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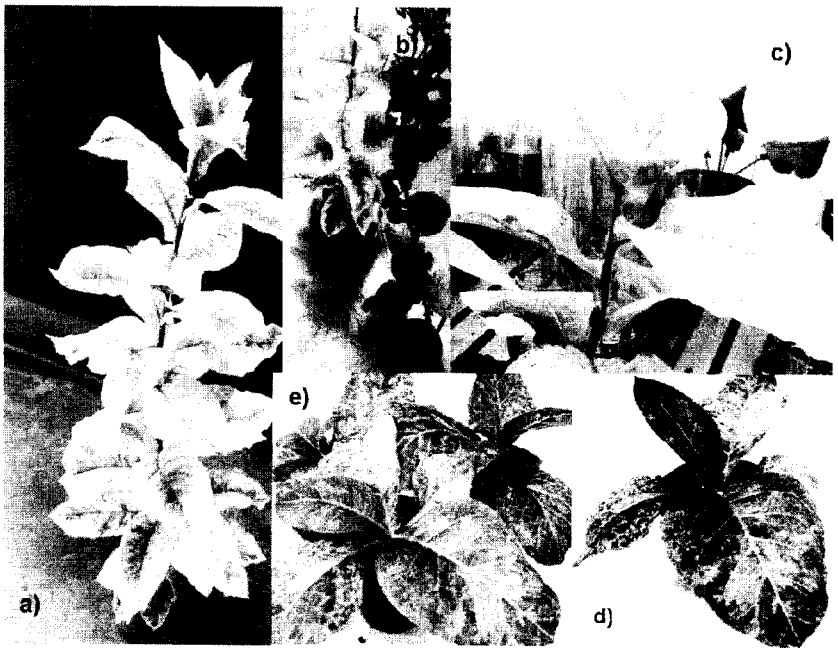
[Continued on next page]

(54) Title: VARIEGATION IN PLANTS

Figure 2



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(57) Abstract: A variegated plant comprising a nucleic acid operatively linked to a regulatory region, the nucleic acid disrupts the expression of Cpn21. The nucleic acid is typically an antisense Cpn21 and the regulatory region may be an inducible regulatory region, a tissue specific regulatory region, or a developmental regulatory region.

VARIEGATION IN PLANTS

FIELD OF INVENTION

[0001] The present invention relates to variegation in plants, and to methods of selecting plants based on this trait.

BACKGROUND OF THE INVENTION

[0002] Variegation in plants is defined as the normal green portion of the plant leaf being replaced by white, cream, yellow, or occasionally other colors, which may be in the form of blotches or stripes. The variegations can occur on the edge of the leaves (marginate variegation), or in the center of the leaf (medio variegation). Due to ornamental nature of variegated plants, these plants are desired by gardeners.

[0003] Variegations have been induced in plants by a variety of methods including the use of transposable elements (for example Itoh et al., 2002, Plant Cell Physiol. 43(5):578-8), plant transformation with variegated and distorted leaf (vdl) gene, located in the nucleus (Wang et al., 2000, Plant Cell. 12(11):2129-42), antisense glutamate 1-semialdehyde aminotransferase transformation, inhibiting chlorophyll synthesis with partial or complete suppression of the GSA-AT leading to severe plant damage (Hofgen et al., 1994, Proc Natl Acad Sci U S A., 91:1726–1730), or spontaneous mutation in the nuclear genes that controlled organelle proteins or mutations in organelle genes (reviewed in Aluru et al., 2006, J. ExpBot. 57:1871-1881. Apuya et al., (2001, *Plant Physiol.* **126,** 717-730) report that a T-DNA mutation in the chaperonin-60α gene (Cpn60) of *Arabidopsis* results in a defect in embryo development, causing developmental arrest before the heart stage.

[0004] Leaf bleaching, along with other abnormal phenotypes such as stunted growth, delayed flowering, reduced root development and the like have been observed in transgenic tobacco plants that constitutively express *Arabidopsis* Cpn60β transcripts in sense and antisense orientation (Zabaleta, E, et al. 1994, Plant Journal, Vol. 6, pp. 425-432).

[0005] Chaperonins are multi-subunit double-ring oligomeric proteins found in bacteria, mitochondria, and plastids. Chaperonins are abundant constitutive proteins that increase in amount after stresses, such as heat shock, bacterial infection of macrophages, and an increase in the cellular content of unfolded proteins.

[0006] Higher plant chloroplasts contain a 21-kDa protein, chaperonin 21 (Cpn21) (Hirohashi T. et al, Biochem Biophys Acta. 1999 1429(2):512-5). The chloroplast Cpn21 polypeptide consists of two Cpn10-like domains fused together in tandem. The cDNA sequence of the Cpn21 (AtCpn21) precursor protein from *Arabidopsis thaliana* is known, and the deduced amino acid sequence of the AtCpn21 precursor protein, 253 amino acids long, shows 61% identity with the spinach Cpn21 protein. The AtCpn21 precursor protein contains a typical chloroplast transit peptide of 51 amino acids at its amino terminus and two Cpn10-like domains, with these two domains exhibiting 46% sequence identity. The predicted, mature polypeptide of AtCpn21 was expressed in Escherichia coli as a soluble 21-kDa protein. Gel-filtration and chemical cross-linking analyses showed that the recombinant mature AtCpn21 protein forms a stable homo-oligomer composed of three or four polypeptides.

[0007] Hanania et al., 2007 (Transgenic Res. 16 :515-525) disclosed that Cpn21 was differentially expressed in seeded and seedless grapes, and may have a role in seed abortion in some plants.

[0008] Sjogren et al., (2004, Plant Physiol, 136: 4114-4126), disclose *Arabidopsis clpC1* T-DNA insertion mutants that lack on average 65% content of a stromal molecular chaperone (ClpC). Mutants display a retarded-growth phenotype, leaves with a homogenous chlorotic appearance throughout all developmental stages. Photosynthetic performance was impaired in knockout lines, with relatively fewer photosystem I and photosystem II complexes, but no changes in ATPase and RuBisCO content.

SUMMARY OF THE INVENTION

[0009] The present invention relates to variegation in plants, and to methods of selecting plants based on this trait.

[0010] It is an object of the invention to provide an improved variegated plant.

[0011] According to the present invention there is provided a variegated plant comprising a nucleic acid operatively linked to a regulatory region, wherein the nucleic acid disrupts the expression of a chaperonin. The chaperonin may be chaperonin 21 (Cpn21). The nucleic acid may be an antisense sequence of Cpn21, a nucleic acid that hybridizes under stringent

conditions with the complement of antisense Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21. Furthermore, the regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region.

[0012] The present invention provides a chimeric construct comprising a first nucleic acid operatively linked to a first regulatory region, the first nucleic acid comprising a sequence that directly or indirectly disrupts the expression of Cpn21, or functional homologue of Cpn21, and a second nucleic acid encoding a protein of interest and operatively linked to a second regulatory region.

[0013] The present invention also provides a method (A) of producing a variegated plant comprising,

i) providing the plant comprising a nucleic acid operatively linked to a regulatory region, the nucleic acid disrupts the expression of Cpn21, and

ii) growing the plant under conditions that results in the expression of the nucleic acid, thereby producing the variegated plant.

[0014] Preferably, the nucleic acid in the step of providing is an antisense Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21. Furthermore, the regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region. The tissue specific regulatory region may be a RuBisCO promoter, or may be a vein-specific promoter. The constitutive regulatory region may comprise a Cauliflower Mosaic Virus 35S (CaMV 35S, or 35S) promoter sequence. The inducible regulatory region may comprise an AlcR sequence and an AlcA promoter.

[0015] The present invention also pertains to a method (B) of selecting a plant comprising a nucleic acid of interest comprising,

i) providing the plant comprising a first nucleic acid operatively linked to a first regulatory region, the first nucleic acid disrupts the expression of Cpn21, and a second

nucleic acid encoding a protein of interest and operatively linked to a second regulatory region,

ii) growing the plant under conditions that result in the expression of the first and second nucleic acids, and

iii) selecting plants that display a variegated phenotype.

[0016] The nucleic acid in the step of providing may be an antisense Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21. Furthermore, the regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region. The tissue specific regulatory region may be a RuBisCO promoter, or may be a vein-specific promoter. The constitutive regulatory region may comprise a Cauliflower Mosaic Virus 35S (CaMV 35S, or 35S) promoter sequence. The inducible regulatory region may comprise an AlcR sequence and an AlcA promoter.

[0017] The present invention also provides a method (C) of producing a variegated plant comprising,

i) transforming a plant with a nucleotide sequence comprising a nucleic acid operatively linked to a regulatory region, the nucleic acid disrupting the expression of Cpn21, and

ii) growing the plant under conditions that results in the expression of the nucleic acid, thereby producing the variegated plant.

[0018] The present invention also provides a method (D) of selecting a plant comprising a nucleic acid of interest comprising,

i) transforming a plant with a nucleotide sequence comprising a first nucleic acid operatively linked to a first regulatory region, the first nucleic acid disrupts the expression of Cpn21, or functional homologue of Cpn21, and a second nucleic acid encoding a protein of interest and operatively linked to a second regulatory region,

ii) growing the plant under conditions that result in the expression of the first and second nucleic acids, and

iii) selecting plants that display a variegated phenotype and expressing the nucleic acid of interest.

[0019] The first nucleic acid in the step of providing or transforming may be an antisense Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21. Furthermore, the regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region.

[0020] The second nucleic acid sequence encoding a protein of interest, may include any nucleic acid, for example, but not limited to, a nucleic acid sequence that encodes a pharmaceutically active protein, for example growth factors, growth regulators, antibodies, antigens, their derivatives useful for immunization or vaccination and the like, interleukins, insulin, G-CSF, GM-CSF, hPG-CSF, M-CSF or combinations thereof, interferons, for example, interferon-alpha, interferon-beta, interferon-gamma, blood clotting factors, for example, Factor VIII, Factor IX, or tPA or combinations thereof. The second nucleic acid sequence may also encode an industrial enzyme, a protein supplement, a nutraceutical, or a value-added product for feed, food, or both feed and food use. Examples of such proteins include, but are not limited to proteases, oxidases, phytases, chitinases, invertases, lipases, cellulases, xylanases, enzymes involved in oil biosynthesis etc.

[0021] The plants produced using the methods defined above exhibited variegated leaves. The methods as described herein may be used to produce plants with variegated leaves for ornamental purposes, or the occurrence of leaf variegation may be used to assist in plant selection, where leaf variegation is trait that is used as a selectable marker to indicate that transformation of a plant occurred. The methods of the present invention can be used within any plant including crop plants, ornamental plants, forage plants and the like, for example but not limited to *Nicotiana*, *Arabidopsis*, Canola, Flax, Hemp, *Brachypodium*, *Oryza*, *Brassica napus*, *Petunia spp.*, *Cyclamen spp*, *Begonia spp.*, *Azalea spp*, and *Spatifilium spp.*, *Artemisia spp.*, *Poplar*, *Rosa spp.*, Rose, *Musa spp* (e.g. Banana), *Coffea Arabica*, Maize, *Glycine spp.* (e.g. Soybean), and the like.

[0022] The present invention further provides a nucleic acid construct comprising a nucleic acid operatively linked to a regulatory region, wherein the nucleic acid disrupts the expression

of Cpn21. The nucleic acid may be an antisense Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21. The regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region.

[0023] The present invention also provides a nucleic acid construct comprising a first nucleic acid operatively linked to a first regulatory region, the first nucleic acid disrupts the expression of Cpn21, and a second nucleic acid encoding a protein of interest. The first nucleic acid may be an antisense Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21. The regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region. The first regulatory region may comprise an AlcR sequence, and the second regulatory region may comprise an AlcA promoter.

[0024] The present invention also includes a vector comprising the nucleic acid construct of the present invention.

[0025] Genetically transformed plants expressing antisense chaperonin 21 exhibit a strong tendency to variegation of the leaves and flowers. An advantage of such a system is that the transformed plants and their progeny are free of heterologous nucleic acid sequences. The plants and methods described herein may be used to produce variegated plants for commercial use, including crop plants of for use within the ornamental market. Furthermore, the variegated phenotype obtained in plants using the methods described herein, may be used as a selectable marker. For example, variegated leaves may provide a visual criteria for selecting a plant encoding a protein of interest.

[0026] Linking the visual marker to a nucleic acid of interest may also be useful for identifying and segregating, or for the identity preservation of industrial or other genetically modified crops that express this trait. Tissue specific variegation (vein, leaf tip, stem etc), may be used so that the marker is expressed in desired parts of the plant to create different patterns in the same plant species and thus identifying and distinguish different genetically modified traits in the plant.

[0027] This summary of the invention does not necessarily describe all features of the invention. Other aspects and features of the present invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0029] **FIGURE 1** shows patterns of variegated leaves from tobacco plants, transformed with Cpn21RNAi 35S. WP – wild type plant (non transformed) – top row, middle.

[0030] **FIGURE 2A-E** shows T1 tobacco plants expressing Cpn21RNAi - 35S constructs. A range of variegation pattern and color intensity is shown in plants. A control plant is shown in the right hand side of Figure 2B.

[0031] **FIGURE 3** shows variegation in the leaves of AlcR Cpn21RNAi transformed tobacco. The AlcA promoter was induced by spraying with a 2% ethanol solution.

[0032] **FIGURE 4** shows a Canola (*Brassica napus*) regenerant on selection media after transformation with Cpn21RNAi - 35S.

[0033] **FIGURE 5** shows patterns of variegation in canola transformed with Cpn21RNAi 35S (WP – untransformed control bottom left leaf).

[0034] **FIGURE 6** shows transgenic rice, transformed with Cpn21RNAi – 35S (right-side pot); left pot is control (untransformed plant).

[0035] **FIGURE 7** shows transgenic *Brachypodium*, transformed with Cpn21RNAi 35S (left-side pot); right pot is control (untransformed plant).

