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- (71) Applicant: E.I. DUPONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).
- (72) Inventors; and
(75) Inventors/Applicants (for US only): HALLAHAN, David, L. [IE/US]; 5117 New Kent Road, Wilmington, DE 19808 (US). KEIPER-HRYNKO, Natalie, M. [US/US]; 3 Galen Court, Bear, DE 19701 (US).
- (74) Agent: JOUNG, Kenneth, J.; E. I. Du Pont de Nemours and Company, Legal Patent Records Center, 4417 Lancaster Pike, Wilmington, DE 19805 (US).
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(54) Title: METHOD FOR MANIPULATING GROWTH, YIELD, AND ARCHITECTURE IN PLANTS

1 80

SEQ ID NO 2 (Hpt2) (1) -----MELVNGRPSVFLGKYMGG

SEQ ID NO 4 (Rpt1) (1) -----MLGSLMSYLPVSDSKTENTDELIATGVLASQNFTECCI

SEQ ID NO 6 (Spt1) (1) MFSLRIPPLVKTTPSPSCYYSHYHYRYRYCYHPFHRSQTSQSLTVSKRGSATAKCHADSVTLRDGGSVLAQESLEPT

SEQ ID NO 21 (Apt5) (1) -----MNTLEEVDESTHIFNMSLMKGF

SEQ ID NO 32 (Apt1) (1) -----MLSLSSDSSLLSLLFLELIPCLFITSYIGFPVFLKLIGLIKIKAAARDNEKRDEGTYVVR

81 160

SEQ ID NO 2 (Hpt2) (24) YSIITPCHITHEFARLDGNERFPRKHKLP-EGGSHKCHFLALNWNKCYBLGMAIIVASTINPRKHKHGYVVD

SEQ ID NO 4 (Rpt1) (40) VAVISYSEMRKHTASLDGNERFPRKHKLP-EGGSHKCHFLALNWNKCYBLGMAIIVASTINPRKHKHGYVVD

SEQ ID NO 6 (Spt1) (81) PASDAEMERKHTASLDGNERFPRKHKLP-EGGSHKCHFLALNWNKCYBLGMAIIVASTINPRKHKHGYVVD

SEQ ID NO 21 (Apt5) (26) FRVLCVSEITPNSISLDGNERFPRKHKLP-EGGSHKCHFLALNWNKCYBLGMAIIVASTINPRKHKHGYVVD

SEQ ID NO 32 (Apt1) (62) EDGLOREIMERKHTASLDGNERFPRKHKLP-EGGSHKCHFLALNWNKCYBLGMAIIVASTINPRKHKHGYVVD

161 240

SEQ ID NO 2 (Hpt2) (103) ALDSEGMIMEESIIAYDICVRVSNKLSSEPVKTAADIRANNSKCVLILACITSTOIVHAEESSELNSN

SEQ ID NO 4 (Rpt1) (119) LPEPPELLENRNVIKVNCKNENWSDMSKSRVRVAPLATAETGLVFSQMPNSTSETVNAVN-----

SEQ ID NO 6 (Spt1) (160) FERTIVSEVQT---FKREGISVITDSSRIFESLKRMIASAEQKQSRFOHVAEGSGKYDVVQACKS-----

SEQ ID NO 21 (Apt5) (106) LLEVEVLAIDQAVSCMNGVSTIACITDSINHFRAHTKHTLDEEARDLIVVVOANSTSEITVHVR-----

SEQ ID NO 32 (Apt1) (141) LIQHYRNKSNIK---FFHRSEVRVSVISKTKIPFSLKEIHEIEEAKGYKNKHIMNDCSGKFDIMACKS-----

241 320

SEQ ID NO 2 (Hpt2) (183) EVCNNQEEANATGSSTIQTESMESYSGIKVILKNNIN---EYCVILRTSESTSNLYLHCTNCILYSPYALN

SEQ ID NO 4 (Rpt1) (191) KVCAER---RD-ILQREDADSVANGVYSDISVALDRHMSAGCGDQIVIRTSSESTSNLYLHCTNCILYSPYALN

SEQ ID NO 6 (Spt1) (230) -----VAKKKGHILHDDINENIIECLENCTEFYPPILIRTSSESTSNLYLHCTNCILYSPYALN

SEQ ID NO 21 (Apt5) (178) -----KSCRRKCTGGDLVLLSEIVSECMYTSIVVDPVIRTSSESTSNLYLHCTNCILYSPYALN

SEQ ID NO 32 (Apt1) (212) -----LVKKSEKGLTREEDVDEAETERELLNCSDQSPDMIRTSSESTSNLYLHCTNCILYSPYALN

321 353

SEQ ID NO 2 (Hpt2) (261) EPIELRVSVINCHSHYSYLEKKHKEYLK---

SEQ ID NO 4 (Rpt1) (267) EPIELRVSVINCHSHYSYLEKKHKEYLK---

SEQ ID NO 6 (Spt1) (296) EPIELRVSVINCHSHYSYLEKKHKEYLK---

SEQ ID NO 21 (Apt5) (243) EPIELRVSVINCHSHYSYLEKKHKEYLK---

SEQ ID NO 32 (Apt1) (278) EPIELRVSVINCHSHYSYLEKKHKEYLK---

