/8h dark cycles; 70 $\mu\text{moles m}^{-2}\text{V}^{-1}$ of white light), short day (SD, 16h light/8 h dark cycles; 140 $\mu\text{moles m}^{-2}\text{V}^{-1}$ of white light) or continuous light (LL, 50 $\mu\text{moles m}^{-2}\text{V}^{-1}$ of white light), depending on the experiment. Flowering time was estimated by counting the number of rosette leaves at the time of bolting. For hypocotyl length measurements, wild-type, *linkl*, *Unk2* and *Hnkl;link2* seedlings were grown on 0.8% agar under complete darkness, continuous white light (LL), short day (8h light/16 h dark) photoperiods, continuous red (100 $\mu\text{moles m}^{-2}\text{V}^{-1}$) or continuous blue light (10 $\mu\text{moles m}^{-2}\text{V}^{-1}$), and the final length of the hypocotyls was measured after 4 days. Light effects on hypocotyl elongation were calculated normalizing hypocotyl length under each light regime relative to hypocotyl length of the same genotypes under constant dark conditions. For leaf movement analysis, plants were grown under 12h light/12h dark cycles, transferred to continuous 20 μm white fluorescent light at 22 °C, and the position of the first pair of leaves was recorded every two hours for 6 days using digital cameras and determined using Image J software.

Expression analysis of *LNK* genes in *link* mutants:

[0270] Plants were grown in soil for 3 weeks and then samples from 4 plants per genotype were collected to reduce biological variation. RNA was obtained using TRIzol reagent (Invitrogen). Resuspended 1µg of RNA was treated with RQ1 RNase-Free DNase (Promega) and subjected to retrotranscription with M-MLV (Invitrogen) and oligo-dT according manufacturer's instructions. The transcripts abundance of LNKs genes was determinate by PCR in the different mutant lines.

Subcellular localization of LNK 1

[0271] Subcellular localization of LNK1 was analyzed infecting three-week old *Nicotiana benthamiana* leaves with *Agrobacterium tumefaciens* GV3101 carrying the 35S:LNK1:YFP construct as described by (45). The 35S:ZN⁷:YFP construct was assembled using the coding region of the *At5g64170.2* gene model, according to the *Arabidopsis* Information Resource (TAIRIO), through Gateway technology (Invitrogen) using the pEarly gate 101 as destination vector (46). The leaves were infected from the abaxial side using a syringe without needle, and 3.5 days later different discs of infected leaves were analyzed. Imaging was completed using a LSM 5 Pascal Axioplan2 confocal microscope equipped with an argon ion (488nm) excitation laser system and a X20 objective lens. Image manipulation was completed with LSM image browser software. Similar localization patterns were observed in different infected leaves.

qRT-PCR

[0272] For time-course analysis, 15 day-old plants were grown under 12h light/12h dark cycles at 22°C and then transferred for three days to continuous white light at 22°C. Samples were collected every 4h for two days, starting 24h after the plants were transferred to constant conditions. Total RNA was obtained from these samples using TRIzol reagent (Invitrogen). 1µg of RNA was treated with RQ1 RNase-Free DNase (Promega) and subjected to retrotranscription with M-MLV (Invitrogen) and oligo-dT according to manufacturer's instructions. Synthesized cDNAs were amplified with FastStart Universal SYBR Green Master (Roche) using the Mx3000P Real Time PCR System (Agilent Technologies)

cycler. *PP2A* transcript was used as house-keeping gene. Primer sequences and PCR conditions are available upon request.

Phylogenetic Analysis:

[0273] Gene homology search was performed using the protein sequence of *Arabidopsis thaliana* LNK1 (splice variant 2) as query in BLASTP tool (worldwideweb at phytozome.net/). Protein sequences were aligned using Clustal Omega program. A maximum likelihood phylogenetic tree was constructed using SeaView Version 4 (Gouy, M. *et al*, 2010). Bootstrap analysis with 1000 replicates was performed. Gouy, M., G RNA-seq reads were estimated using Illumina Pipeline version 1.3. Reads were quality-filtered using the standard Illumina process and demultiplexed with 2 allowed barcode mismatches. Sequence files were generated in FASTQ format. Table 1 provides a summary table of main read count statistics. Sequence data was made available from GEO repository (accession number: GSE43865). The TopHat suite [Trapnell C, Pachter L, Salzberg S. "TopHat: discovering splice junctions with RNA-Seq". *Bioinformatics* doi:10.1093/bioinformatics/btpl20] was used to map reads to the *A. thaliana* TAIR10 reference genome [The Arabidopsis Genome Initiative 2000]. Along with the pre-built *A. thaliana* index, the reference genome was downloaded from ENSEMBL (December 2012). Default values for TopHat parameters were used with the exception of maximum intron length parameter, that was set to a value of 5000 nt following estimated values reported in [Hong *et al*, *D. Mol. Biol. Evol.* 23:2392-2404 (2006)]. Transcript abundances were estimated using Cufflink [Trapnell *et al.*, "Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks", *Nature Protocols* 7:562-578 (2012)] with default parameter setting.

Table 1

	Minimum library size	Maximum Library size	Median Library size	Correlation between replicates	Correlation between conditions
LL	14074859	18297931	15855443	(0.9893, 0.9986)	(0.9677, 0.9986)
Time Course	9176985	13408078	11350789.67	(0.8758, 0.9995)	(0.827, 0.9995)

Microarray analysis

- [0274] Total RNA was extracted from aerial tissue of 15 day-old wild-type plants grown under 12h light/ 12h dark cycles at 22°C. Triplicate samples were collected after a 1h white light treatment (70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the middle of the night or subjective day, and control samples kept in darkness were collected at the same time. Each biological replicate consisted of 10-12 plants, to reduce biological variation. Total RNA was processed and hybridized to Affymetrix GeneChip Arabidopsis ATH1 Genome Arrays, according to the manufacturer's instructions. Data were analyzed using MA 10, and ANOVA was used to identify differentially expressed genes (q-value < 0.0005; fold change >2). Genes were then classified in six groups, depending on whether they were induced or repressed by light, and whether the effect of light was proportionally larger, smaller or relatively similar during the night compared to the subjective day.
- [0275] Growth conditions, cDNA library preparation and high-throughput sequencing protocols
- [0276] Seeds were sown onto solid Murashige and Skoog medium containing 0.8% agarose, stratified for four days in the dark at 4°C, and then grown at 22°C in continuous light or long day conditions (16h light and 8h dark cycles) depending on the experiment. Nine-day-old seedlings were harvested and total RNA was extracted with RNeasy Plant Mini Kit (QIAGEN) following the manufacturer's protocols. To estimate the concentration and quality of samples, NanoDrop 2000c (Thermo Scientific) and Agilent 2100 Bioanalyzer (Agilent Technologies) with Agilent RNA 6000 NanoKit were employed, respectively. Libraries were prepared following the TruSeq™ RNA Sample Preparation Guide (Illumina). Briefly, 3 μg of total RNA were polyA-purified, fragmented and first-strand cDNA synthesized by reverse transcriptase (Superscript II-Invitrogen) and random hexamers. This was followed by RNA degradation and second strand cDNA synthesis. End repair process and addition of a single 'A' nucleotide to the 3' ends allowed ligation of multiple indexing adapters. Then, an enrichment step of 12 cycles of PCR was performed. Library validation included quality control and quantification. Samples were pooled to create 17-multiplexed DNA libraries and they were single-end

sequenced with an Illumina Genome Analyzer II kit on the Illumina GAIIx platform, providing 100bp sequences.

RNA-seq reads processing

[0277] RNA-seq reads were estimated using Illumina Pipeline version 1.3. Reads were quality-filtered using the standard Illumina process and demultiplexed with 2 allowed barcode mismatches. Sequence files were generated in FASTQ format. Table 1 provides a summary table of main read count statistics. Sequence data was made available from GEO repository (accession number: GSE43865). The TopHat suite [Trapnell *et al.*, "TopHat: discovering splice junctions with RNA-Seq". *Bioinformatics* doi:10.1093/bioinformatics/btpl20] was used to map reads to the *A. thaliana* TAIR10 reference genome [The Arabidopsis Genome Initiative 2000]. Along with the pre-built *A. thaliana* index, the reference genome was downloaded from ENSEMBL (December 2012). Default values for TopHat parameters were used with the exception of maximum intron length parameter, that was set to a value of 5000 nt following estimated values reported in [Hong *et al.*, *Mol. Biol. Evol.* 2006 23:2392-2404]. Transcript abundances were estimated using Cufflink [Trapnell *et al.*, *Nature Protocols* 7:562-578 (2012)] with default parameter setting.

RNA-seq data processing and differential expression analysis

[0278] The normalization and statistical analysis of count data was performed using the package *edgeR* (version 3.04) [M.D. Robinson, *et al.*, "edgeR: a Bioconductor package for differential expression analysis of digital gene expression data", *Bioinformatics*. 2010 January 1:26(1):139—140] from the Bioconductor library (version 2.11) of the R (version 2.15) statistical analysis framework. The package *easyRNAseq* [N. Delhomme *et al.*, "easyRNASeq: a Bioconductor package for processing RNA-Seq data.", *Bioinformatics*, 2012] was used to generate read count tables at the gene level. A non-specific prefiltering step was conducted in order to filter out genes with less than two counts per million reads in at least three samples resulting in 21143 (22628) - out from 33602 genes - that were considered for further analysis, in the Col vs *linklink2* (time-course)

experiment. Differences in the RNA composition for each library, was taken into account through a normalization step using the trimmed mean of M-values (TMM) methodology. Estimates of the dispersion parameter for each transcript were obtained in a two-step procedure: a first trended dispersion estimation - that depends on transcript abundances - was further refined with an empirical Bayes procedure for each transcript, with expression levels specified by a log-linear model [Robinson *et al.*, "Moderated statistical tests for assessing differences in tag abundance", *Bioinformatics* 23:2881-2887]. In order to assess for differential expression a negative binomial generalized log-linear model was fitted to the read counts for each gene. We found 806 genes differentially expressed between *link1;link2* and WT Col conditions (Benjamini-Hochberg FDR adjusted p-value < 0.05). On the other hand, for the more complex time-course experiment, statistical significance tests were performed for mean differences between WT Col and *link1;link2* mutant timecourses, along with genotype-time interaction contrasts for time points: 6hs, 10hs, 14hs, 18hs, and 22hs. We focused on genes that simultaneously fulfilled the following two conditions. Transcripts should present large (fold-change larger than 50%) and significant (Benjamini-Hochberg FDR adjusted p-value less than 0.0001) *link1;link2* vs WT Col mean expression level differences along the time-course, and at least one significant (Benjamini-Hochberg FDR adjusted p-value less than 0.0001) genotype-time interaction contrast. In this way, a subset of 387 transcripts were identified and considered for follow-up analysis.

Expression profile clustering

[0279] For each transcript, concatenated WT Col and *link1;link2* time course profiles were considered. Clusters of similar expression patterns could be detected using a correlation based distance metric and a hierarchical clustering (complete linkage) procedure followed by a hybrid adaptive dendrogram cut step [Langfelder *et al.*, "Defining clusters from a hierarchical cluster tree", *Bioinformatics* 2008] in order to identify well-defined clusters.

EXAMPLE 2

LNK genes integrate light and clock signaling networks at core of
Arabidopsis oscillator

[0280] Light signaling pathways and the circadian clock interact to help organisms synchronize physiological and developmental processes with periodic environmental cycles. The plant photoreceptors responsible for clock resetting have been characterized but signaling components that link the photoreceptors to the clock remain to be identified. Here we describe a family of night-light inducible and clock regulated genes (LNK) that play a key role in the control of daily and seasonal rhythms in *Arabidopsis thaliana*. A genome-wide transcriptome analysis revealed that most light induced genes respond more strongly to light during the subjective day, which is consistent with the diurnal nature of most physiological processes in plants. However, a handful of genes, including the homologous genes *LNK1* and *LNK2*, are more strongly induced by light in the middle of the night, when the clock is most responsive to this signal. Further analysis revealed that the morning phased *LNK1* and *LNK2* genes control circadian rhythms and photomorphogenic responses. In addition, because they regulate the expression of a subset of clock and flowering time genes in the afternoon, they are required for photoperiodic dependent flowering. *LNK1* and *LNK2* themselves are directly repressed by members of the *TIMING OF CAB1 EXPRESSION (TOC1yPSEUDO RESPONSE REGULATOR (PRR1))* family of core-clock genes in the afternoon and early night. Thus, *LNK1* and *LNK2* integrate early light signals with temporal information provided by core oscillator components to control the expression of afternoon genes, allowing plants to keep track of seasonal changes in day-length.

[0281] The rotation of the earth around its own axis along with its movement around the sun cause daily and seasonal oscillations in light intensity on our planet. The profound impact of these environmental changes on biological processes strongly contributed to the evolution of circadian clocks (1). Therefore, it is not surprising that circadian and light signaling networks are intimately connected. Indeed, while circadian rhythms normally persist in the absence of environmental

cues with a period of approximately 24h, light/dark cycles entrain the clock and thereby ensure appropriate phasing of circadian rhythms in relation to changing sunrise and sunset throughout the year (2).

[0282] The effect of light on the clock is mediated by specific photoreceptors, such as phytochromes, cryptochromes and members of the ZEITLUPE protein family in plants (3-5), cryptochromes in flies (6), or melanopsin in mice (7). In mammals, cryptochromes are central clock components rather than circadian photoreceptors (8,9). In contrast, neither cryptochromes nor phytochromes are essential for circadian oscillations in plants (10-12), but circadian regulation of phototransduction pathways generates tight links between these two signaling networks (13, 14). This phenomenon, known as gating, was originally described for the light regulated activity of the promoter of the *CHLOROPHYLL A/B BINDING PROTEIN II (CABII)* gene (15). *CABII* expression is acutely induced by red light pulses, but the effectiveness of this treatment oscillates during a 24-hr day, with maximal effects when photosynthetic activity is expected to be at its peak during the day, and minimal effects during the night (15-17). Clock regulation of light signaling also influences physiological processes such as germination (18), stem elongation (19, 20), and the clock itself (16, 21, 22). In plants grown under light/dark cycles and then transferred to constant darkness, brief light pulses are most effective in resetting the phase of circadian rhythms during the night rather than during the subjective day (*i.e.* the phase that would have been illuminated if the plants were kept under light/dark cycles) (21-23). This phenomenon is shared across kingdoms suggesting that it is critical for the appropriate adjustment of circadian rhythms to the environment (24).

[0283] Despite the importance of the interactions between light and the circadian clock in the control of biological activities in plants, a comprehensive analysis of these interactions has been lacking. Light signaling and circadian networks operate primarily by transcriptional control (13, 25-28). In order to characterize transcriptional interactions in *Arabidopsis* we employed a genomics approach. This analysis allowed us to identify a new family of light and clock regulated morning genes. These genes control both the pace of circadian rhythms and the

photoperiodic regulation of flowering time, apparently by promoting the expression of a subset of core-clock and clock-output genes in the afternoon.

RESULTS

Light treatments are more effective during the subjective day

[0284] To investigate if and how time of day affects light regulation of gene expression at a global level, we evaluated the response of the *Arabidopsis* transcriptome to a 1h light treatment given either in the middle of the subjective day or in the middle of the night (Figure 1A). The effect of a brief light pulse in the middle of the subjective day should reveal genes acutely regulated by light on a daily basis throughout the year. In contrast, the night-light treatment simulates either the earlier sunrise or later sunset that plants normally experience on a typical spring day at high latitudes, and may reveal genes involved in clock resetting and/or seasonal adjustment.

[0285] Many light-regulated genes showed a stronger response to the light pulse given during the subjective day compared to a similar treatment given during the night (Figure 1B). Among a total of 2237 light induced genes, 1537 responded at least twice as strongly to the light pulse given in the middle of the subjective day, and only 65 genes showed a stronger response during the night (data not provided). Thus, almost 70% of light-induced genes behaved similarly to what had been reported for *CABII* (15). This group was enriched in genes associated with metabolism, chloroplast components, responses to environmental stimuli, as well as with abiotic and biotic stress responses (data not provided). The influence of time of day was less pronounced for light-repressed genes. Among a total of 1672 light-repressed genes, only 607 responded at least twice as much during the subjective day compared to the night, and 78 showed the opposite response (data not provided). The group more strongly repressed by light during the subjective day was mostly enriched in genes involved in amino acid catabolism, while those more responsive to light during the night were associated with hormonal regulation, among other processes (data not provided).

Night-light is more effective in inducing the expression of a subset of core-clock genes

[0286] Clock entrainment is most sensitive to light pulses given during the night, a treatment that simulates seasonal changes in day-length. Consistent with this, the subset of 65 genes responding at least twice as strongly to the night-light treatment was significantly enriched in clock genes, a phenomenon that was specific for this particular class of light regulated genes (data not provided). Clock genes are also enriched among those with oscillations that are robust to different experimental conditions, such as continuous light, continuous darkness, short days, long days, temperature cycles, etc. (26). Thus, we reasoned that the list of genes that are more effectively induced by night-light and also cycle under multiple conditions should contain new candidate clock regulators. Thirteen genes fulfilled both criteria, a 30-fold enrichment over what would have been expected by chance ($p < 1 \times 10^{-15}$, hypergeometric distribution) (Figure 1C). This group included the clock genes *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)*, *PRR7*, and *GIGANTEA (GI)*, six genes involved in the control of stem elongation, flowering time or photosynthesis, as well as four genes that constitute a new family of plant specific proteins, which we named *LNK 1-4*, for night-light inducible and clock regulated genes 1-4 (Figure 1D).

[0287] LNK1 (AT5G64170) and LNK2 (AT3G54500) proteins are of medium size, about 66 kDa, with 35% sequence similarity across their entire length. LNK3 (AT3G12320) and LNK4 (AT5G06980) proteins are smaller, with 60% sequence similarity, and with a third of conserved positions also shared with LNK1/LNK2 (Figure 6). *LNK* homologs can be found throughout land-plants, including non-vascular plants. *LNK3* and *LNK4* appear to be the result of a recent duplication event within the Brassicaceae. (Figure 7). Since *LNK1* responded most strongly to the night-light treatment (Table 6), we focused on *LNK1* and its closest homolog, *LNK2*. qRT PCR analyses of wild-type (WT) and mutants indicated that these two genes were induced by a light pulse in the middle of the night through the phytochrome family of red/far-red light photoreceptors, and that they were rhythmically expressed with maximum levels in the subjective morning (Figure 1 E and F). Although *LNK1* and *LNK2* expression is highly correlated, a close

inspection of their expression patterns across different conditions using publicly available microarray data revealed some differences. In particular, the phase of *LNK1* expression is delayed by four hours in comparison to that of *LNK2* under 8 or 12 hour photoperiods, with *LNK1* reaching maximum levels in the middle rather than the beginning of the photoperiod under these conditions (Figure 8).

LNK1 and *LNK2* regulate light signaling and biological timing

[0288] To determine if *LNK1* and *LNK2* affect light and clock regulated developmental and physiological processes, several mutants with T-DNA insertions in these two genes were identified and characterized in detail (Figure 9). An early developmental phenomenon under control of light and the circadian clock is the elongation of the hypocotyl, the embryonic stem. No significant differences in hypocotyl length were observed among WT plants and *link1*, *link2* or *Unk1;link2* mutants grown in complete darkness (Figure 10). In contrast, *link1* mutants had longer hypocotyls than WT plants under continuous white light (Figure 2A), under continuous red light or under 8 hr white-light/16 hr dark cycles (Figure 10). *Unk2* mutants also had longer hypocotyls than WT plants in red light (Figure 10), whilst the differences in hypocotyl length were not statistically significant under most other light conditions (Figures 2A and 10). The *link1;link2* double mutant had significantly longer hypocotyls than either single mutant or WT seedlings under most light conditions tested, and the phenotype was stronger under red or white light than blue- light (Figures 2A and 10). Taken together, these results indicate that *LNK1* and *LNK2* mediate light inhibition of hypocotyl elongation, in particular that triggered by the phytochrome family of red/far-red light photoreceptors.

[0289] Another physiological process that depends on interactions between light signaling and the circadian clock is photoperiod dependent flowering (29). *link1;link2* double mutant flowered later than WT plants, *link1* or *Unk2* single mutants under long days (LD; 16 hr light/8 hr dark) (Figure 2B and 2C). Under short days (SD; 8 hr light/16 hr dark), no delay in flowering was observed (Figure 2D), confirming that *LNK1* and *LNK2* are indeed only required for long-day acceleration of flowering, rather than the transition to flowering *per se*.

[0290] To observe circadian behavior directly, we monitored the circadian rhythm of leaf movement in WT plants, *linkl*, *Unk2*, and *linkl ;link2* mutants by time lapse photography. Leaf movement of *Unk2* mutants had longer circadian period than WT or single *linkl* mutant plants (Figures 2E and 11), and the *linkl ;link2* double mutant was even more strongly affected (Figures 2F and 11). Similar photomorphogenic and circadian phenotypes were observed in two additional mutant alleles of *LNK1* and *LNK2* (Figure 11), confirming that these two genes play important and partially redundant roles controlling light and clock regulated processes in *Arabidopsis*.

LNK1 and LNK2 activate clock controlled genes with afternoon peak

[0291] LNK proteins lack known functional domains, but transiently expressed LNKLYFP localized to the nuclei of *Nicotiana benthamiana* cells, suggesting a role in the regulation of gene expression (Figure 3A). To identify genes controlled by LNK1 and LNK2, we compared the transcriptome of WT and *linkl ;link2* mutant plants using RNA-seq. In plants grown under constant light and temperature, we found 806 genes differentially expressed using an FDR adjusted p-value of 0.05 as cut-off (data not provided). Genes down-regulated in *linkl ;link2* mutants were significantly enriched for genes that peak in LD at Zeitgeber time 10 (ZT10), *i.e.* 10 h after lights-on. Up-regulated genes were slightly enriched for genes that peak late at night (Fig 3B).

[0292] To learn more about LNK1/LNK2 target genes, we characterized the daily transcriptome of LD-grown WT and *Unkl;link2* mutant plants. Using stringent criteria aimed at identifying genes with altered overall mRNA levels, and not simply changed temporal patterns of expression, we identified 387 genes that differed between WT and *Unkl;link2* mutant plants. A cluster analysis revealed that most of the genes down-regulated in *Unkl;link2* mutant oscillated in WT plants with peak expression in the afternoon or early night (Figure 12), with the largest cluster peaking at ZT10 (Figure 3C), providing independent support for the initial phase enrichment analysis, which had suggested that LNK1/LNK2 activity is maximal in the afternoon (Figure 3B).

[0293] To identify genes likely to be responsible for the phenotypic defects in *link1;link2* mutants, we focused our analysis on the 113 down-regulated and the 47 up-regulated genes in both RNA-seq data-sets. Down-regulated genes included two core-clock genes, *PRR5* (Figure 3E) and *EARLY FLOWERING 4 (ELF4;* Figure 3F), which were present in the cluster of genes with peak expression in WT plants at ZT10 (Figure 3C), and might be primary targets of LNK1/LNK2 activity. Other clock and light signaling genes were also miss-regulated in *link1;link2* mutants (data not shown). However, these genes were affected more subtly, suggesting that they are possibly secondary targets of LNK1/LNK2 activity. Down-regulated genes also included the flowering time genes *FLAVINBINDING KELCH REPEAT F-BOX 1 (FKF1;* Figure 3D), which was also present in the cluster of genes with peak expression at ZT10 (Figure 3C), as well as *FLOWERING LOCUS T (FT)* and *SUPPRESSOR OF CONSTANS OVEREXPRESSION 1 (SOCI)* (Figure 13). All three genes are positive regulators of flowering time, with *FKF1* acting upstream of *FT* and *SOCI*. Therefore, the late flowering of *link1;link2* mutants under LD is likely due to reduced *FKF1* expression, which in turn leads to reduced *FT* and *SOCI* mRNA levels (Figure 13).

PRR5 expression is severely affected in *link1;link2* mutants under free-running conditions

[0294] To investigate the effect of LNK1 and LNK2 on the central clock in more detail, we analyzed the expression of clock components in plants that had been entrained under 12h light/12h dark cycles at 22°C and were then transferred to constant light and temperature (*i.e.* free-running) conditions. The plant circadian clock is based on interlocking transcriptional feedback loops in which the morning clock factors CCA1 and **LATE ELONGATED HYPOCOTYL (LHY)** repress the expression of evening clock genes such as *TOC1/PRR1* (30). In addition, CCA1 and **LHY** also promote the expression of *PRR9* and *PRR7(3\)* which, sequentially with *PRR5* and *TOC1/PRR1*, repress *CCA1* and *LHY* expression throughout the remaining of the day and early night(32-34). Finally, **ELF4, ELF3** and **LUX ARPvHYTHMO(LUX)** proteins form a complex during the evening that represses *TOC1* expression, allowing *CCA1* and *LHY* mRNA levels to start rising again in the late part of the night (19).

[0295] We observed a substantial delay in the phase of *CCA1*, *LHY*, *PRR9* and *PRR7* expression during the second day in continuous light. The delay increased to eight hours on the third day, consistent with a lengthening of circadian period by approximately 2.5 hours in the *link1;link2* mutant compared to WT plants (Figure 4 A-D). In contrast, the phase of *CCA1* expression was delayed less than four hours in *link2* single mutants on the third day (Figure 14), consistent with an increase in period length of rhythmic leaf movement of approximately one hour (Figure 2E).

[0296] In spite of the strong effect of *LNK1* and *LNK2* on the period and/or phase of circadian oscillations, the overall mRNA levels of morning and early afternoon clock components were largely unaffected in *Unkl;link2* mutants. In contrast, significant down-regulation coupled with a much longer delay in the phase of expression, *i.e.* close to 12 hours on the third day, was observed for *PRR5*, which is normally expressed in the afternoon (Figure 4E). A similar phase delay, but without differences in the overall mRNA levels, was observed for the *TOC1/PRR1* gene that is expressed slightly after *PRR5* (Figure 4F). Taken together, these results suggest that *LNK1* and *LNK2* act initially as activators of a subset of genes with peak expression in the afternoon, such as *PRR5* (Figures 3F and 4E), *ELF4* (Figure 3F) and *FKF1* (Figure 3D), which later affect the rhythmic expression of other core-clock and clock-output genes.

LNK1 and *LNK2* are repressed by members of the *TOC1/PRR1* family of clock genes

[0297] Many clock-regulated genes with peak expression in the morning are repressed throughout the day and during the early night by members of the *TOC1/PRR1* family of clock proteins. To determine if the *LNKs* were regulated by members of this protein family, we reexamined data describing *TOC1/PRR1* and *PRR5* binding sites in the *Arabidopsis* genome using chromatin immunoprecipitation followed by sequencing (ChIP-seq) (33, 35). Indeed, we found that the regulatory region of *LNK3* was directly bound by *TOC1/PRR1* (33). Furthermore, *PRR5*, *PRR7* and *PRR9* were also found to bind directly to the regulatory regions of *LNK 1-4* (35).

[0298] To evaluate the functional consequence of the binding of these factors to *LNK1-4* promoters, we compared the expression patterns of *LNK1* and *LNK2* in WT, *tocl*, or *prp9;prp7* mutant plants, entrained under light/dark cycles and then transferred to constant light conditions. In addition to progressively larger delays in the phase of the circadian oscillations of *LNK1* and *LNK2*, their mRNA levels at the trough of the circadian oscillations were increased in the *prp9;prp7* double mutant (Figure 5A and B). A larger overall increase in *LNK1* and *LNK2* mRNA levels, coupled with progressive phase advances, was observed in the short period mutant *tocl* over the entire time course (Figure 5C and D), indicating that TOC1 is a direct repressor of these genes.