[0036] **FIGURE 8A**: SEQ ID NO: 1 – sequence encoding *A. thaliana* cpn21; **Figure 8B**: SEQ ID NO: 4 – Cpn21 antisense 35S; **Figure 8C**: SEQ ID NO: 5 – Cpn21 RNAi fragment with intron; **Figure 8D**: SEQ ID NO: 6 – Cpn21RNAi RBC; **Figure 8E**: SEQ ID NO: 7 – Cpn21 RNAiRBC; **Figure 8F**: SEQ ID NO: 8 – AlcR Cpn21 RNAi Sequence; **Figure 8G**: SEQ ID NO: 9 35S promoter; **Figure 8H**: SEQ ID NO: 10 – AlcA promoter (nucleotides 1-

232) with minimal ; **Figure 8I**: SEQ ID NO: 11 – *N. tabacum* RuBisCO (RBC) promoter sequence; **Figure 8J**: SEQ ID NO: 12 – AlcR fragment with minimal 35S promoter sequence; **Figure 8K**: SEQ ID NO: 23 Cpn21 Antisense; **Figure 8L**: PCR – amplified fragment of *A. thaliana* Cpn21 (SEQ ID NO: 2); **Figure 8M**: SEQ ID NO: 28 – pGreen0029-RBC construct; **Figure 8N**: SEQ ID NO: 29 pUC57-RBC construct. SEQ ID NO: 4 – nucleotides 103-866 comprise an Acpn21 antisense sequence; nucleotides 904-1156 comprise a NOS terminator sequence; nucleotides 10232-10648 comprise a 35S promoter sequence. SEQ ID NO: 5 – nucleotides 1-427 comprise ACpn RNAi fragment 1; nucleotides 938-2017 comprise an intron of the ACpn RNAi construct; nucleotides 2156-2627 comprise ACpn RNAi fragment 2. SEQ ID NO: 6 – nucleotides 8140-9175 comprise a 35 S promoter sequence; nucleotides 9324-9795 comprise ACpn RNAi fragment 1; nucleotides 9934-11013 comprise an intron of the ACpn RNAi construct; nucleotides 11479-11948 comprise ACpn RNAi fragment 2. SEQ ID NO: 7 – nucleotides 2-1026, RBC promoter; nucleotides 1280-1747 comprise ACpnRNAi fragment 1; nucleotides 1839-3193 comprise an intron of the ACpnRNAi construct; nucleotides 3349-3816 comprise ACpnRNAi fragment 2. SEQ ID NO: 8 – nucleotides 717-1502 comprise a 35 S promoter sequence; nucleotides 1529-4123 comprise an AlcR sequence; nucleotides 4130-4388 comprise a NOS terminator sequence; nucleotides 4389-4406 comprise an AlcA promoter sequence; nucleotides 5058-5525 comprise ACpnRNAi fragment 1; nucleotides 5617-6971 comprise an intron of the ACpnRNAi construct; nucleotides 7127-7594 comprise ACpnRNAi fragment 2.

DETAILED DESCRIPTION

[0037] The present invention relates to variegation in plants, and to methods of selecting plants based on this trait.

[0038] The present invention provides a variegated plant comprising a nucleic acid operatively linked to a regulatory region, wherein the nucleic acid disrupts the expression of chaperonin 21 (Cpn21), or a functional homologue of Cpn21, for example Cpn10. The nucleic acid that disrupts the expression of Cpn21 or a functional homologue of Cpn21, may include but is not limited to, an antisense sequence of Cpn21 or a functional homologue of Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21 or a functional homologue of Cpn21, *in vitro*, or an RNAi that disrupts the expression of Cpn21 or a functional homologue of Cpn21. The regulatory region may be an

inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region.

[0039] The Cpn21 sequence may comprise SEQ ID NO: 1, or a fragment of SEQ ID NO: 1 that exhibits Cpn21 activity. SEQ ID NO: 1 corresponds to an *Arabidopsis* chaperonin 21 (cpn21, Acpn21) sequence (NCBI GenBank Accession No. AF428366.1 gi:16226910 Arabidopsis thaliana AT5g20720/T1M15_120 mRNA). A fragment of SEQ ID NO. 1 may comprise nucleotides 77-842 of SEQ ID NO: 1. However, the chaperonin sequence may also comprise, for example, *Vitis vinifera* cpn21 (NCBI GenBank Accession No. AY680699), spinach Cpn21 (Baneyz et al. 1995, JBC 270:10695-10702). Furthermore, Cpn21 polypeptide consists of two Cpn10-like domains fused together in tandem, and Cpn21 is considered a functional homologue of Cpn10 (accession number:AF059037; Bertsch U., et al., 1992, PNAS 89:8696-8700; Hirohashi T., et al.., 1999, Biochem Biophys Acta 1429:512-515), therefore, the chaperonin sequence of the present invention may also include spinach chaperonin cpn10 (NCBI GenBank Accession No. M87646), *Helicosporidium sp.* ex Simulium jonesii cpn10 (NCBI GenBank Accession No. AY596494), or RbcX (Emlyn-Jones et al., 2006 Plant & Cell Physiology 47:1630-1640), or other functional homologue of Cpn21. Other examples of chaperonins, from both prokaryotes and eukaryotes (including plants) may also be found in cpndb- A chaperonin database (for example at URL: cpndb.cbr.nrc.ca/ ; Hill et al 2004. Genome Research 14:1669-1657, herein incorporated by reference). Chaperonin 21 may be indicated by the abbreviation "cpn21" or "Cpn21".

[0040] The method as described herein involves the use of a nucleic acid sequence to disrupt or reduce or eliminates the expression of Cpn21, or a functional homologue of Cpn21, for example Cpn10. This may be achieved, for example but not limited to, by using a silencing nucleic acid as described below. An example of a silencing nucleic acid includes, but is not limited to, an antisense Cpn21, or a functional homologue of Cpn21, sequence, a nucleic acid that hybridizes under stringent conditions with the complement of an antisense Cpn21, or a functional homologue of Cpn21, sequence *in vitro*, or an RNAi that disrupts expression of Cpn21, or a functional homologue of Cpn21.

[0041] Examples of silencing nucleic acids include those comprising a sequence according to: a reverse-complement of SEQ ID NO: 1, 2, or a fragment thereof, SEQ ID NO: 4 or a fragment thereof, SEQ ID NO: 5 or a fragment thereof, SEQ ID NO: 23 or a fragment thereof,

nucleotides 2078-2518 of SEQ ID NO: 5, or the reverse complement of nucleotides 223-663 of SEQ ID NO: 2. Sequences encoding Cpn10 may also be used as Cpn10 may be considered a fragment of cpn21 (Bertsch U., et al., 1992, PNAS 89:8696-8700; Hirohashi T., et. al., 1999, Biochem Biophys Acta 1429:512-515).

[0042] The regulatory region used in the method and construct described above may be a constitutive regulatory region, an inducible regulatory region, a tissue specific regulatory region, or a developmental regulatory region.

[0043] The present invention therefore provides a method for reducing the level of Cpn21 in a plant or a tissue within the plant comprising,

i) introducing a nucleic acid sequence into a plant, the nucleic acid sequence comprising a regulatory region operatively associated with a silencing nucleotide sequence, wherein expression of the silencing nucleotide sequence reduces or eliminates the expression of Cpn21, and

ii) expressing the silencing nucleotide sequence within the plant or the tissue within the plant. The amount of the Cpn21 may be determined by comparing the level of Cpn21 expression in the plant, or a tissue of the plant, with a level of Cpn21 expression in a second plant, or the tissue from the second plant, that does not express the silencing nucleic acid sequence. The amount of Cpn21 may be determined using standard techniques including northern analysis, western analysis, SDS-PAGE and the like.

[0044] The present invention further provides a method for producing a variegated plant, comprising,

i) introducing a silencing nucleic acid sequence into the plant, the silencing nucleic acid sequence comprising a regulatory region operatively associated with the silencing nucleic acid sequence. The silencing nucleic acid sequence may comprise a reverse compliment of SEQ ID NO's: 1 or 2, a fragment of a reverse compliment of SEQ ID NO: 1, a sequence that hybridizes to the complement of the antisense of SEQ ID NO's: 1 or 2 or a sequence that hybridizes to a fragment of the complement of the antisense of SEQ ID NO: 1, and

ii) growing the plant.

[0045] Other silencing nucleic acids may be used in the method above, including SEQ ID NO's: 4, 5 or 23, or fragment thereof, nucleotides 2078-2518 of SEQ ID NO: 5, or the reverse complement of nucleotides 223-663 of SEQ ID NO: 2. Sequences encoding Cpn10 may also be used .

[0046] The regulatory region may be a constitutive regulatory region, an inducible regulatory region, a tissue specific regulatory region, or a developmental regulatory region. If an inducible regulatory region is used, then the method further includes a step of subjecting the plant to a chemical agent or environmental condition that induces the regulatory region and results in expression of the silencing nucleic acid.

[0047] In embodiments where chaperonin expression is disrupted, the level of chaperonin expression may be reduced by about 10% to about 100%, or any amount therebetween, when compared to the level of chaperonin expression obtained from a second plant that does not express the silencing nucleotide sequence. For example, Cpn21 expression may be reduced by from about 10% to about 60% or any amount therebetween, about 10% to about 50% or any amount therebetween, about 10% to about 40% or any amount therebetween, or from about 10% to about 30%, or any amount therebetween, or about 10% to about 20% or any amount therebetween.

[0048] By the term “expression” it is meant the production of a functional RNA, protein or both, from a nucleic acid molecule.

[0049] A “silencing nucleotide sequence” or "silencing nucleic acid sequence" refers to a sequence that when transcribed results in disrupting, reducing or eliminating expression of a target sequence. A silencing nucleotide sequence may encode for example, but not limited to, an antisense nucleotide, an RNAi, or an siRNA.

[0050] By “reduction of gene expression” or “reduction of expression” “disruption of expression” or "elimination of expression", it is meant a decrease in the level of mRNA, protein, or both mRNA and protein, encoded by a nucleic acid sequence. Expression may be reduced by from 10 to about 100%, or any amount therebetween, for example, from about 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 ,50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, and100%, or any amount therebetween. Reduction of expression may arise as a result of the lack of production

of full length RNA, for example mRNA, or through the expression of a silencing nucleic acid sequence, and result in cleaving mRNA, for example with a ribozyme (e.g. see Methods in Molecular Biology, vol 74 *Ribozyme Protocols*, P.C. Turner, *ed*, 1997, Humana Press), siRNA, or RNAi (e.g. see *Gene Silencing by RNA Interference, Technology and Application*, M. Sohail *ed*, 2005, CRC Press), or otherwise reducing the half-life of RNA, using antisense (e.g. see *Antisense Technology, A Practical Approach*, C. Lichtenstien and W. Nellen eds., 1997, Oxford University Press), ribozyme, siRNA, or RNAi techniques, or other related methods known in the art.

[0051] Antisense nucleic acid molecules interact with complementary strands of nucleotide sequences and when present, may modify the expression of a target nucleic acid. An antisense nucleic acid may be DNA or RNA and may be delivered as a composition, or transcribed *in vivo* or *in situ* from a template nucleic acid, a vector or other construct such as an artificial chromosome, or a transgenic sequence introduced into the genome of the host cell or organism. Antisense nucleic acids may be of varying length from a few tens of nucleotides, to several hundred nucleotides and may comprise antisense sequences that may interact with one, or more than one mRNA.

[0052] An antisense nucleic acid may be transcribed from a template nucleic acid, a plasmid or other genetic construct as a single RNA strand that self-anneals to form a hairpin-loop structure. The transcribed RNA strand may comprise two palindromic sequences that are capable of annealing to form a double-strand, separated by a loop, or unpaired section of RNA. The double stranded region may be from about 15 base pairs to several hundred base pairs in length, or any length therebetween, and, for example, may result in the formation of short double-stranded fragments (siRNA) when processed by DICER or RISC-like enzymes or enzyme complexes present in the plant. The short double stranded fragments may include, for example, but not limited to, 21-mers, with a 19 base pair double stranded portion and a two base overhang at the 3' end, as is generally known in the art.

[0053] Examples of an antisense sequence of SEQ ID NO: 1 include SEQ ID NO: 23, or a fragment thereof, nucleotides 2078-2518 of SEQ ID NO: 5 or a fragment thereof, or the reverse complement of nucleotides 223-663 of SEQ ID NO: 2, or a fragment thereof, the reverse complement of SEQ ID NO: 1, or a fragment thereof.

[0054] By "operatively linked" it is meant that the particular sequences interact either directly or indirectly to carry out an intended function, such as mediation or modulation of gene expression. The interaction of operatively linked sequences may, for example, be mediated by proteins that interact with the operatively linked sequences. A coding region of interest may also be introduced within a vector along with other sequences, typically heterologous, to produce a chimeric construct. A transcriptional regulatory region and a sequence of interest are "operably linked" when the sequences are functionally connected so as to permit transcription of the sequence of interest to be mediated or modulated by the transcriptional regulatory region.

[0055] As described below, variegated plants were produced using the methods as described herein with *Nicotiana tabacum* L. cv. SR1, *Arabidopsis thaliana* cv Landsberg, *Brassica napus* cv. DH12075 *Oryza sativa* and *Brachypodium distachyon* cv. Doublon Blue Sky plants as model systems. However, other plants may also be modified using the methods described herein, including a crop plant, an ornamental plant, a forage plant, and the like, for example but not limited to *Nicotiana*, *Arabidopsis*, Canola, Flax, Hemp, *Brachypodium*, *Oryza*, *Brassica napus*, *Petunia spp.*, *Cyclamen spp*, *Begonia spp.*, *Azalea spp*, *Spatifilium spp.* alfalfa, corn, barley, rice, tobacco, Arabidopsis, canola, flax, hemp , for example but not limited to *Cannabis sativa*, potato. Other examples of plants include species from the genera *Anacardium*, *Arachis*, *Asparagus*, *Atropa*, *Avena*, *Brassica*, *Brachypodium*, *Citrus. Citrullus*, *Capsicum*, *Carthamus*, *Cocos*, *Coffea*, *Cucumis*, *Cucurbita*, *Daucus*, *Elaeis*, *Fragaria*, *Glycine*, *Gossypium*, *Helianthus*, *Heterocallis*, *Hordeum*, *Hyoscyamus*, *Lactuca*, *Linum*, *Lolium*, *Lupinus*, *Lycopersicon*, *Malus*, *Manihot*, *Majorana*, *Medicago*, *Nicotiana*, *Olea*, *Oryza*, *Panieum*, *Pannesetum*, *Persea*, *Phaseolus*, *Pistachia*, *Pisum*, *Pyrus*, *Prunus*, *Raphanus*, *Ricinus*, *Secale*, *Senecio*, *Sinapis*, *Solanum*, *Sorghum*, *Theobromus*, *Trigonella*, *Triticum*, *Vicia*, *Vitis*, *Vigna*, and *Zea*.

[0056] Constructs used for plant transformation are set out in Table 4 and include:

Cpn21AS (a nucleic acid sequence encoding Cpn21 in antisense orientation under transcriptional control of a constitutive, CaMV 35S promoter),

Cpn21RNAi - 35S (Cpn21 RNAi hairpin-loop under transcriptional control of the 35S promoter),

Cpn21 RNAi RBC (Cpn21 RNAi hairpin-loop under transcriptional control of a tissue specific, RuBisCO (RBC) promoter),

Cpn21AS RBC a nucleic acid sequence encoding Cpn21 in antisense orientation under transcriptional control of the RBC promoter)

AlcRCpn21RNAi (Cpn21 RNAi hairpin-loop under transcriptional control of an alcohol inducible promoter).

[0057] Tobacco plants with varying levels of leaf variegation were produced using antisense expression of Cpn21 under the control of 35S promoter. Variegated tobacco plants were generated using on kanamycin selection medium or hygromycin selection medium, as well as on selection-free medium. Variegated *Arabidopsis*, canola (*B. napus*), *O. sativa* and *B. distachyon* plants were identified on kanamycin–free selection medium following transformation with Cpn21 gene in antisense orientation or RNAi.

[0058] The methods as described herein may be used to produce plants with variegated leaves for ornamental purposes, or the occurrence of leaf variegation may be used to assist in plant selection, where leaf variegation is trait that is used as a selectable marker to indicate that transformation of a plant occurred.

[0059] Therefore, the present invention provides a chimeric construct comprising a nucleic acid operatively linked to a regulatory region, the nucleic acid comprising a sequence that directly or indirectly disrupts the expression of Cpn21, or functional homologue of Cpn21, in a plant. The present invention also provides a method of producing a variegated plant comprising, transforming a plant with the construct just described, and growing the plant under conditions that results in the expression of the nucleic acid, thereby producing the variegated plant.