(57) Abstract: This invention pertains to a method for manipulating the growth rate and/or yield and/or architecture of a genetically modified plant, as compared to a corresponding wild-type plant. The method relies on over-expression of an endogenous or exogenous gene encoding a *cis*-prenyltransferase enzyme. In a preferred embodiment, transgenic plants obtained by this method exhibit increased growth rates to maturity, increased seed production, and increased height, siliques, and leave area.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLEMETHOD FOR MANIPULATING GROWTH, YIELD, AND
ARCHITECTURE IN PLANTS

5 This application claims the benefit of U.S. Provisional Application No. 60/472,813, filed May 22, 2003, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

10 This invention is in the field of plant molecular biology. More specifically, this invention provides a method of manipulating the growth rate, yield, and architecture of a genetically modified plant, as compared to a corresponding wild-type plant. The method relies on expression of *cis*-prenyltransferase genes in the plant host.

15 BACKGROUND OF THE INVENTION

 Plants exhibit growth rates dependent on the interplay of their genetics (genotypes) and the environmental conditions to which they are exposed during development. Under most conditions, the maximum growth potential of a plant is not realized. However, it has been
20 demonstrated that manipulation of plant growth rate can be achieved by modification of the plant's genetic makeup, i.e., cultivated plant species may be bred to exhibit enhanced growth rates.

 Genetic engineering of plants involves the isolation and manipulation of genetic material (e.g., DNA or RNA), followed by
25 introduction of that genetic material into plants or plant cells. This means of "genetic enhancement" provides a methodology for rapid and directed alteration of the genetic makeup of cultivated plants, and this methodology represents an advance over traditional breeding methods that rely on selection of varieties or cultivars possessing and exhibiting the desired
30 traits. In recent years, many beneficial traits have been introduced into plants using genetic engineering techniques (which often involve introduction of exogenous genes into the plant host), including: increased yield, pest and pathogen resistance, herbicide resistance, stress (e.g., drought) tolerance, etc.

35 One trait of particular interest to agricultural science is that of accelerated or increased growth rate to maturity. Such a trait is desirable, since plants would be capable of maturing in shorter growing seasons. This ability would permit expansion of the geographic range of cultivated

species into climatic zones with shorter growing seasons, to thereby allow increased seed sales for the producer, bring benefit in the areas where the plants were newly adapted, and enable additional economic advantage to producers and consumers.