DISCUSSION

[0299] Light and the circadian clock interact to regulate many biological processes in plants, such as flowering time (29) and stem growth (19, 20). In addition, this interaction is also required for robust functioning of the circadian clock itself (16, 21, 22). Our genome-wide analysis revealed that these physiological interactions are mirrored by global interactions at the transcriptional level. In particular, we found that 70% of light induced genes responded more strongly to a light pulse during the subjective day than at night, most likely optimizing the energy spent on light-dependent biological processes that have maximal activity at midday, when light intensity is at its peak under natural conditions. At the same time, a light stimulus during the night preferentially promoted the expression of certain key clock components, consistent with the general observation that light present at the beginning or end of the photoperiod adjusts the circadian clock to seasonal changes in day-length (2, 24).

[0300] The characterization of genes that are preferentially induced by light at night and are also rhythmic across multiple conditions led to the identification of *LNK* genes, a partially redundant family of plant-specific genes that control photomorphogenic and photoperiodic responses, as well as circadian rhythms. *LNK1* and *LNK2* are regulated by the phytochrome photoreceptors and predominantly affect responses to red light, pointing to an important role in phytochrome signaling. In turn, they are expressed rhythmically with peak

expression in the morning or at noon, most likely as a result of their repression by members of the TOC1/PRR1 family of core clock regulators during the afternoon and early night. Thus, LNK1 and LNK2 link phytochrome and circadian signaling to regulate many physiological processes, including time keeping by the clock itself.

[0301] A comparison of *LNK* genes with other morning expressed clock genes is informative. Like *LNK* genes, *CCA1* and *LHY* are light induced genes whose mRNAs reach peak levels in the early morning. *CCA1* and *LHY*, however, lengthen the period of circadian rhythms, while *LNK1* and *LNK2* shorten it (36, 37). In contrast, *PRR9* and *PRR7* are similar to *LNK1* and *LNK2* in that they are expressed during the morning and early afternoon, are induced by light, and that they decrease period length and promote flowering (31). However, different from *LNK1* and *LNK2*, which at least under constant light do not seem to be required for normal *CCA1* and *LHY* expression (Figure 4), *PRR9* and *PRR7* are repressors of *CCA1* and *LHY*(31).

[0302] LNK1 and LNK2 are plant-specific proteins without recognizable functional domains. This is reminiscent of the clock components ELF3 and ELF4, which only very recently were shown to participate in an evening phased protein complex that represses the expression of *TOC1* and *PRR9* (19). We note that *LNK1* and *LNK2* mRNAs reach maximum levels in the morning or at noon, but that their positive effects on gene expression appear to be concentrated in the afternoon. Together with the nuclear localization of LNK1, this suggests that LNK1 and/or LNK2 are regulatory proteins under post-transcriptional and/or post-translational control, similar to several other clock proteins.

[0303] In summary, our work together with previous results support a model in which light perceived through phytochromes activates the expression of the *CCA1* and *LHY* transcription factors (40), as well as that of the LNKs, in the early morning. *CCA1* and *LHY* then promote the expression of *PRR9* and *PRR7* (31), while LNK1 and LNK2 act at later during the day to activate clock genes with peak expression in the afternoon, such as *PRR5* and *ELF4*. Simultaneously, members of the TOC1/PRR family repress these morning genes throughout the afternoon and beginning of the night (32-34, 41). Finally, the progressive reduction

in TOC1/PRR levels due to the evening complex (19) leave *CCA1*, *LHY* and the *LNK* genes poised to respond again to light signals that reset the clock every morning (Figure 5E).

EXAMPLE 3

Identification of LNK homologs in *Oryza sativa*

[0304] Ortholog proteins to each of the 4 known *Arabidopsis thaliana* LNK proteins (LNK1, At5g64170; LNK2, At3g5400; LNK3, At5g06980; LNK4, At3g12320) were searched in rice using the OsGDB BlastP service of PlantGDB (<http://www.plantgdb.org/OsGDB/cgi-bin/blastGDB.pl>). The protein query sequences were obtained from the longest isoform of each gene from The Arabidopsis Information Resource, release 10 (TAIR10, <http://-wwwv'.arabidopsis.org/>). The coding sequences of the *OsLNK* genes and the corresponding proteins are the following:

[0305] >OsLNK1_gnl|OsGDB|LOC_Os03g27019.2 (SEQ ID NO: 17)
 ATTCTTTTACTTGCTGGCTACTACTACTACTTGCCTCCACGCCCTCATC
 CCATCGACCCACCCATCTCTCCTATTCTCTCTTCAAAAAAAAAATCAGGA
 AATTTAATCGCGAGGAGGAGAGAGAAAAGAAAAAAAAAAGGAAATTC
 GCAGGAGAAATCCCTGGAAGCCTCTAGAGACCGCGCGAACAGGAGAC
 CCCGAGGAGCAGAGCAGAGCAGAGCAGAGGCGGCCAGCTTTGGGA
 GCTCTCGGATTGATCAAGAACTGGGGATCCTGAGTTGAATATGATATC
 AAATCCGGTGAATTGGTACAGTTTCGGTCCCGGATAGAATTTGTAGTGA
 TGCCAGATTGGAGGGTGGGCGAGTTTGAAGGTAAGCTCAAGGATGGAT
 TTGCTCGGAGCAATAATAGCGAACATGAAAATGGAGCCGGGACTGTGA
 GTATATCCAGCAAGAAGTCGAAGCATCGGGTTGACAGTGAAAAAAAAAC
 CCCATGTTGACATCTCTGGAGTGATTGATTCAGATTCGCAGAAATGTAA
 TTCAGAGCAAATCCATTCAGCTAATGGGATAGTATCCCGGGATGTCAA
 CCATGATCACATAGAAAAGTCAAAGTTGAATCAAATGATTTCCCTTG
 AATACTATCTCTGAAACAAGATATCCAACAGACAATTGGAACAGTTCT
 CAGTTTGCTCTGAGCAATGATGGTTCACCTGTTCTCAATAATCAGAGTA
 CTCCACAAACTGGTCATGGCTATGGAGATAATGATCTAACCTACATTGA

TTGGCCTGCTATTGATAATTTTGAGGACGTTCGACAACCTTATTCAGGAGG
TGTGATTCCACGTACGGTCAGCAGCAACTTCCGAACACGGATGAACTG
TCATGGATTCCCTTCTTCAGATGCCATGTACTCTTCTGATGTTGCTATGCA
ACCAGGGTTTGAATCCTCGTATTCTGATTATGGTATCCTGGATGACCTC
TCCGCATTCAATTGTACGGAAGATAAATCCCTGACAACAGCTGATCCTT
CAAGTGCTGTATGTGACGAGCAATTTCGATGACTCTTATCTGTTCAATGA
GCAAAAACCTGAAGATGTTTATGGCGAACAGGCTTACCAAAGGGATGC
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AGATATGATTGGAAATCGATATTCATCGGAAAATGCCATGGAACAGCC
TGAAGATCAGAAGTTTTCTATAGCTTCAGGTTCTCAACTAAGCTCTTCT
CAGAATTTATTGAACCAAAAAGAACCACTTGGATTCAACTTCACCAAGT
AACATAACTTCTGAGTCCTATCCAGAAAGAAATTGCCAGATCATTCCAT
CTGGGGCTTCCTTTGCAGAGAGAACTTGAAGGTGCAGAAGAAAGTGG
CCAGTTCGGCATCAGGACAACCTTATCAGTGATAATGTGACTGGACATC
CTGGTCATCAAACATTGACAAGAAGGGCTTCATATCCTTGTGAGAATC
ATGAAATTGGAAAGAGGTCCTTAGGAAAAAGAGGTTTGGGTCATTCTG
ATGTTGCAATGGGCACCTCAATGGTAGTTGATGGGTCCTTCGTATCGTC
TATTTCTTCTGATAACTCAGTTGAAGAGAACAGCTTTCGGCAGCTTCAG
GATGCAGTTAGTCAGTTGGATGTGAAAACAAAAATGTGCATTTCGAGAT
GGGTTATACCGCCTGGCAAGAAGTGCGCAGAATAGGCAAGTCTTCCCA
AATACAATGAACAACAATGGAGATAGCCACAATGTAAAGGACATGCA
AAATGCAGAACTTCGGGAAAGTTTGTGATCCTGGGAGCATCGAGAC
ACAGACAAATCCAATTGATCGGTCCATAGCTCTGTTGCTTTTCCATCAG
CCATCAGAGCATGTCACTGGAGCTGTTGATGAAGCGGCTTCACTAAAG
TCACATAATGATAACCACCAGGCTGCTGCTAAAAACCAAAGAGTGATG
CATGCTTCATCTGTACATTCACCAAGAGGGCAGGGAGACCCCATGGAT
GCAAAATCTTGCAGAAACAACCTGAAGTTATCTCATGAAGCTTCAACAA
CTGTGACTGTTAATATTGTACTTGTGCTGCAAACCTTCTCCGTTTTGA
TCCGATCATGCAGTTAGCAGCTGTGTAGTGTGTACTACTACTCTAGTCG
ATTAGTCAGGTACAGGCAAACCTTGTAAACCAATTTAACAAGTTAGAT
TGGCTTTTGACCATTAGCTTTGTATCTTAATTATTGAGAAGGTCGTCTGT
GCCGTGGCCATTCGTGTCTGTTCTCACTCACATTTTGTGTAATCTCAAAC

ATAGATTTTTTTTTCTGTTTTAGCTAACTATTTTTATTTCTGAACTAA
CACCATTTTCATGTTGTAAAACAAGCCAACCTCTGTACTTCTGTTTTAA
TGATTGCTGGGGTGTGTTACATGTCAGTCATTC

[0306]

>OsLNK2_gnl|OsGDB|LOC_Os01g31360.3

ATGCGCCCCCTCCACACGTGGCCCTCCCGCACCTCAACCCCGACCGCCC
GCCCAATCATCTTGCACCACGCGTCCGCCCCCTCCCTGGACTCCTCTCGG
TGGGTGGGAAGGGAGAGGAGAGAGGGAGAGGGGGAGAGGGAAGCG
CAGACGACGACAACACAGGTCGCGAGTGGAGGAGGAGGAGAAGCGAG
CAAGCGCAACGCGATTCTCCGCCGCGTCACCGAATTGGAGGATTTTCTG
CCCTTCCCTTTTGCCGTGGCTGGAGATGCAACCGTCGGGACCCGGGGG
GCAGCGCGTATCGGGCTCTCGTTCGGGTGCGGCCAGCGGCGGCCGC
CACCTGAGGAGGATGTTTCGAGTGAACGACGACCAGCAGCAGGGGATT
TTAGTGCTCGAAGAAGCAGCTGATGATTGTGAGCGAGCGAGCGAGGAT
GATAGGATTATTTCCAAGGAGGCTCACCTAGCAGAGAGAGTCTTGTGG
GCATTTAGATGCTACCGCGTGCGGTTGGGTTGTGCTAGGTCTCATAAGG
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[0308] >osLNK1_(Os03g27019.2)

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 RGQGDPMDAKSCRNN

[0309] >osLNK2_(Os01g3 1360.3)

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 SALVSVGDQKKNDEETDNIPGLTERSSSGQTEFPVLEKQPASQASEHYSAT
 QLDVESWPDLP LNATLDRNYSDDNIASTYLDFSSAPSLEKVTGNTTVQLD
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 ALIAIQQQQQFGQDGSQSDTMVPQAYSPKSKNPDSLGSVVVIDDNANKV
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 LAHSVSRQITSDRSSANKSNKDDDEVEDTSKRRSPASEAETNTNPIDRIV
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[0310] >osLNK3_(Os02g5 1760.1)

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 TKSILSSVSDTTSAEPLFLDQNNMANPINIQPPSKGRSSATLNHEALAC
 SSGEIERFSQHSDDVDFYFPDNVTSSERISGCEGLEAIFCTNQEMLAPTSSI
 MCDDEIVSSSTFSAPDLVATYVPRSMKRSHDPLNGTPDMILDEMAGNPLE
 MYFPPSLTAYEHPEHLNNTLTQTHQFPEGFAGDDVLKSADLQFLSKGKT
 SADLCVNPCSPILILEAVPVKDLGFHKLQEGMNQLDVASKARIRDALYRLA
 NCVEHRHRIASTTEWNQLGVMESSASKRWREIQMMNPMDRSVAQLLQ
 KPLHHKSPDPSALGIGP

EXAMPLE 4

Phylogenetic analysis of LNK genes in monocots

[0311] In addition to the AtLNK homologs in *Oryza sativa*, we also searched for orthologs in *Sorghum bicolor* and *Glycine max* using the BlastP algorithm of PlantGDB for sorghum (<http://www.plantgdb.org/SbGDB/cgi-bin/blastGDB.pl>) and soy (<http://www.plantgdb.org/GmGDB/cgi-bin/blastGDB.pl>), respectively.

[0312] The sets of ortholog proteins from *Arabidopsis thaliana*, *Oryza sativa*, *Sorghum bicolor* and *Glycine max* were aligned by MEGA6. The evolutionary history of taxa was inferred by the neighbor-joining method according to Saitou N

and Nei M. (Saitou N. and Nei M. (1987). *The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution* 4:406-425). The bootstrap consensus tree inferred from 1000 replicates [Felsenstein J. (1985). *Confidence limits on phylogenies: An approach using the bootstrap. Evolution* 39:783-791] is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method [Zuckerkandl E. and Pauling L. (1965). *Evolutionary divergence and convergence in proteins*. Edited in *Evolving Genes and Proteins* by V. Bryson and H.J. Vogel, pp. 97-166. *Academic Press, New York*] and are in the units of the number of amino acid substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.05). The analysis involved 23 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1028 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013). *MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution* 30: 2725-2729]. (Figure 15).

EXAMPLE 5

LNK function is conserved in dicots and monocots

[0313] To determine if the function of LNK genes is conserved between monocots and dicots, we compared the regulation and function of LNK genes in rice, a model monocot species, and Arabidopsis, a model eudicot species. For this, wild type rice plants and a rice mutant line with a T-DNA insertion in OsLNK2 (PGF_2C-50209.L, developed by Gynheung An, see Jeong *et al.* and Jeon *et. al* 2000) and gene expression patterns of rice LNK1 and LNK2 homologs under a long day (LD, 16 h light /8 h darkness) cycle were characterized. Fresh leaves were harvested every 4 hours on days 30 and 31 after germination for a total of 9

time points, starting at ZT2 (2 hours after lights on). Leaves were immediately frozen in liquid Nitrogen and stored at -80°C until sample processing. All samples were processed on the same day. Total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA (1 µg) was amplified with the dT18 primer by using the MMLV Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. The reaction product (2 µl aliquot) was then diluted 1/50 and used for qRT-PCR analysis. We carried out qRT-PCR in a total volume of 10 µl containing 2 µl of the reverse-transcribed product above, 0.25 mM gene-specific primers, and 5 µl FastStart Universal SYBR Green Master Mix Rox (Roche) on a Stratagene Mx3005P Real-Time PCR System according to the manufacturer's instructions. The measurements were obtained using the relative quantification method (Livak and Schmittgen, 2001) using the rice ubiquitin gene (OsUBQ) as an internal control. The expression of OsUBQ, OsLNK1, OsLNK2 and OsLNK3 was measured by qRT-PCR. As observed previously in Arabidopsis, OsLNK1 and OsLNK2 rice genes display diurnal oscillations in gene expression with peak levels in the early morning (Figure 16). Thus, similarly to what we observed in Arabidopsis, the expression of the rice LNK gene orthologs is regulated by the circadian clock.

[0314] Next we examined the molecular function of OsLNK2 in the control of the rice circadian clock as well as in the regulation of flowering time in this species using wild type rice plants and the mutant line PGF_2C-50209.L (Figure 17). Then, we grew plants from this mutant background side by side with wild-type plants under LD conditions (16 h light /8 h darkness). In Arabidopsis, LNK1 and LNK2 genes regulate flowering time under long day conditions promoting the expression of the flowering time gene FKF1. In rice, we found lower mRNA levels of OsFKF1 in the Oslnk2 mutant, indicating that OsLNK2 also functions promoting the expression of this gene. Thus, LNK genes have a conserved function in the control of FKF1, a key flowering time gene in rice and Arabidopsis.

[0315] Finally, we analyzed the role of OsLNK2 in the regulation of rice clock genes. In Arabidopsis, LNK1 and LNK2 genes accelerate the pace of the circadian clock activating the expression of clock genes peaking in the afternoon or early evening, such as PRR5. In rice, we found lower mRNA levels of OsPR95 in the Oslnk2 mutant, indicating that OsLNK2 also functions promoting the expression of this gene (Figure 17). Thus, LNK genes have a conserved function in the control of OsPR95, a key core clock gene in rice and Arabidopsis, indicating that the role of LNK genes in the control of the plant circadian clock is conserved between monocots and dicots.

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[0316] *LNK1* cDNA, genomic and protein sequence accessible at AT5G64170 (last modified April 27, 2007. The start codon (atg) at position 302 is double-underlined and in bold. *LNK1* cDNA sequence (AT5G64170.2; SEQ ID NO: 1):

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1  GTCAAAAAAA  AAAAAATAGG  AAGCCTTCAT  CGTCTTCTGC  CGAACATCTC
51  TAGAAAGTCC  TACACAGTTT  TTTTFTTCGA  TCTAGCTTCG  ATGAATTGAT
101  ACAATCTCTT  CTATCTGCTG  AGTTTTTTGT  TTTCTGGTAA  ACAATTTTAT
151  CTGTGGAGTT  TTCTGGAGTT  GCTAGTGGAA  GAATCTGTCT  CACAGAACCA
201  GAAATAGTGT  TTGTGGGAGT  TACATGTTTT  GCAATAGAGA  TGACATATCG
251  GGGTAATGTG  CATGATTTTA  GTGGTGATTA  AAGAGTCTGA  AGTTGGGAGG
301  AATGTCGGAC  TTGTACATTC  ATGAGCTAGG  CGATTATCTT  TCGGATGAAT
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401  GGTCAGTTTC  CAATTCTAGT  TAGTAACAGG  AAGAAAC GAA  GAAATGATGA
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601  ATTGGAGGAT  ACAAATA CTT  CAGATCATGG  TTTCAATGGT  GGCCACGTAG
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1451  TCAAAT CAT G  CACAATCAAT  CGAGAGCTTA  CAAGGTCCAA  CTGTTGATGA
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2151 AATGATGTCA AATAAAACTG GCTCTTTCAT TGCTGAAGCA TTCGAGATAT
2201 TGTTGTAAAC ACTAATCTCG ATTTTGGCTA AGCGAAATTG CTCAGGTTAG
2251 TFACTAAAAAT CCTGACTGCT TCTTCATTAG AATCATGGAT TTTGTACAC
2301 AGTTGTATTT ATGTGTTGAG ATAACCTTCT GGTCTAGATT TCGGTTGCGAA
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2401 AACCAGGCTT TTAAGATATG TTCTTTACCT TTGTAATCTG TTCAAGTTGT
2451 TAAGTAGAGG AAGAAGTAGA GACATAGCAA TTCTGTAGAT AAAGATGTTA
2501 GTTCGTTTTG TAATGCGATA AAATATTCTG CTGTGTATTT ACTCTCTTTT
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[0317] *LNK1* polypeptide sequence (AT5G64170.2; SEQ ID NO:2):

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101 LEDTNTSDHG FNGGHVDWE NFSTGDPMLC DTSAAATNDGV YNYSLNSIPD
151 AENDLSFFDN GDKEKNDLFY GWGDIIGNFED VDNMLRSCDS TFGLDSLNNNE
201 GDLGWFSSAQ PNEETAGAMT DDLKPKMLE NQRTAMLQVE DFLNNSEPNH
251 AVEDEYGYTI EDDSAQ GKSS QNVFDTSLQK KDILMLDVEA NLEKKQTDHL
301 HHLDGKSDGF SENSFTLQHS GISREIMDTN QYYPPSAFQQ RDVPYSHFNC
351 EQPSVQVSAC ESKSGIKSEN KPSPSSASNE SYTSNHAQSI ESLQGPVDD
401 RFRKVFETRA NLLPGQDMPP SFAANTKKSS KTDSMVFPDA APIQKIGLEN
451 DHRKAATELE TSNMQGSSCV SSWDDISLE ATFRQLQOV LEQLDVRTKL
501 CIRDSLRLA KSAEQRHHGG NRPEKGAGSH LVTGEADKYA GFMDIETDTN
551 PIDRSIAHLL FHRPSDSSLS SDNNVLSYKS HPMIPQPNS PSLRIEKQEE
601 TTELPRPEAEV VTSDDN
    
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[0318] *LNK1* cDNA sequence (AT5G64170.1; SEQ ID NO:5):

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1 GTCAAAAAAA AAAAAATAGG AAGCCTTCAT CGTCTTCTGC CGAACATCTC
51 TAGAAAGTCC TACACAGTTT TTTTTCGTA TCTAGCTTCG ATGAATTGAT
101 ACAATCTCTT CTATCTGCTG AGTTTTTGT TTTCTGGTAA ACAATTTTAT
151 CTGTGGAGTT TTCTGGAGTT GCTAGTGGAA GAATCTGTCT CACAGCTAGG
201 CGATTATCTT TCGGATGAAT TTCATGGGAA CGATGATGGT ATAGTGCCAG
251 ACTCAGCGTA TGAGGATGGA GGTCAGTTTC CAATCTAGT TAGTAACAGG
301 AAGAAACGAA GAAATGATGA TATGGGTAGT GGAACAAACC ATCTAAAGAG
351 TAATACTTTT ATCAAGAGAG AGGCAAACAT GTTAGGAAAA AATCCATGGC
401 CTGAGAAAGA TAGTGGTGGC TCTTCGGTTT CTCGTGATAC GGAACAGGA
451 AAAGATGTTC AGGATATGAC ATTGGAGGAT ACAAATACTT CAGATCATGG
501 TTTCAATGGT GGCCACGTAG ATGTGGTTGA AAATTTCTCC ACTGGGGATC
551 CCATGTTGTG TGACACTTCC GCTGCAACGA ATGATGGCGT ATATAATTAT
601 TCCCTCAACA GCATTCGGGA TGCTGAAAAT GATCTTAGCT TTTTCGACAA
651 TGGAGATAAA GAAAAAATG ATCTCTTCTA TGGCTGGGGT GACA TAGGAA
701 ATTTTGAGGA TGTGGACAAC ATGCTTAGGA GTTGTGATTC AACATTCGGC
751 TTGGATAGTC TTAATAATGA AGGTGACTTA GGGTGGTTCT CTTCTGCCCA
801 GCCAAATGAG GAGACAGCAG GCGCGATGAC AGATGATCTC AAACAGACA
851 AAATGTTGGA AAATCAAAGA ACCGCTATGT TGCAGGTTGA AGACTTTTGT
901 AACACAGTGA AGCCCAACCA TGCTGTTGAA GATGAGTATG GATATACTAT
951 TGAGGACGAT TCAGCTCAAG GAAAATCATC ACAAATGTA TTCGATACAA
1001 GTTTGCAAAA GAAGGATATA TTGATGCTGG ATGTAGAGGC AAATCTTGAG
1051 AAGAAGCAGA CTGATCATCT TCATCATCTG GATGGGAAAA GTGATGGGTT
1101 CTCGGAGAAC AGTTTTACTT TACA GCACAG TGGTATTTCA AGGGAATAAA
1151 TGGATA CCAA CCAGTATTAC CCTCCCTCCG CATTCCAACA GCGAGATGTG
1201 CCATACTCGC ATTTCAATTG TGAGCAGCCT TCCGTTCAAG TATCAGCATG
1251 TGAAAGTAAA TCTGGTATTA AATCTGAGAA CAAACCCAGT CCTTCTCTG
    
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1301 CTTCAAATGA GTCATACACA TCAAATCATG CACAATCAAT CGAGAGCTTA
 1351 CAAGGTCCAA CTGTTGATGA TCGGTTTAGG AAGGTCTTTG AAACGAGAGC
 1401 CAACTTGCTG CCTGGGCAAG ATATGCCTCC TTCTTTTGCT GCAAATACCA
 1451 AGAAGAGTAG TAAGACAGAC TCCATGGTGT TTCCTGATGC TGCTCTATC
 1501 CAGAAAGATT GGCTTGAGAA TGACCACAGA AAAGCTGCAA CTGAATTGGA
 1551 AACATCAAAT ATGCAGGGAA GTTCTTGTGT TAGCTCTGTT GTGGATGACA
 1601 TTTCACTGGA AGCAACAAGT TTTCGCCAGC TTCAACAAGT TATAGAACAG
 1651 TTGGATGTTA GGACAAAGCT TTGCATAAGA GACAGCTTAT ACCGATTAGC
 1701 AAAAAAGTGCG GAACAGAGAC ATCATGGTGG AAACAGACCG GAGAAAGGCG
 1751 CAGGCTCGCA TCTCGTGA CTGTTGACT GGTGAAGCAG ACAAGTATGC TGGATTGATG
 1801 GATATCGAAA CTGATACAAA CCCGATAGAT CGATCGATAG CTCATTGCT
 1851 GTTTCACAGG CCCTCAGACT CATCTCTTTC ATCCGATAAC AATGTCTGT
 1901 CTTATAAATC TCATCCAATG ATTCCTCAGC CCAATAGTAG TCCGAGCCTG
 1951 AGGATAGAGA AACAAAGAGGA AACTACAGAA CTTAGACCCG AAGCAGAAGT
 2001 TTGTAACAAGT GACAACAATT AATGATGTCA AATAAAACTG GCTCTTTCAT
 2051 TGCTGAAGCA TTCGAGATAT TGTGTGTAAC ACTAATCTCG ATTTTGGCTA
 2101 AGCGAAATTG CTCAGGTTAG TTAATAAAAT CCTGACTGCT TCTTCATTAG
 2151 AATCATGGAT TTTGTAACAC AGTTGTATTT ATGTGTTGAG ATAACTTTCT
 2201 GGCTAGATT TCGGTTGCAA CCTGATTGTA CACCTGGTTG CTCGAACCTG
 2251 ATTGTACACC TGGTTGCTCG AACCAGGCT TTAAGATATG TTCTTTACCT
 2301 TTGTAATCTG TTCAAGTTGT TAAGTAGAGG AAGAAGTAGA GACATAGCAA
 2351 TTCTGTAGAT AAAGATGTTA GTTCGTTTTG TAATGCGATA AAATATTCTG
 2401 CTGTGTATT ACTCTCTTTT TTCAAAATA GCGAATTTTA GGGTTTTATT
 2451 TTCCAATAAA

[0319] *LNK1* polypeptide sequence (AT5G64170.1; SEQ ID NO:6):

1 MGSNTNHLKS NTFIKREANM LGKNPWPEKD SGGSSVSRDT GTGKDVQDMT
 51 LEDTNTSDHG FNGGHVDWE NFSTGDPMLC DTSAAATNDGV YNYSLNS I PD
 101 AENDLS FFDN GDKEKNDLFY GWGDI GN FED VDNMLRSCDS TFGLDSL NNE
 151 GDLGWFS SAQ PNEETAGAMT DDLKPKMLE NQRTAMLQVE DFLNNSEPNH
 201 AVEDEYGYTI EDDSAQGKS S QNVFDTSLQK KDI LMLDVEA NLEKKQTDHL
 251 HHLDGKSDGF SENS FTLQHS GI SREIMDTN QYYPPSAFQQ RDVPYSHFNC
 301 EQPSVQVSAC ESKSGI KSEN KPS P S SASNE SYTSNHAQS I ESLQGPTVDD
 351 RFRKVFETRA NLLPGQDMPP SFAANTKKS S KTDSMVFPA APIQKIGLEN
 401 DHRKAATELE TSNMQGS SCV SSWDDI SLE ATS FRQLQQV I EQLDVRTKL
 451 CI RDSLYRLA KSAEQRHHGG NRPEKGAGSH LVTGEADKYA GFMDI ETDN
 501 PI DRS IAHL FHRPSDS SLS SDNNVLSYKS HPMI PQPNS S PSLRI EKQEE
 551 TTEL RPEAEV VTS DNN