[0060] The nucleic acid may be an antisense Cpn21 or an antisense functional homologue of Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of an antisense Cpn21, or the complement of an antisense functional homologue of Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21, or functional homologue of Cpn21. Furthermore, the regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region.

[0061] The present invention further provides a chimeric construct comprising a first nucleic acid operatively linked to a first regulatory region, the first nucleic acid comprising a sequence that directly or indirectly disrupts the expression of Cpn21, or functional homologue of Cpn21, and a second nucleic acid encoding a protein of interest and operatively linked to a second regulatory region.

[0062] The present invention also provides a method of selecting a plant comprising a nucleic acid of interest comprising,

i) transforming a plant with a nucleotide sequence comprising a first nucleic acid operatively linked to a first regulatory region, the first nucleic acid disrupts the expression of Cpn21, or functional homologue of Cpn21, and a second nucleic acid encoding a protein of interest and operatively linked to a second regulatory region,

ii) growing the plant under conditions that result in the expression of the first and second nucleic acids, and

iii) selecting plants that display a variegated phenotype and express the nucleic acid of interest.

[0063] The present invention also provides a method of selecting a plant comprising a nucleic acid of interest comprising,

i) providing the plant comprising a first nucleic acid operatively linked to a first regulatory region, the first nucleic acid disrupts the expression of Cpn21, or functional homologue of Cpn21, and a second nucleic acid encoding a protein of interest and operatively linked to a second regulatory region,

ii) growing the plant under conditions that result in the expression of the first and second nucleic acids, and

iii) selecting plants that display a variegated phenotype and express the nucleic acid of interest.

[0064] The first nucleic acid (in the step of providing or transforming) may be an antisense Cpn21 or an antisense functional homologue of Cpn21, a nucleic acid that hybridizes under stringent conditions with the complement of an antisense Cpn21, or the complement of an

antisense functional homologue of Cpn21 *in vitro*, or an RNAi that disrupts the expression of Cpn21, or functional homologue of Cpn21. Furthermore, the regulatory region may be an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region, or a constitutive regulatory region.

[0065] The second nucleic acid sequence encoding a protein of interest, may include any nucleic acid, for example, but not limited to, a nucleic acid sequence that encodes a pharmaceutically active protein, for example growth factors, growth regulators, antibodies, antigens, their derivatives useful for immunization or vaccination and the like, interleukins, insulin, G-CSF, GM-CSF, hPG-CSF, M-CSF or combinations thereof, interferons, for example, interferon-alpha, interferon-beta, interferon-gamma, blood clotting factors, for example, Factor VIII, Factor IX, or tPA or combinations thereof. The second nucleic acid sequence may also encode an industrial enzyme, a protein supplement, a nutraceutical, or a value-added product for feed, food, or both feed and food use. Examples of such proteins include, but are not limited to proteases, oxidases, phytases, chitinases, invertases, lipases, cellulases, xylanases, enzymes involved in oil biosynthesis etc.

[0066] The present invention includes nucleotide sequences having antisense cpn21 activity and that hybridize to SEQ ID NO: 1, or that hybridized to the complement of antisense Cpn21 under stringent hybridization conditions (see Maniatis et al., in Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982) p. 387 to 389; Ausubel, *et al.* (eds), 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3; both of which are herein incorporated herein by reference). Non-limiting examples of stringent hybridization conditions include hybridization in 4XSSC at 65°C for 8-16 hours, followed by washing in 0.1XSSC at 65°C for an hour or hybridization in 5XSSC and 50% formamide at 42°C for 8 to 16 hours, followed by washing in about 0.5XSSC to about 0.2XSSC at 65°C for one hour. However, hybridization conditions may be modified in accordance with known methods depending on the sequence of interest (see Tijssen, 1993, *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; which is incorporated herein by reference). Generally, but not wishing to be limiting, stringent conditions are selected to be about 5°C lower than the thermal melting point for the specific sequence at a defined ionic strength and pH.

[0067] As would be understood to one of skill in the art, nucleotide sequences of varying length that hybridize to SEQ ID NO: 1 or the complement of SEQ ID NO: 1, when transcribed *in vivo*, may exhibit the property of reducing cpn21 expression. Such nucleotide sequences may be from about 15 to about 900 nucleotides in length, or any length therebetween. Non-limiting examples of such sequences (including fragments of such sequences) include: GenBank Accession No. AF059037 (cpn10), GenBank Accession No. NM_180714 (cpn20), GenBank Accession No.AF510565.1 (hda2), and SEQ ID NO: 4.

[0068] The present invention also includes nucleotide sequences having RNAi activity for Cpn21. For example, SEQ ID NO: 5 comprises a 440 nucleotide sequence (nucleotides 223-663 of SEQ ID NO: 2), and a reverse, complement nucleotide sequence of nucleotides 223-663 of SEQ ID NO: 2) separated by an intron. When a nucleic acid having the sequence of SEQ ID NO: 5 is transcribed, the RNA forms a hairpin-loop structure (the intron is the loop, the nucleotides corresponding to SEQ ID NO: 2 form a paired region). As would be understood by one of skill in the art, the double-stranded region is cleaved into shorter double-stranded RNAi molecules specific for Cpn21.

[0069] Also included are sequences having from about 75 to about 100% sequence identity, or from about 80% to about 100% sequence identity, with the antisense Cpn21 sequence defined herein, provided that the sequences have antisense cpn21 activity. The identity determinations may be made using oligonucleotide alignment algorithms for example, but not limited to, BLAST (Altschul et al 1990. J. Mol Biol 215:403-410; which is incorporated herein by reference). Software for performing BLAST analyses is available through the National Center for Biotechnology Information (GenBank URL: ncbi.nlm.nih.gov/cgi-bin/BLAST/). Default parameters include: Program: blastn; Database: nr; Expect 10; filter: default; Alignment: pairwise; Query genetic Codes: Standard(1)). Another example of an algorithm suitable for determining percent sequence identity and sequence similarity is FASTA (Pearson et al 1988. Proc Natl Acad Sci USA 85:2444-8).

[0070] A fragment or portion of a nucleic acid may comprise from about 60% to about 100%, of the length of the nucleic acid or nucleotide sequence, or any amount therebetween. For example, from about 60% to about 100%, from about 70% to about 100%, from about 80% to about 100%, from about 90% to about 100%, from about 95% to about 100%, of the length of the nucleic acid or nucleotide sequence, or any amount therebetween. Alternately, a

fragment or portion may be from about 150 to about 500 nucleotides, or any amount therebetween. For example, a fragment may be from 150 to about 500 nucleotides, or any amount therebetween, from about 200 to about 500 nucleotides, or any amount therebetween, from about 250 to about 500 nucleotides, or any amount therebetween, from about 300 to about 500 or any amount therebetween, from about 350 to about 500 nucleotides, or any amount therebetween, from about 400 to about 500 or any amount therebetween, from about 450 to about 500 or any amount therebetween. For example, about 5, 10, 20, 30, 40 or 50 nucleotides, or any amount therebetween may be removed from the 5' end, the 3' end or both the 5' and 3' end of the nucleic acid or nucleotide sequence.

[0071] By "regulatory region" (or regulatory element) it is meant a nucleic acid sequence that has the property of controlling the expression of a sequence that is operatively linked with the regulatory region. Such regulatory regions may include promoter or enhancer regions, and other regulatory elements recognized by one of skill in the art. By "promoter" it is meant the nucleotide sequences at the 5' end of a coding region, or fragment thereof, that contain all the signals essential for the initiation of transcription and for the regulation of the rate of transcription.

[0072] There are several types of regulatory elements, including those that are developmentally regulated, inducible, tissue-specific, constitutive or the like. A regulatory element that is developmentally regulated, is activated within certain organs or tissues of an organ at specific times during the development of that organ or tissue, and regulates the differential expression of a gene. However, some regulatory elements that are developmentally regulated may preferentially be active within certain organs or tissues at specific developmental stages, or may also be active in a developmentally regulated manner, or at a basal level in other organs or tissues within the plant as well. Such regulatory elements may be found in any organ, for example but not limited to, leaves, root, stem, buds, fruit, seeds, flowers, tubers, ovules, embryos or the like.

[0073] A regulatory element may be derived from any suitable source provided that the regulatory element is active in the host plant. A regulatory element may be derived from the same species or type of plant or plant cell in which it is used, or it may be obtained from a different plant source. A regulatory element may also be an animal nucleic acid sequence, a bacterial nucleic acid sequence, a viral nucleic acid sequence, a protozoan nucleic acid

sequence, or a cyanobacterial nucleic acid sequence, provided that the regulatory element functions within the host plant in which it is used. A regulatory element may comprise, in whole or in part, synthetic nucleic acid sequences not found in nature (a synthetic regulatory element).

[0074] A tissue-specific regulatory element is one that is capable of directly or indirectly activating transcription of one or more nucleotide sequences or genes in a tissue-specific manner. Such regulatory elements may be leaf-specific, guard cell specific, stem-specific, root specific, green tissue specific, organelle-specific or the like. Non limiting examples of guard-cell specific regulatory elements include promoter sequences of the *Arabidopsis* TGG1 gene (Husebye et al 2002. Plant Physiol 128:1180-1188; Plesch et al 2000 Gene 249:83-9; PCT Patent Application No. WO 93/018169; which are incorporated herein by reference). Non-limiting examples of organelle-specific regulatory elements include promoter sequences of the pea chlorophyll a/b binding protein gene AB80 (Zabaleta et al 1994. Plant J. 6:425-432; which is incorporated herein by reference). Non-limiting examples of leaf-specific regulatory elements are described in PCT Patent Publications WO 02/077248; WO02/036786; WO 98/00533 (which are incorporated herein by reference). Non-limiting examples of root-specific or root-active regulatory elements are described in PCT Patent Publications. WO 06/066193; WO 05/085449 (which are incorporated herein by reference).

[0075] An inducible regulatory element is one that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor, that binds specifically to an inducible regulatory element to activate transcription, may be present in an inactive form which is then directly or indirectly converted to the active form by the inducer. However, the protein factor may also be absent. The inducer can be a chemical agent such as a protein, metabolite, growth regulator, herbicide or phenolic compound or a physiological stress imposed directly by heat, cold, salt, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus. A plant cell containing an inducible regulatory element may be exposed to an inducer by externally applying the inducer to the cell or plant such as by spraying, watering, heating or similar methods. Inducible elements may be derived from either plant or non-plant genes (e.g. Gatz, C. and Lenk, I.R.P.,1998, Trends Plant Sci. 3, 352-358; which is incorporated by reference). Examples, of potential inducible promoters include, but not limited to, steroid

inducible promoter, for example the estradiol promoter, tetracycline-inducible promoter (Gatz, C.,1997, Ann. Rev. Plant Physiol. Plant Mol. Biol. 48, 89-108; which is incorporated by reference), steroid inducible promoter (Aoyama, T. and Chua, N.H.,1997, Plant J. 2, 397-404; which is incorporated by reference), alcohol or ethanol-inducible promoter (Salter, M.G., et al, 1998, Plant Journal 16, 127-132; Caddick, M.X., et al,1998, Nature Biotech. 16, 177-180, which are incorporated by reference) cytokinin inducible *IB6* and *CKI1* genes (Brandstatter, I. and Kieber, J.J.,1998, Plant Cell 10, 1009-1019; Kakimoto, T., 1996, Science 274, 982-985; which are incorporated by reference), auxin inducible element, DR5 (Ulmasov, T., et al., 1997, Plant Cell 9, 1963-1971; which is incorporated by reference), and a dexamethasone inducible promoter (Sablowski et al., 1998. Cell 92:93-103).

[0076] A constitutive regulatory element directs the expression of a gene throughout the various parts of a plant and continuously throughout plant development. Examples of known constitutive regulatory elements include promoters associated with the CaMV 35S transcript (Odell et al., 1985, *Nature*, **313**: 810-812), octopine synthase promoter (Fromm et al 1989. Plant Cell 1:977-984), the nopaline synthase (NOS) promoter (Lam et al 1990. J. Biol Chem. 265:9909-9913), the rice actin 1 (Zhang et al, 1991, *Plant Cell*, **3**: 1155-1165) and triosephosphate isomerase 1 (Xu et al, 1994, *Plant Physiol.* **106**: 459-467) genes, the maize ubiquitin 1 gene (Cornejo et al, 1993, *Plant Mol. Biol.* **29**: 637-646), the *Arabidopsis* ubiquitin 1 and 6 genes (Holtorf et al, 1995, *Plant Mol. Biol.* **29**: 637-646), tobacco t-CUP promoter (WO/99/67389; US 5,824,872), the HPL promoter (WO 02/50291), and the tobacco translational initiation factor 4A gene (Mandel et al, 1995 *Plant Mol. Biol.* **29**: 995-1004), all of which are herein incorporated by reference. The term "constitutive" as used herein does not necessarily indicate that a gene under control of the constitutive regulatory element is expressed at the same level in all cell types, but that the gene is expressed in a wide range of cell types even though variation in abundance is often observed.

[0077] Other non-limiting examples of developmentally regulated, inducible, tissue-specific, or constitutive regulatory elements are found in Table 1, and references therein, all of which are herein incorporated by reference.

[0078] Table 1: Regulatory elements

Regulatory Element	**Associated gene or marker**	**Reference**

Regulatory Element	Associated gene or marker	Reference
Green tissue specific promoter	RbsS3A	Panguluri et al 2005. Indian J. Exp Biol 43(4):369-372
Green leaf specific promoter	AtSTP3	Buttner et al 2000. Plant Cell Env 23(2):175-184
Leaf primordial promoter	CAB	US 5,639,952
Light repressible promoter	Pra2 (pea)	AU 765413, CA2328139
	Pra2 (maize)	US 5639952, US 5656496
Vein promoter	AtSUC2	Imlau et al 1999. The Plant Cell 11:309-322
Heat shock promoter	sigma32 heat shock regulator	GenPept gi91070642 Chen et al 2005. PNAS 103(15):5977-5982
Alcohol-inducible promoter (Alc sequence)	alcR	Caddick et al., 1998
Stem-specific promoter	Stem-specific protein	WO 01/18211
Estradiol-inducible promoter	Per-8	US 6,784,340
Heatshock promoter	Gmshp17.3	Holtorf et al 1995. Plant Mol Biol 29(4):637-646
Pathogen-related promoter	PR1	Beilmann et al 1992. Plant Mol Biol 18(1):65-78
Cotyledon-specific promoter	At2S1/ast2S2	Guerche et al 1990. Plant Cell 2(5):469-478
Vascular tissue-specific promoter	RolC	Matsuki et al 1989. Molecular Genetics and Genomics 220:12-16
Flower-specific promoter	ChsA	Koch et al 2001. Mol Biol and Evol. 18:1882-1891
Light-inducible promoter	ST-LS1 (L700)	Stockhaus et al 1987. PNAS 84(22):7943-7947
RuBisCO	CbbL, cbbM	Giri et al 2004. Appl Environ Microbiol 70(6):3443-3448
Cold-inducible promoter	cspA	US 6,479,260
Light-specific promoter	ssu	US5750385
Estrogen-inducible promoter	xve	Zua et al 2000. Plant J. 24(2):265-273
ABA-responsive promoter	CdeT27-45	Michel et al., 1993 Plant J. 4:29-40
Epidermis-specific	NtItp1	Canevascini et al., 1996. Plant Physiol 112:513-24

[0079] Other non-limiting examples of regulatory elements may comprise particular sequences or response elements. For example, a regulatory element may comprise one or more cis-acting motifs, such as an abscisic acid response element, an Sph/RY element, a Myb recognition element or an Myc recognition element. These and/or other regulatory elements may be combined to provide a non-naturally occurring promoter, and inserted in –cis, 5' to the desired nucleic acid that is to be expressed (for example, the antisense cpn21, or the RNAi cpn21 as described herein).