5 Accelerated growth to maturity of cultivated plants would be a distinct advantage in numerous applications. For example:

1. In silviculture, growth rate is a key economic property due to the generally slow-growing nature of trees. Applications of growth-influencing chemicals, currently extensively used, could be
10 reduced or eliminated if genetically enhanced trees were available that exhibited properties of increased growth.
2. In agriculture, a focus of traditional breeding is expansion of the range of domesticated plants into cooler climatic zones with shorter growing seasons, with respect to the climatic zone in
15 which the plant originated. Cereals originating in the Middle East (i.e., wheat) and Central America (i.e., corn) are now cultivated worldwide in different climatic zones. Numerous other plant species could be modified to exhibit accelerated growth to maturity, thus conferring advantages to the world's human
20 population.
3. In cell culture, where plants are generated for the production of valuable metabolites (e.g., drugs, drug precursors, vaccines, food additives), rapid cell growth is desirable to rapidly achieve high titers of the desired products.
- 25 4. In processes of plant genetic transformation, reduction in the overall time required for plant growth would be a significant improvement.

Of course, the value of a trait for accelerated or increased growth rate would be significantly reduced if accompanied by deleterious effects in the
30 plants so modified (e.g., reduction in seed number/ fertility, negative alterations in the aesthetic or other commercially important aspects of the plant).

Previous work has recognized the need for a trait that confers accelerated or increased growth to maturity in plants. For example, U.S.
35 6,252,139 describes a method of producing a genetically enhanced plant with increased root growth and yield as a consequence of increased expression of genes encoding cyclin proteins. The transgenic plants

exhibited increased main and lateral root growth rates, but effects on other tissues of the transgenic plants were not described.

WO 98/04725 describes a method for modulating the rate of plant development by modulating the amount of DNA methylation; in particular, an increase in methylated DNA (mediated by cytosine methyl transferase gene products) was found to correlate with an increased rate of growth to maturity.

WO 00/56905 describes a method for modifying plant growth, yield or architecture by increased expression of at least two cell cycle proteins. Specifically, unexpected alterations to plant architecture are described, as a consequence of overexpression of a protein kinase and a cyclin which interact together as a complex (e.g., plants overexpressing both genes exhibited increases in both root and shoot growth of between 10% and 30%). The results appeared to be based on an increase in cell number arising from an increased rate of cell division.

Finally, WO 01/66777 describes improvements in the growth rate and biomass of transgenic hybrid aspen trees following overexpression of a gibberellic acid 20-oxidase gene involved in gibberellin biosynthesis. Gibberellins are plant hormones well known for their effects on plant growth.

Although each of these above methods serves to modify plant growth, the art would be advanced by the identification of a novel gene sequence that conferred accelerated growth to maturity and increased yield to a genetically engineered plant, as compared to a corresponding wild-type plant. It would be particularly desirable to be able to manipulate the growth rate, and/or the yield, and/or the architecture of an entire plant, or specific target organs thereof.

Cis-prenyltransferase genes, which are known to catalyze the sequential addition of C₅ units to polyprenols and rubbers in *cis* 1-4 orientation, have not been previously recognized as capable of modifying plant phenotype and functioning to modify the growth rate to maturity, and/or yield, and/or architecture of a transformed plant, as compared to a corresponding wild-type plant.

SUMMARY OF THE INVENTION

The invention provides a method for producing a transformed plant having an altered growth phenotype as compared with an untransformed plant comprising:

- 5 a) transforming a plant cell with a an isolated nucleic acid molecule encoding a *cis*-prenyltransferase under the control of suitable regulatory sequences;
- b) recovering a transformed plant cell produced in step (a);
- c) regenerating a plant from the transformed plant cell of step (b); and
- 10 d) growing the transformed plant produced in step (c) under conditions wherein the isolated nucleic acid molecule encoding a *cis*-prenyltransferase is expressed and the growth phenotype of the transformed plant is altered.

Preferred *cis*-prenyltransferase of the invention are those that comprise the domains as identified in SEQ ID NOs: 7-10 and SEQ ID NOs: 11-13.