[0320] *LNK1* promoter sequence (SEQ ID NO:7):

CCCAAGCCTGTTAAACCTTTTGTATTAGTTGCCACGTGTTGATATCATGGTCACTTGTC
 TTTTGATTCTCCCGAAACAAAAACAATAGTTAATTAATAATTTAAAAATGTTTGTCAAAA
 AAAAAAAAAATAGGAAGCCTTCATCGTCTTCTGCCGAACATCTCTAGAAAAGTCTACACA
 GTTTTTTTTTTCGATCTAGCTTCGATGAATTGATACAACTCTCTATCTGCTGAGTTT
 TTGTTTTCTGGTAAACAATTTTATCTGTGGAGTTTTCTGGAGTTGCTAGTGGAAGAAT
 CTGTCTCACAGGTGAGCTTATTTTCGTTCCGCTTTGATGGAATCTATTTGATTGAATTT
 AATTAGAAAATTTGGTTCTTATCCTCAGTGTTCATAGCAGCGGTAGCTGAGGATTTT
 TCAGATTTGATTGTTTTTTTTTTTGTGTCAGTTAGTTTCATTGAATGCCTCATTTCCT
 GGATTTTAGTTGTTTTTGTAAACTCTCGGTTAATAATGATAGTTTAACTCGATTTTGC
 TAGAAAATTACGAGCATAAAATGGTTGAATCCTACTATCTGATTTTTCTCCTACTTTGTT
 TTTGTGCTACTTGCTATGAGATTTGACTTTTGGCTGTAGAATTTGAGTCTAATTTCTG
 GAATTCATGAATAGTTGAAAGAATGGGTAACTCTTAGGGTTTG GATTACATGTATTTCT
 GGGTTTCATGGGATTTGATTCTGGATAAGAAAAGTCAAGTTTTCTTATATGCTGGGTTT
 GATTCTCTATTATTTGGAAGCTGTTAATAATTTATATTTCTATCCAGAACCAGAAA
 TAGTGTGTTGGGAGTTACATGTTTGCAGTATGATGACATATCGGGGTAATGTGCAT
 GATTTTAGTGGT GATTAAGAGTCTGAAGTTGGGAGGAATG

[0321] *LNK2* cDNA, genomic and protein sequence accessible at AT3G54500.
LNK2 cDNA (splice variants): including splice variants *e.g.*, AT3G54500.2;
 AT3G54500.1 or AT3G54500.4: *LNK2* cDNA sequence (AT3G54500.3; SEQ ID
 NO:3). The start codon (atg) is double-underlined and in bold:

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1  AAAAAAAAACT CTTTTTCTT TTTTCCACTG TAACTCCTTT CTCTTCCCAT
51 ATCCTTTGAG CACGAAGGAG GATTCTGCTG AATCAAGGTT TTTCAAAGCT
101 TGTTGCTTTT TCAGTTTAAT ATTCTTTTAT TTATCGTTTT TTCCACTTTA
151 TTATTGTGTG TGGATTTACG TTATCCGATT TGTTGATCTT GTTGTTTTTT
201 CCTGGATTTT TTCGTTGTG GAGCTGGCGA TGTGTGGAGC AATCAGGGAG
251 CTTGATTTTG GGTCTGTGAT TCGATTTTGT TTCTCCAGTC ATGTTTGATT
301 GGGGAAGAAGA AGAGCTTACT AATATGATAT GGGGTGATGA TGCTGAGACA
351 GGCACCATA TTGTGCCTTT TAAAGT CAGA AGT GAACAAC TTAACAAAAA
401 GGAACAGATT GAGGAATCTA AGACAGCTGA GCAAAAGAT A ACTGGGACTA
451 AAATTGACCT CCAT GATAAAA AATTTGGGGA GCAGTTCGAG CCATAATGTT
501 GATGAGGGGC TTCCTCAGCC AGATTTCTGT ATGAGCTCAT GGCCTGACAC
551 GTCGCTAACT AATGCTACAA AGGTTGATCA AGATTTGAGT GCGACTGAAC
601 TTTCAAATG CTAGCTGAG CCAGTCAGAT ATGATTCAAC AAGAGGTGGT
651 GCGTTTCTCT TGAAACAGTC ATGTTTTACT TGGGTACGTT CTTTCCAATC
701 TAATCATTTT AAAAGTTGTG TGCTCACTCT GTTTTTACCA GAGAAAACGT
751 CTGAAGTTGG GAAAGGCCCT GATATTTTC ATAGCTCTGA TGAGAGTAAA
801 GAGCAAGGTG ATTTTGTGTA CTACAGCTGG GCCAACATTG GTAGTTTGA
851 TGATCTTGAT CGAATGTTCA GCAATGATGT CCCTATATTT GGTGATGGCA
901 GTCTCAGCGG TGGTGATGAG TTATGGTCAT CTCTAAAGA TGTATCCAAT
951 AGCCCAAAAT CAT TAT CATC AATGTTGGAC TCTCAAGATC TAGGATTGGA
1001 TATTAGAACT GAGTTTGAGC AACAAGAGAA CCAGCAATTT CCATTGACTG
1051 GAAAAGCCAA TGGTCTGTCA TCCCAAAGTG TCCCAAAGTGT ACGTGTAACT
1101 CTTAAGGCTG ACCAATATCG TGAGCATAAG GGTCAACCCT CAGTTGAGGA
1151 CCAGCCATAT CAACAAAATA AAATGATGAA GTTTTCGAAA ATGCCTGGGA
1201 CTTCTGAGGC AAGGCCCTTT CAAGAGCTGT ACGGTCAGAG GATTCCATTT
1251 AGTAATTCAG CTGAAAATG TGTAATCAG TTGGCACCGC CACAATCGTC
1301 TCTGATGGCT GTTAATCTCC TGAGTGAGTC TGAGGGGTCT GGGACATCAC
1351 ACTATTCACA TATGCCAAAC CAATACATGG CTAATTCTGC TTTTGTAAAT
1401 CTTGCAAATC CATATTCTAG TGTGCCTGTA ATTTCCGGCCG TCCAACATCC
1451 TGATGTTAGG AATCAGTTGA TGCATCCTTC CTACAATCCT GCTACTGCTA
1501 CCTCAGTAAA CAT GGCAACA GATGCCTCTG CACGGCCTTC AACAAATGACA
1551 CCA CA GAAA AAC TAGAAA ACTTAGACGC AGAC AGCAA TGCAAGCAAT
1601 GCTTGCCATT CAGAGACAAC AGCA GCAATT TTCTCATCAG GTGCCTGTAG
1651 CAGAT CAAT C CATTACTCAA AACTGTCTCC AAGATATTCC ACTCCAGCTT
1701 GTCGACAAAA CTAATCTCCA AGGGCTAACT GCAATGCCTT CCTTTGATCC
1751 TAGTTCATCT TTGGAACAAG ATGATTCTGG CAAATTTGCT GCTGCTGTTG
1801 ATAATTCAGC AGAATTCGCA GTTCTTTATC GGCTTCAGGA TGTGTAGCA
1851 AAGTTGGATA TGGGAACACG GACTTGTATA CGGGATAGT TATTCGGTT
1901 GGCTGGTAGT GCAGCTCAGA GACA TTA CA C TAGT GATACA TCCCACAGTA
1951 ACAAGACTAG CCAGGATGAC CAGGAAGTCA TTCCCAGAGA AGAATCCAGA
2001 TATAGAT ATG CTGGGATGCC AGATACAGAA GCAGTGACCA ACCCCACAGA
2051 CAGAACTGTG GCTCATTTGC TCTTTCATAG GCCTTTTGAT ATGTTGGCGG
2101 CAAAGCGAAT GGAAGGACCA GAATCACCCG CTTCTTCGAA GATGGGAACT
2151 GAAGAAAAAG GGAATTTTCC TAAATGCAGC ATACGAGAGA CTCACTTAAC
2201 TAAGCAGAAA GCTCAAAGG AAGAAGGACC TGCAGATTCA CTTGCTTTGG
2251 GGAATGCACC CAACTCTGGA TCTAGTAGTA CTGTTGGTGA GAGGTTGTT
2301 GAAGCATCCC AAGGAAACAA AAGAAAATTG TGAATTTAG AAGTTTAGGT
2351 TGCCGTGTTA GAGAGGAGCT TTGGGTATTT CTGCAGACTA GACAGCATCT
2401 TTCTGCGTCT CTTGAAACAA TCTCTGACT GCTCCTTTCT TGTGAAGCTC
2451 TTATTCTTGT AATTAGATTT TTTTTTTTTT GTTACTTTTC GAAAACCTCT
2501 TTTGTTATAC GTATCTCCAT GACAACTACA TTTTGGAAAA CGTGTAGTTT
    
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2 5 5 1 GCAATGTTTT TCCCCCATTC GAAACCAGAT TCAATCTCTT AATACAATTG
 2 6 0 1 TAAGCACACA CCAAGGACAC ATAAAGTACT GTTTTGAGG

[0322] *LNK2* polypeptide sequence (AT3G54500.3; SEQ ID NO:4):

1 MIWGDDAETG DHIVPFKVRV EQLNKKEQI E ESKTAEQKIT GTKI DLHDKN
 5 1 LGS SSSHNV D EGLPQPDFCM SSWPDTSLTN ATKVDQDLSA TELSKCLAEP
 1 0 1 VRYDSTRGGA FLLKQSCFTW VRS FQSNHFK SCVLTFLPE KTSELGKGPD
 1 5 1 I FHSSDESKE QGDFDDYSWA NIGS FDDLDR MFSNDVPI FG DGSLSGGDEL
 2 0 1 ws SSKDVSNS PKSLS SMLDS QDLGLDIRTE FEQQENQQFP LTGKANGLS S
 2 5 1 QSVPSVRVTL KADQYREHKG QPSVEDQPYQ QNKMMKFSKM PGTSEARPFQ
 3 0 1 ELYGQRI PFS NSAGKCVNQL APPQS SLMAV NLLSESESG TSHYSHMPNQ
 3 5 1 YMANSAFGNL ANPYS SVPVI SAVQHPDVRN QLMHPSYN PA TATSVNMATD
 4 0 1 ASARPS'TMTP QEKLEKLRRL QMQAMLAIQ RQQQQFSHQV PVADQSITQN
 4 5 1 CLQDI PLQLV DKTNLQGLTA MPS FDPSS SL EQDDSGKFAA AVDNSAEFAV
 5 0 1 LYRLQDWAK LDMGTRTCI R DSLFRLAGSA AQRHYTSDTS HSNKTSQDDQ
 5 5 1 EVI PREESRY RYAGMPDTEA VTNPTDRTVA HLLFHRPFDM LAAKRMEGPE
 6 0 1 SPASSKMGTE EKNFPC I RETHLTKQKA QKEEGPADSL ALGNAPNSGS
 6 5 1 SSTVGERWE ASQGNKRKL

[0323] *LNK2* cDNA sequence (AT3G54500.1; SEQ ID NO:8). The start codon

(atg) is double-underlined and in bold:

1 CCCATATCCT TTGAGCACGA AGGAGGATTC TGCTGAATCA AGCTGGCGAT
 5 1 GTGTGGAGCA ATCAGGGAGC TTGATTTTGG GTCTGTGATT CGATTTTGTT
 1 0 1 TCTCCAGTCA **ATG**TTTGATTG GGAAGAAGAA GAGCTTACTA ATATGATATG
 1 5 1 GGGTGATGAT GCTGAGACAG GCGACCATAT TGTGCCTTTT AAAGTCAGAA
 2 0 1 GTGAACAAC TAACAAAAAG GAACAGATTG AGGAATCTAA GACAGCTGAG
 2 5 1 CAAAAGATAA CTGGGACTAA AATTGACCTC CATGATAAAA ATTTGGGGAG
 3 0 1 CAGTTCGAGC CATAATGTTG ATGAGGGGCT TCCTCAGCCA GATTTCTGTA
 3 5 1 TGAGCTCATG GCCTGACACG TCGCTAACTA ATGCTACAAA GGTGATCAA
 4 0 1 GATTTGAGTG CGACTGAACT TTCAAAATGC TTAGCTGAGC CAGTCAGATA
 4 5 1 TGATTCAACA AGAGAGAAAA CGTCTGAACT TGGGAAAAGC CCTGATATT
 5 0 1 TTCATAGCTC TGATGAGAGT AAAGAGCAAG GTGATTTTGA TGA CTACAGC
 5 5 1 TGGGCCAACA TTGGTAGTTT TGATGATCTT GATCGAATGT TCAGCAATGA
 6 0 1 TGTCCCTATA TTTGGTGATG GCAGTCTCAG CGGTGGTGAT GAGTTATGGT
 6 5 1 CATCTTCTAA AGATGTATCC AATAGCCCAA AATCATTATC ATCAATGTTG
 7 0 1 GACTCTCAAG ATCTAGGATT GGATATTAGA ACTGAGTTTG AGCAACAAGA
 7 5 1 GAACCAGCAA TTTCCATTGA CTGGAAAAGC CAATGGTCTG TCATCCCAA
 8 0 1 GTGTCCCAAG TGTACGTGTA ACTCTTAAGG CTGACCAATA TCGTGAGCAT
 8 5 1 AAGGGTCAAC CCTCAGTTGA GGACCAGCCA TATCAACAAA ATAAAATGAT
 9 0 1 GAAGTTTTTCG AAAATGCCTG GGACTTCTGA GGCAAGGCC TTTCAAGAGC
 9 5 1 TGTACGGTCA GAGGATTCCA TTTAGTAATT CAGCTGGAAA ATGTGTAAT
 1 0 0 1 CAGTTGGCAC CGCCACAATC GTCTCTGATG GCTGTTAATC TCCTGAGTGA
 1 0 5 1 GTCTGAGGGG TCTGGGACAT CACACTATTC ACATATGCCA AACCAATACA
 1 1 0 1 TGGCTAATTC TGCTTTTGGT AATCTTGCAA ATCCATATTC TAGTGTGCCT
 1 1 5 1 GTAATTTTCGG CCGTCCAACA TCCTGATGTT AGGAATCAGT TGATGCATCC
 1 2 0 1 TTCCTACAAT CCTGCTACTG CTACCTCAGT AAACATGGCA ACAGATGCCT
 1 2 5 1 CTGCACGGCC TTCAACAATG ACACCACAGG AAAA ACTAGA AAAACTAGA
 1 3 0 1 CGCAGCAGC AATGCAGGC AATGCTTGCC ATTCAGAGAC AACAGCAGCA
 1 3 5 1 ATTTTCTCAT CAGGTGCTG TAGCAGATCA ATCCATTACT CAAA ACTGTC
 1 4 0 1 TCCAAGATAT TCCACTCCAG CTGTGCGACA AA ACTAATCT CCAAGGGCTA
 1 4 5 1 ACTGCAATGC CTTCTTTGA TCCTAGTTCA TCTTTGGAAC AAGATGATTC
 1 5 0 1 TGGCAAATTT GCTGCTGCTG TTGATAATTC AGCAGAAATC GCAGTCTTT
 1 5 5 1 ATCGGCTTCA GGATGTTGTA GCAAAGTTGG ATATGGGAAC ACGGACTTGT
 1 6 0 1 ATACGGGATA GCTTATTCCG GTTGGCTGGT AGTGAGCTC AGAGACATTA
 1 6 5 1 CACTAGTGAT ACATCCCACA GTAACAAGAC TAGCCAGGAT GACCAGGAAG
 1 7 0 1 TCATTTCCAG AGAAGAATCC AGATATAGAT ATGCTGGGAT GCCAGATACA

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1751 GAAGCAGTGA CCAACCCAC AGACAGAACT GTGGCTCATT TGCTCTTTCA
1801 TAGGCCTTTT GATATGTTGG CGGCAAAGCG AATGGAAGGA CCAGAATCAC
1851 CCGCTTCTTC GAAGATGGGA ACT GAAGAAA AAGGGAATTT TCCTAAATGC
1901 AGCATA CGAG AGACTCACTT AACTAAGCAG AAAGCTCAAA AGGAAGAAGG
1951 ACCTGCAGAT TCACTTGCTT TGGGGAATGC ACCCAACTCT GGATCTAGTA
2001 G TACTGTTGG TGAGAGGGTT GTTGAAGCAT CCCAAGGAAA CAAAAGAAAA
2051 TTGTGAAATT TAGAAGTTTA GGTGCCGTG TTAGAGAGGA GCTTTGGGTA
2101 TTTCTGCAGA CTAGACAGCA TCTTTCTGCG TCTCTGAAA CAATCTCTG
2151 ACTGCTCCTT TCTTGTGAAG CTCTTATTCT TGTAATTAGA TTTTTTTTTT
2201 TTTGTTACTT TTCGAAAACC TCTTTGTGTTA TACGTATCTC CATGACAACT
2251 ACATTTTGA AAACGTGTAG TTTGCAATGT TTTTCCCCA TTCGAAACCA
2301 GATTCAATCT CTTAATACAA TTGTAAGCAC
    
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[0324] *LNK2* polypeptide sequence (AT3G54500.1; SEQ ID NO:9):

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1 MFDWEEELT NMIWGDDAET GDHIVPFKVR SEQLNKKEQI EESKTAEQKI
51 TGTKIDLHDK NLGSSSSSHNV DEGLPQPDFC MSSWPDTSLT NATKVDQDLS
101 ATELSKCLAE PVRYDSTREK TSELGKGPDI FHSSDESKEQ GDFDDYSWAN
151 IGSFDDLDRM FSNDVPI FGD GSLSGGDELW SSSKDVSNP KSLSSMLDSQ
201 DLGLDIRTEF EQQENQQFPL TGKANGLSSQ SVPSVRVTLK ADQYREHKQ
251 PSVEDQPYQQ NKMMKFSKMP GTSEARPFQE LYGQRIPFSN SAGKCVNQLA
301 PPQSSLMAVN LLSESESGT SHYSHMPNQY MANSAFGNLA NPYSSVPVIS
351 AVQHPDVRNQ LMHPSYNPAT ATSVNMTDA SARPSTMTPO EKLEKLRRRQ
401 QMQAMLAIQR QQQQFSHQVP VADQSITQNC LQDI PLQLVD KTNLQGLTAM
451 PSFDPSSSLE QDDSGKFAAA VDNSAEFAVL YRLQDWAKL DMGTRTCIRD
501 SLFRLAGSAA QRHYTSDTSH SNKTSQDDQE V I PREESRYR YAGMPDTEAV
551 TNPTDRTVAH LLFHRPFDM L AAKRMEGPES PASSKMGT EE KGNFPKCSIR
601 ETHLTKQKAQ KEEGPADSLA LGNAPNSGSS STVGERWEA SQGNKRKL
    
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[0325] *LNK2* cDNA sequence (AT3G54500.2; SEQ ID NO: 10). The start codon

(atg) is double-underlined and in bold:

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1 CATATCCTTT GAGCA CGAAG GAGGATTCTG CTGAATCAAG CTGGCGATGT
51 GTGGAGCAAT CAGGGAGCTT GATTTTGGGT CTGTGATTCG ATTTTGTFTT
101 TCCAGTCATG TTTGATTGGG AAGAAGAAGA GGCGACCATA TTGTGCCTTT
151 TAAAGTCAGA AGT GAACAAC TTAACAAAAA GGAACAGATT GAGGAATCTA
201 AGACAGCTGA GCAAAAGATA ACTGGGACTA AAATTGACCT CCATGATAAA
251 AATTTGGGGA GCAGTTCGAG CCATAATGTT GATGAGGGGC TTCTCAGCC
301 AGATTTCTGT ATGA GCTCAT GGCCTGACAC GTCGCTAAT AATGCTACAA
351 AGGTTGATCA AGATTTGAGT GCGACTGAAC TTTCAAAATG CTTAGCTGAG
401 CCAGTCAGAT ATGATTCAAC AAGAG GTGAG AAAACGTCTG AACTTGGGAA
451 AGGCCCTGAT ATTTTTCATA GCTCTGATGA GAGTAAAGAG CAAGGTGATT
501 TTGATGACTA CAGCTGGGCC AACATTGGTA GTTTTGATGA TCTTGATCGA
551 ATGTTTCAGCA ATGATGTCCC TATATTTGGT GATGGCAGTC TCAGCGGTGG
601 TGATGAGTTA TGGTCATCTT CTAAAAGATGT ATCCAATAGC CCAAAT CAT
651 TATCATCAAT GTTGGACTCT CAAGATCTAG GATTGGATAT TAGAACTGAG
701 TTTGAGCAAC AAGAGAAC CA GCAATTTCCA TTGACTGGAA AAGCCAATGG
751 TCTGTATCC CAAAGTGTC CAAGTGACG TGTAACCTT AAGGCTGACC
801 AATATCGTGA GCATAAGGGT CAACCCTCAG TTGAGGACCA GCCATATCAA
851 CAAAATAAAA TGATGAAGTT TTCGAAAATG CCTGGGACTT CTGAGGCAAG
901 GCCCTTTCAA GAGCTGTACG GTCAGAGGAT TCCATTTAGT AATTCAGCTG
951 GAAAATGTGT AAATCAGTTG GCACCGCCAC AATCGTCTCT GATGGCTGTT
1001 AATCTCCTGA GTGAGTCTGA GGGGTCTGGG ACATCACACT ATTCACATAT
1051 GCCAAACCAA TACATGGCTA ATTCTGCTTT TGGTAATCTT GCAAATCCAT
1101 ATTCTAGTGT GCCTGTAATT TCGGCCGTCC AACATCCTGA TGTTAGGAAT
1151 CAGTTGATGC ATCCTTCCTA CAATCCTGCT ACTGCTACCT CAGTAAACAT
1201 GGCAACAGAT GCCTCTGCAC GGCCTTCAAC AATGACACCA CAGGAAAAAC
1251 TAGAAAAACT TAGACGCAGA CAGCAAATGC AGGCAATGCT TGCCATTCAG
    
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1301 AGACAACAGC AGCAATTTTC TCATCAGGTG CCTGTAGCAG ATCAATCCAT
1351 TACTCAAAAC TGTCTCCAAG ATATTCCACT CCAGCTTGTC GACAAAAC TA
1401 ATCTCCAAGG GCTAACTGCA ATGCCTTCCCT TTGATCCTAG TTCATCTTTG
1451 GAACAAGATG ATTCTGGCAA ATTTGCTGCT GCTGTTGATA ATTCAGCAGA
1501 ATTCGCAGTT CTTTATCGGC TTCAGGATGT TGTAGCAAAG TTGGATATGG
1551 GAACA CGGAC TTGTATACGG GATAGCTTAT TCCGGTTGGC TGGTAGTGCA
1601 GCTCAGAGAC ATTACACTAG TGATACATCC CACAGTAACA AGAC TA GCCA
1651 GGAT GAC CA G GAAGTCATTC CCAGAGAAGA ATCCAGATAT AGATATGCTG
1701 GGATGCCAGA TA CAGAAG CA GTGACCAACC CCACAGACAG AACTGTGGCT
1751 CATTGTCTCT TTCATAGGCC TTTTGATATG TTGGCGGCAA AGCGAATGGA
1801 AGGAC CAGAA TCACCCGCTT CTTGGAAGAT GGGAACTGAA GAAAAAGGGA
1851 ATTTTCCTAA ATGCA GCATA CGAGAGACTC ACTTAACTAA GCAGAAAGCT
1901 CAAAAGGAAG AAGGACCTGC AGATTCACTT GCTTTGGGGA ATGCACCCAA
1951 CTCTGGATCT AGTAGTACTG TTGGTGAGAG GGTGTTGAA GCATCCCAA
2001 GAAACAAAAG AAAATTGTGA AATTAGAAG GTTGTATTT CCATATGATA
2051 ACTCTATAAA TCGTTTCCAT GCCCTTTTGT ATACGTGCAT AGAGCTTGTA
2101 TACAGATTGT AACTAATACG CAGGTGTTGC TTTTGCTTTC AATCAGTTTA
2151 GGTGCGCGTG TTAGAGAGGA GCTTTGGGTA TTTCTGCAGA CTAGACAGCA
2201 TCTTTCTGCG TCTCTTGAAA CAATCTCCTG ACTGCTCCTT TCTTGTGAAG
2251 CTCTTATTCT TGTAATTAGA TTTTTTTTTT TTTGTTACTT TTCGAAAACC
2301 TCTTTTGTTA TACGTATCTC CATGACAACT ACATTTTGGA AAACGTGTAG
2351 TTGCAATGT TTTTCCCCCA TTCGAAACCA GATTCAATCT CTTAATACAA
2401 TTGTAAGC
    
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[0326] LNK2 polypeptide sequence (AT3G54500.2; SEQ ID NO: 11) :

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1  MSSWPDTSLT NATKVDQDLS ATELSKCLAE PVRYDSTRGE KTSELGKGPD
51  IFHSSDESKE QGDFDDYSWA NIGSFDDLDR MFSNDVPIFG DGSLSGGDEL
101 WSSSKDVSNS PKSLSSMLDS QDLGLDIRTE FEQQENQQFP LTGKANGLSS
151 QSVPSVRVTL KADQYREHKG QPSVEDQPYQ QNKMMKFSKM PGTSEARPFQ
201 ELYGQRI PFS NSAGKCVNQL APPQSSLMV NLLSESESGS TSHYSHMPNQ
251 YMANSAFGNL ANPYSSVPI SAVQHPDVRN QLMHPSYN PA TATSVNMATD
301 ASARPSTMTQ QEKLEKLRRR QQMQAMLAI Q RQQQQFSHQV PVADQSITQN
351 CLQDI PLQLV DKTNLQGLTA MPSPDPSSSL EQDMSGKFAS AVDNSAEFAV
401 LYRLQDWAK LDMGTRTCIR DSLFRLAGSA AQRHYTSDTS HSNKTSQDDQ
451 EVI PREESRY RYAGMPDTEA VTNPTRDTVA HLLFHRPFDM LAAKRMGEPE
501 SPASSKMGTE EKGNFPKCSI RETHLTKQKA QKEEGPADSL ALGNAPNSGS
551 SSTVGERWE ASQGNKRKL
    
```

[0327] LNK2 promoter sequence (SEQ ID NO: 12). The start codon (atg) is double-underlined and in bold:

```

TTAATATTTTTATTTATTTTGTATACAACATAATTTTTTTCTTGTACCAGTTTCTT
AGAAACATA TTCTCTAGCGTCTTT CATGAT TGTAGAT CACTAAAAT CTTGCGCAT GTAAC
AAGT A CAAAT TTAT CAAGT CA GCAATAAC GGGTTTGTAG CACA TTTCTAT TTTTGATA TA
TA CA CTAAT TTGCTTTTGTCT CAGAC TCAC TCTAT CTAT GAT CTA TCA CAAG CTAGCAAC
AATGTTTACTGCTTAATATGTGTCTAACGTAACCTCGTGATCATTTCTGAAACAAGAGTAA
CCCCTA CCAACAAT TCGTTTAAAT CTCGAAATA CAAAGT A CAAACA CAAGAT AAACAAAC
GCATAAAACAAT CGAGGCA CAAAG CCTAT TAACAAG GTCTGAAAAT TTTCAAC CTAGT CAT
CAAG GAC GAAAAC GAT TTTTATATAAT TGAAAAATAAAC A CACT TAT TAAAAAT A TA TA
TCAAT TGCAAC CAT TTTATA TCCACTGTTTCGT TAT GAAC TGTTCAGGATAAC GCAT TAGT
TCCTTTTCAATAAACTGATCCAAGCAAATACTTTTGAAAAAGTGATATCAATGAAAGCT
AAAT TAGT TAAAAC A TA TAAGAT AAAAAT TGTGGTGCCTAT GAACTCAAAC CCCAC CAAA
    