[0080] The nucleic acid constructs of the present invention may be introduced into any desired plant, including forage plants, food crops, ornamental plants, or other plants depending upon the need. Thus, embodiments of the invention have use over a broad range of plants. Examples of such plants include, but not limited to, alfalfa, corn, barley, rice, tobacco, *Arabidopsis*, canola, wheat, oat, *Brassica*, *Oryza*, hemp, soybean, pea, ginseng, flax, hemp, maize, for example but not limited to *Cannabis sativa*, potato and ornamental plants, for example, but not limited to, *Petunia spp.*, *Cyclamen ssp*, *Begonia ssp*, *Azalea spp.*, *Spatifilium spp*. Other examples include species from the genera *Anacardium*, *Arachis*, *Artemesia*, *Asparagus*, *Atropa*, *Avena*, *Brassica*, *Brachypodium*, *Citrus*. *Citrullus*, *Capsicum*, *Carthamus*, *Cocos*, *Coffea (e.g. C. arabica, C. robusta)*, *Cucumis*, *Cucurbita*, *Daucus*, *Elaeis*, *Fragaria*, *Glycine*, *Gossypium*, *Helianthus*, *Heterocallis*, *Hordeum*, *Hyoscyamus*, *Lactuca*, *Linum*, *Lolium*, *Lupinus*, *Lycopersicon*, *Malus*, *Manihot*, *Majorana*, *Medicago*, *Musa* (e.g. Banana), *Nicotiana*, *Olea*, *Oryza*, *Panieum*, *Pannesetum*, *Persea*, *Phaseolus*, *Pistachia*, *Pisum*, *Pyrus*, *Prunus*, *Raphanus*, *Ricinus*, *Rosa*, *Secale*, *Senecio*, *Sinapis*, *Solanum*, *Sorghum*, *Theobromus*, *Trigonella*, *Triticum*, *Vicia*, *Vitis*, *Vigna*, *Zea* and the like.

[0081] In the experiments outlined below, tobacco, *Arabidopsis* , Canola, rice or *Brachypodium* were used as the test organism for the expression of the constructs as defined herein, however it is to be understood that the constructs of the present invention may be introduced and expressed in any plant.

[0082] Also considered part of this invention are transgenic plants comprising a construct, vector or nucleic acid as described herein.

[0083] Methods of regenerating whole plants from plant cells are known in the art. In general, transformed plant cells are cultured in an appropriate medium, which may contain

selective agents such as antibiotics, where selectable markers are used to facilitate identification of transformed plant cells. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be used to establish repetitive generations, either from seeds or using vegetative propagation techniques.

[0084] To aid in identification of transformed plant cells, the constructs of this invention may be further manipulated to include plant selectable markers. Selectable markers may include but are not limited to enzymes which provide for resistance to an antibiotic such as gentamycin, hygromycin, kanamycin, and the like. Similarly, enzymes providing for production of a compound identifiable by colour change such as GUS (beta-glucuronidase), luminescence, such as luciferase, metabolism of a particular carbon source (preventing growth of nontransformants) such as phosphomannose isomerase, are useful.

[0085] Nucleic acids, antisense molecules, silencing nucleotide sequences, vectors or other genetic constructs (collectively referred to as "constructs" or "nucleic acid constructs") may be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, micro-injection, electroporation, floral dip, biolistic particle gun, or the like. For reviews of such techniques see, for example, Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academy Press, New York VIII, pp. 421-463 (1988); Geierson and Corey, *Plant Molecular Biology*, 2d Ed. (1988); and Miki and Iyer, Fundamentals of Gene Transfer in Plants. In *Plant Metabolism*, 2nd edition DT. Dennis, et al., (editors), Addison Wesley, Langmans Ltd. London, pp. 561-579 (1997); Clough and Bent, 1998 (Plant J. 16, 735-743). Hemp, for example but not limited to *Cannabis sativa* L. may be transformed using *Agrobacterium tumifasciens*, as described by Feeny and Punja, 2006. (Methods Mol Biol 344: 373-382; which is incorporated herein by reference)

[0086] The present invention further includes a suitable vector comprising the nucleic acid construct.

[0087] Also considered part of this invention are transgenic plants and their progeny that contain a nucleic acid construct of the present invention. Methods of regenerating whole plants from plant cells are known in the art, and the method of obtaining transformed and

regenerated plants is not critical to this invention. In general, transformed plant cells are cultured in an appropriate medium, which may contain selective agents such as antibiotics, where selectable markers are used to facilitate identification of transformed plant cells. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be used to establish repetitive generations, either from seeds or using vegetative propagation techniques. Plant regeneration from cultured protoplasts is described in Evans et al. (Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, pp. 124-176, MacMillian Publishing Company, New York, 1983; and Binding, Regeneration of Plants, Plant Protoplasts, pp. 21-73, CRC Press, Boca Raton, 1985; which are incorporated herein by reference). Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al. 1987 (Ann. Rev. Plant Phys. 38:467-486; which is incorporated herein by reference).

[0088] The silencing nucleic acid sequence or construct as described herein may also be introduced into a plant by crossing a first plant with a second plant that comprises the silencing nucleic acid sequence or construct, and selecting for expression of the nucleic acid.

[0089] Table 2. List of Sequences and primers according to some embodiments of the invention.

SEQ ID NO:	Description	Table/Figure/Sequence
1	Arabidopsis Cpn21	Figure 8A
2	Cpn21 fragment	Figure 8L
4	Cpn21 antisense 35S	Figure 8B
5	Cpn21 RNAi fragment with intron	Figure 8C
6	Cpn21 RNAi 35S	Figure 8D
7	Cpn21RNAi RBC	Figure 8E
8	AlcR Cpn21 RNAi Sequence	Figure 8F

SEQ ID NO:	Description	Table/Figure/Sequence
9	35S Promoter	Figure 8G
10	AlcA promoter	Figure 8H
11	RBC Promoter	Figure 8I
12	AlcR fragment with required 35S Promoter	Figure 8J
13	35SSF	AAAGGAAGGTGGCTCCTAC
14	35SSSR	CCATCTTTGCCCTTGAAGTC
15	CpnR2	GGAGTTCCTCCACTTCAGCAACGGC
16	35SF3	CTACGAGGAGCACCCACCCCC
17	35S-ASF	AAGGAAAGGCCATCGTTG
18	35S-ASR	CAAATACGCAGGAACTGAGG
19	RBCRnaiR	GAGCAGTGGATGGAAGTAAG
20	RBCRnaiF	ACTTTAGGGCACGATGTC
21	35S RNAIF	CTATCACCGTGCCAATAG
22	AlcR	GAGCCGTATAGAGCAGAGAC
23	Cpn 21 Antisense	Figure 8K
24	ACpn 21 RNAiF*	GGGGACCACTTTGTACAAGAAAGCTGGG TGGGAGGCAGAGGAGAAGACTTTAG
25	ACpn 21 RNAi R*	GGGGACAAGTTTGTACAAAAAAGCAGGCT GGCTTCCGGTTGATACTGGTAGAGG
26	ACpn 21 Antisense F	GGCACGTGAAATGGCGGCGACTCAACTTAC
27	ACpn 21 Antisense R	GGACTAGTCCAGATTATGCTCAGGCCGTTTAC
28	pGreen0029-RBC	Figure 8M
29	pUC57-RBC	Figure 8N

* Underlined sequences correspond to Gateway™ (Invitrogen) extensions

[0090] The present invention will be further illustrated in the following examples. However it is to be understood that these examples are for illustrative purposes only, and should not be used to limit the scope of the present invention in any manner.

Examples

Methods

Vector construction

[0091] *Arabidopsis* Chaperonin 21 sequence (GenBank Accession No. AF428366.1 gi:16226910 Arabidopsis thaliana AT5g20720/T1M15_120 mRNA; SEQ ID NO: 1) was used to design PCR primers incorporating Pml1 and Spe1 sites for cloning purposes.

[0092] **Cpn21 Antisense under 35S promoter (cpn21AS) (SEQ ID NO: 4)** A sequence encoding cpn21 was amplified from *A. thaliana* cDNA using primers ACpn 21 Antisense F and ACpn 21 Antisense R (SEQ ID NO: 26 and 27) , incorporating Spe1 (at the 5' end) and Pml1 (at the 3' end) restriction enzyme recognition sequences for cloning purposes. The resulting PCR product was ligated into pGEM-T and verified by sequencing (resulting construct Acpn21 antisense pGEM-T). ACpn21 antisense pGEM-T was digested with PmlI/ SpeI and the resulting 800bp fragment containing Cpn21 was ligated into a pCambia 1302 vector cut with PmlI/ SpeI, and verified by sequencing.

[0093] The pCambia vector (available from CAMBIA) was derived from a pPZP backbone (Hajdukiewicz et al 1994. Plant Mol Biol 25:989-994; GenBank Accession No: AF234301).

[0094] The "Cpn21 RNAi-35S" (SEQ ID NO: 6) construct places expression of the RNAi sequence of Cpn21 under control of the 35S promoter (SEQ ID NO: 9). To make the Cpn21 RNAi constructs a 500bp fragment from the middle of the Cpn21 gene was amplified by PCR using primers containing the Gateway® (Invitrogen) attB1 (forward) and attB2 (reverse) 5' extensions (ACpn 21 RNAi F and ACpn 21 RNAi R, SEQ ID NO: 24 and 25), following manufacturers' instructions. The Gateway® BP recombination reaction was used to insert the Cpn21 PCR product into a pDONR™221 vector, and the resulting Cpn21 pDONR construct verified by sequencing. The Gateway® LR recombination reaction was again used to insert the Cpn21 sequence from Cpn21 pDONR into pKGW1WG2II (Karimi et al., 2002 Trends Plant Sci 7(5):193-195)in an RNAi format (sense and antisense sequences flanking an intron; when

transcribed, the nucleic acid forms a hairpin-loop structure) (SEQ ID NO: 5) and verified by sequencing.

[0095] The "cpn21 RNAi-RBC" construct places expression of the RNAi sequence of Cpn21 under control of the RuBisCO (RBC) promoter (SEQ ID NO: 11). To make cpn21 RNAi-RBC (SEQ ID NO: 7), the cpn21 insert in RNAi orientation from pKGW1WG2II was excised with XmnI, and ligated into pGreen0029-RBC (SEQ ID NO: 28), linearized with XmnI/SmaI. pGreen0029-RBC comprises an *N. tabacum* RBC promoter (nucleotides 699-1744 of SEQ ID NO: 28) a multiple cloning site (nucleotides 1745-1841 of SEQ ID NO: 28), a sequence encoding a NOS terminator (nucleotides 1842-2122 of SEQ ID NO: 28), and was made by excising a KpnI/ApaI fragment of pUC57-RBC (SEQ ID NO: 29) comprising an RBC promoter, and ligating this fragment into a pGreen0029 vector that had been linearized with KpnI/ApaI. pGreen plasmids are known in the art and are described in, for example, references by Hellens et al., 2000 Plant Mol Biol 42:819-832; Hellens et al., 2000 Trends in Plant Sci 5:10:446-451; GenBank Accession No; Y09374).

[0096] The pUC57 vector is known and available from Genscript (GenScript USA Inc. 120 Centennial Ave Piscataway, NJ. Catalog # SD1176-50ug). pUC57-RBC comprises a *N. tabacum* RBC promoter (nucleotides 21-1066 of SEQ ID NO: 29), a multiple cloning site (nucleotides 1067-1163 of SEQ ID NO: 29) and a sequence encoding a NOS terminator (nucleotides 1164-1444 of SEQ ID NO: 29).

[0097] The resulting cpn21 RNAi-RBC construct was digested with BamHI/ XmnI to check for the presence of the insert.

[0098] The "Cpn21AS-RBC" construct places expression of the sense orientation of Cpn21 under control of the RBC promoter. To make Cpn21AS-RBC, the RBC promoter (SEQ ID NO: 11) was removed from pUC57 RBC (SEQ ID NO: 29) by digestion with HindIII and SpeI and inserted into pCambia 1302 AAS6 (from CAMBIA; cut with HindIII/SpeI) comprising a Cpn21 sequence in an antisense orientation. The resulting construct was digested with HindIII/ SpeI to check for correct insert size.

[0099] Inducible constructs comprising the Alc were also made. The Alc regulon is describe in Caddick 1998 et al. Briefly, the minimal regulon includes an alcR sequence and an AlcA promoter. The AlcR polypeptide (transcription factor) responds to the inducer molecule

(ethanol) when the plant is exposed to ethanol; the AlcR polypeptide binds the AlcA promoter, stimulating expression of the open reading frame operably linked to the AlcA promoter sequence.

[00100] The "AlcR Cpn21 RNAi" construct (SEQ ID NO: 8) places expression of the RNA format of Cpn21 under control of an alcohol-inducible promoter (AlcA – SEQ ID NO: 10). To make AlcR Cpn21 RNAi, a nucleic acid sequence comprising Cpn21 in RNAi configuration (sense and antisense sequences flanking an intron; when transcribed, the nucleic acid forms a hairpin-loop structure) from pKGW1WG2II Cpn21 RNAi digested with PspOMI/ XmnI, was ligated into a backbone containing an AlcA promoter from AIR-AIA pGreen 0029 cut with SwaI/ NotI. The construct was digested with BglII to check for correct banding pattern. The A1cR-A1cA pGreen0029 construct was obtained from GenScript. A1R sequence (GenBank Accession No. XM_677155) and A1A promoter sequences (GenBank Accession No. M16196) are known in the art.

Transformation of tobacco plants (*Nicotiana tabacum* L. cv. SR1)

[00101] Tobacco plants grown *in vitro* were utilised for *Agrobacterium* transformation with the above constructs (Cpn21AS, Cpn21 RNAi-RBC, Cpn21AS-RBC, AlcR Cpn21 RNAi). *A. tumifasciens* carrying the construct of interest were grown in suspension culture (400 ml) overnight in LB medium with antibiotics and agitation (28°C, 200 rpm). The bacterial suspension was centrifuged and resuspended in 5% sucrose (OD_{600} ~ 1.0), supplemented with Silwet L-77 to a concentration of 0.02% (200 microliter (µL)/L). Leaf discs from *in vitro* growing plants were immersed for 30 minutes in the resuspended *A. tumifasciens*, followed by two days co-cultivation on solidified CT medium (Sigma-Aldrich). Co-cultivation was carried out in the dark 22°C and 70% humidity. Transformants were selected using selection medium (Murashige & Skoog – "MS") in the presence or absence of selection agents (kanamycin or hygromycin) or induction agents (alcohol), according to the construct used in transformation (see Tables 3, 4 for media, selection agents, inducing agents). Plants demonstrating variegation were rooted and transferred to soil.