- 15 In particular the expression of *cis*-prenyltransferase genes in plants has been demonstrated to affect plant growth rate, which may result in, decreased time to germination, increased root growth rate, increased shoot growth rate, decreased time to flowering, decreased time for fruit maturation, and decreased time of seed setting; increased yield as defined by increased total biomass, increased root growth, increased
- 20 shoot growth, increased seed set, increased seed production, increased grain yield, increased fruit size, increased nitrogen fixing capacity, increased nodule size, increased tuber formation, increased stem thickness, increased endosperm size, and an increased number of fruit per plant; and modified plant architectural traits as defined by
- 25 modifications in the shape, size, number, color, texture, arrangement and patternation of the root, leaf, shoot, fruit, petiole, trichome, flower, sepals, petal, hypocotyl, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre nodule, cambium, wood, heartwood, parenchyma, sclerenchyma, seive element, phloem, or
- 30 vascular tissue.

In an alternative embodiment the invention provides a method for altering the growth phenotype of a plant as compared with an untransformed plant comprising:

- 35 a) providing a plant comprising a gene encoding a *cis*-prenyltransferase; and
- b) upregulating the gene of (a) wherein the growth phenotype of the plant is altered.

Plants, produced by the methods of the invention are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS
AND SEQUENCE DESCRIPTIONS

5 Figure 1 shows an alignment of the deduced amino acid sequences of the Hevea, rice, soybean and *Arabidopsis* Apt5 genes with that of *Arabidopsis* Apt1.

 Figure 2 is a gel showing the results of a reverse-transcriptase PCR of *Arabidopsis* lines transgenic for plant *cis*-prenyltransferase genes.

10 Figure 3 visually compares the growth of a transgenic *Arabidopsis* expressing a Hpt2 *cis*-prenyltransferase and a wild-type plant, each 35 days after sowing, and grown under identical conditions.

 Figure 4 visually compares the growth of transgenic *Arabidopsis* lines expressing a Spt1 *cis*-prenyltransferase and a wild-type plant, each
15 35 days after sowing, and grown under identical conditions.

 Figure 5 visually compares the growth of a transgenic *Arabidopsis* expressing a Rpt1 *cis*-prenyltransferase and a wild-type plant, each 35 days after sowing, and grown under identical conditions.

 Figure 6 is a comparison between transgenic *Arabidopsis*
20 expressing *cis*-prenyltransferases (Spt1, Rpt1, Hpt2, and Apt5, respectively) and wild-type plants, grown under identical conditions.

 Figure 7 visually compares the growth of a transgenic *Arabidopsis* plant expressing the *cis*-prenyltransferase Hpt2 and a wild-type plant, each 18 days after sowing, and grown under identical conditions.

25 Figure 8 visually compares the growth of transgenic *Arabidopsis* plants expressing *cis*-prenyltransferases and a wild-type plant, each 28 days after sowing, and grown under identical conditions.

 The invention can be more fully understood from the following detailed description and the accompanying sequence descriptions, which
30 form a part of this application.

 The following sequences comply with 37 C.F.R. 1.821-1.825 ("Requirements for Patent Applications Containing Nucleotide Sequences and/or Amino Acid Sequence Disclosures - the Sequence Rules") and are consistent with World Intellectual Property Organization (WIPO) Standard
35 ST.25 (1998) and the sequence listing requirements of the EPO and PCT (Rules 5.2 and 49.5(a-bis), and Section 208 and Annex C of the Administrative Instructions). The symbols and format used for nucleotide

and amino acid sequence data comply with the rules set forth in
37 C.F.R. §1.822.

SEQ ID NOs: 1-6, 20, 21 and 32 are genes or proteins as identified
in Table 1.

Table 1
Summary of Gene and Protein SEQ ID Numbers

Gene Name	Clone ID number and Description	Organism	SEQ ID Nucleic acid	SEQ ID Peptide
Hpt2	ehb2c.pk001.d17	<i>Hevea brasiliensis</i>	1	2
Rpt1	rr1.pk0050.h8	<i>Oryza sativa</i> (rice)	3	4
Spt1	sl1.pk0128.h7	<i>Glycine max</i> (soybean)	5	6
Apt5	--	<i>Arabidopsis thaliana</i> (GenBank AB011483)	20	21
Apt1	--	<i>Arabidopsis thaliana</i>		32

5 SEQ ID NOs:7-10 are consensus sequences representing conserved Domains I, II, III and V, as described by Apfel et al. (*J. Bact.* 182(2):483-492 (1999)).