```

TCTAAAAGT TGAACACT GGT TCCACT CTTTTAAAAACAT A TTTAC CCAAT TGTGTGTCTC
 TTCTTTTCTC GAACA CTAAT CCGATA TAACA TGAAAATA TTA AAAAAAC GTCAACAAAAG
 ACCACAGCCAGGGAAAGTCAAATGAGCTGTCATGGAATCAAGTCGTGTTACTCTCAGC
 CACATGTTTAGATACGGCCTTCTCGTCTGACGTGGCAACCCGTTCTCTCTCAGCCACTCG
 TTTGGCACGTGACCATTT CATA CAAAAAAACTCTTTTTTCTTTTTTCCACTGTAACTC
 CTTTCTTTCCCATATCCTTTGAGCACGAAGGAGGATTCTGCTGAATCAAGGTTTTTCAA
 GCTTGTGCTTTTT CAGTTAATATTCTTTTATTTATCGTTTTTCCACTTTATTATTGTG
 TGTGGATTACGTTATCCGATTTGTTGATCTTGTGTTTTTCTGGATTTTTTCTGTTGT
 GGAGCTGGCGATGTGTGGAGCAATCAGGGAGCTTGATTTTGGGTCTGTGATTTCGATTTTGT
 TTCTCCAGT CATGTTTGAT TGGGAAGAAGAAGAG CTTA CTAAT ATG

[0328] *LNK3cONA*, genomic and protein sequence accessible at AT3G12320:

LNK3 cDNA sequence (AT3G12320.1; SEQ ID NO: 13). The start codon (atg) is double-underlined and in bold:

1	ACATTACAAC	AAACA CATT C	GTCTTCTTCT	CTCTCTCTTT	CTCTTTGGTC
51	TTTTTTTTGC	TACTGTTGA	AGAAAATTTA	AACAAAGGCT	TCTCTTGATT
101	TTTGAACCAA	TCATCTTTAC	TTCATCTGAG	GGTTTCTGAT	TTGGGTCTGA
151	TTCAAAACTC	TCTCATCCGT	TCA CTACAAA	GTTGATAGAT	CGATCGATTG
201	ATTTTATG <u>GA</u>	TTGTTATGCT	GAAGAGCTTG	TTGTGCCTAA	TTATCAAGAA
251	TCAT CAT CAG	AGACA TA CCC	ATCTACTGGT	ATGTGGGGTG	GATGGAGCAT
301	GAGCTCCCT	GAAGCAGCTG	AGAAATGTTT	CGAT TAC GAT	GGTTTCAATG
351	GAGAAG GAAT	GAT GTA TAGT	CAGAT GAGTA	TGAGGACTAG	TGAAGAAGAA
401	GAAGAGT CTA	AAAGAT CAAA	GGCTTTCTAT	GGTGTCTCTT	CACCTCACGA
451	TTTCGAAGGC	ATCGAACAGA	TGGAT GATA T	GTTCTTAAGT	TCAATTTTGG
501	AGGATGTTCC	AGAGGATGAT	GGAGATGTTT	ACCGCGCGAC	CAGTAGCAAT
551	AACAGTGTG	GTCTTCTTCT	TATGTATGGT	GGTGGTAGGG	AAGTCCCTAT
601	GTTCCATTGT	CAT GACAT GT	CTTCAAGGA	GGAAGCTCCA	TTTACAATCT
651	CGGATCTGTC	TGAAGAGAAC	ATGTTAGATT	CAAACATATG	GGAT GA ACTG
701	TCTTCTGAAG	AATTCGTGTT	GCAGGATCTA	CAAAGGGCTT	CACAAAAGTT
751	GACT GAT GAG	ACACGCAAAT	GTTTCCGGGA	TACTTTTTAC	CGACTTGCAA
801	GGAGCTCACA	AGATAAATCT	GATTCAGTCA	GCCCCAACTC	GGAAGAGCTG
851	CTTATGCAAA	CGTCTAGATA	TGATTATGGT	GATGGAAACA	GGTTTAGTAG
901	AGAGGAAGAG	ATAGAATCTG	AAAC GAATTC	AAT CGATAGA	GCTGTCGCAA
951	ACCTCACATT	CAACAAAATG	GAAT CCAACA	TAAGCAATTT	TCCTCTGTCTG
1001	GAAAGAGTAC	AGTAGCTGCC	GCTGATCAAG	ATCAAC CAT T	GGCGCCTCAT
1051	CATCTTCCTA	TGAAGTTTTT	TGTTACCATA	TGTGTATAAA	TGTGTGTATA
1101	TATAGTTGAC	TTAATGTTGC	AAGCTTCAGT	TTTGATCTGT	CAAAAGAATC
1151	TGGTCCCCTT	TTAATTCCT	GCAGTATGCT	TTCTACAAAC	ACTACCTGAT
1201	GGCCTTGAGA	CTTACTTT			

[0329] *LNK3* polypeptide sequence (AT3G12320.1; SEQ ID NO: 14):

1	MDCYAEELW	PNYQESSSET	YPSTGMWGGW	SMSSPEAAEK	CFDYDGFNGE
51	GMMYSQMSMR	TSEEEEEESKR	SKAFYGASSL	HDFEGIEQMD	DMFLSSILED
101	VPEDDGDVHR	ATSSNNSVGS	SSMYGGGREV	PMFHCHDMSF	KEEAPFTISD
151	LSEENMLDSN	YGDELSSEEF	VLQDLQRASQ	KLTDETRKCF	RDTFYRLARS
201	SQDKSDSVSP	NSEELLMQTS	RYDYGDGNRF	SREEEIESET	NSIDRAVANL
251	TFNKMESNIS	NFPLSERVQ			

[0330] *LNK4* cDNA, genomic and protein sequence accessible at AT5G06980.

LNK4 cDNA sequence (AT5G06980.4; SEQ ID NO: 15). The start codon (atg) is double-underlined and in bold:

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1 GTGGACCAAT GAAAGAGTTG CTTTAGATGG AGGAGTAATC TAAGCCACAA
51 ATCTCGTTTG TGAATTCTAA GCCACTAACT AAAGTTCAAA CTCACGCGCT
101 CTCCACTTCC TCTCTCAAC TTGCTTCTTT GTCTTCTCTC TCTTCTCTCC
151 TTCGCACTCA CAAATCTACA GAAGATAGAA GAAGATAGAC TCATAATCTT
201 TTGGATTTGG ATTTAGATCT GATCATAAAG GTTAAAGTAT CATTC ATGGA
251 TCGTTATTCG AGGAGGAATT TAGAGGATCT TGTGTGCCT AACTATCAAG
301 AGACATCAGA TTCGTACCCT TCTCCTGATA TGTGGGGTAC TGGATGGAGC
351 ATGAACTCTT CAGAAGCTGC TGAGAAATGT TTTGATTATG ATGTGATTCA
401 CAATGGTTTC AGTGGAGGAT TATACAGTCA GATGGAGATG GATATGGGAA
451 CTAGT GAACA AGTAGAAGAA GAAACTAAGA AGTTAAAGGC TAGTGTTGT
501 TTTGACCGTT CGCTTCATGA TTTTGATGAA ATCCAACACA TGGATGACAT
551 GTTCTTTTCC ATTTTGGAGG ATGTTCCAGG GAATGAGAAT TTTCTTTCTT