Table 3 Composition of tissue culture media utilised for plant variegation *in* vitro

Media composition	CT plant cocultivation medium	½ strain MS seeds germination medium	MS tobacco shoots medium	MMO shoot initiation medium	N6 callus Induction medium	N6 Rice Regeneration medium	LS-Brachypodium Embryo Induction medium	LSR-Brachypodium Regeneration medium	MS basal medium
MS salts with vitamins	-	2.2 g/l	4.4 g/l	-	-	-	-	-	4.4 g/l
MS minimal organics	-	-	-	4.4 g/l	-	-	-	-	-
N6 salts	-	-	-	-	3.98 g/l	3.98 g/l	3.98 g/l	3.98 g/l	-
N6 Vitamins	-	-	-	-	1 ml/l	1 ml/l	1 ml/l	1 ml/l	-
Sucrose	-	10 g/l	30 g/l	30 g/l	30 g/l	30 g/l	30 g/l	-	30 g/l
Glucose	30 g/l	-	-	-	-	-	-	-	-
Maltose			-	-	-	-	-	30 g/l	-
KH_2PO_4	0.2 g/l	-	-	-	-	-	-	-	-
2.4 D	1 mg/l*	-	-	-	2 mg/l	-	5 mg/l	-	-
NAA			-		-	1 mg/l	-	-	-
IAA	0.8 mg/l**	-	0.8mg/l	-	-		-	-	-
BA	-	-	-	4.5mg/l****	-	1 mg/l	-	-	-
kinetin	2.0 mg/l**	-	2.0 mg/l	-	-	-	-	0.2 mg/l	-
DTT	75 mg/l	-	75 mg/l	75 mg/l	75 mg/l	75 mg/l	75 mg/l	75 mg/l	-
Vitamin C	150 mg/l	-	-	-	-	-	-	-	-
acetosyringone	150 um	-	-	-	-	-	-	-	-
agar	6.5 g/l***	6.5 g/l	6.5 g/l	6.5 g/l	6.5 g/l	6.5 g/l	6.5 g/l	6.5 g/l	6.5 g/l
pH	5.5	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7

* Only for canola transformation

** Only for tobacco transformation

*** The same media without agar (liquid one) was used to dissolve Agrobacterium after overnight grow and cocultivation with explants prior CT agar.

Table 4. Constructs, inducing and selection agents, promoters and plants tested.

Construct	Inducing Agent	Selection Agent	Promoter	plants tested
Acpn21 antisense pGEMT	-	-	-	-
Cpn21 AS	none	hyg	35S	T, C, A
AlcR Cpn21RNAi	ethanol	kan	AlcA	T
pDONR221	-	-	-	-
Cpn21 RNAi 35S	none	kan	35S	T, C, R, B, A
Cpn21 RNAi RBC	none	kan	RBC	T
Cpn21 AS-RBC	none	kan	RBC	T
pKGW1WG211	-	-		
pGreen0029 RBC NARNRT	-	-		-
pUC57 RBC	-	-		-
pCambia 1302 AS6	-	-		-
pCambia 1302 AAS6	-	-		-
AIR-AIA pGreen 0029	-	-		-

T= *Nicotiana tabacum*
C= *Brassica napus* (Canola)
A= *Arabidopsis thaliana*
R= *Oryza sativa*
B= *Brachypodium distachyion*

[00102] Hygromycin B (Invitrogen) and kanamycin (Sigma/Aldrich) were used as selection agents. Selection media was also supplemented with Timentin (GlaxoSmithKline). 2-4-D (Sigma), BA (Sigma), indole-e-acetic acid (IAA) (Invitrogen) and kinetin (Sigma) were used for induction of callus and/or shoot regeneration.

Transformation of Arabidopsis

[00103] Transformation of Arabidopsis was according to the method of Clough and Bent, 1998. Briefly, *Arabidopsis* plants were grown until the flowering stage under long days light condition (16/8 hours photoperiod, 70% humidity, and 22°C), in potsoil. *A.tumifasciens* carrying the construct of interest (Cpn21AS or Cpn21 RNAi RBC) was grown in suspension culture (400 ml) overnight in LB medium with antibiotics, with agitation. The bacterial suspension was centrifuged and resuspended in 5% sucrose (OD_{600} ~ 0.8), supplemented with Silwet L-77 to a concentration of 0.02% (200 µL/L). Flowering plants were immersed for 2-3 sec in the *A.tumifasciens* suspension and placed under plastic wrap for 24 hours. Dry matured seeds were collected, sterilized and germinated *in vitro* on selection medium (50 mg/l hygromycin) or selection-free medium (lacking hygromycin).

Transformation of Canola (*Brassica napus*)

[00104] *B. napus* cultivar DH 12075 characterised by high regeneration efficiency was used for chaperonin-transformation experiments. Seeds were germinated on 1/2 MS media for 6 days under long day light conditions (22°C, 16/8 hours photoperiod, 70% humidity). Cotyledons along with petioles from six day old explants were used for transformation experiments.

[00105] *A. tumifasciens* carrying the construct of interest (cpn21AS or Cpn21RNAi - 35S) was grown in suspension culture (400 ml) overnight in LB medium with antibiotics, with agitation (22°C, 200 rpm). Bacteria were collected by centrifugation and resuspended in CT medium at an OD_{600}=0.4. Cotyledons along with a portion of petioles were detached from the plantlets and the cut side of the tissue dipped in the Agrobacterium suspension.

[00106] Explants were co-cultivated for 48 hours on CT agar medium supplemented with 100 um acetosyringone, 1 mg/l 2.4-D and 75 mg/l DTT. Explants were then transferred

to MMO selection media enriched with either with 4.5 mg/l BA, 200 mg/l timentin and with or without 5 mg/l hygromycin and with or without 50 mg/l kanamycin.

[00107] Well developed shoots were detached from the explants and transferred to fresh medium. Rooting was carried out on MS medium lacking growth regulators, supplemented with 200 mg/l timentin, with or without hygromycin. Well rooted plants were hardened in soil. Plants were grown to flowering stage in an isolator at 23°C with 16/8 hour photoperiod. Mature seeds were harvested, sterilized, and germinated *in vitro* on selection media supplemented with 10 mg/ l hygromycin or 100 mg/ml kanamycin. Young leaves were harvested for PCR analysis.

Example 1: Transformation of *N.tabacum* with Cpn21AS

[00108] *Nicotiana tabacum* L. cv. SR1 plants were transformed with cpn21AS as described. The Cpn21 antisense sequence was expressed constitutively under control of the 35S promoter, as described.

[00109] Over 200 plants demonstrating various variegation patterns were selected following transformation in antisense orientation or RNAi. T0 plants at flowering stage were grown under isolators in growth cabinet (23°C day/19°C night; 16/8 hour photoperiod). Seeds from 15 randomly chosen variegated plants were harvested and sterilised and germinated *in vitro* on ½ MS media with hygromycin (50 mg/l) or hygromycin free MS media (Tables 3& 4). Three weeks after germination the number of Hyg^R and Hyg^S seedlings was determined. T0 and T1 plants were tested using PCR with primers 35S-ASR and 35S-ASF, to confirm the presence of the Cpn21 antisense sequence.

[00110] Shoots grown on hygromycin selection media were subcultured for further shoot elongation onto MS medium supplemented with hygromycin. At the shoot elongation stage, some leaves were lighter green, with small portions of dark green tissue at the edges while others exhibited variegation over the entire leaf. Variegated plants demonstrated rooting in hygromycin selection media, while those with bleached leaves demonstrated less viability, and reduced growth. Seeds from bleached plants either were not produced, or if they were, did not germinate upon cultivation *in vitro*. Partially variegated plants were grown under reduced light intensity (800 Lux) and produced viable seeds. 67.4% of regenerated

shoots were rooted on hygromycin media. 12.2% of all hygromycin-resistant rooted plants were visibly variegated.

[00111] Variegated *N. tabacum* plants were also identified on hygromycin-free medium (MS-hyg). A plant was considered variegated if one or more regions of one or more leaves displayed differential coloration. The efficiency of transformation was slightly decreased when plants were regenerated without selection pressure - 11.3% of regenerated plants were variegated and 4.4% were rooted after 3 weeks. These results demonstrate the variegation effect may be used as a marker of a transformed plant in the absence of artificial selection pressure.

[00112] T1 plants (produced by self fertilization of T0 plants) demonstrated variegation in ~25% of plants that germinated on hygromycin medium.

[00113] Nontransformed plants do not degrade hygromycin, and the surviving shoots on the selection medium (hygromycin-containing) were albino. Transformed plants grown on selection medium were green, or had some green tissue.

Example 2: Transformation of Tobacco with cpnRNAi-35S

[00114] Three weeks following transformation of leaf tissue with cpnRNAi-35S as described, shoots were assessed for variegation, coloration and rooting.

[00115] During the stage of shoot regeneration (initiation and elongation) bleaching was observed in some shoots under both selection and non-selection conditions (+/- kanamycin regeneration media).Well-growing shoots were subcultured for further elongation and rooting to MS selection medium. At this stage of shoot elongation, leaf coloration patterns included light green colouration over the entire leaf, variegated patterns, or yellow edges (Figure 1). 67.4% of regenerated shoots were rooted on kanamycin media, including variegated plants. Plants with bleached leaves were less robust, and demonstrated reduced growth. Overall, 43.7% of kanamycin rooted plants demonstrated variegation.

[00116] Variegated plants were also grown on media lacking kanamycin selection. Transformation efficiency was decreased when plants were regenerated without selection pressure, with 23.3% of the explants having regenerated at least one variegated plant.

[00117] T0 plants at flowering stage were grown and the seeds harvested. Bleached plants either failed to produce seed, or produced seed that did not germinate. Partially variegated plants were grown under reduced light intensity (800 Lux) and produced viable seeds.

[00118] Seeds harvested from variegated plants were sterilised and germinated *in vitro* on ½ MS media with appropriate antibiotics (e.g. kanamycin at 500 mg/l) or antibiotic free MS media to produce T1 progeny, and the number of kanamycin resistant (Kan^R) and kanamycin sensitive (Kan^S) seedlings were tallied at 3 weeks' growth. Self-fertilisation of T0 regenerants yielded either near to 3:1 or 2:1 segregation ratio of kanamycin positive/negative plants, and a 3:1 ratio of variegation positive/variegation negative plants. Variegated T1 plants were easily identified on kanamycin negative media, and a variety of variegation patterns were observed, including bleached or light green leaf edges, with darker leaf tissue, light veination with darker leaf tissue, or speckled/spotted variegation over the entire leaf (Figure 2A-E).

[00119] PCR was used on tissues from visibly variegated T1 progeny to confirm the transgenic status of the regenerants transformed with cpnRNAi-35S, however a significant rate of sterility in T1 seeds was observed. Seeds were germinated on MS basal cultivation media without selection, with 42 % of the seeds were germinated and produced viable plants.

Example 3: Transformation of tobacco with Cpn21RNAi-RBC

[00120] Transformation efficiency of tobacco plants transformed with Cpn21RNAi-RBC under tissue specific promoter was similar to those transformed with Cpn21RNAi - 35S. Leaves were generally brighter comparing to the control (wild type), with variegation in transformed plants observed at later growth stages. Stem colour, viability and morphology during vegetative development and flowering stage of the transformed plants was similar to untransformed plants, and seeds were produced. PCR was used on tissues from visibly variegated T1 progeny plants to confirm the transgenic status of the regenerants.

Example 4: Transformation of Tobacco with Cpn21RNAi-AlcR

[00121] Transformation efficiency of tobacco plants transformed with Cpn21RNAi-AlcR was similar to those transformed with Cpn21RNAi - 35S or Cpn21RNAi-RBC.. Regenerated plants demonstrated normal morphology and seed viability. (78% germination for T0 generation; 81% for wild type control).

[00122] During the stage of shoot elongation ethanol in concentration of 2% was added to the selection media if the plants were transformed with Cpn21 under ethanol inducible promoter. The new developed leaves demonstrated variegated morphology (Figure 3). Some older leaves demonstrated variegation in the new growth meristematic areas.

[00123] Expression from the AlcR promoter may be induced by any of several methods: supplement of the cultivation media with 2% ethanol; exposure of regenerants to ethanol vapour (placement of a small test tube containing 99% ethanol inside the magenta jar with growing shoots); spraying leaves (*in vitro* or *in vivo*) with 2% ethanol. All three methods induced variegation in developing tissues, including leaves and stems.

[00124] About 2 % of the plants growing in ethanol free media also developed variegated pattern.

[00125] 34.2% of rooted plants regenerated on antibiotic free media (supplemented with ethanol during shoot elongation) were visibly variegated. Regenerated plants were transferred to the soil and seeds were produced, harvested, sterilised and germinated *in vitro*. A germination ratio of about 3:1 (germinated :non-germinated) was observed on antibiotic selection (kanamycin) media. When ethanol was added to the regeneration media, 12.8% of the plants demonstrated variegation.

Example 5: Transformation of *Arabidopsis* with Cpn21S, Cpn21AS, Cpn21RNAi - 35S

[00126] *Arabidopsis* was transformed with Cpn21AS and transgenic progeny grown as described.

[00127] Less than 1% (0.4%) efficiency was observed in *Arabidopsis* transformed with cpn21AS or Cpn21RNAi - 35S constructs, and only 0.2% demonstrated variegation. The

seedling transformants demonstrated bleaching, and lacked any significant amount of green colouration.

[00128] Transformants comprising Cpn21RNAi - 35S were rooted on kanamycin selection media and viable seeds were produced by some variegated plants. T1 segregation of kanamycin resistant:sensitive was about 1:1; about 63.2% of regenerants were variegated. PCR was used on tissues from visibly variegated T1 progeny to confirm the transgenic status of the regenerants transformed with Cpn21RNAi - 35S.

Example 6: Transformation of Canola (*Brassica napus*) with Cpn21RNAi - 35S, cpn21AS

[00129] The transformation efficiency with Cpn21RNAi - 35S was low and few variegated plants were identified in the media without kanamycin selection. Approximately 32 variegated shoots (1.2% of total amount of the regenerants) were regenerated on kanamycin free media. T0 Plants developed roots but the growth was inhibited. Seed development was delayed relative to untransformed plants however viable seed was produced, and germinated seeds demonstrated variegation in early leaves and were distinguishable from untransformed plants (Figure 4 and 5).

[00130] PCR was used on tissues from visibly variegated T1 progeny plants to confirm the transgenic status of the regenerants.

Example 7: Constructs and transgenic plants comprising vein-specific promoter.

[00131] A promoter for vein-specific expression of a chaperonin construct may be used to transform plants. The Cpn21AS construct may be digested with restriction enzymes to remove the 35S promoter, according to standard methods. An AtSUC2 promoter, amplified as described in Imlau et al 1999 (Cell 11:309-322) may be ligated into the cut vector, and the construct verified by sequencing. Briefly, PCR amplification may be performed using the primers HP-SUC2P, comprising HindIII and PmlI sites (SEQ ID NO: 19) and SNN-SUC2P SacI, NotI, and NcoI sites (SEQ ID NO: 20). The resulting plasmid comprising the vein-specific promoter may be transformed into plant tissue of interest using known methods, such as those exemplified herein.