 SEQ ID NOs: 11-13 are consensus sequences representing modified conserved Domains I, IV, and V, that are indicative of the
10 subfamily of *cis*-prenyltransferases associated with rubber-producing plants. These were described by Hallahan and Keiper-Hrynko in PCT/US03/36164.

 SEQ ID NOs:14-19 are the primers HW8, HW12, JK1, JK2, JK3, and JK4, respectively.

15 SEQ ID NOs:22-25 are the primers Apt5/XbaI, Apt5/KpnI, Apt5s, Apt5as, respectively.

 SEQ ID NOs:26-31 are the primers H2s, H2as, NHK33, NHK34, NHK35, and NHK36, respectively.

 SEQ ID NO:33 is the peptide 'ELVISLIVES'

20 DETAILED DESCRIPTION OF THE INVENTION

 The present invention provides a method for producing a genetically modified plant characterized as having a modified growth phenotype (as compared to a plant of the same species not genetically modified (i.e., a 'wild-type' plant)), by elevating the expression of *cis*-
25 prenyltransferase gene(s) in the plant. The method comprises

transforming a plant cell with a an isolated nucleic acid molecule encoding a *cis*-prenyltransferase under the control of suitable regulatory sequences; recovering a transformed plant cell produced; regenerating a plant from the transformed plant; and growing the transformed plant produced in step
5 (c) under conditions wherein the isolated nucleic acid molecule encoding a *cis*-prenyltransferase is expressed and the growth phenotype of the transformed plant is altered. Genetically modified plants of this invention are fertile (i.e., capable of self- or cross-pollination with other plants of the same species to produce seed) and seeds so produced are capable of
10 germination and growth. Furthermore, other plant properties are not affected in ways deleterious to agriculture, silviculture, horticulture, floriculture or cell culture, when the plants are transformed according to the present invention.

Definitions

15 The following definitions are provided for the full understanding of terms and abbreviations used in this specification:

"Polymerase chain reaction" is abbreviated PCR.

"Open reading frame" is abbreviated ORF.

"Expressed sequence tag" is abbreviated EST.

20 "SDS polyacrylamide gel electrophoresis" is abbreviated SDS-PAGE.

"Polyisoprenoids" refer to a variety of hydrocarbons produced by plants that are built up of isoprene units (C₅H₈) (Tanaka, Y. In *Rubber and Related Polyprenols. Methods in Plant Biochemistry*, Dey, P. M. and
25 Harborne, J. B., Eds., Academic Press: San Diego, CA (1991); Vol. 7, pp 519-536). Those with 45 to 115 carbon atoms and varying numbers of *cis*- and *trans*- (Z- and E-) double bonds are termed "polyprenols", while those polyisoprenoids of longer chain length are termed natural "rubbers" (Tanaka, Y. In *Minor Classes of Terpenoids. Methods in Plant*
30 *Biochemistry*, Dey, P. M. and Harborne, J. B., Eds., Academic: San Diego, CA (1991); Vol. 7, pp 537-542). There are several suggested functions for plant polyisoprenoids; however, the specific roles of the C₄₅-C₁₁₅ polyprenols remain unidentified (although as with most secondary metabolites they too most likely function in plant defense). Short-chain
35 polyprenols may also be involved in protein glycosylation in plants, by analogy with the role of dolichols in animal metabolism.

The term "*cis*-prenyltransferase" refers generally to a class of enzymes (E.C. 2.5.1.31) capable of catalyzing the sequential addition of

C₅ isopentenyl diphosphate (IPP) units to polyprenols and rubbers in *cis* 1-4 orientation. Two examples of *cis*-prenyltransferases are the undecaprenyl diphosphate synthase (EC 2.5.1.31) (Shimizu et al., *J. Biol. Chem.* 273:19476-19481 (1998); Apfel et al., *J. Bacteriol.* 181:483-492 (1999)) and yeast dehydrodolichyl diphosphate synthase (Sato et al., *Mol. Cell. Biol.* 19:471-483 (1999)).