601 TCAAGGAGTC GGATAATAAC AACAGTCCA GTTCTCCTA TCTCGACACA
651 ACTGATGCA GGGAAAGTCCC TTTGTTCCAT TACAAGTGGG AAACATGTCA
701 AGATATGCCA CTTATGGAGG AGGATGCACC TAT GAATCTG TGTGAGGAGA
751 ACAAGGAGGA GGCATCTGCT GAAGAAGTTG TGTTCAGGA TCTACAAAGG
801 GCTACAGAAA TGTTGACTGA TGATACCCGC AAGTGCTTCC GCGATACCTT
851 TTACCGGCTT GCAAAGAAGT CACAACAGAA GTCAGACTCC AACTCGGATG
901 AGTTCCTTGA GGATAGAACC AGTTCTAATG ACTCATCACC ATCTATGACG
951 TTTTGTTCAG TAGGGAAACT GAATTTGAAA CCAAACCTCA TCGACAGAGC
1001 CGTGGCCAAT CTCACATTCA ACAAGATGGA ATCCAACATG AGAAATATGC
1051 CTCCACCGAA GAGACTTTCT TCTGTTCAAG GATAAGAGAG TTTTCATCATA
1101 TTTGGTAATA CCATATGTGT ATATAATATA TATGTGTGTG TGTATGTGTG
1151 CTTGGCTTGA CTTTTTCCAA GCTTCAGTTT TGATCTGTTT CATTAGACTC
1201 TGACTCTGCT TCTGGATTTC CAATTTCTTA TGCTATATGT TATTTACCGA
1251 GTGAACCTGT GAATGTACAT TACCCCATGT AGAATCTGTT GTTTCAATGT
1301 ATCAAAAAAG ATATTCTTCA GAC

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[0331] *LNK4* polypeptide sequence (AT5G06980.4; SEQ ID NO: 16):

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1 MDRYSRRNLE DLWPNYQET SDSYPS PDMW GTGWSMNS SE AAEKCFDYDV
51 IHNGFSGGLY SQMEMDMGTS EQVEEETKKL KASGCFDRSL HDFDEIQHMD
101 DMFFS I LEDV PGNENFLS FK ESDNNNSS SS SYLDTTDGRE VPLFHYNWET
151 CQDMPLMEED APMNLCEENK EEASAEWL QDLQRATEML TDDTRKCFRD
201 TFYRLAKNSQ QKSDSNSDEF LEDRTS SNDS SPSMTFLSVG KLNLKPNS I D
251 PAVANLTFNK MESNMRNMPP PKRLS SVQG

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[0332] All publications, patents, patent applications, internet sites, and accession numbers/database sequences (including both polynucleotide and polypeptide sequences) cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

WHAT IS CLAIMED IS:

1. A transgenic plant stably transformed with an isolated nucleic acid encoding a polypeptide selected from the group consisting of:

- (a) a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2;
- (b) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2;
- (c) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2;
- (d) a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4;
- (e) an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4; and
- (f) an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4.

2. The transgenic plant of claim 1, wherein the nucleic acid sequence encodes the LNK1 polypeptide of SEQ ID NO:2.

3. The transgenic plant of claim 1, wherein the nucleic acid sequence encodes the LNK2 polypeptide of SEQ ID NO:4.

4. A transgenic plant stably transformed with an isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence of SEQ ID NO: 1;

- (b) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof;
- (c) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof;
- (d) the nucleotide sequence of SEQ ID NO:3;
- (e) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof;
- (f) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof;
- (g) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a), (c), (d) or (f);
- (h) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a), (c), (d) or (f) under stringent hybridization conditions; and
- (i) a nucleotide sequence that differs from the nucleotide sequence of any of (a), (c), (d) or (f)-(h) due to the degeneracy of the genetic code.

5. The transgenic plant of claim 4, wherein the nucleic acid comprises at least 10, at least 15, at least 20, or at least 25 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 1 or the complementary strand thereof.

6. The transgenic plant of claim 4, wherein the nucleic acid sequence comprises at least 10, at least 15, at least 20, or at least 25 consecutive nucleotides of the nucleotide sequence of SEQ ID NO: 3 or the complementary strand thereof.