Example 8: Transformation of *Oryza sativa* (indica) with Cpn21RNAi -35S

[00132] An embryogenic culture was initiated from mature indica rice seeds (cv. Nipponbare) on 2xN6 medium supplemented with 2 mg/l 2-4D. The embryogenic culture was maintained by subculturing on the fresh medium every 3 weeks. For *Agrobacterium* mediated transformation, the protocol of Kumar et al., 2005 (Plant. Mol. Biol. Rep. 232: 67-73) was used. Regenerated shoots were obtained four to six weeks after the transfer of embryonic callus to regeneration media (supplemented with kanamycin 100 mg/l and 200 mg/l timentin). Elongated shoots were rooted on selection media. Well rooted plants were subsequently transferred to pots (Figure 6) for growth, maturation and production of seed. Self-pollinated and T1 seeds were germinated in kanamycin (500 mg/l) selection medium. PCR was used on tissues from visibly variegated T1 progeny plants to confirm the transgenic status of the regenerants.

Example 9: Transformation of *Brachypodium distachyon* with Cpn21RNAi - 35S

[00133] Immature embryos were utilized as a source of primary explants for embryogenic culture, using the protocol of Draper et al., (2001). (Plant Physiol. 127: 1539-1555). Embryo initiation was on media (LS) supplemented with 5 mg/l 2-4D (callus-inducing medium, CIM). The embryogenic culture was maintained by subculturing on CIM fresh medium every 3 weeks. For *Agrobacterium* mediated transformation, the protocol of Vogel et al., 2006 (Cell, Tissue and Organ Culture 84: 199-211) was used. Regenerated shoots were obtained four to six weeks after the transfer of embryonic callus to regeneration media, supplemented with 0.2 mg/l kinetin, 50 mg/l kanamycin and 150 mg/l timentin. Elongated shoots were rooted on selection media. Well rooted plants were transferred to pots (Figure 7) for growth, maturation and production of seed. Self-pollinated and T1 seeds germinated in kanamycin (500 mg/l) selection medium. PCR was used on tissues from visibly variegated T1 progeny plants to confirm the transgenic status of the regenerants.

[00134] All citations are herein incorporated by reference, as if each individual publication was specifically and individually indicated to be incorporated by reference herein and as though it were fully set forth herein. Citation of references herein is not to be construed nor considered as an admission that such references are prior art to the present invention.

[00135] One or more currently preferred embodiments of the invention have been described by way of example. The invention includes all embodiments, modifications and variations substantially as hereinbefore described and with reference to the examples and figures. It will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims. Examples of such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way.

WHAT IS CLAIMED IS:

1. A variegated plant comprising a nucleic acid operatively linked to a regulatory region, wherein the nucleic acid disrupts the expression of Cpn21.

2. The variegated plant of claim 1, wherein the nucleic acid is an antisense Cpn21, or a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21.

3. The variegated plant of claim 1, wherein the regulatory region is an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region or a constitutive regulatory region.

4. The variegated plant of claim 3, wherein the inducible regulatory region is an alcohol-inducible promoter.

5. The variegated plant of claim 3, wherein the tissue specific regulatory region is a RuBisCO promoter.

6. The variegated plant of claim 1, wherein the nucleic acid is an RNAi.

7. The variegated plant of claim 6, wherein the RNAi comprises a fragment of SEQ ID NO: 1.

8. The variegated plant of claim 6 wherein the RNAi comprises SEQ ID NO: 5, or a fragment or portion thereof.

9. A method of producing a variegated plant comprising,

i) providing the plant a nucleic acid operatively linked to a regulatory region, the nucleic acid disrupting the expression of Cpn21, and

ii) growing the plant under conditions that results in the expression of the nucleic acid, thereby producing the variegated plant.

10. The method of claim 9, wherein the nucleic acid is an antisense Cpn21 or a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21.

11. The method of claim 9, wherein the regulatory region is an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region or a constitutive regulatory region.

12. A method of selecting a plant comprising a gene of interest comprising,

i) providing the plant comprising a first nucleic acid operatively linked to a first regulatory region, wherein the first nucleic acid disrupts the expression of Cpn21, and a second nucleic acid encoding the gene of interest and operatively linked to a second regulatory region,

ii) growing the plant under conditions that result in the expression of the first and second nucleic acids, and

iii) selecting plants that display a variegated phenotype and express the nucleic acid of interest.

13. The method of claim 12, wherein the nucleic acid is an antisense Cpn21 or a nucleic acid that hybridizes under stringent conditions with the complement of antisense Cpn21.

14. The method of claim 12, wherein the regulatory region is an inducible regulatory region, a tissue specific regulatory region, a developmental regulatory region or a constitutive regulatory region.

15. The method of claim 14 wherein the inducible regulatory region is alcohol-inducible.

16. The method of claim 12, wherein the first regulatory region comprises an AlcR sequence and the second regulatory region comprises an AlcA promoter.

17. The method of claim 9 wherein the regulatory region comprises a RuBisCO promoter.

18. The method of claim 9 wherein the regulatory region comprises an AlcR sequence and an AlcA promoter.

19. The method of claim 9 wherein the regulatory region comprises a vein-specific promoter.

20. A chimeric construct comprising a nucleic acid operatively linked to a regulatory region, the nucleic acid comprising a sequence that directly or indirectly disrupts the expression of Cpn21, or functional homologue of Cpn21.

21. The chimeric construct of claim 20, wherein the construct further comprises a second nucleic acid encoding a protein of interest and operatively linked to a second regulatory region.

22. A method of selecting a plant comprising a gene of interest comprising, transforming the plant with a nucleotide sequence comprising the chimeric construct of claim 21, growing the plant under conditions that result in the expression of the first and second nucleic acids, and selecting plants that display a variegated phenotype and express the nucleic acid of interest.

Figure 1

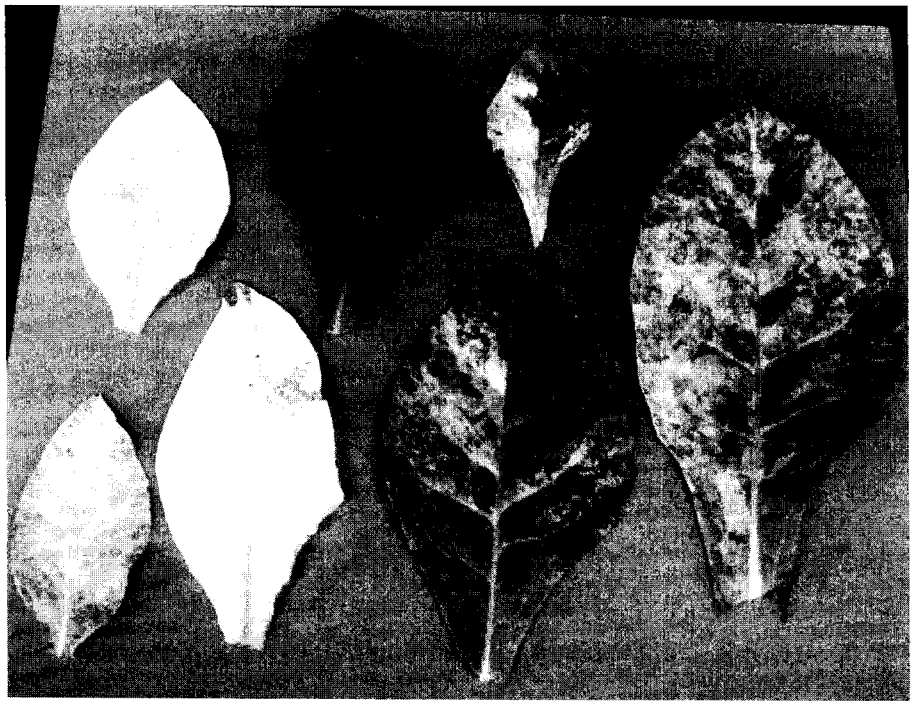

Figure 2

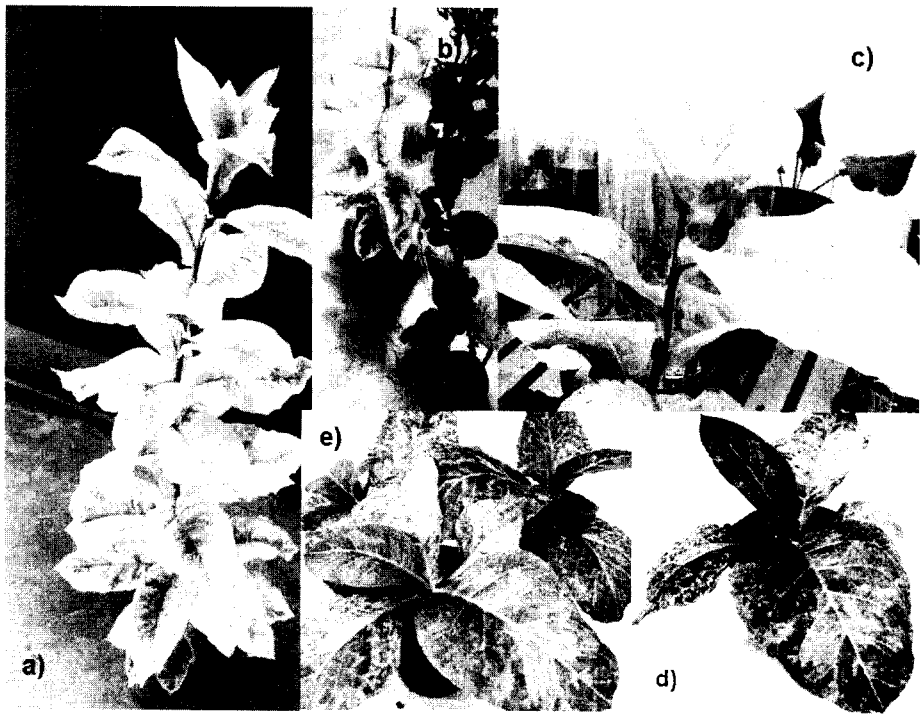

Figure 3

Figure 4

Figure 5

Figure 6

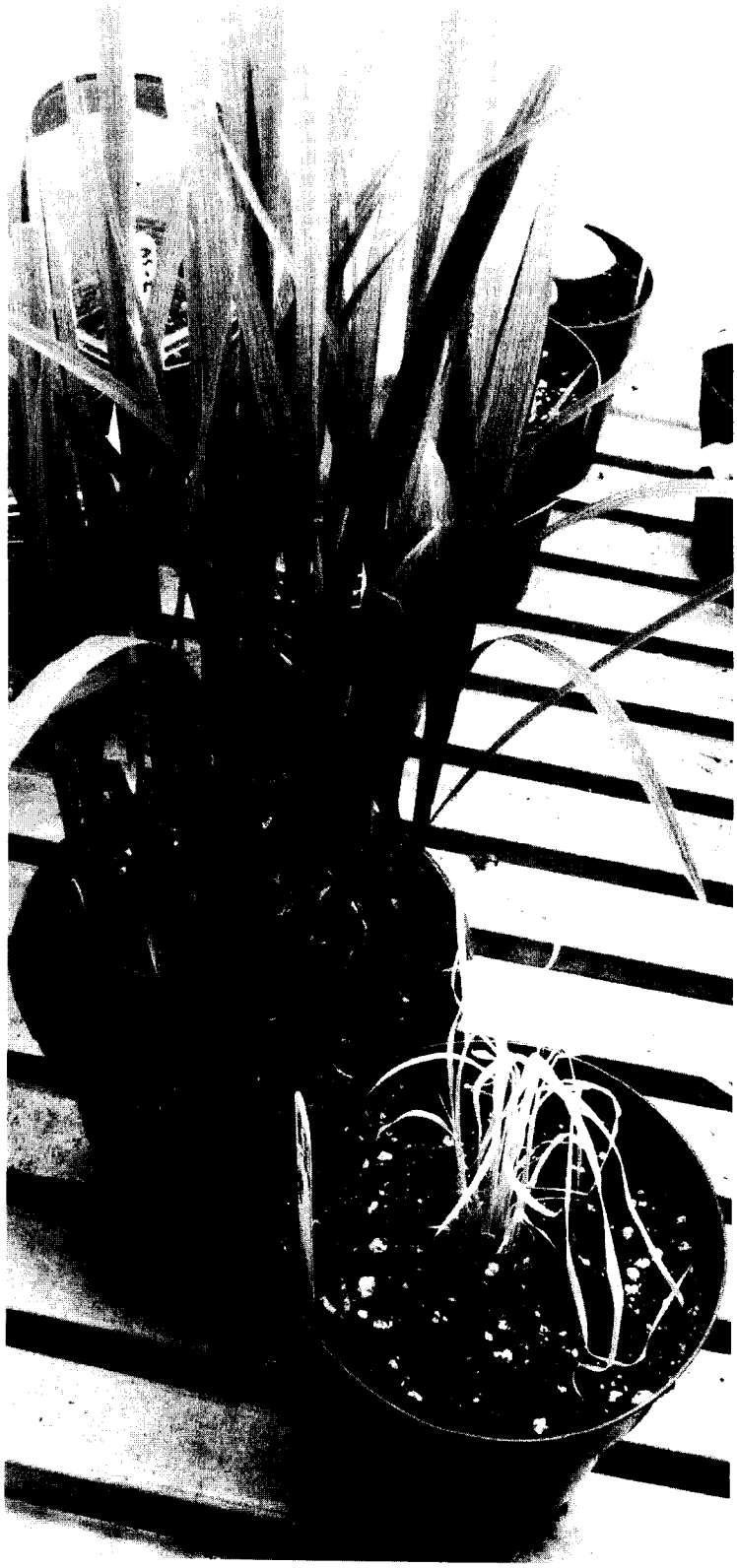

Figure 7

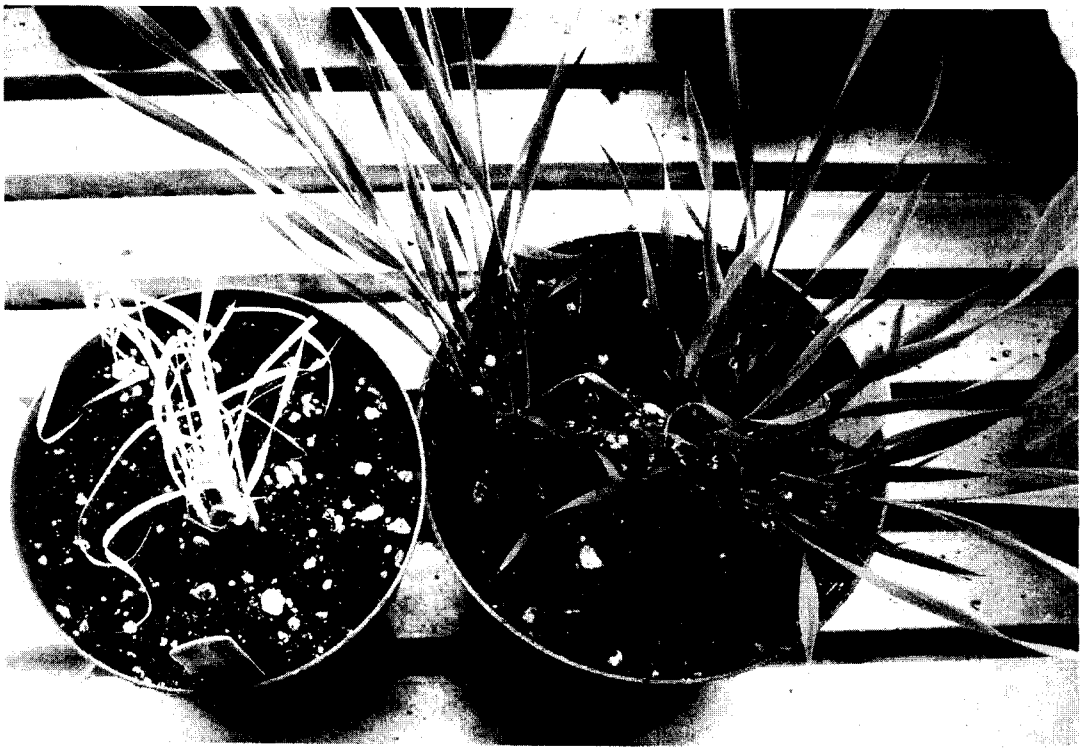

Figure 8A

SEQ ID NO: 1

gi|16226910|gb|AF428366.1|AF428366 Arabidopsis thaliana AT5g20720/T1M15_120 mRNA, complete cds