The term "genetic modification" as used herein refers to the introduction of one or more exogenous nucleic acid sequences, e.g., *cis*-prenyltransferase encoding sequences, as well as regulatory sequences, into one or more plant cells, which can generate whole, sexually competent, viable plants. The term "genetically modified plant" as used herein refers to a plant that has been generated through the aforementioned process. Genetically modified plants of the invention are capable of self-pollinating or cross-pollinating with other plants of the same species so that the foreign gene, carried in the germ line, can be inserted into or bred into useful plant varieties.

The term "altered growth phenotype" refers to a plant having a changed phenotype as relating to the growth of the plant. A plant will have an altered growth phenotype when it exhibits changes in growth of the total plant, specific tissues or organs of the plant, or the yield. Additionally, the term "altered growth phenotype" will encompass changes in the rate of development or size or characteristics of plant architecture.

The term "enhanced growth" is a concept well known to the person skilled in the art of plant biology and includes increased crop growth and/or enhanced biomass.

The term "maturity" in general refers to plants which have initiated the transition from vegetative to a reproductive phase of growth, but may also refer to fruit maturation or ripening.

The term "increased yield" or "increased plant yield" refers to an increase in harvestable material resulting from, for example, increased crop growth, increased biomass, or increased seed/fruit yield. Increases can result, for example, from an increased overall growth rate, increased root or tuber size, increased shoot growth, increased leaf biomass, and/or increased seed/fruit growth/number.

The term "plant architecture" refers to any trait of morphology of a plant. Structural features encompassed by the term may include shape, size, number, colour, texture, arrangement and patternation of any cell,

tissue or organ or groups of cells, tissues, or organs of plants (e.g., shoots, roots, calli, tumors, flowers, leaves).

The term "plant" refers to a whole plant, a plant tissue, a plant organ, or a portion thereof. Plantlets are also included within the meaning of "plant".

The term "plant tissue" or "plant organ" may refer to any part of a plant, including, but not limited to: the root, leaf, shoot, fruit, petiole, trichome, flower, sepals, petal, hypocotyl, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre, nodule, cambium, wood, heartwood, parenchyma, sclerenchyma, sieve element, phloem, or vascular tissue, or parts thereof.

The term "plant cell" as used herein refers to any cell of plant origin, including protoplasts, gamete-producing cells, and cells which regenerate into whole plants.

As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

The term "fragment" refers to a DNA or amino acid sequence comprising a subsequence of a *cis*-prenyltransferase nucleic acid sequence or protein. However, an active fragment of the present invention comprises a sufficient portion of the protein to maintain activity.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA molecule, when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. (Molecular Cloning: A Laboratory Manual, 2nd ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1989 (hereinafter "Maniatis"), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. Stringency conditions can be adjusted to screen for moderately similar fragments (such as homologous sequences from distantly related organisms), to highly similar fragments (such as genes that duplicate functional enzymes from closely related organisms). Post-hybridization washes determine stringency

conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C. An additional set of stringent conditions include hybridization at 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS, for example.

Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (see Maniatus, *supra*, 9.50-9.51). For hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Maniatus, *supra*, 11.7-11.8). In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferably a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

The term "complementary" is used to describe the relationship between nucleotide bases that are capable of hybridizing to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine.

The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including (but not limited to) those described in: 1.) Computational Molecular Biology; Lesk, A. M., Ed.; Oxford University: NY, 1988; 2.) Biocomputing: Informatics and Genome Projects; Smith, D. W., Ed.; Academic: NY, 1993; 3.) Computer Analysis of Sequence Data, Part I; Griffin, A. M., and Griffin, H. G., Eds.; Humana: NJ, 1994; 4.) Sequence Analysis in Molecular Biology; von Heinje, G., Ed.; Academic, 1987; and 5.) Sequence Analysis Primer; Gribskov, M. and Devereux, J., Eds.; Stockton: NY, 1991. Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the AlignX program of the Vector NTI bioinformatics computing suite (InforMax Inc., North Bethesda, MD). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp, *CABIOS*. 5:151-153 (1989)) with the default parameters (GAP OPENING PENALTY=10, GAP EXTENSION PENALTY=0.1). Default parameters for pairwise alignments using the Clustal method were KTUPLE SIZE=1, GAP PENALTY=3, WINDOW SIZE=5 and NUMBER OF BEST DIAGONALS=5.