7. The transgenic plant of any of claims 1-6, wherein the transgenic plant is stably transformed with an expression cassette comprising the isolated nucleic acid operably associated with a promoter.

8. The transgenic plant of claim 7, wherein the promoter is a heterologous promoter.

9. The transgenic plant of any of claims 7-8, wherein the expression cassette comprises a selectable marker.

10. An isolated nucleic acid selected from the group consisting of:

- (a) a nucleic acid encoding a polypeptide comprising the LNK1 amino acid sequence of SEQ ID NO:2;
- (b) a nucleic acid encoding a polypeptide having an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:2;
- (c) a nucleic acid encoding a polypeptide having an amino acid sequence comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2;
- (d) a nucleic acid encoding a polypeptide comprising the LNK2 amino acid sequence of SEQ ID NO:4;
- (e) a nucleic acid encoding a polypeptide having an amino acid sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:4;
- (f) a nucleic acid encoding a polypeptide comprising at least 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4;

- (g) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof;
- (h) a nucleotide sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45 or 50 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof;
- (i) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:1 or the complementary strand thereof;
- (j) a nucleotide sequence comprising at least 100 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:3 or the complementary strand thereof;
- (k) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of (a)- (i) or (j); and
- (l) a nucleotide sequence that hybridizes to the complete complement of the nucleotide sequence of (a)-(i) or (j) under stringent hybridization conditions.

11. A method of modulating a circadian response of a plant, the method comprising (a) stably transforming a plant cell with the isolated nucleic acid of claim 10.

12. A method of modulating the flowering time of a plant, the method comprising (a) stably transforming a plant cell with the isolated nucleic acid of claim 10.

13. The method of claim 12 wherein the flowering time of the plant is accelerated.

14. The method of claim 12 wherein the flowering time of the plant is delayed.

15. A method of modulating the biomass of a plant of a plant, the method comprising (a) stably transforming a plant cell with the isolated nucleic acid of claim 10.

16. The method of claim 15 wherein the biomass of the plant is increased.
17. The method of claim 15 wherein the biomass of the plant is decreased.
18. The method of any of claims 11-17, which further comprises regenerating a stably transformed plant from the stably transformed plant cell of (a); and expressing the nucleic acid sequence in the plant.
19. An expression cassette comprising the isolated nucleic acid of claim 10.
20. The expression cassette of claim 19 wherein the nucleic acid is operably associated with a promoter.
21. The expression cassette of claim 20, wherein the promoter is a heterologous promoter.
22. The expression cassette of claims 19-21, wherein the expression cassette comprises a selectable marker.
23. A plant comprising the expression cassette of any of claims 19-22.
24. The plant of any of claims 1-9 or 23, wherein the plant is a monocot.
25. The plant of any of claims 1-9 or 23, wherein the plant is a dicot.