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CTTAGCCTCGCTGGATGGTCTCAGAGCTTCGAGTGTCAAGTTTTCATCTTTGAAACCAGGGAC
CCTTAGACAGAGCCAGTTCCGTCGTTTGGTTGTCAAAGCTGCTTCTGTTGTTGCCCCTAAGTAT
ACTTCAATTAAGCCATTGGGAGATCGAGTTTTGGTGAAGATCAAGGAGGCAGAGGAGAAGAC
TTTAGGTGGTATCTTACTTCCATCCACTGCTCAATCAAAACCTCAAGGAGGTGAAGTCGTTGC
CGTGGGTGAAGGAAGAACTATTGGGAAGAACAAAATTGATATCACTGTCCCTACTGGAGCAC
AAATTATCTACTCCAAATACGCAGGAACTGAGGTGGAGTTCAATGATGTGAAGCATCTTATCC
TCAAGGAAGATGATATTGTTGGCATTCTTGAGACAGAGGACATCAAAGATCTCAAACCTTTGA
ATGACCGAGTCTTTATTAAGGTTGCTGAGGCGGAGGAGAAAACAGCTGGAGGGTTGTTGTTA
ACCGAGACTACCAAAGAGAAGCCTTCTATTGGCACGGTGATAGCAGTTGGACCGGGTTCCCTA
GACGAGGAAGGTAAAATTACGCCTCTACCAGTATCAACCGGAAGCACAGTACTTTACTCCAA
GTATGCTGGTAACGACTTCAAGGGCAAAGATGGTTCCAACTACATTGCCCTCAGAGCTTCAGA
TGTGATGGCTATACTTTCTTAGTTATGTTATATCTTTGTAATCTGCAACTTGTATCCCAATTGTG
GAAATTTTTTCCGTAAACGGCCTGAGCATAATCTGGAATAAAGACTTGAGTTTGAAAATGTGA
TTTTATTGCC

Figure 8B
SEQ DI NO: 4
Cpn21 Antisense 35S

TGACCATGGTAGATCTGACTAGTCCAGATTATGCTCAGGCCGTTTACGGAAAAAATTTCCACA
ATTGGGATACAAGTTGCAGATTACAAAGATATAACATAACTAAGAAAGTATAGCCATCACAT
CTGAAGCTCTGAGGGCAATGTAGTTGGAACCATCTTTGCCCTTGAAGTCGTTACCAGCATACT
TGGAGTAAAGTACTGTGCTTCCGGTTGATACTGGTAGAGGCGTAATTTTACCTTCCTCGTCTAG
GGAACCCGGTCCAACTGCTATCACCGTGCCAATAGAAGGCTTCTCTTTGGTAGTCTCGGTTAA
CAACAACCCTCCAGCTGTTTTCTCCTCCGCCTCAGCAACCTTAATAAAGACTCGGTCATTCAAA
GGTTTGAGATCTTTGATGTCCTCTGTCTCAAGAATGCCAACAATATCATCTTCCTTGAGGATAA
GATGCTTCACATCATTGAACTCCACCTCAGTTCCTGCGTATTTGGAGTAGATAATTTGTGCTCC
AGTAGGGACAGTGATATCAATTTTGTTCTTCCCAATAGTTCTTCCTTCACCCACGGCAACGACT
TCACCTCCTTGAGGTTTTGATTGAGCAGTGGATGGAAGTAAGATACCACCTAAAGTCTTCTCC
TCTGCCTCCTTGATCTTCACCAAAACTCGATCTCCCAATGGCTTAATTGAAGTATACTTAGGGG
CAACAACAGAAGCAGCTTTGACAACCAAACGACGGAACTGGCTCTGTCTAAGGGTCCCTGGT
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GACATAGTCACTGGTGACGCTGTAAGTTGAGTCGCCGCCATTTCACGTGTGAATTGGTGACCA
GCTCGAATTTCCCCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGC
CGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATG
TAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAAT
ACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCT
ATGTTACTAGATCGGGAATTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAACCTAA
GAGAAAAGAGCGTTTATTAGAATAACGGATATTTAAAAGGGCGTGAAAAGGTTTATCCGTTC
GTCCATTTGTATGTGCATGCCAACCACAGGGTTCCCCTCGGGATCAAAGTACTTTGATCCAAC
CCCTCCGCTGCTATAGTGCAGTCGGCTTCTGACGTTCAGTGCAGCCGTCTTCTGAAAACGACA
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CGCGTGTTTTAGTCGCATAAAGTAGAATACTTGCGACTAGAACCGGAGACATTACGCCATGAA
CAAGAGCGCCGCCGCTGGCCTGCTGGGCTATGCCCGCGTCAGCACCGACGACCAGGACTTGA
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GGCACCAGGCGCGACCGCCCGGAGCTGGCCAGGATGCTTGACCACCTACGCCCTGGCGACGT
TGTGACAGTGACCAGGCTAGACCGCCTGGCCCGCAGCACCCGCGACCTACTGGACATTGCCG
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TAAAGAGAAAATGAGCAAAAGCACAAACACGCTAAGTGCCGGCCGTCCGAGCGCACGCAGC
AGCAAGGCTGCAACGTTGGCCAGCCTGGCAGACACGCCAGCCATGAAGCGGGTCAACTTTCA

Figure 8B continued

GTTGCCGGCGGAGGATCACACCAAGCTGAAGATGTACGCGGTACGCCAAGGCAAGACCATTA
CCGAGCTGCTATCTGAATACATCGCGCAGCTACCAGAGTAAATGAGCAAATGAATAAATGAG
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CCGTGGAATGCCCCATGTGTGGAGGAACGGGCGGTTGGCCAGGCGTAAGCGGCTGGGTTGTC
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CATCCGGCCCGGTACAAATCGGCGCGGCGCTGGGTGATGACCTGGTGGAGAAGTTGAAGGCC
GCGCAGGCCGCCCAGCGGCAACGCATCGAGGCAGAAGCACGCCCCGGTGAATCGTGGCAAGC
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GGAAGCCGCCCAAGGGCGACGAGCAACCAGATTTTTTCGTTCCGATGCTCTATGACGTGGGCA
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CTGGCGAGGTGATCCGCTACGAGCTTCCAGACGGGCACGTAGAGGTTTCCGCAGGGCCGGCC
GGCATGGCCAGTGTGTGGGATTACGACCTGGTACTGATGGCGGTTTCCCATCTAACCGAATCC
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ATCGTTATTCAGTAAGTAATCCAATTCGGCTAAGCGGCTGTCTAAGCTATTCGTATAGGGACA

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CTGAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGATTGTCGTTTCCCGCCTTCAGTTT
AGCTTCATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTAAAGACTGGCGA

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GTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATA
AAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCC
ACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATG
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Figure 8 C
SEQ ID NO: 5
Cpn21 RNAi fragment with intron

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CTTCTATTGGCACGGTGATAGCAGTTGGACCGGGTTCCCTAGACGAGGAAGGTAAAATTACGC
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CTCGGTCATTCAAAGGTTTGAGATCTTTGATGTCCTCTGTCTCAAGAATGCCAACAATATCATC
TTCCTTGAGGATAAGATGCTTCACATCATTGAACTCCACCTCAGTTCCTGCGTATTTGGAGTAG
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Figure 8 D
SEQ ID NO: 6
Cpn21 RNAi 35S

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GCCGATGACGCGGGACAAGCCGTTTTACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAG
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GCGTTCAAAAGTCGCCTAAGGTCACTATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCAC
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GGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCG
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TGATCTCGCGGAGGGTAGCATGTTGATTGTAACGATGACAGAGCGTTGCTGCCTGTGATCAAT
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Figure 8 E
SEQ ID NO: 7
RBC Cpn21 RNAi Sequence

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Figure 8 F
SEQ ID NO: 8
AlcR Cpn21 RNAi Sequence

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Figure 8 G
SEQ ID NO: 9
35S Promoter

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Figure 8 H
SEQ ID NO: 10
AlcA promoter

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Figure 8 I
SEQ ID NO: 11
RBC Promoter

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TAGCTATCAAATTAGTTTCCACTTCTAAACCATGAATATTAGGAAAAACAAGAAACAAAACA
AATATACATAAACAATACGGCTAAAGCCAAGGAAAAGGGACTCTAAAAAAATTAACCAACCT
CAATCACACATTCATATCCTCTTCCTACCCCATCTAGGATGAGATAAGATTACTAGGTCTTACA
CGTGGCACCTCCATTGTGGTGACTAAATGAAGAGTGGCTTAGCTCAAAATATAATTTTCCAAC
CTTTCATGTGTGGATATTAAGTTTTGTGTAGTGAATCAAGAACCACATAATCCAATGGTTAGCT
TTATTCCAAGATGAGGGGGTTGTTGATTTTTGTCCGTCAGATATAGGAAATATGTAAAACCTT
ATCATTATATATAGGGTGGTGGGCAACTATGCAATGACCATATTGGAAGTTAAAGGAAAAGA
GAGAAAGAGAAAT

Figure 8 J
SEQ ID NO: 12
AlcR fragment with required 35s Promotor

CATGGTGGAGCACGACACTCTCGTCTACTCCAAGAATATCAAAGATACAGTCTCAGAAGACC
AAAGGGCTATTGAGACTTTTCAACAAAGGGTAATATCGGGAAACCTCCTCGGATTCCATTGCC
CAGCTATCTGTCACTTCATCAAAAGGACAGTAGAAAAGGAAGGTGGCACCTACAAATGCCAT
CATTGCGATAAAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGG
ACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACTCTCGTCTACTCCAAGAATATCAAAGATA
CAGTCTCAGAAGACCAAAGGGCTATTGAGACTTTTCAACAAAGGGTAATATCGGGAAACCTC
CTCGGATTCCATTGCCCAGCTATCTGTCACTTCATCAAAAGGACAGTAGAAAAGGAAGGTGGC
ACCTACAAATGCCATCATTGCGATAAAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGT
GGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCAC
GTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCA
CTATCCTTCGCAAGACCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGACACGCTGA
AATCACCAGTCTCTCTCTACAAATCTATCTCTCTCGAGCTTTCGCAGATCCCGGGGAGTACTAT
GTGCTGGAACCCCGCATGCTCCAGGCCTCATTCCCCAGATTTCGACTCCATGGCTGACAGCAT
CCAGACCGCACGTTTTTGGACTTCTGATACCATCTATACAGATATCACTGTCGATATTCTCCTG
CACACAGCATGGCAGATACGCGCCGACGCCAGAATCATAGCTGCGATCCCTGTCGCAAGGGC
AAGCGACGCTGTGATGCCCCGAAACGAGGCCAATGAAAACGGCTGGGTTTCGTGTTCAAATT
GCAAGCGTTGGAACAAGGATTGTACCTTCAATTGGCTCTCATCCCAACGCTCCAAGGCAAAAG
GGGCTGCACCTAGAGCGAGAACAAAGAAAGCCAGGACCGCAACAACCACCAGTGAACCATC
AACTTCAGCTGCAACAATCCCTACACCGGAAAGTGACAATCACGATGCGCCTCCAGTCATAAA
CTCTCACGACGCGCTCCCGAGCTGGACTCAGGGGCTACTCTCCCACCCCGGCGACCTTTTCGA
TTTCAGCCACTCTGCTATTCCCGCAAATGCAGAAGATGCGGCCAACGTGCAGTCAGACGCACC
TTTTCCGTGGGATCTAGCCATCCCCGGTGATTTCAGCATGGGCCAACAGCTCGAGAAACCTCT
CAGTCCGCTCAGTTTTCAAGCAGTCCTTCTTCCGCCCCATAGCCCGAACACGGATGACCTCATT
CGCGAGCTGGAAGAGCAGACTACGGATCCGGACTCGGTTACCGATACTAATAGTGTACAACA
GGTCGCTCAAGATGGATCGCTATGGTCTGATCGGCAGTCGCCGCTACTGCCTGAGAACAGTCT
GTGCATGGCCTCAGACAGCACAGCACGGCGATATGCCCGTTCCACAATGACGAAGAATCTGA
TGCGAATCTACCACGATAGTATGGAGAATGCACTGTCCTGCTGGCTGACAGAGCACAATTGTC
CATACTCCGACCAGATCAGCTACCTGCCGCCCAAGCAGCGGGCGGAATGGGGCCCGAACTGG
TCAAACAGGATGTGCATCCGGGTGTGCCGGCTAGATCGCGTATCTACCTCATTACGCGGGCGC
GCCCTGAGTGCGGAAGAGGACAAAGCCGCAGCCCGAGCCCTGCATCTGGCGATCGTAGCTTT
TGCGTCGCAATGGACGCAGCATGCGCAGAGGGGGGCTGGGCTAAATGTTCCTGCAGACATAG
CCGCCGATGAGAGGTCCATCCGGAGGAACGCCTGGAATGAAGCACGCCATGCCTTGCAGCAC

ACGACAGGGATTCCATCATTCCGGGTTATATTTGCGAATATCATCTTTTCTCTCACGCAGAGTG
TGCTGGATGATGATGAGCAGCACGGTATGGGTGCACGTCTAGACAAGCTACTCGAAAATGAC
GGTGCGCCCGTGTTCCTGGAAACCGCGAACCGTCAGCTTTATACATTCCGACATAAGTTTGCA
CGAATGCAACGCCGCGGTAAGGCTTTCAACAGGCTCCCGGGAGGATCTGTCGCATCGACATTC
GCCGGTATTTTCGAGACACCGACGCCGTCGTCTGAAAGCCCACAGCTTGACCCGGTTGTGGCC
AGTGAGGAGCATCGCAGTACATTAAGCCTTATGTTCTGGCTAGGGATCATGTTCGATACACTA
AGCGCTGCAATGTACCAGCGACCACTCGTGGTGTCAGATGAGGATAGCCAGATATCATCGGC
ATCTCCACCAAGGCGCGGCGCTGAAACGCCGATCAACCTAGACTGCTGGGAGCCCCCGAGAC
AGGTCCCGAGCAATCAAGAAAAGAGCGACGTATGGGGCGACCTCTTCCTCCGCACCTCGGAC
TCTCTCCCAGATCACGAATCCCACACACAAATCTCTCAGCCAGCGGCTCGATGGCCCTGCACC
TACGAACAGGCCGCCGCCGCTCTCTCCTCTGCAACGCCCGTCAAAGTCCTCCTCTACCGCCGC
GTCACGCAGCTCCAAACCCTCCTCTATCGCGGCGCCAGCCCTGCCCGCCTTGAAGCGGCCATC
CAGAGAACGCTCTACGTTTATAATCACTGGACAGCGAAGTACCAACCATTTATGCAGGACTGC
GTTGCTAACCACGAGCTCCTCCCTTCGCGCATCCAGTCTTGGTACGTCATTCTAGACGGTCACT
GGCATCTAGCCGCGATGTTGCTAGCGGACGTTTTGGAGAGCATCGACCGCGATTCGTACTCTG
ATATCAACCACATCGACCTTGTAACAAAGCTAAGGCTCGATAATGCACTAGCAGTTAGTGCCC
TTGCGCGCTCTTCACTCCGAGGCCAGGAGCTGGACCCGGGCAAAGCATCTCCGATGTATCGCC
ATTTCCATGATTCTCTGACCGAGGTGGCATTCCTGGTAGAACCGTGGACCGTCGTTCTTATTCA
CTCGTTTGCCAAAGCTGCGTATATCTTGCTGGACTGTTTAGATCTGGACGGCCAAGGAAATGC
ACTAGCGGGGTACCTGCAGCTGCGGCAAAATTGCAACTACTGCATTCGGGCGCTGCAATTTCT
GGGCAGGAAGTCGGATATGGCGGCGCTGGTTGCGAAGGATTTAGAGAGAGGTTTGAATGGGA
AAGTTGACAGCTTTTTGTA