"Synthetic genes" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments that are then enzymatically assembled to construct the entire gene. "Chemically synthesized", as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of DNA may be accomplished using well-established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon

usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

5 "Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not
10 found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural
15 location in the genome of an organism. A "foreign" or "exogenous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome
20 by a transformation procedure.

"Coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence,
25 and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding sites, stem-loop structures, or any other gene expression control
30 elements which are known to activate gene expression and/or increase the amount of gene products.

The "3' non-coding sequences" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals
35 capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of

different 3' non-coding sequences is exemplified by Ingelbrecht et al. (*Plant Cell*, 1:671-680 (1989)).

“Promoter” refers to a nucleotide sequence, usually upstream (5') to its coding sequence, capable of controlling the expression of a coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

The term “promoter” includes a minimal promoter that is a short DNA sequence comprised of a TATA-box and other sequences that serve to specify the site of transcription initiation, to which regulatory elements are added for control of expression. “Promoter” also refers to a nucleotide sequence that includes DNA sequences that are involved in the binding of protein factors which control the effectiveness of transcription initiation in response to physiological or developmental conditions. Additionally, “promoter” also refers to a nucleotide sequence that includes regulatory elements that are capable of controlling the expression of a coding sequence or functional RNA. This type of promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a DNA sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Enhancers are capable of operating in both orientations (normal or flipped), and are capable of functioning even when moved either upstream or downstream from the promoter. Both enhancers and other upstream promoter elements bind sequence-specific DNA-binding proteins that mediate their effects.

“Constitutive promoter” refers to promoters that direct gene expression in all tissues and at all times. “Regulated promoter” refers to promoters that direct gene expression not constitutively but in a

temporally- and/or spatially-regulated manner and include tissue-specific, developmental stage-specific, and inducible promoters. It includes natural and synthetic sequences, as well as sequences which may be a combination of synthetic and natural sequences. Different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro et al. (*Biochemistry of Plants* 15:1-82 (1989); see also WO 00/56905, Tables 3-4). Since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity. Typical regulated promoters useful in plants include, but are not limited to: safener-inducible promoters, promoters derived from the tetracycline-inducible system, promoters derived from salicylate-inducible systems, promoters derived from alcohol-inducible systems, promoters derived from glucocorticoid-inducible systems, promoters derived from pathogen-inducible systems, and promoters derived from ecdysone-inducible systems.

"Tissue-specific promoter" refers to regulated promoters that are not expressed in all plant cells but only in one or more cell types in specific organs (e.g., leaves, shoot apical meristem, flower, or seeds), specific tissues (e.g., embryo or cotyledon), or specific cell types (e.g., leaf parenchyma, pollen, egg cell, microspore- or megaspore mother cells, or seed storage cells). These also include "developmental-stage specific promoters" that are temporally regulated, such as in early or late embryogenesis, during fruit ripening in developing seeds or fruit, in fully differentiated leaf, or at the onset of senescence. It is understood that the developmental specificity of the activation of a promoter (and, hence, of the expression of the coding sequence under its control) in a transgene may be altered with respect to its endogenous expression. For example, when a transgene under the control of a floral promoter is transformed into a plant, even when it is the same species from which the promoter was isolated, the expression specificity of the transgene will vary in different transgenic lines due to its insertion in different locations of the chromosomes.

"Plant developmental stage-specific promoter" refers to a promoter that is expressed not constitutively but at a specific plant developmental

stage or stages. Plant development goes through different stages; for example, in the context of this invention, the germline goes through different developmental stages starting, say, from fertilization through development of embryo, vegetative shoot apical meristem, floral shoot apical meristem, anther and pistil primordia, anther and pistil, micro- and macrospore mother cells, and macrospore (egg) and microspore (pollen).