26. The plant of any of claims 1-9 or 23-25 wherein the plant is sunflower, wheat, maize, soybean, rice, sorghum, alfalfa or *Arabidopsis*.
27. A product harvested from the plant of any of claims 1-9 or 23-26.
28. A processed product produced from the harvested product of claim 27.
29. A crop comprising a plurality of the plant of any of claims 1-9 or 23-26.
30. A method of introducing a nucleic acid into a plant, plant part or plant cell, the method comprising transforming the plant, plant part or plant cell with the expression cassette of any of claims 19-22.

31. A stably transformed transgenic plant produced by the method of any of claims 11-18.

32. Seed produced from the transgenic plant of any of claims 1-9 or 23-26, wherein the seed comprises the isolated nucleic acid stably incorporated in its genome.

Fig. 1

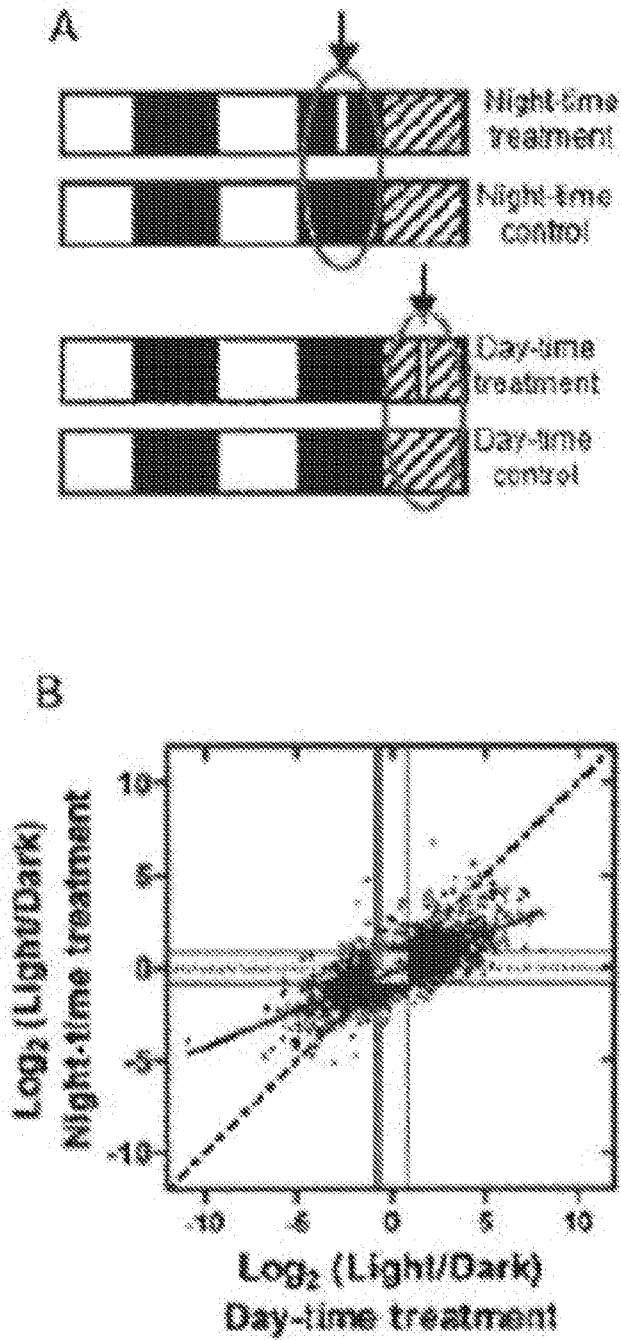


Fig. 1 continued

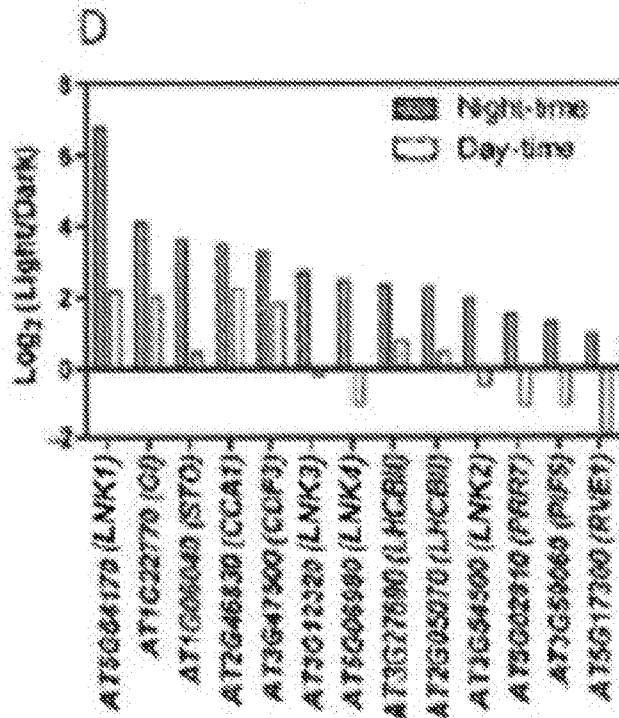
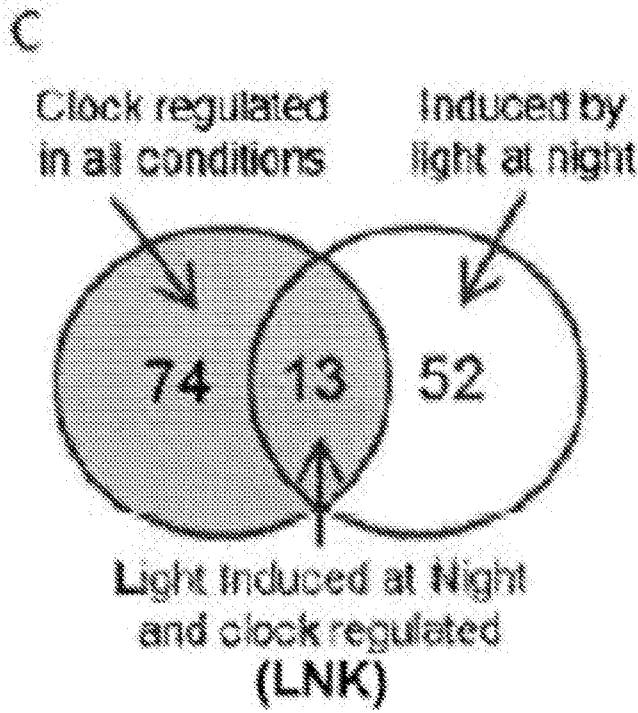


Fig. 1 continued

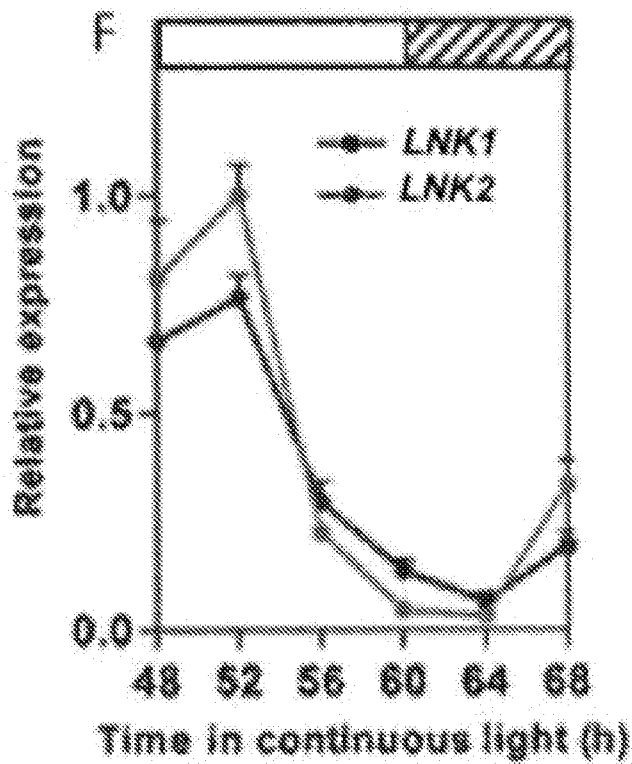
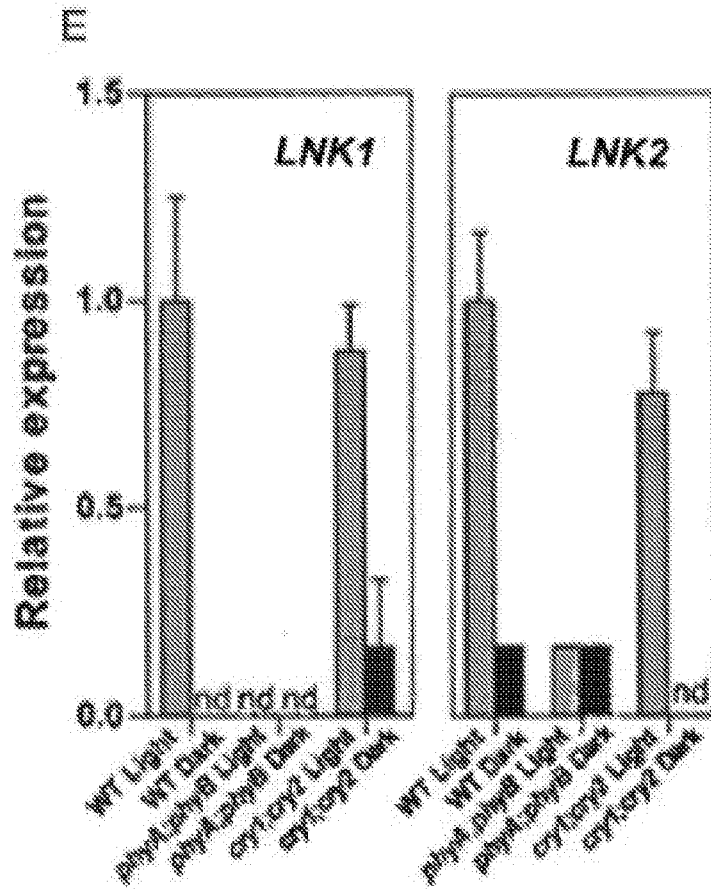


Fig. 2

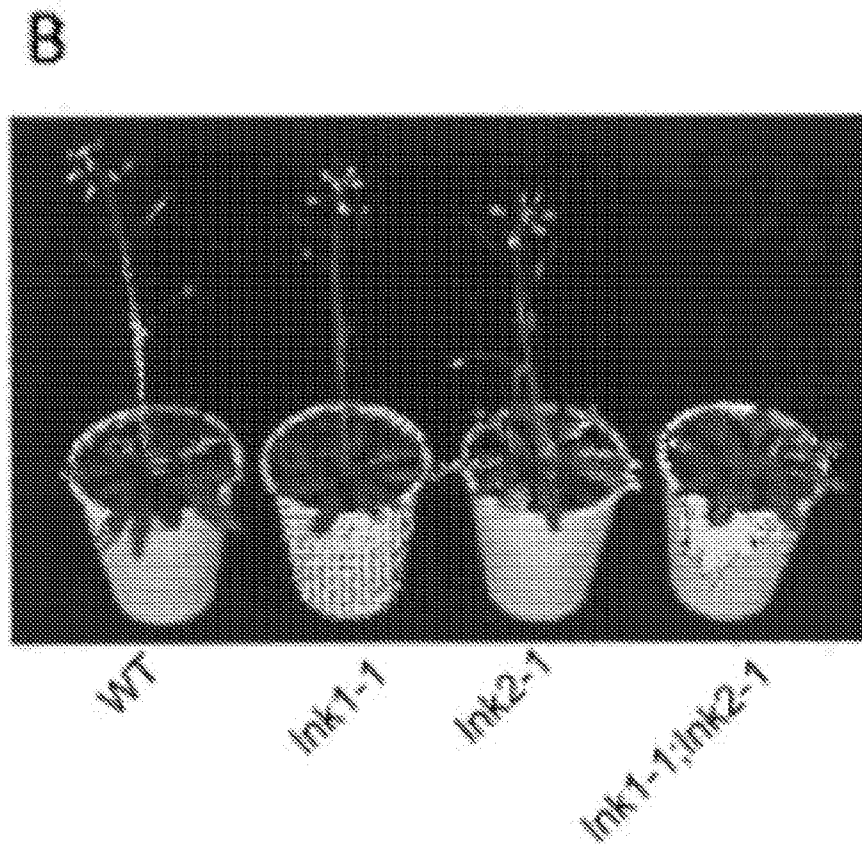
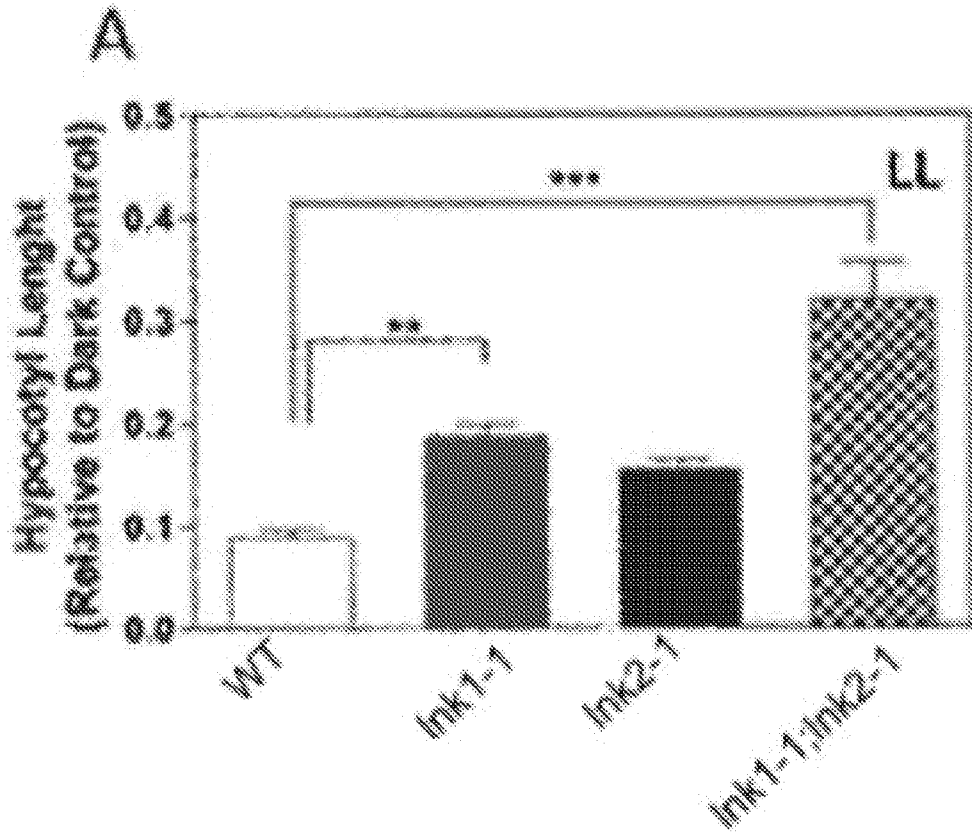


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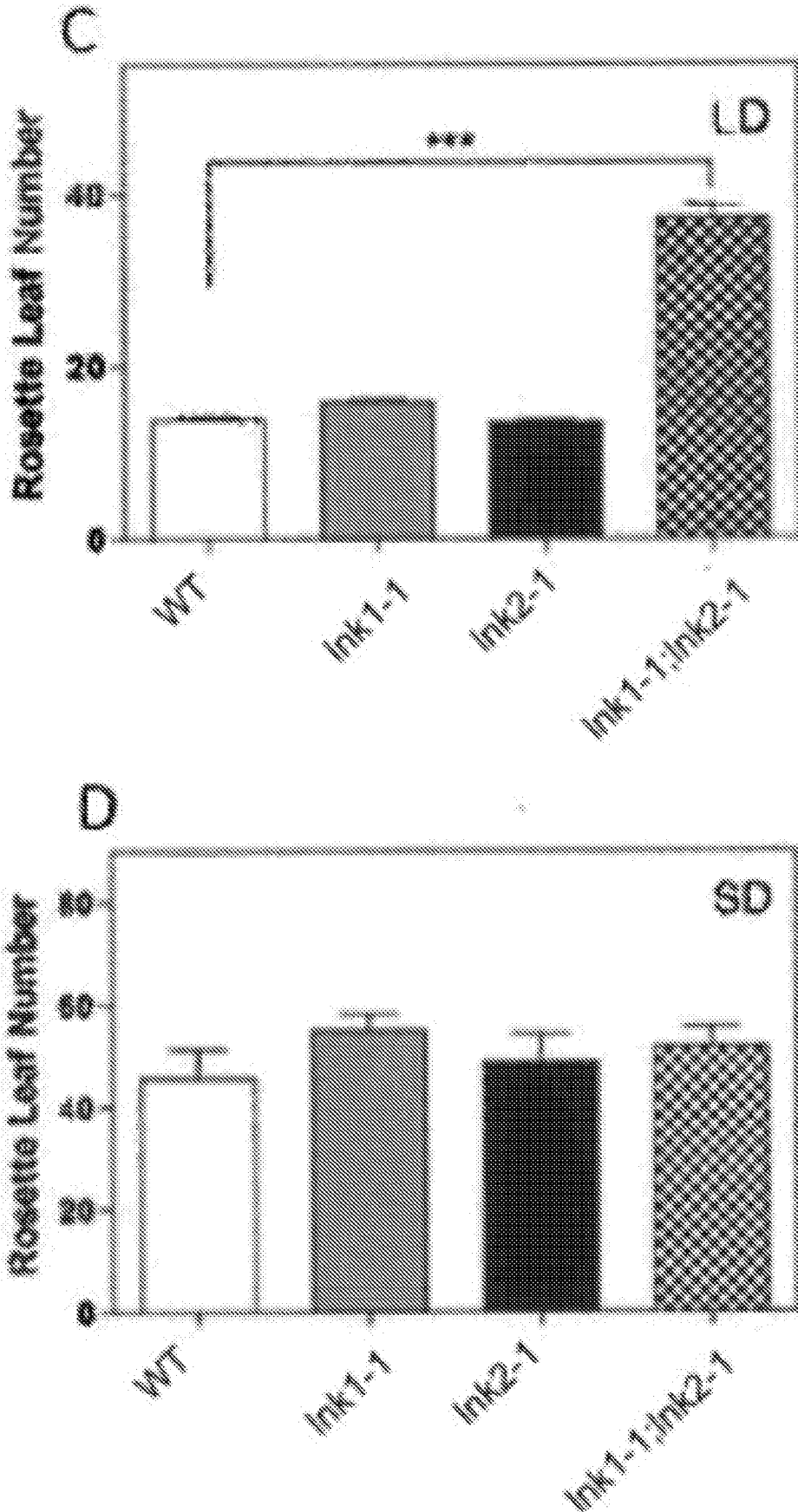


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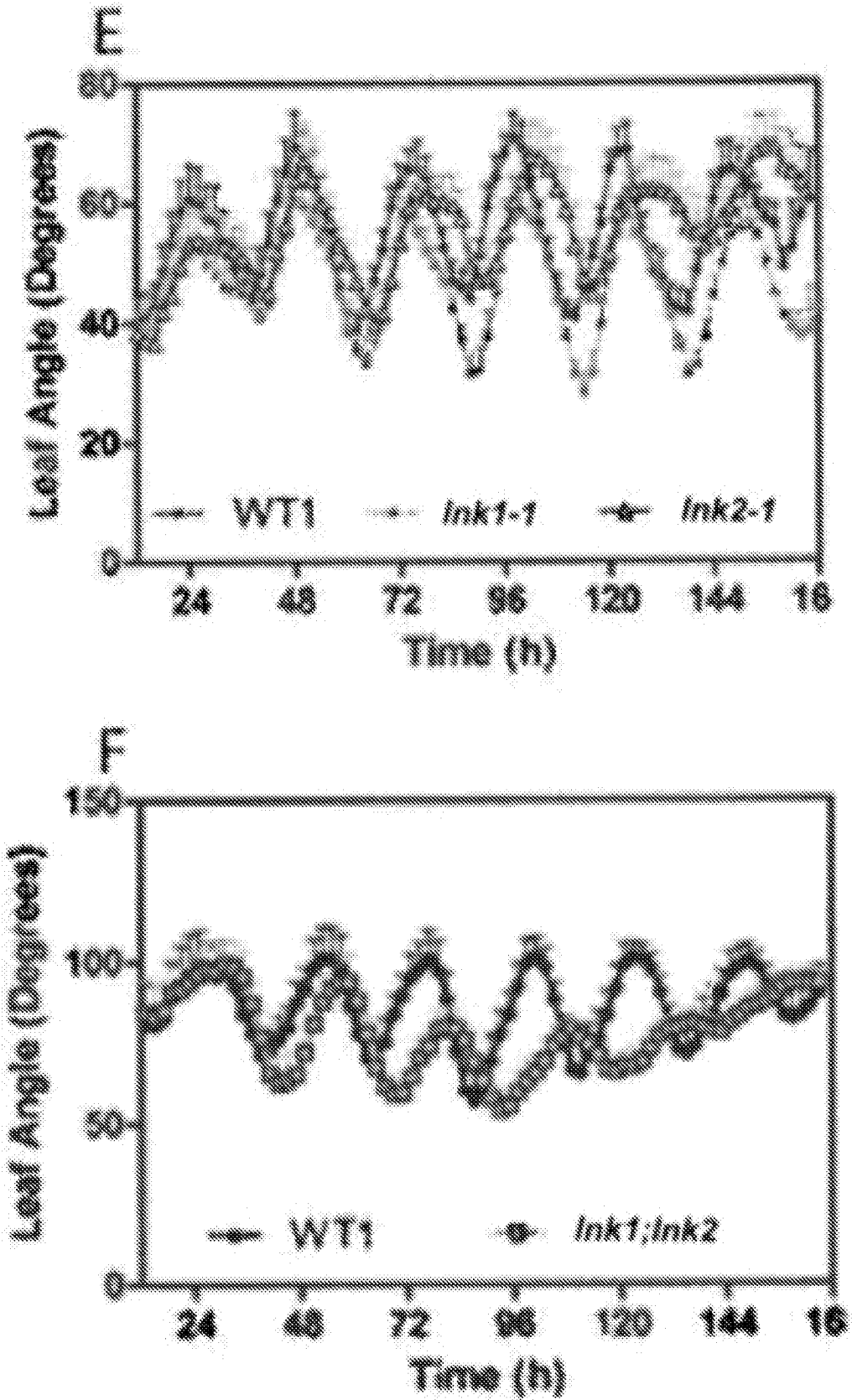


Fig. 3

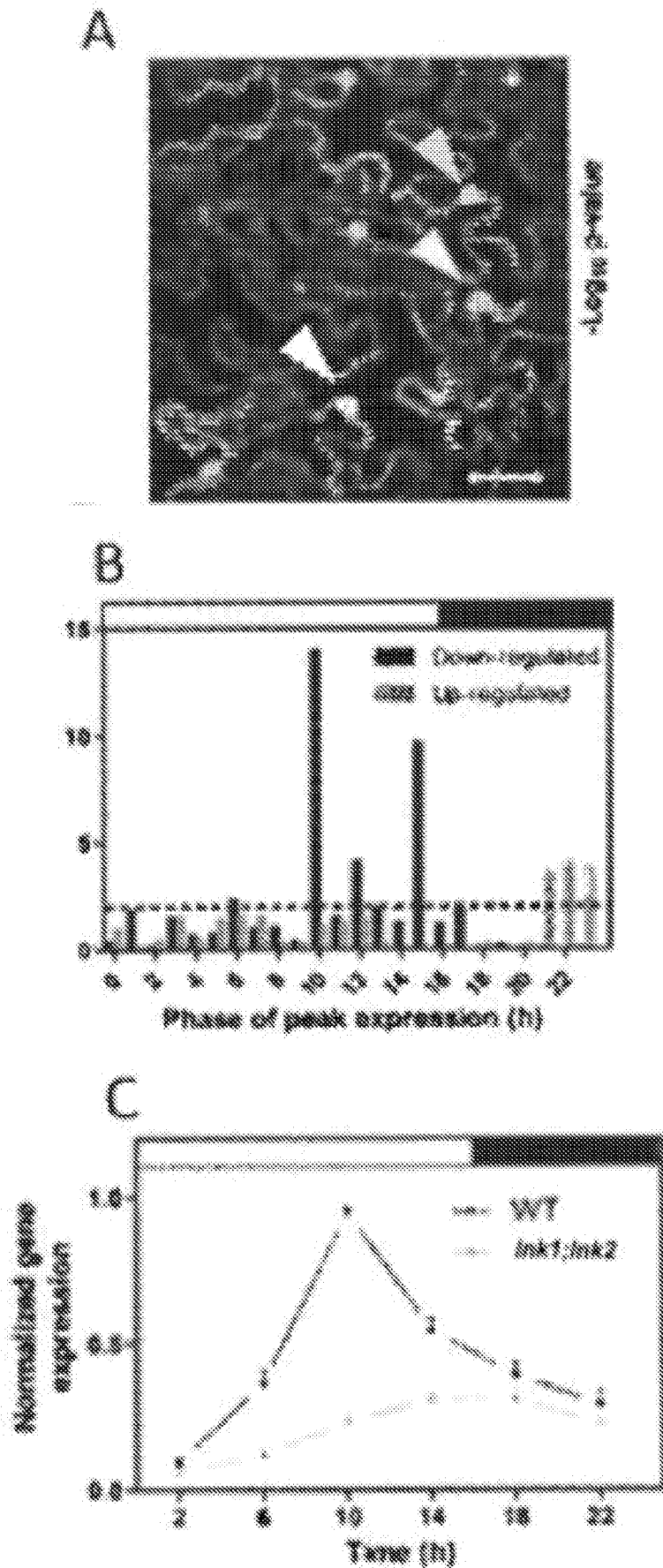


Fig. 3 continued

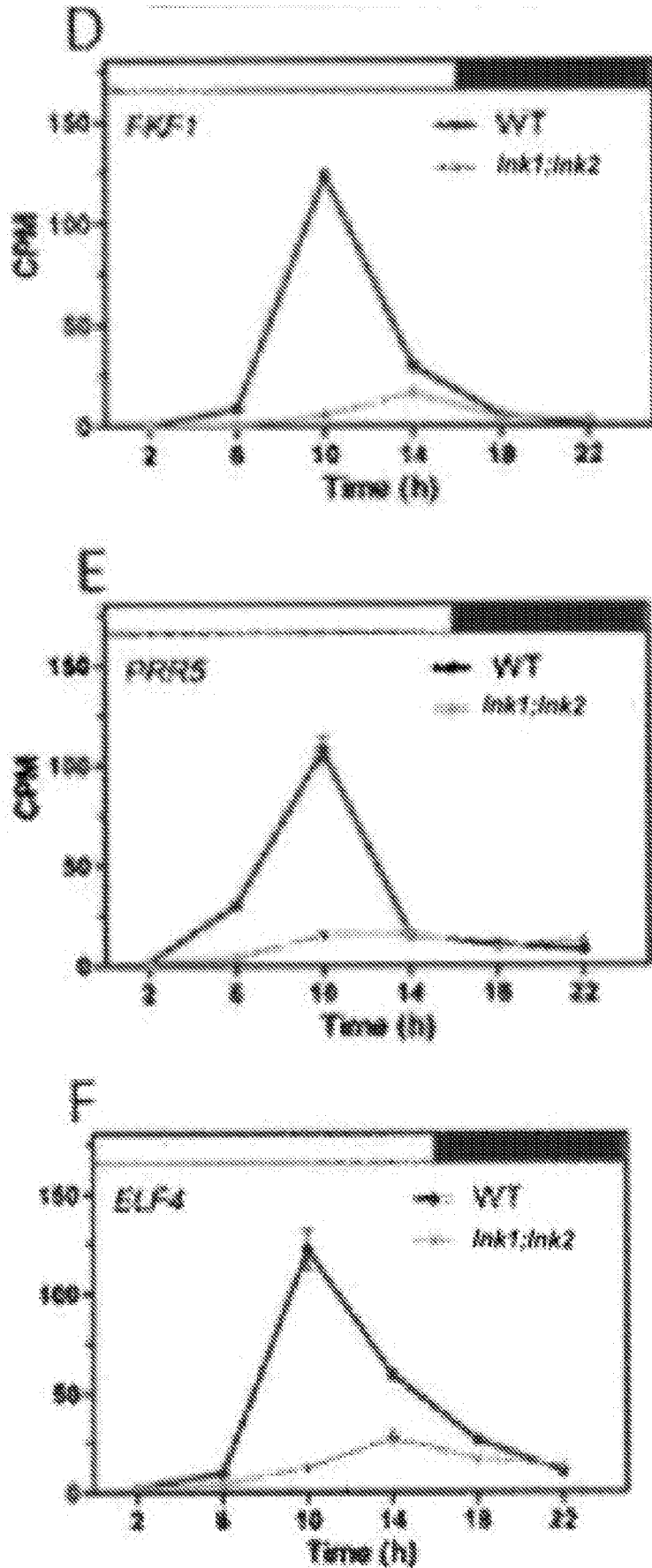


Fig. 4

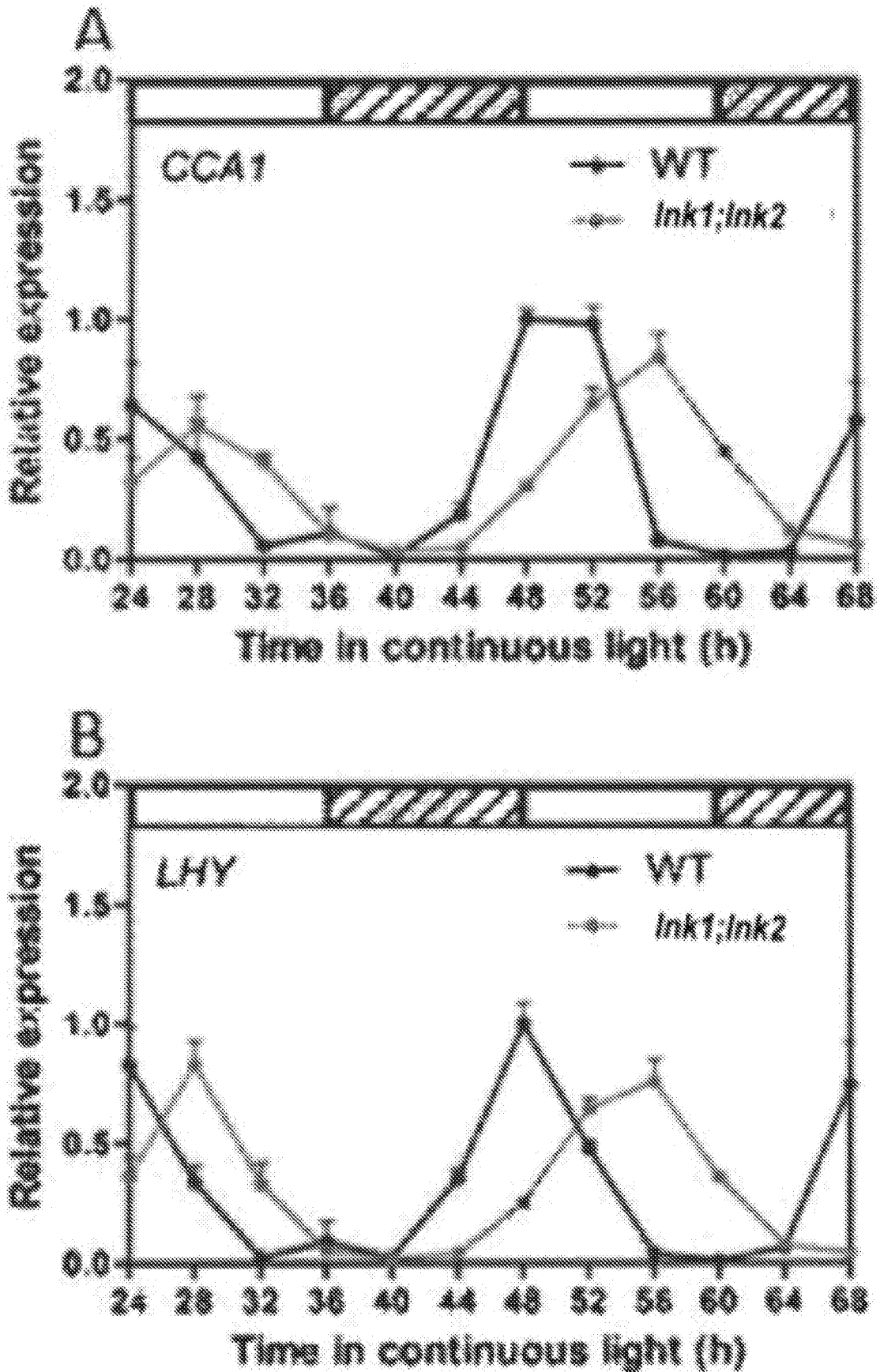


Fig. 4 continued

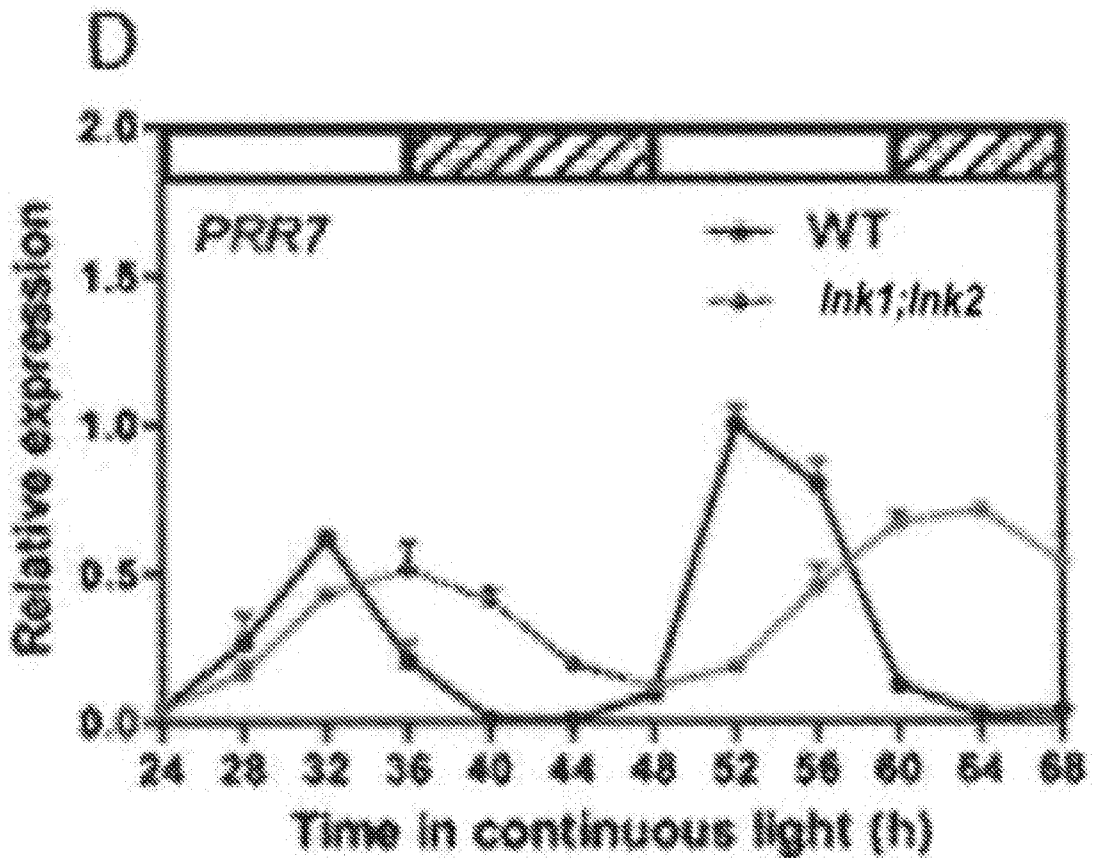
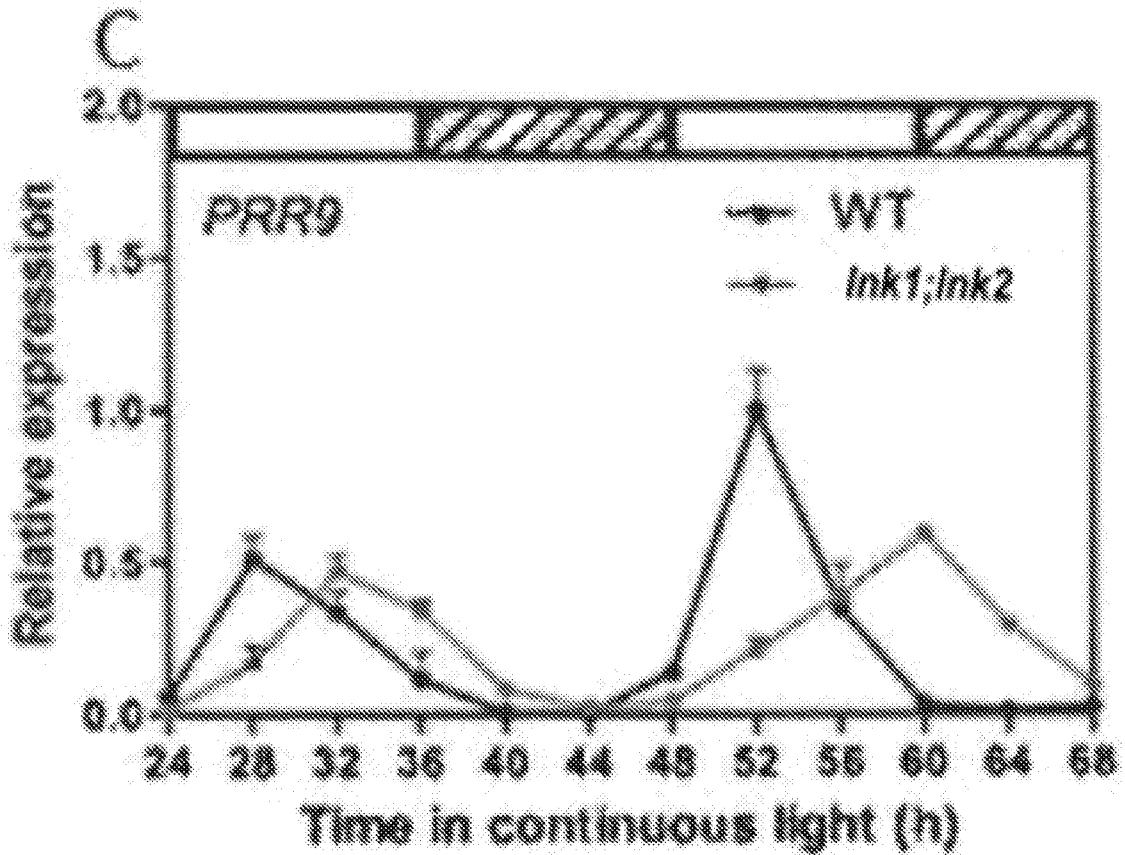


Fig. 4 continued

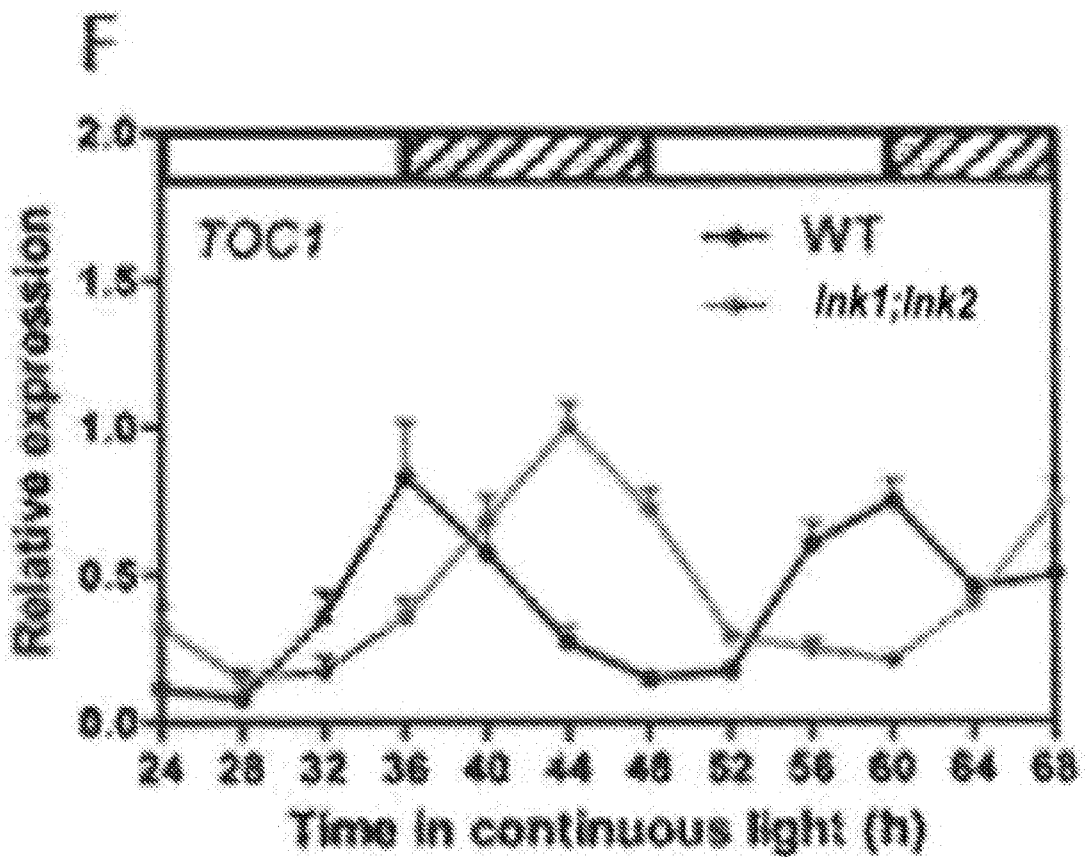
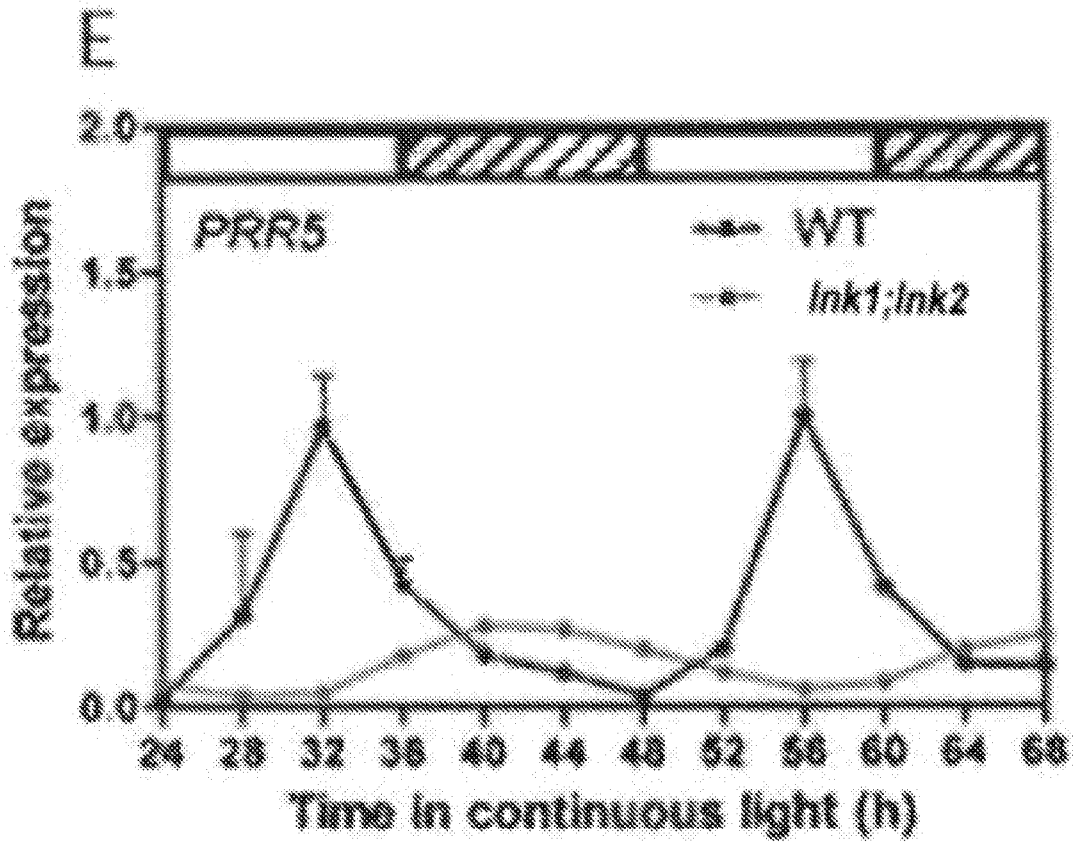


Fig. 5

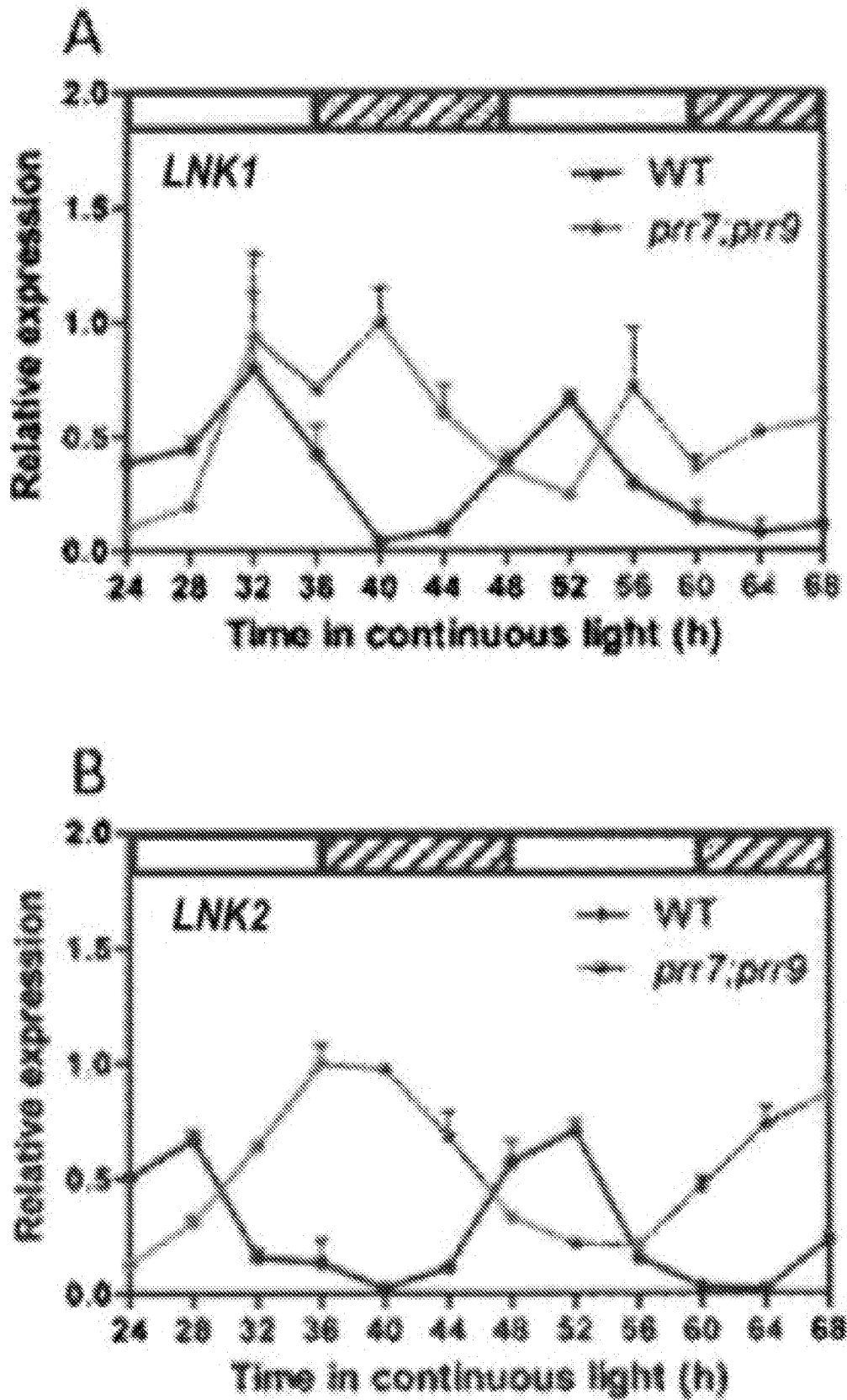


Fig. 5 continued

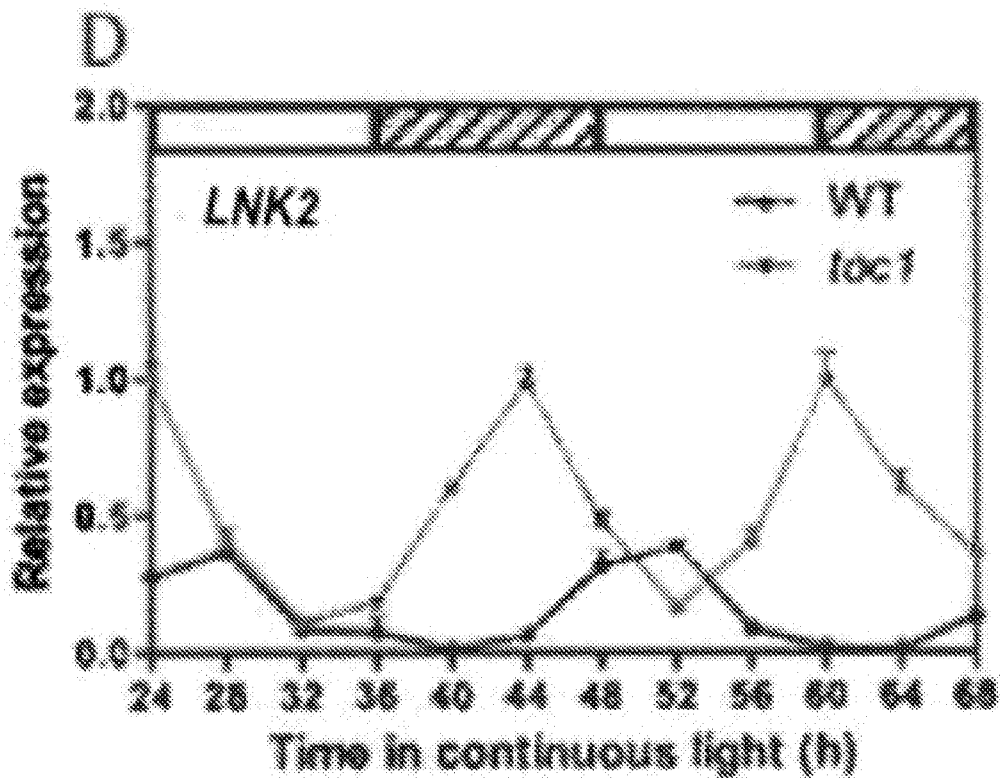
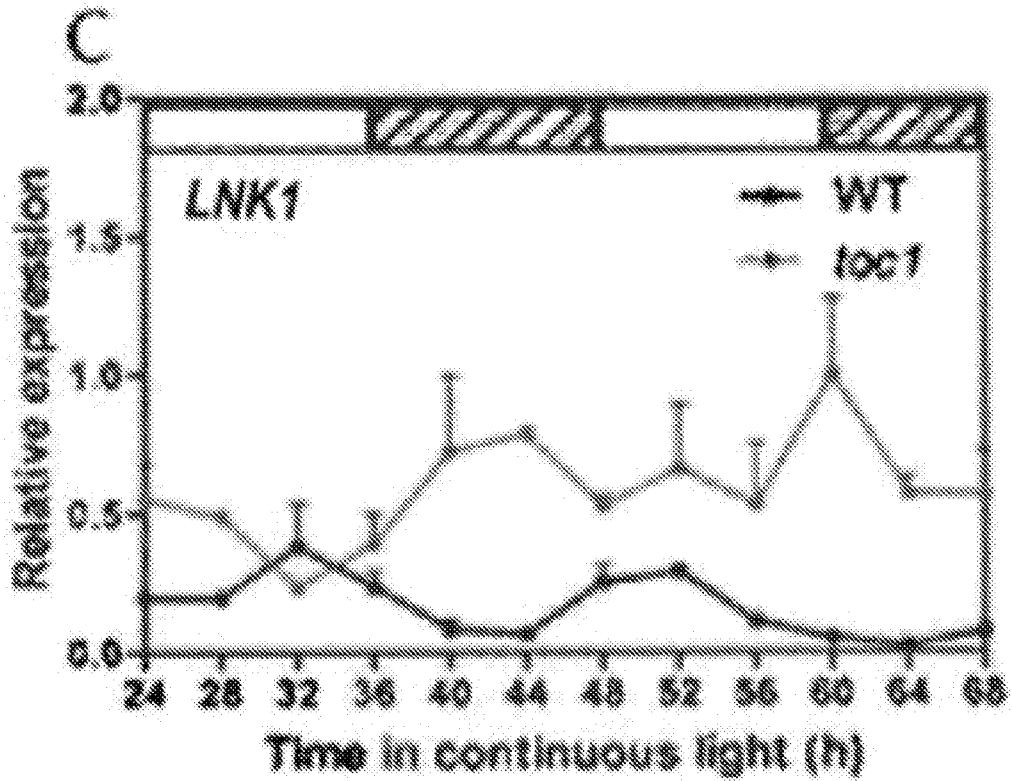


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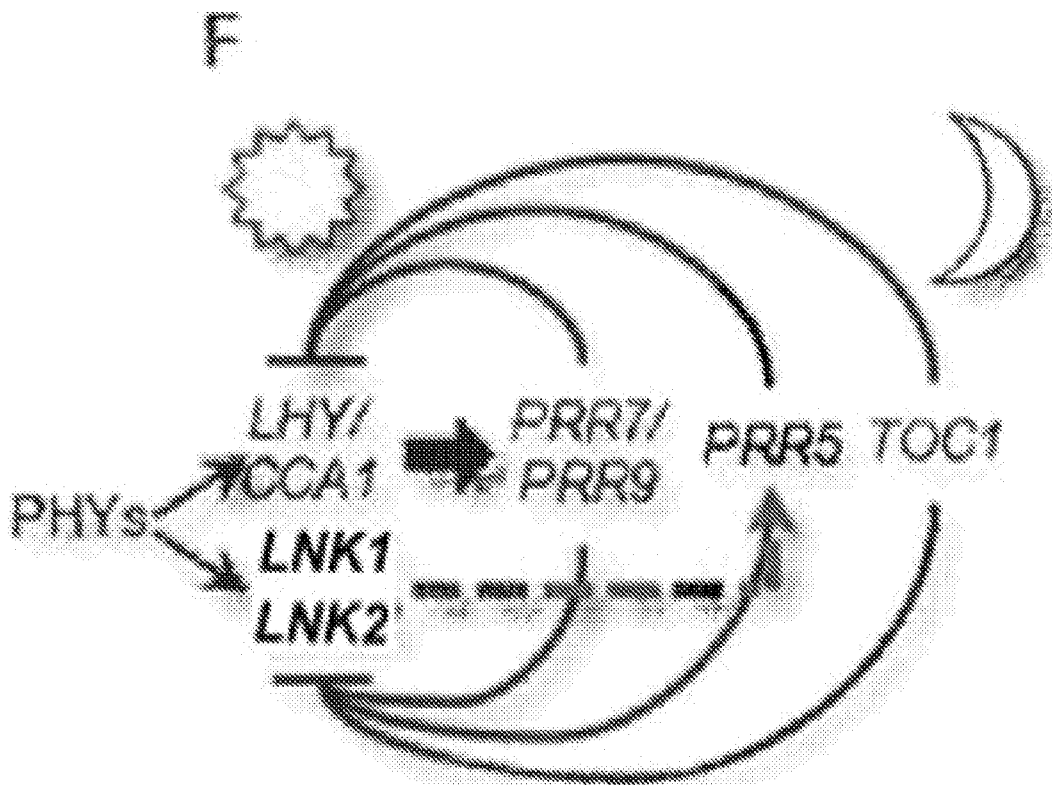
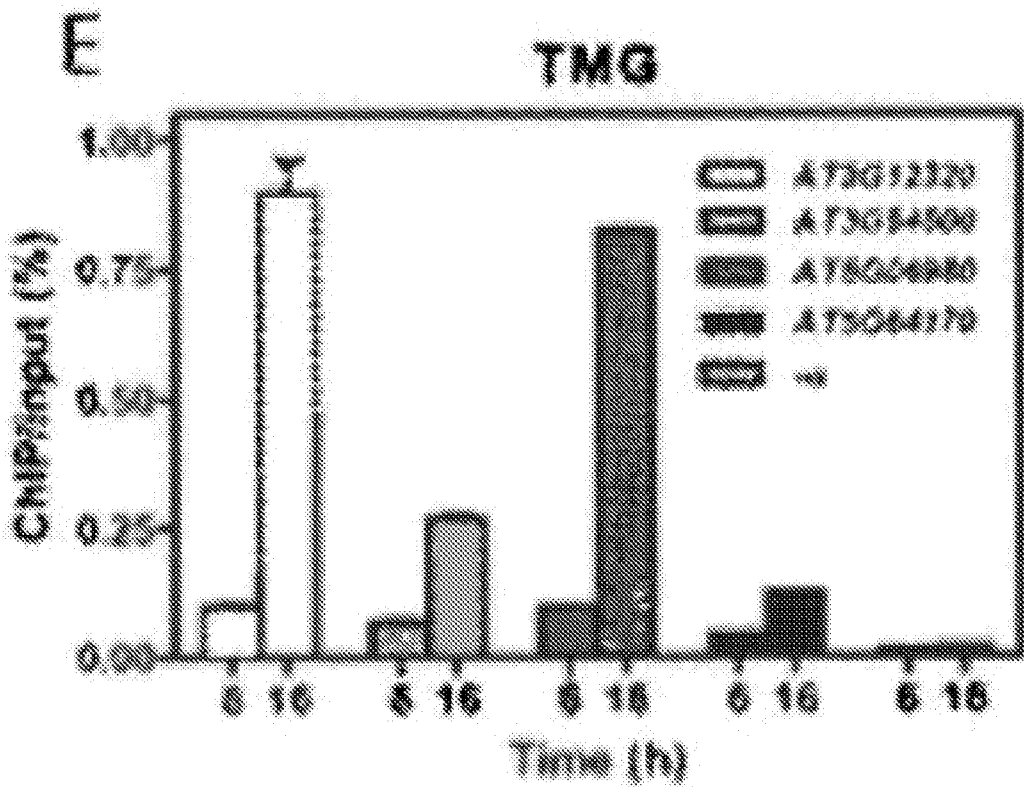


Fig. 6

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LAK171-616 MSGLYIHELGDYLSDEFHNDGDIYEDSAYEGGGPILVSRKKRR.....N 48
LAK21-648 MFRREZEELTMMIWDQDAEYGHVVE.....FKVRSQLMKKEQIEEKYAEQKIYQYKIDLND 88
LAK31-269 .....
LAK41-279 .....

LAK171-616 DOMGGDTNMLKSNYFIKREANMLGKNPHEKDSGSSVSRDTGTGKOVQDMTLEDNTYSDHGPNQGNVDF 128
LAK21-648 KNLGSSSNVDEGLP...QPDFCS...DTSLTNAI.....K 88
LAK31-269 .....
LAK41-279 .....

LAK171-616 VENTSTGDFMLCOTYBAATNDQVYMYSLNSIPDAENGLSFDNG...DKEKNDLFYONGDIQNFEDVDNML 188
LAK21-648 DQDLGATELSKGLAEPV...RYDSTHEKYSSELGKGPDI#HSSDESKKGGDFDYSWARIQSFDLDRMF 181
LAK31-269 .....
LAK41-279 .....

LAK171-616 RSCDSTFOLDSEHNEGLGPFSAQPNEETAGAMTDDLKPKMLENGRTAMLQVEDLNNSEPNNAVDE 228
LAK21-648 SNOVPIEDDGLSGKDELSSKNDVNSPKSLB...SMLDSDDLGLDIRTEEQEN.....278
LAK31-269 .....
LAK41-279 .....

LAK171-616 YGYTIEDCSAQKKEGNY.....FDTSLKKDILMLDVEANLEKKGTJNLHHLG 304
LAK21-648 QQFPLTCKANGLSSQVPSVRYTLKADQYREHKGQPSVEDQPYGKMKKFSKM...PQYEAQPFQELY 282
LAK31-269 .....CYABELVVPNI...ESSLETYETQMN 27
LAK41-279 .....MORYSRNLEDLVVPNI...ETSDSTSPDMN 30

LAK171-616 K...SDGFEN...SF.....TLQHSIERSIMDINGYPPSFGQRDVPYSHFNCEQ 352
LAK21-648 Q...RIPFNGAGVNCQLAPPQSSLMAYNLLSEESGDTSHYSHMPNLYMANSEFGLANDYSEVPYIS 350
LAK31-269 .....
LAK41-279 .....

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Fig. 6 continued

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436 440 444 448 452 456 460 464 468 472 476 480
LNK21-616 P8VQV5ACE5K521K3ENKP8P8S8AS8NE5YTS8NHAQS1E8LQ...G8T...V8DRF8R8V8E8TR...ANL 412
LNK21-648 AVQ...HP2V8R8Q...L8M8P8Y8N8P8A8T8S8V8N8A8Y8D8A8R8E8S8T8M8P8Q8E8K8E8...L 398
LNK21-269 ...D8F8N8Q...E8M8Y8S8C8S8R8E8E8...E8E8K8...R8S8A8F8Y8G8A8S8E8H8C8F 83