Figure 8 K
SEQ ID NO: 23
Cpn21AS

CACACGTGCCAGATTATGCTCAGGCCGTTTACGGAAAAAATTTCCACAATTGGGATACAAGTT
GCAGATTACAAAGATATAACATAACTAAGAAAGTATAGCCATCACATCTGAAGCTCTGAGGG
CAATGTAGTTGGAACCATCTTTGCCCTTGAAGTCGTTACCAGCATACTTGGAGTAAAGTACTG
TGCTTCCGGTTGATACTGGTAGAGGCGTAATTTTACCTTCCTCGTCTAGGGAACCCGGTCCAAC
TGCTATCACCGTGCCAATAGAAGGCTTCTCTTTGGTAGTCTCGGTTAACAACAACCCTCCAGCT
GTTTTCTCCTCCGCCTCAGCAACCTTAATAAAGACTCGGTCATTCAAAGGTTTGAGATCTTTGA
TGTCCTCTGTCTCAAGAATGCCAACAATATCATCTTCCTTGAGGATAAGATGCTTCACATCATT
GAACTCCACCTCAGTTCCTGCGTATTTGGAGTAGATAATTTGTGCTCCAGTAGGGACAGTGAT
ATCAATTTTGTTCTTCCCAATAGTTCTTCCTTCACCCACGGCAACGACTTCACCTCCTTGAGGT
TTTGATTGAGCAGTGGATGGAAGTAAGATACCACCTAAAGTCTTCTCCTCTGCCTCCTTGATCT
TCACCAAAACTCGATCTCCCAATGGCTTAATTGAAGTATACTTAGGGGCAACAACAGAAGCA
GCTTTGACAACCAAACGACGGAACTGGCTCTGTCTAAGGGTCCCTGGTTTCAAAGATGAAAAC
TTGACACTCGAAGCTCTGAGACCATCCAGCGAGGCTAAGCTCCTTGCTGACATAGTCACTGGT
GACGCTGTAAGTTGAGTCGCCGCCATT

Figure 8 L

SEQ ID NO: 2

AATGGCGGCGACTCAACTTACAGCGTCACCAGTGACTATGTCAGCAAGGAGCTTAGCCTCGCT
GGATGGTCTCAGAGCTTCGAGTGTCAAGTTTTCATCTTTGAAACCAGGGACCCTTAGACAGAG
CCAGTTCCGTCGTTTGGTTGTCAAAGCTGCTTCTGTTGTTGCCCCTAAGTATACTTCAATTAAG
CCATTGGGAGATCGAGTTTTGGTGAAGATCAAGGAGGCAGAGGAGAAGACTTTAGGTGGTAT
CTTACTTCCATCCACTGCTCAATCAAAACCTCAAGGAGGTGAAGTCGTTGCCGTGGGTGAAGG
AAGAACTATTGGGAAGAACAAAATTGATATCACTGTCCCTACTGGAGCACAAATTATCTACTC
CAAATACGCAGGAACTGAGGTGGAGTTCAATGATGTGAAGCATCTTATCCTCAAGGAAGATG

ATATTGTTGGCATTCTTGAGACAGAGGACATCAAAGATCTCAAACCTTTGAATGACCGAGTCT
TTATTAAGGTTGCTGAGGCGGAGGAGAAAACAGCTGGAGGGTTGTTGTTAACCGAGACTACC
AAAGAGAAGCCTTCTATTGGCACGGTGATAGCAGTTGGACCGGGTTCCCTAGACGAGGAAGG
TAAAATTACGCCTCTACCAGTATCAACCGGAAGCACAGTACTTTACTCCAAGTATGCTGGTAA
CGACTTCAAGGGCAAAGATGGTTCCAACTACATTGCCCTCAGAGCTTCAGATGTGATGGCTAT
ACTTTCTTAGTTATGTTATATCTTTGTAATCTGCAACTTGTATCCCAATTGTGGAAATTTTTTCC
GTAAACGGCCTGAGCATAATCTGGCACGTGTG

Figure 8M
SEQ ID NO: 28
pGreen0029-RBC

ATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCC
GGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCT
CCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATCATGCCTCGATCGAGTT
GAGAGTGAATATGAGACTCTAATTGGATACCGAGGGGAATTTATGGAACGTCAGTGGAGCAT
TTTTGACAAGAAATATTTGCTAGCTGATAGTGACCTTAGGCGACTTTTGAACGCGCAATAATG
GTTTCTGACGTATGTGCTTAGCTCATTAAACTCCAGAAACCCGCGGCTGAGTGGCTCCTTCAA
CGTTGCGGTTCTGTCAGTTCCAAACGTAAAACGGCTTGTCCCGCGTCATCGGCGGGGGTCATA
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TTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTG
GCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG
ACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGGTACCTC
TGCAGATAAGCTTGTGGGAACGAGATAAGGGCGAAGTGCGCTAGTAGCCTGCTATTTAAAAT
ATATCCACAATTTATAATGTATTTGAAGATTAGTCAATTCGTCCAAAATTCAGGACTAAGTAT
CTTGAATTTTTGTATCCTGAATTTTTGGGCTACTAATTTGGAACTCAGGACTTAATGTCCTAAA
TTTTTGAGCCGCTAATTTGAAATTCAGGACTAAGTGTTTTGAATTTTTGAACTGCTTATTCGAA
ATGCAAGACTAAGTGACATGAATTTTTGAACTGCTAATTTAAAATTCAGGACATAAGATTTGA
ATTTTCAAACATAATTTTTTAACTTTAGGGCACGATGTCCTGAAGTTTGAATCTTGAGATCTAA
ACTTCAAGATGCAGCGTCTTGAAGTTTGAGTGAACTGGCTAATCTTTAAATACTTGTAAACTG
TGGATACATTTTTAAATAATATATTTAAAAGCGGCTACCTGGTATCATCTTCACGAGAATTTTC
CAAGTTAATTGTAAAGGAAATAGTGGTGTTGCATCAAGTTATGGACAATATAAGGAAGCAAA
CAGTACTCTAGCTATCAAATTAGTTTCCACTTCTAAACCATGAATATTAGGAAAAACAAGAAA
CAAAACAAATATACATAAACAATACGGCTAAAGCCAAGGAAAAGGGACTCTAAAAAAATTA
ACCAACCTCAATCACACATTCATATCCTCTTCCTACCCCATCTAGGATGAGATAAGATTACTA
GGTCTTACACGTGGCACCTCCATTGTGGTGACTAAATGAAGAGTGGCTTAGCTCAAAATATAA
TTTTCCAACCTTTCATGTGTGGATATTAAGTTTTGTGTAGTGAATCAAGAACCACATAATCCAA
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GAAAAGAGAGAAAGAGAAATCTTTCTGTCTAAGTGTAATTAACTTCTAGATACATGTCTCGAG
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CGATAAGCTTGGCGTCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTT
GCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACA
TGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTA
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CCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCCGGGGGATCCA
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TTCCACACAACATACGAGCCGGAAGHCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAG
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CTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCT
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CTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCT
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CCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAA
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GGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAAGAGTTGGTAGCTCTTGATCCGGC
AAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAA
AAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAAC
TCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT
AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGTGTAACATTGGTCTAGTGATTAGAAAA
ACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTG
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CCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTC
AAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCA
AAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATC
ACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATC
GCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCG
CATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCTGG
GATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAA
GAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACAACATTGGCAACGC
TACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGGTAGATTGT
CGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTT
GGAATTTAATCGCGGCCTTGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGT
ATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATCTTGTGCAATGT
AACATCAGAGATTTTGAGACACAACGTGGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGA
AGGATCAGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAA
ATCACCAACTGGTCCACCTACAACAAAGCTCTCATCAACCGTGGCTCCCTCACTTTCTGGCTG
GATGATGGGGCGATTCAGGCGATCCCCATCCAACAGCCCGCCGTCGAGCGGGCTTTTTTATCC
CCGGAAGCCTGTGGATAGAGGGTAGTTATCCACGTGAAACCGCTAATGCCCCGCAAAGCCTT
GATTCACGGGGCTTTCCGGCCCGCTCCAAAAACTATCCACGTGAAATCGCTAATCAGGGTACG
TGAAATCGCTAATCGGAGTACGTGAAATCGCTAATAAGGTCACGTGAAATCGCTAATCAAAA
AGGCACGTGAGAACGCTAATAGCCCTTTCAGATCAACAGCTTGCAAACACCCCTCGCTCCGGC
AAGTAGTTACAGCAAGTAGTATGTTCAATTAGCTTTTCAATTATGAATATATATATCAATTATT
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CGGTTGCCCACCGTCGAGCGCCAGCGCCTTTGCCCACAACCCGGCGGCCGGCCGCAACAGATC
GTTTTATAAATTTTTTTTTTTGAAAAAGAAAAAGCCCGAAAGGCGGCAACCTCTCGGGCTTCT
GGATTTCCGATCCCCGGAATTAGAGATCTTGGCAGGATATATTGTGGTGTAACGTTATCAGCT
TGCATGCCGGTCGATCTAGTAACATAGATGACACCGCGCGCGATAATTTATCCTAGTTTGCGC
GCTATATTTTGTTTTCTATCGCGTATTAAATGTATAATTGCGGGACTCTAATCAAAAAACCCAT
CTCATAAATAACGTCATGCATTACATGTTAATTATTACATGCTTAACGTAATTCAACAGAAATT
ATATGATAATCATCGCAAGACCGGCAACAGGATTCAATCTTAAGAAACTTTATTGCCAAATGT
TTGAACGATCTGCTTGACTCTAGCTAGAGTCCGAACCCCAGAGTCCCGCTCAGAAGAACTCGT
CAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAG
GAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTC
CTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTC
CACCATGATATTCGGCAAGCAGGCATCGCCCTGGGTCACGACGAGATCCTCGCCGTCGGGCAT
CCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATC
ATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTCCTCGCTCGATGCGATGTTTCGCTTG
GTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGA
TGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCA
ATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCC
GTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCTTGGAGTTC

Figure 8N
SEQ ID NO :29
pUC57-RBC

TATGGGGTACCTCTGCAGATAAGCTTGTGGGAACGAGATAAGGGCGAAGTGCGCTAGTAGCC
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AGGACTAAGTATCTTGAATTTTTGTATCCTGAATTTTTGGGCTACTAATTTGGAACTCAGGACT
TAATGTCCTAAATTTTTGAGCCGCTAATTTGAAATTCAGGACTAAGTGTTTTGAATTTTTGAAC
TGCTTATTCGAAATGCAAGACTAAGTGACATGAATTTTTGAACTGCTAATTTAAAATTCAGGA
CATAAGATTTGAATTTTCAAACATAATTTTTTAACTTTAGGGCACGATGTCCTGAAGTTTGAAT
CTTGAGATCTAAACTTCAAGATGCAGCGTCTTGAAGTTTGAGTGAACTGGCTAATCTTTAAAT
ACTTGTAAACTGTGGATACATTTTTAAATAATATATTTAAAAGCGGCTACCTGGTATCATCTTC
ACGAGAATTTTCCAAGTTAATTGTAAAGGAAATAGTGGTGTTGCATCAAGTTATGGACAATAT
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AAAACAAGAAACAAAACAAATATACATAAACAATACGGCTAAAGCCAAGGAAAAGGGACTC
TAAAAAAATTAACCAACCTCAATCACACATTCATATCCTCTTCCTACCCCATCTAGGATGAGA
TAAGATTACTAGGTCTTACACGTGGCACCTCCATTGTGGTGACTAAATGAAGAGTGGCTTAGC
TCAAAATATAATTTTCCAACCTTTCATGTGTGGATATTAAGTTTTGTGTAGTGAATCAAGAACC
ACATAATCCAATGGTTAGCTTTATTCCAAGATGAGGGGGTTGTTGATTTTTGTCCGTCAGATAT
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ATCCGAGCTCATCGATAAGCTTGGCGTCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAG
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CCCCGGGGAGGGCCCGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAA
ATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGG
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GAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGT
ATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAG
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CGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGT
GGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT
CTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGC
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TGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGT
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GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGA
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TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATT
ACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAG
TGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAG
ATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCT
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CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTA
ATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCA
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ACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCC
TCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCA
TAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAG
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ACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAA
ACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTG
ATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGC
CGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATA
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CATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCG
TTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
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GGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCA

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/001853

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12N 15/82*** (2006.01) , ***A01H 1/04*** (2006.01) , ***A01H 5/00*** (2006.01) , ***C07K 14/415*** (2006.01) , ***C12N 15/29*** (2006.01) , ***C12Q 1/68*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N* (2006.01), A01H* (2006.01), C07K* (2006.01), C12Q* (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Canadian Patent Database, TotalPatent, Pubmed, SCOPUS, GenBank

Keywords: chaperonin, cpn21, cpn20, cpn10, variegation, variegated plants, antisense, RNAi, selection method

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	HANANIA et al. Silencing of chaperonin 21, that was differentially expressed in inflorescence of seedless and seeded grapes, promoted seed abortion in tobacco and tomato fruits. Transgenic Research. August 2007 (08-2007). Vol. 16, No. 4, pages 515-525. ISSN:0962-8819 *whole document*	1-6, 9-11, 17-21 7, 8
Y	HIROHASHI et al. cDNA sequence and overexpression of chloroplast chaperonin 21 from *Arabidopsis thaliana*. Biochimica et Biophysica Acta. 11 January 1999 (11-01-1999). Vol. 1429, No. 2, pages 512-515. ISSN:0167-4838 *figure 1*	7, 8

[ ] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 January 2011 (17-01-2011)	16 February 2011 (16-02-2011)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Scott Gurd (819) 994-4157

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/001853

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   [X] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [X] subsequently to this Authority for the purposes of search

2. [X] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments :

The original electronic copy of the sequence listing submitted with the application was corrupted so another copy was requested for the purposes of search.