LNK21-279 ...D8V8I8H8Q...F8S8O8L8Y8Q8E8N8D8M8G8T8E8...Q8V8E8E8Y8K8K8A8S8C8F8O8R8E8L8H8D8F 93

400 404 408 412 416 420 424 428 432 436 440 444 448 452 456 460 464 468 472 476 480
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LNK21-648 R8R8N8Q8...G8A8L8I8C8R8Q...Q8Q8F8...H8C8V8A8D8S8I8T8Q8N8C8L8O8I8P8L8Q8L8V8D 440
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LNK21-279 D8E8I8N8...D8D8N8F8...I8E8D8V8P8C8H8E8N8F8L8S8F8K8E8D8N8N8S8S8S8Y8L8D8T8D8O8R8E8...L8M8Y8N8...E8T8C8O8M8P8L8E8D 180

476 480 484 488 492 496 500 504 508 512 516 520 524 528 532 536 540 544 548 552 556 560 564 568 572 576 580 584 588 592 596 600 604 608 612 616 620 624 628 632 636 640 644 648 652 656 660 664 668 672 676 680 684 688 692 696 700 704 708 712 716 720 724 728 732 736 740 744 748 752 756 760 764 768 772 776 780 784 788 792 796 800 804 808 812 816 820 824 828 832 836 840 844 848 852 856 860 864 868 872 876 880 884 888 892 896 900 904 908 912 916 920 924 928 932 936 940 944 948 952 956 960 964 968 972 976 980 984 988 992 996 1000
LNK11-616 Y...3...M8C8O8S8C8V8Q8...V8V8D8I...L8A8T8S8F8R8Q8V8I8E8Q8D8V8R8K8L8I8S8L8Y8...M8S8A 813
LNK21-648 K...T...L8L8L8L8T8A8M8P8F8O8P8S8S8U8E8Q8O8S8O8K8F8A8A8Y8O8N8...F8A8V8Y8R8...D8V8A8...D8M8C8...I8S8L8F8...S8E8A 509
LNK21-269 ...F8T8I8S...D8L8E8E8N8M8L8D8E8N8Y8O8E8L8S8...S8P8V8I8O8N8...R8A8S8O8N8...Y8E8H8K8...F8T8F8...R8E8S 281
LNK21-279 ...A8P8M8L8...C8E8E8N8K8E8A8...S8V8I8C8D8...P8A8T8...M8T8D8...K8K8...F8T8F8...K8N8 309

640 644 648 652 656 660 664 668 672 676 680 684 688 692 696 700 704 708 712 716 720 724 728 732 736 740 744 748 752 756 760 764 768 772 776 780 784 788 792 796 800 804 808 812 816 820 824 828 832 836 840 844 848 852 856 860 864 868 872 876 880 884 888 892 896 900 904 908 912 916 920 924 928 932 936 940 944 948 952 956 960 964 968 972 976 980 984 988 992 996 1000
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LNK21-279 G8K8S8D8N8D8...E8F8...L8E8D8T8S8N8D8S8S8P8M8T8F8L8Y8C8K8L8N8L8K8P8S8...A8V8...N8T8...K8M8E8S8M8R8... 287

716 720 724 728 732 736 740 744 748 752 756 760 764 768 772 776 780 784 788 792 796 800 804 808 812 816 820 824 828 832 836 840 844 848 852 856 860 864 868 872 876 880 884 888 892 896 900 904 908 912 916 920 924 928 932 936 940 944 948 952 956 960 964 968 972 976 980 984 988 992 996 1000
LNK11-616 Y8K8...H8P8I8...P8Q8P8I8S8P8L8R8I8...E8K8E8T8T8E8L8R8E8A8E8Y8T8O8N8... 818
LNK21-648 S8A8S8K8M8G8T8E8K8O8N8F8K8C8I8R8E8T8H8T8K8O8K8A8Q8E8C8P8A8D8L8A8L8Q8M8P8S8O8S8S8T8V8O8R8V8E8A8S8O8N8K8K8L 846
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LNK21-279 M8P8P8K8L8S8V8... 278

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Fig. 7

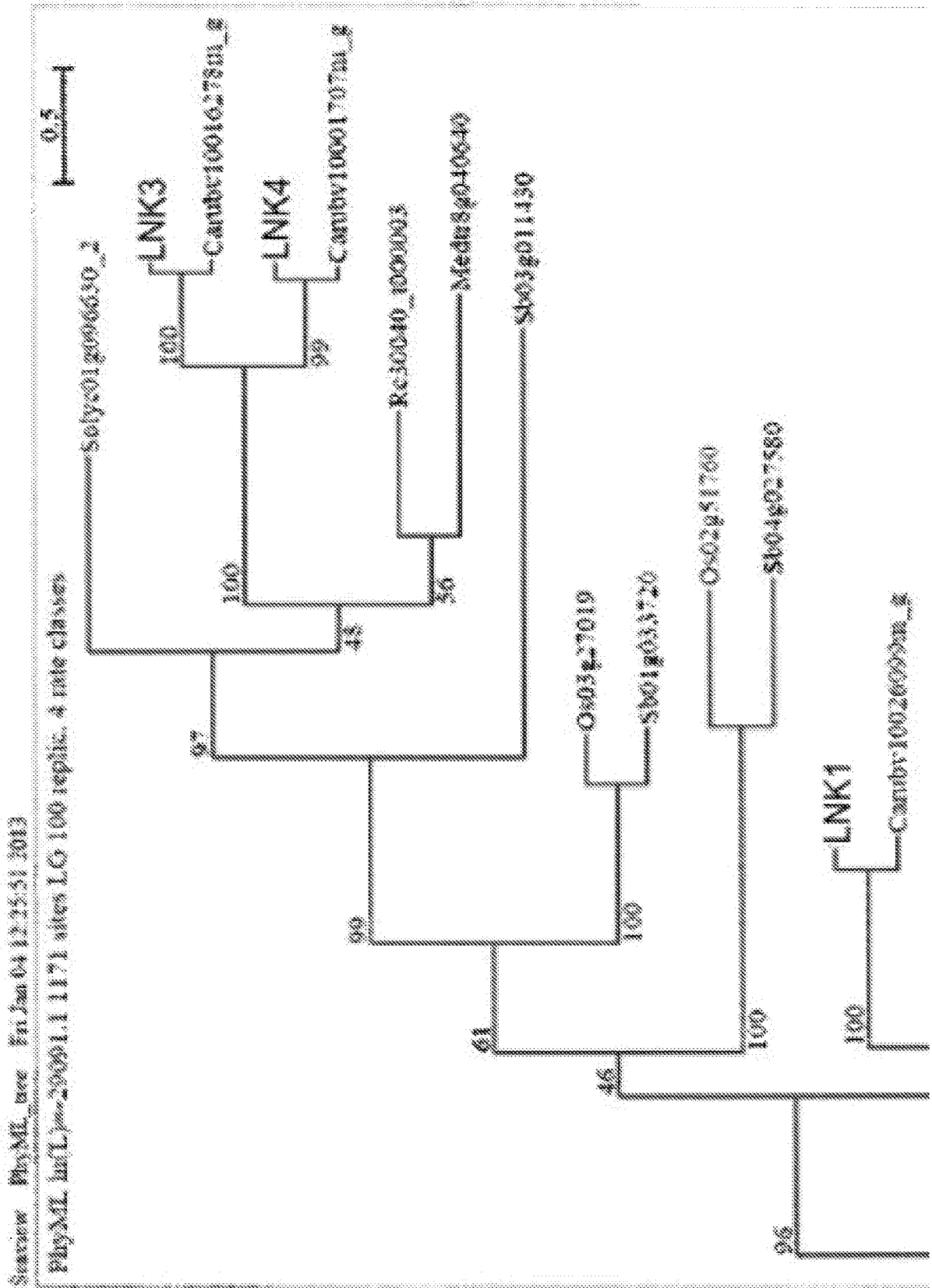


Fig. 7 continued

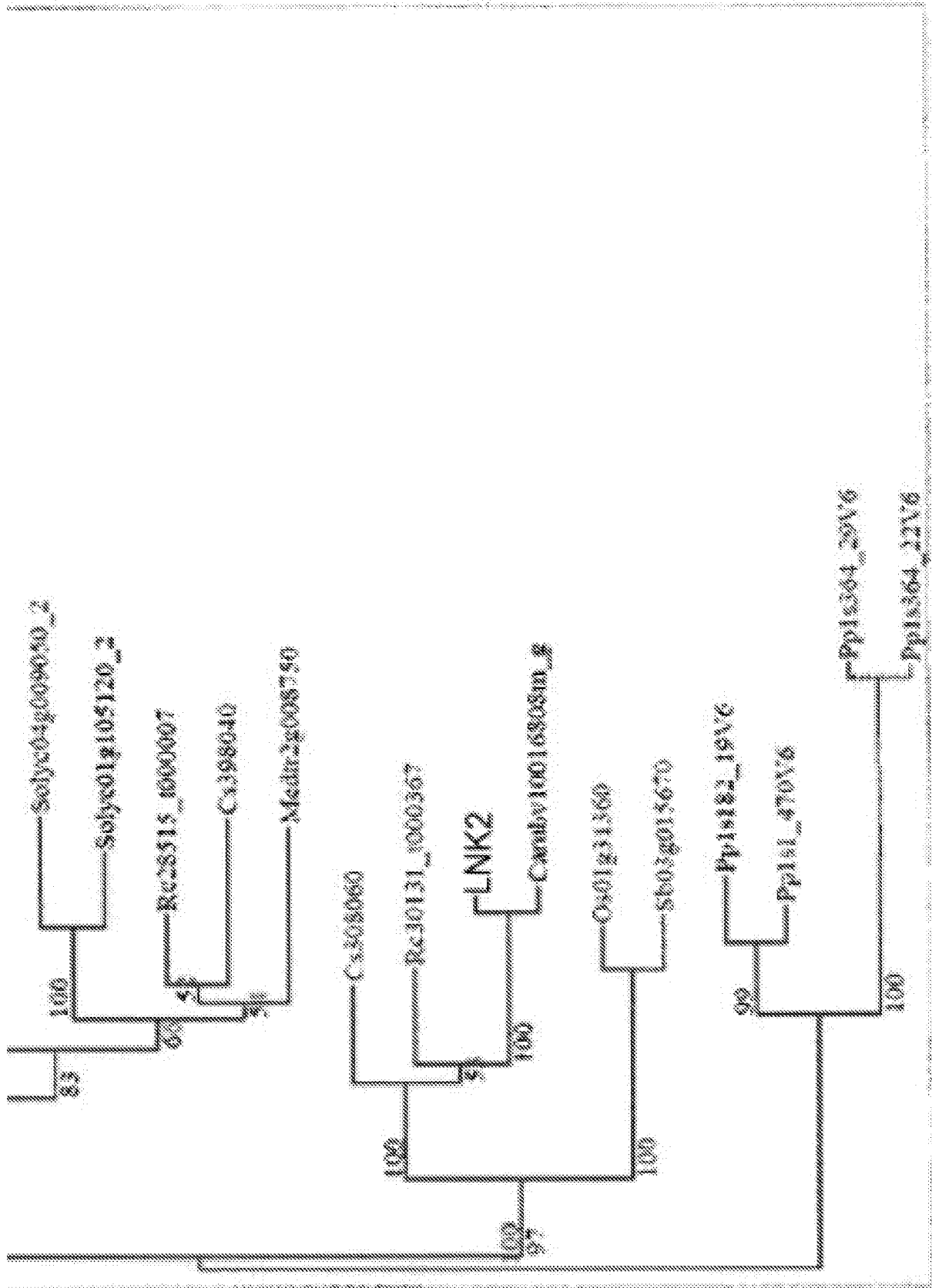


Fig. 8

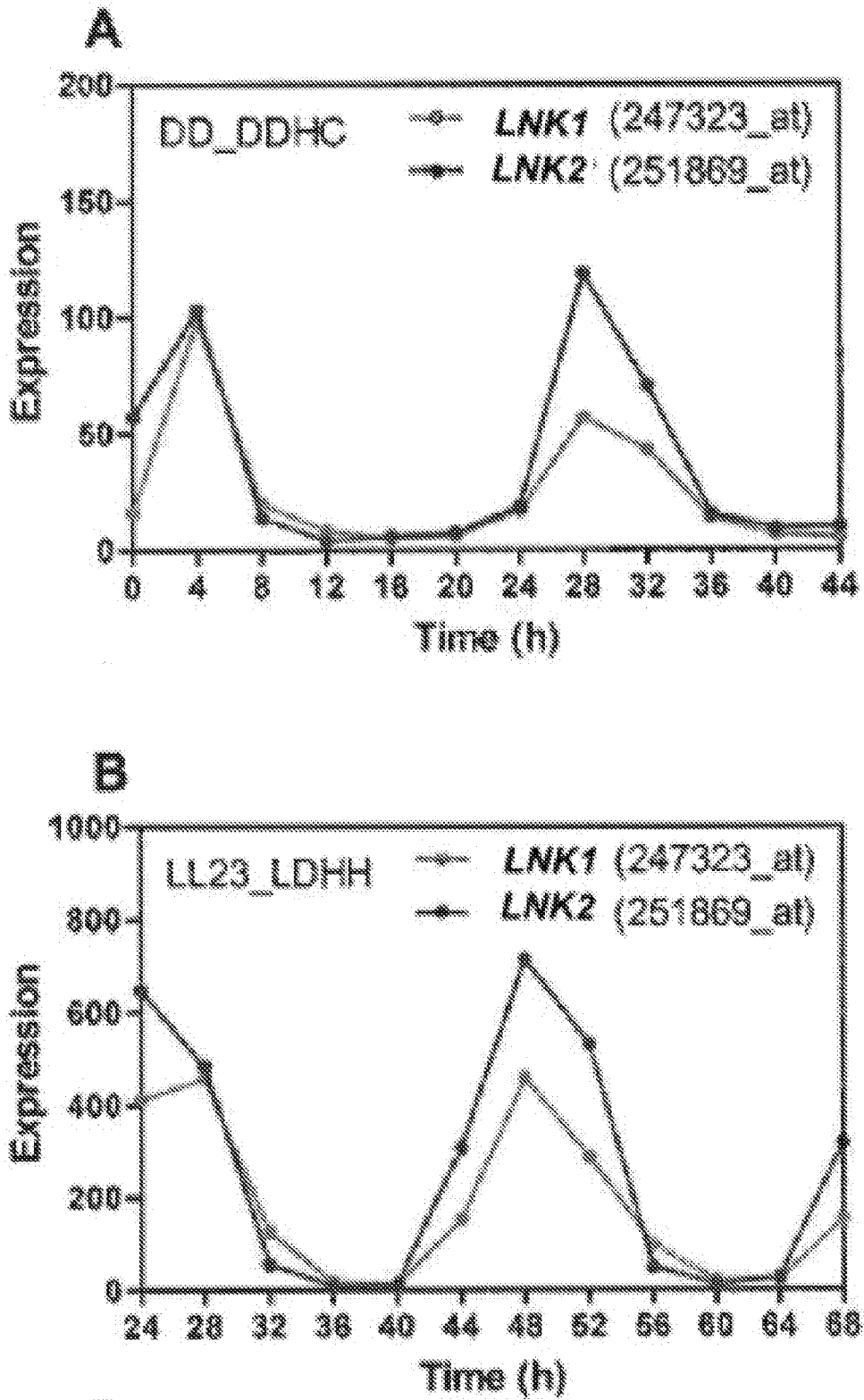


Fig. 8 continued

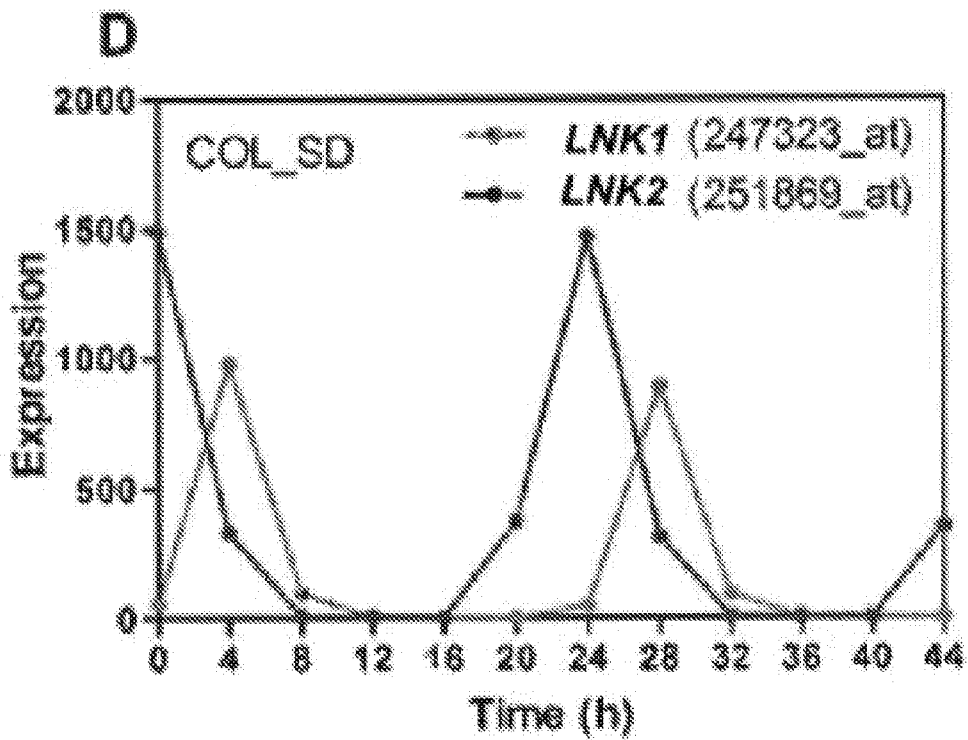
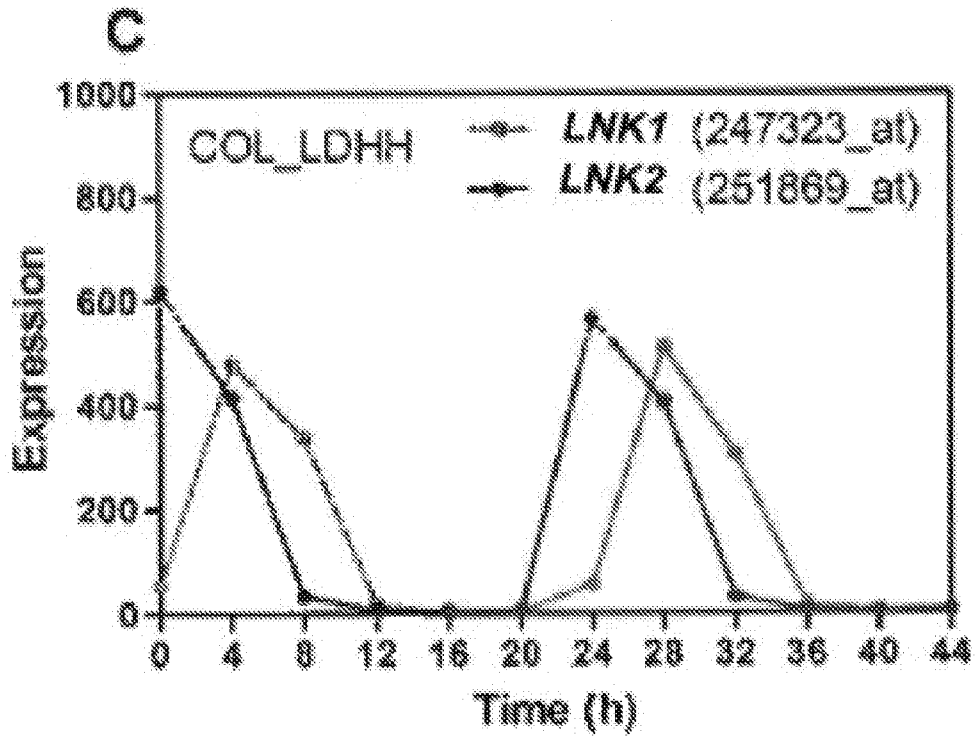


Fig. 9

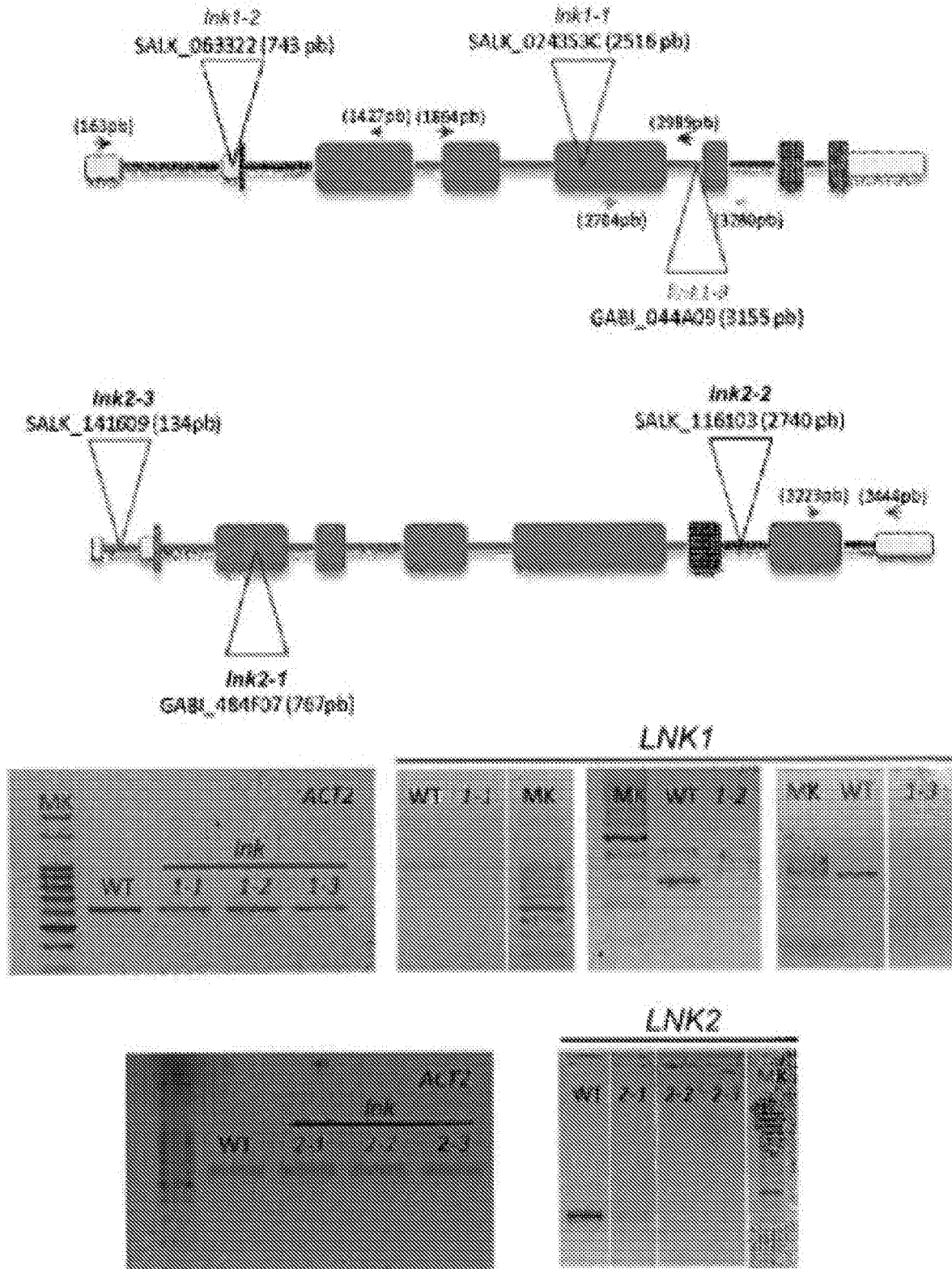


Fig. 10

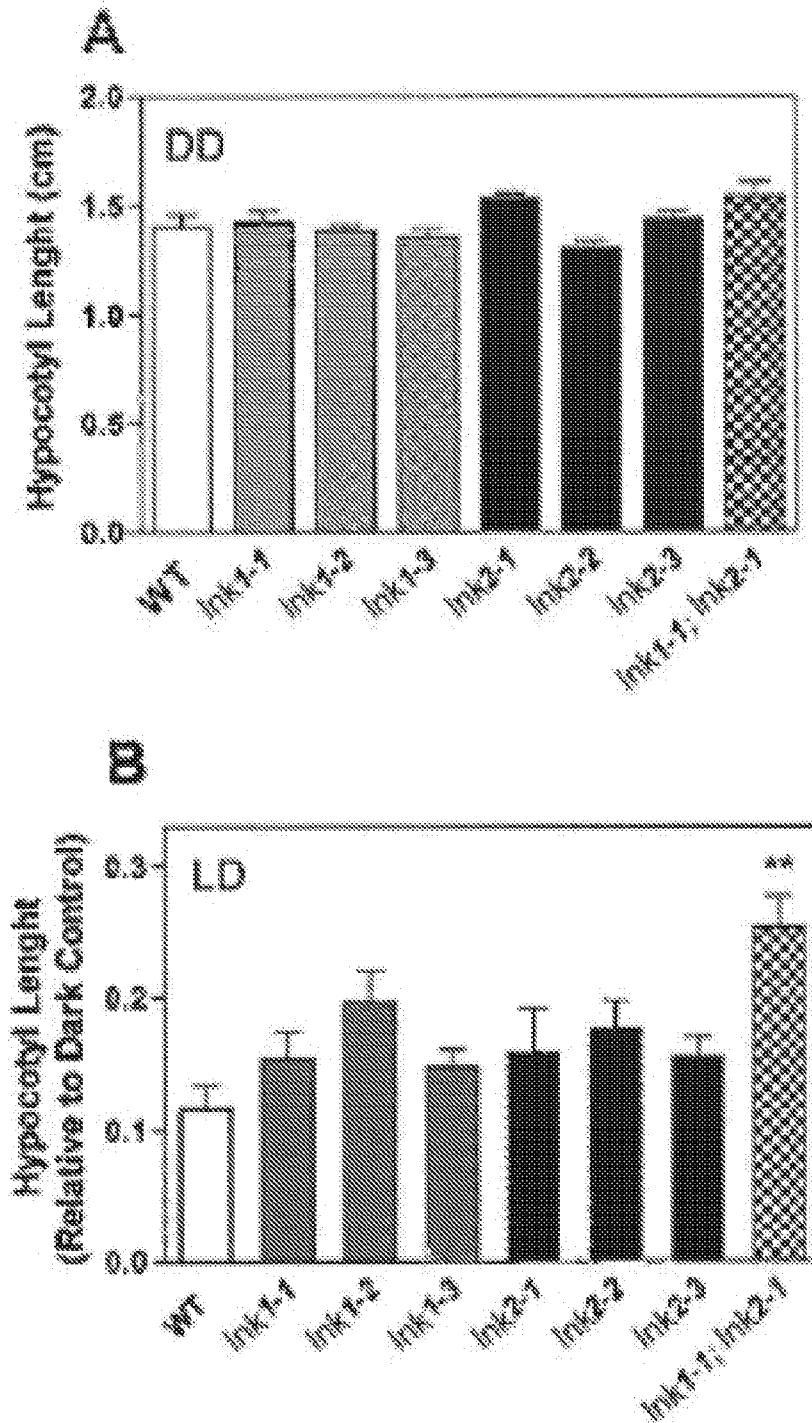


Fig. 10 continued

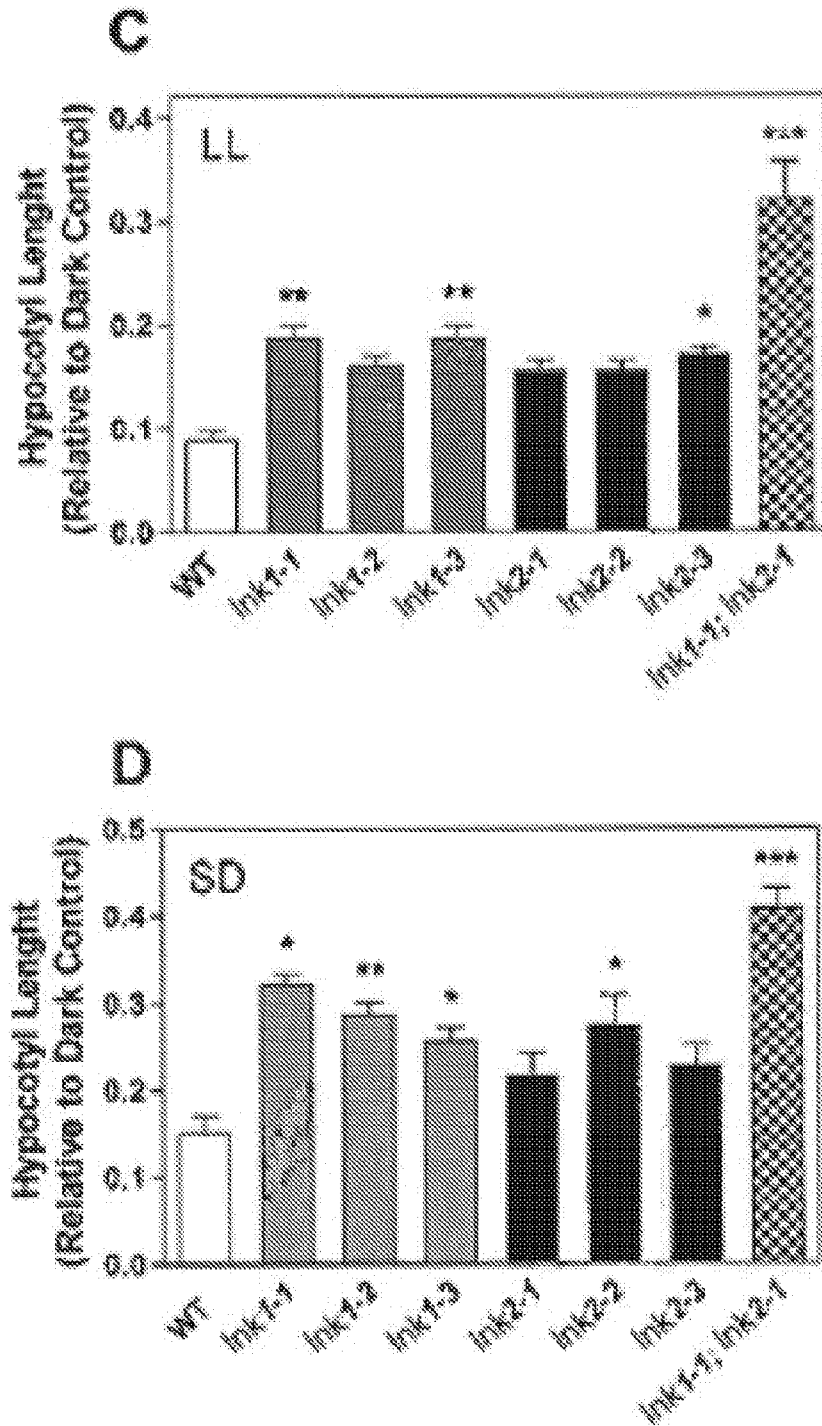


Fig. 10 continued

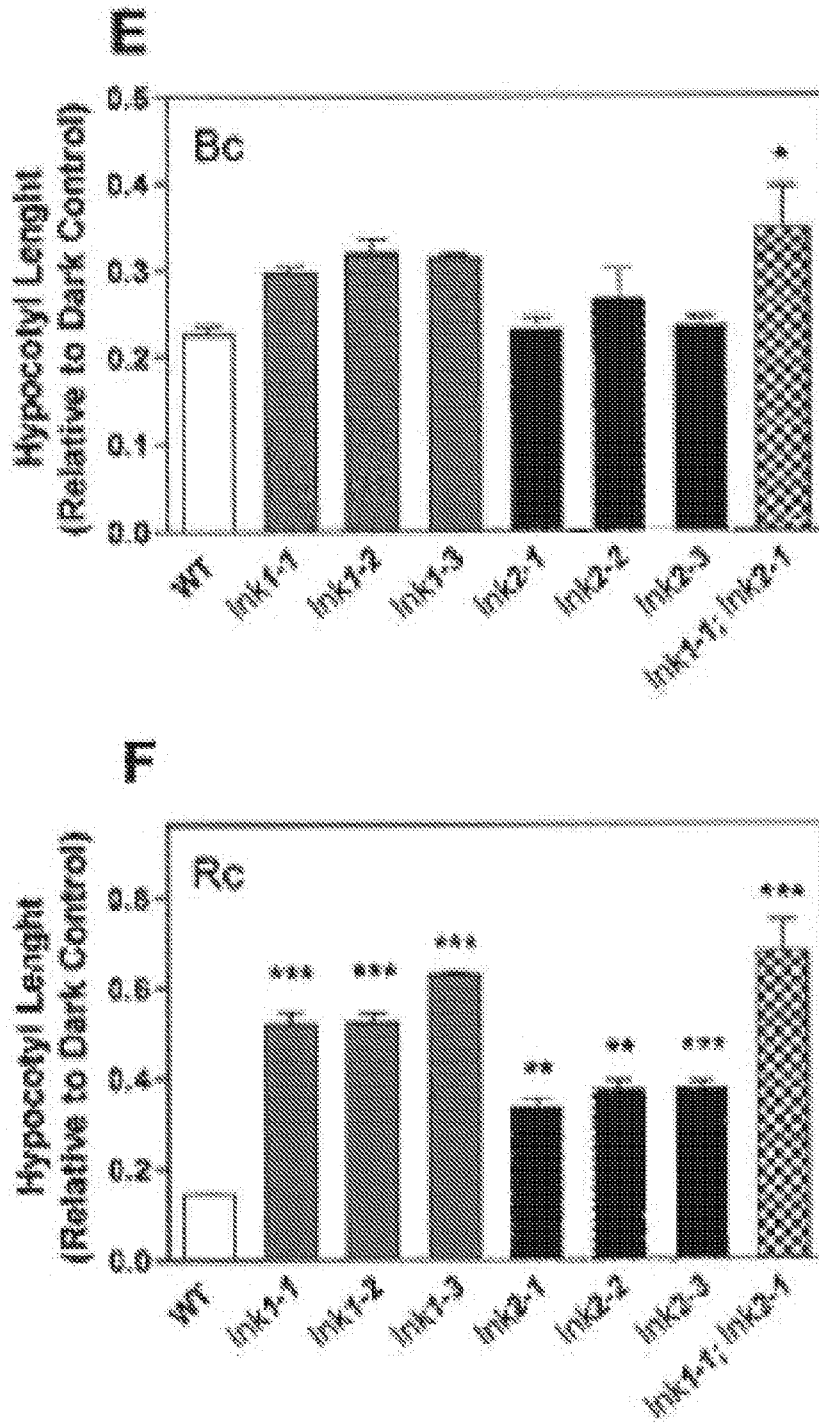


Fig. 11

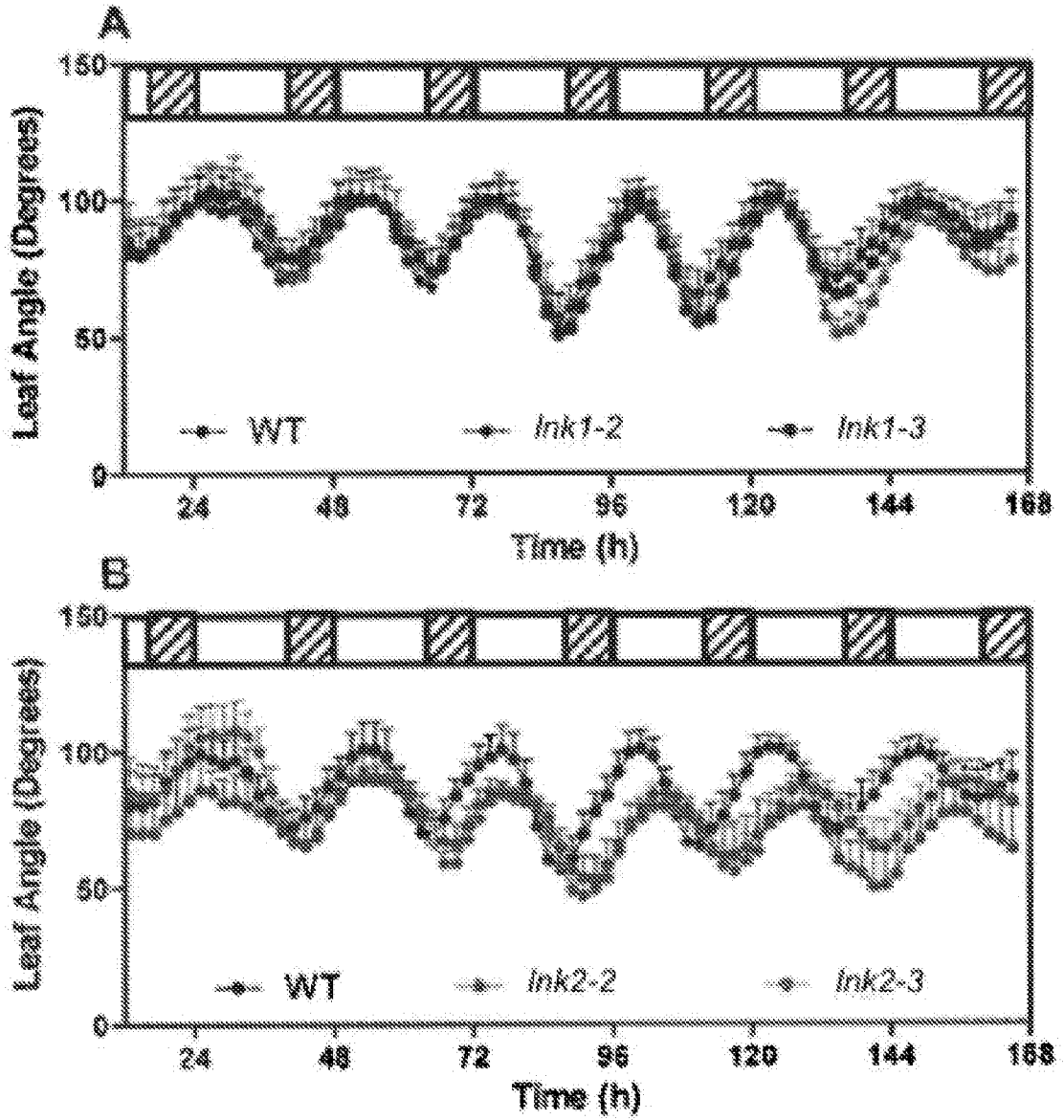


Fig. 12

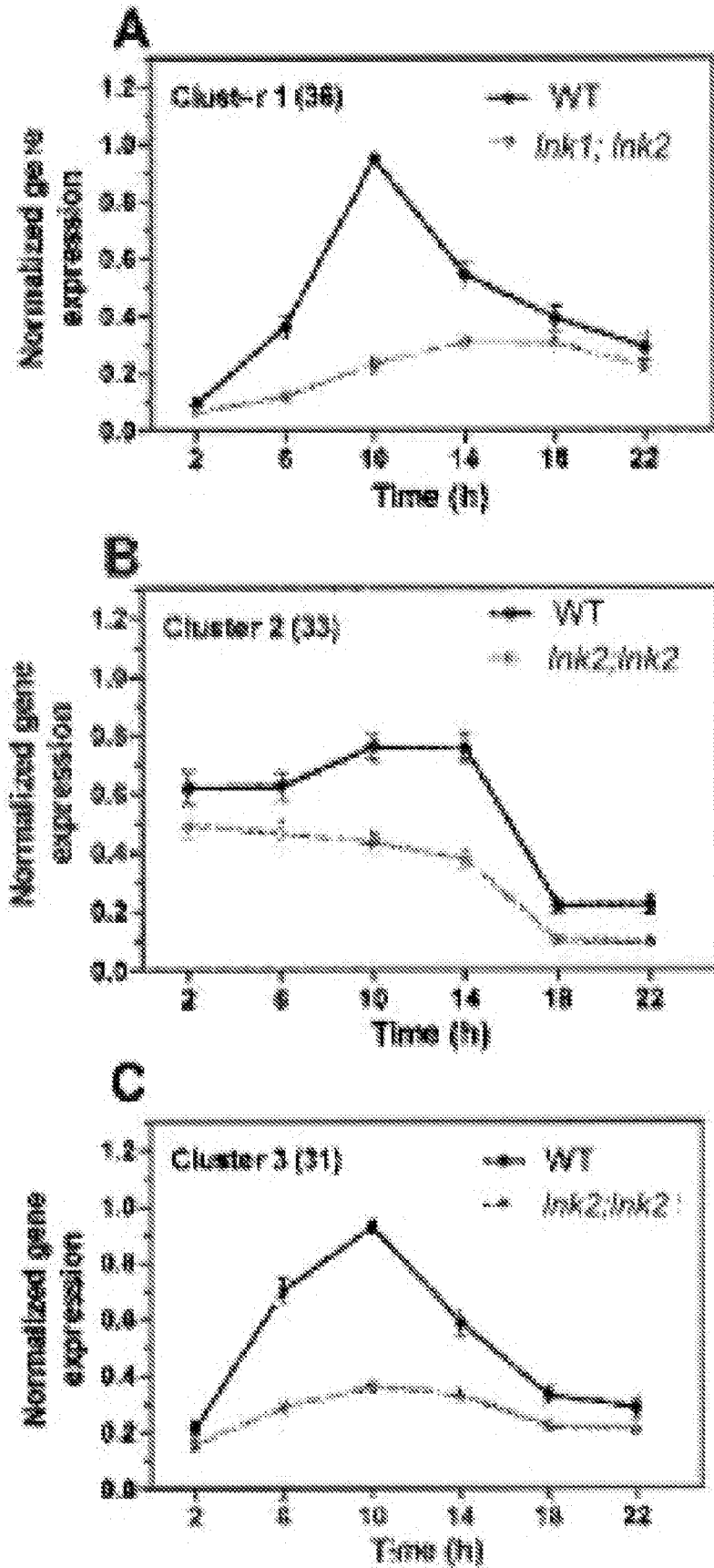


Fig. 12 continued

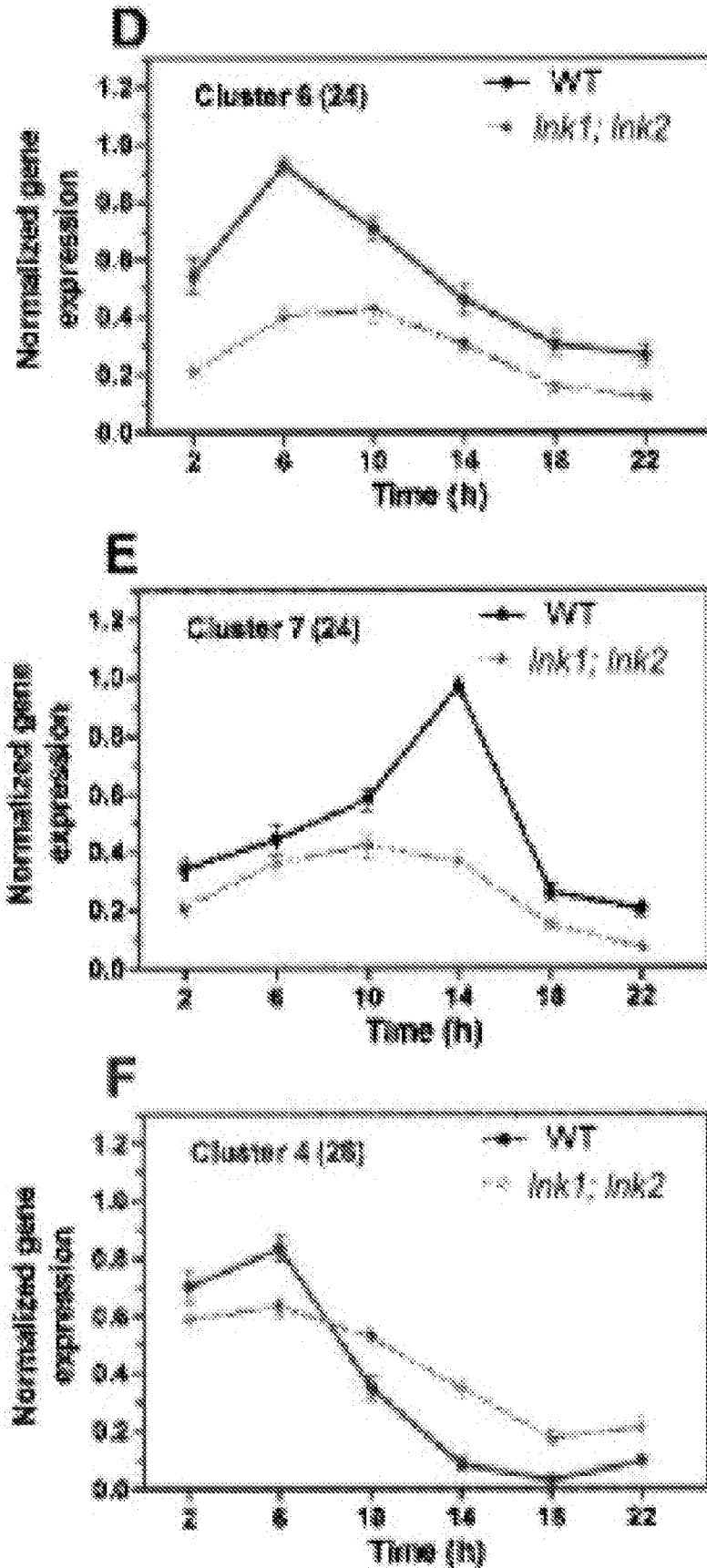


Fig. 12 continued

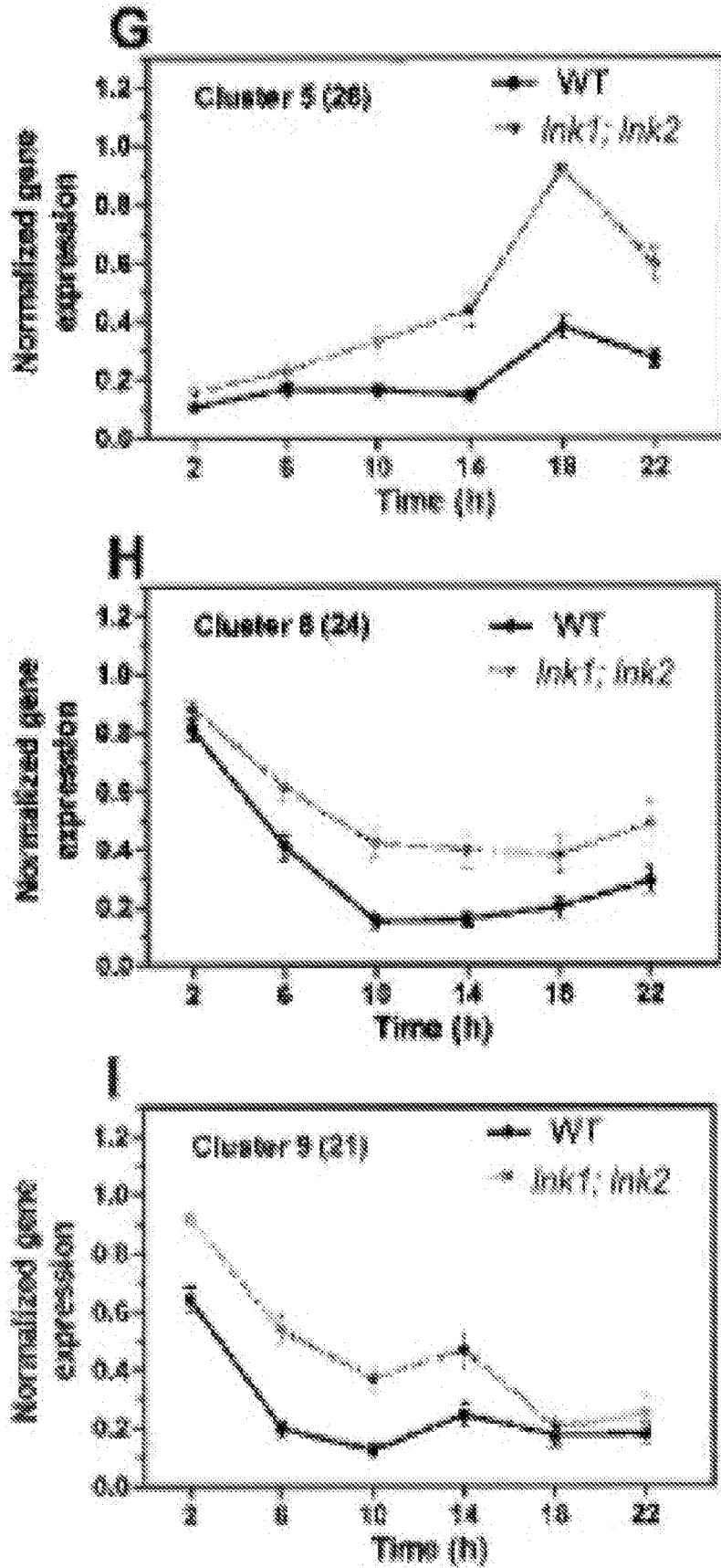


Fig. 13

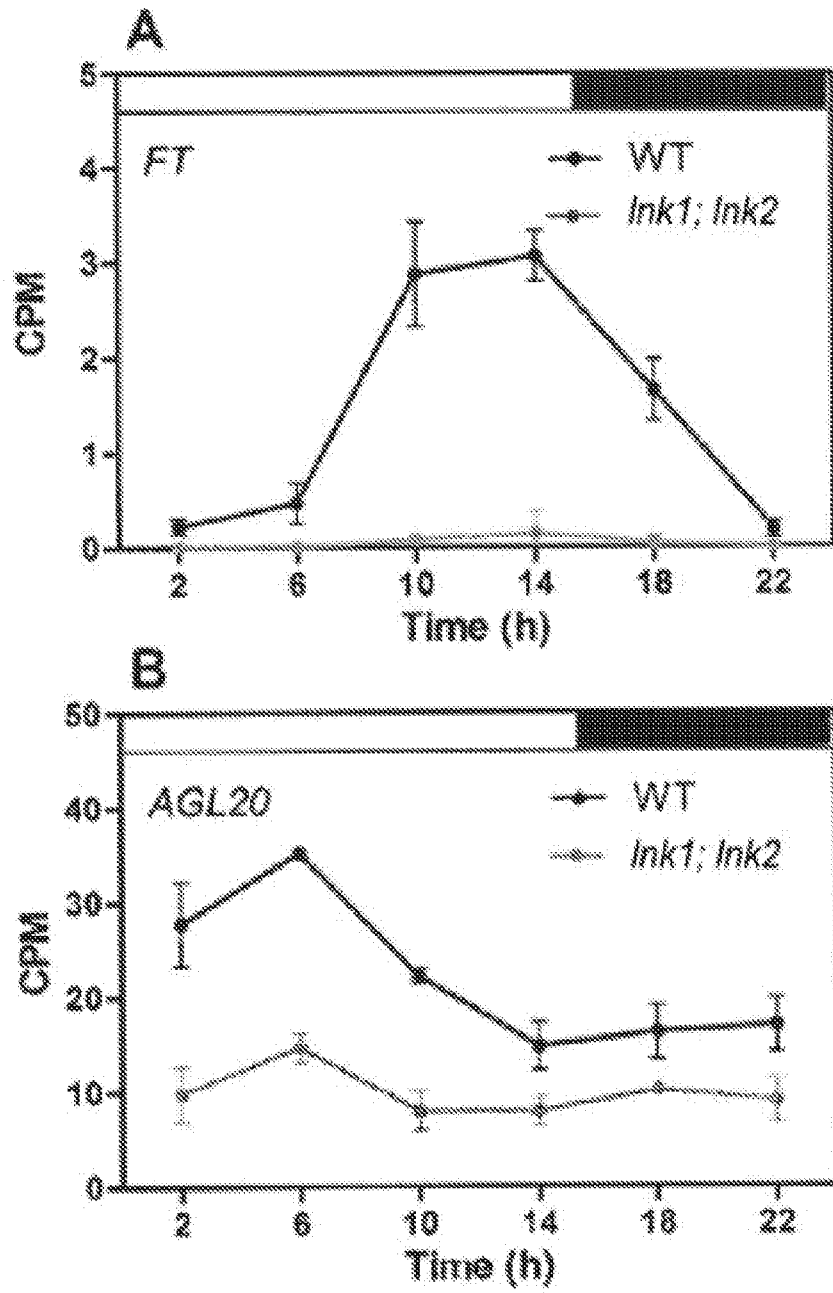


Fig. 14

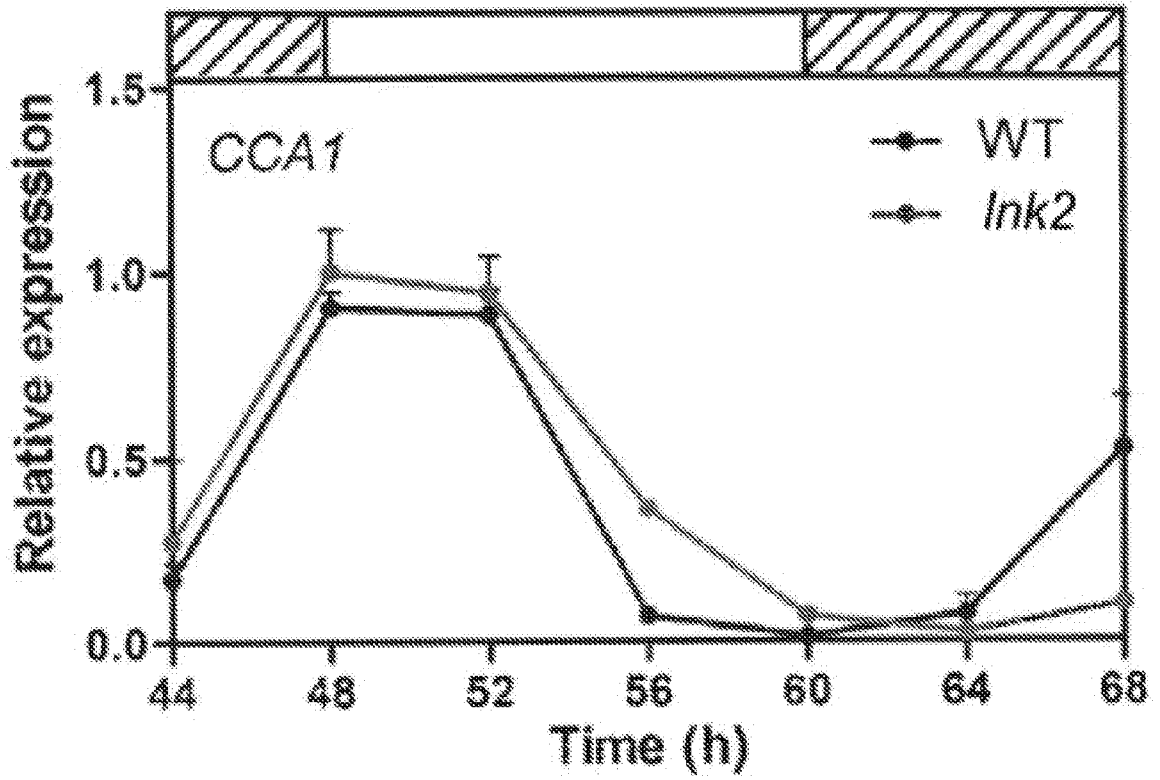


Fig. 15

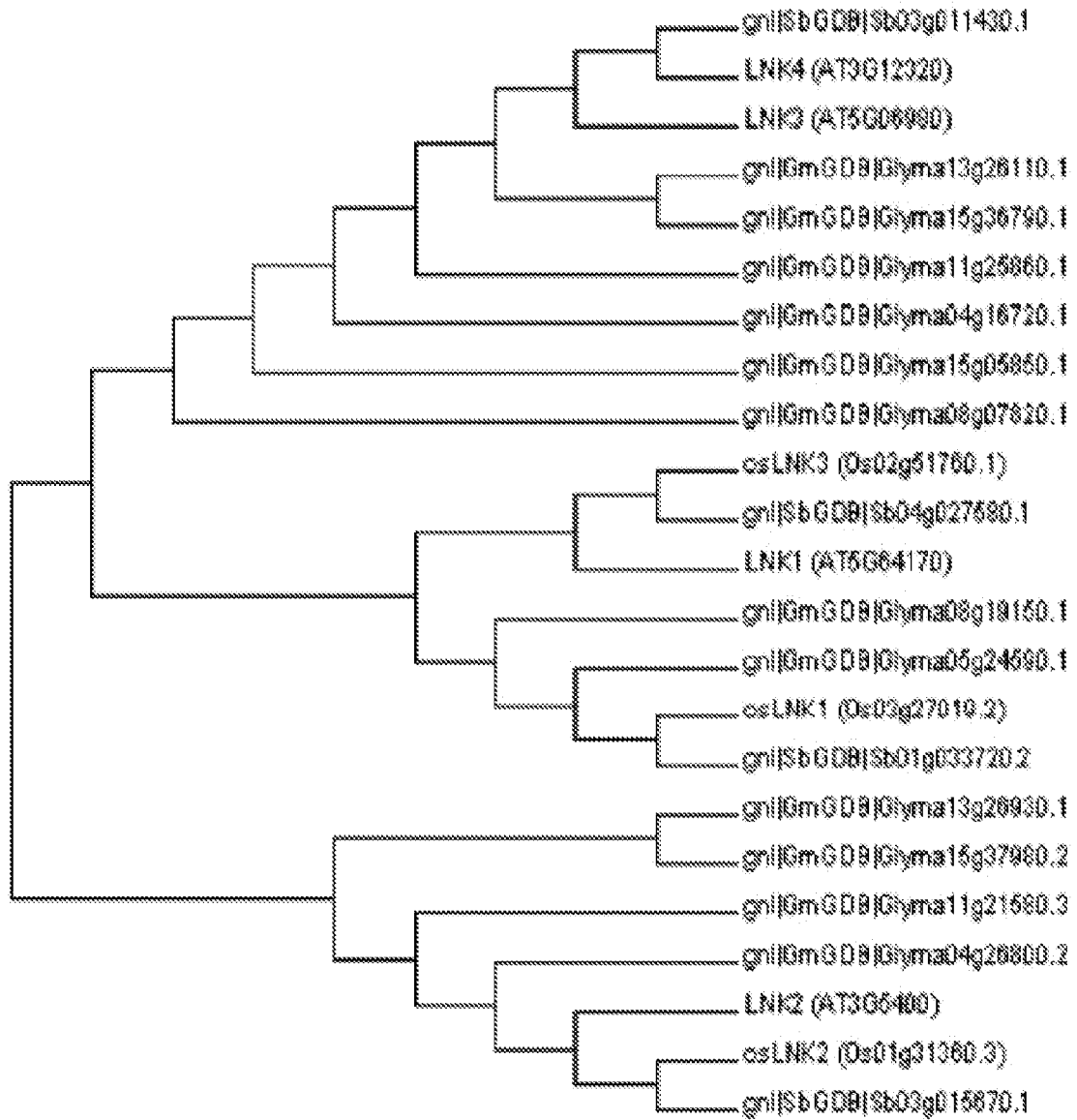


Fig. 16

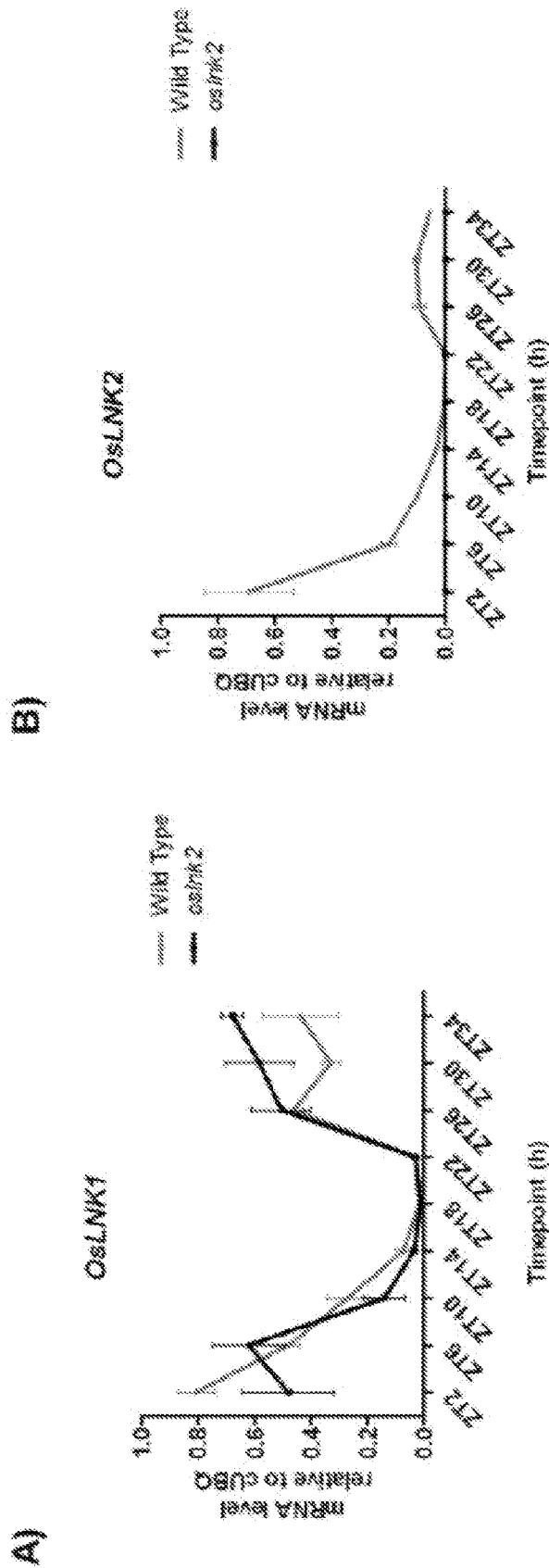


Fig. 17