"Inducible promoter" refers to those regulated promoters that can be turned on in one or more cell types by a stimulus external to the plant, such as a chemical, light, hormone, stress, or a pathogen.

"Promoter activation" means that the promoter has become activated (or turned "on") so that it functions to drive the expression of a downstream genetic element. Constitutive promoters are continually activated. A regulated promoter may be activated by virtue of its responsiveness to various external stimuli (inducible promoter), or developmental signals during plant growth and differentiation, such as tissue specificity (floral-specific, anther-specific, pollen-specific, seed-specific, etc.) and development-stage specificity (vegetative-specific or floral-, shoot-, or apical meristem-specific, male germline-specific, female germline-specific, etc).

"Operably-linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably-linked with a coding sequence or functional RNA when it is capable of affecting the expression of that coding sequence or functional RNA (i.e., that the coding sequence or functional RNA is under the transcriptional control of the promoter). Coding sequences can be operably-linked to regulatory sequences in sense or antisense orientation. "Unlinked" means that the associated genetic elements are not closely associated with one another and the function of one does not affect the other.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense RNA" refers to RNA transcript that

includes the mRNA and so can be translated into protein by the cell.

"Antisense RNA" refers to RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Patent No. 5,107,065; WO 9928508). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

10 The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragments of the present invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of
15 suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or
20 endogenous genes (U.S. 5,231,020).

"Constitutive expression" refers to expression using a constitutive or regulated promoter. "Conditional" and "regulated expression" refer to expression controlled by a regulated promoter. "Transient" expression in the context of this invention refers to expression only in specific
25 developmental stages or tissue in one or two generations. "Non-specific expression" refers to constitutive expression or low level, basal ('leaky') expression in nondesired cells, tissues, or generations.

The term "altered biological activity" will refer to an activity, associated with a protein encoded by a nucleotide sequence which can be
30 measured by an assay method, where that activity is either greater than or less than the activity associated with the native sequence. "Enhanced biological activity" refers to an altered activity that is greater than that associated with the native sequence. "Diminished biological activity" is an altered activity that is less than that associated with the native sequence.

35 "Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and

propeptides still present. Pre- and propeptides may be (but are not limited to) intracellular localization signals.

The term "sporophyte" means the diploid phase or cells of a plant.

5 The term "gametophyte" means the haploid phase or cells of a plant. This is the stage in a plant's life cycle between meiosis and fertilization. The male gametophyte includes the haploid phase or cells of the pollen and the female gametophyte includes the haploid phase or cells of the egg cell.

10 The term "plant life cycle" means a complete sequence of developmental events in the life of a plant, such as from fertilization to the next fertilization or from flowering in one generation to the next. The term "generation" means a plant life cycle starting from fertilization to fertilization.

15 "Primary transformant" and "T₀ generation" refer to transgenic plants that are of the same genetic generation as the tissue which was initially transformed (i.e., not having gone through meiosis and fertilization since transformation).

20 "Secondary transformants" and the "T₁, T₂, T₃, etc. generations" refer to transgenic plants derived from primary transformants through one or more meiotic and fertilization cycles. They may be derived by self-fertilization of primary or secondary transformants or by crosses of primary or secondary transformants with other transformed or untransformed plants.

25 "Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. The polynucleotide may be transiently or stably introduced into the host cell and may be maintained in a non-integrated fashion (e.g., as a plasmid) or alternatively, may be integrated into the host genome. The resulting transformed plant cell or plant tissue can then be used to
30 regenerate a transformed plant in a manner known by a skilled person. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

35 "Regeneration" means growing a whole plant from a plant cell, a group of plant cells, a plant part (including seeds), or a plant piece (e.g., from a protoplast, callus, or tissue part).

The terms "plasmid", "vector" and "cassette" refer to an extra chromosomal element often carrying genes which are not part of the central metabolism of the cell, and usually in the form of circular double-

stranded DNA fragments. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell. "Transformation cassette" refers to a specific vector containing a foreign gene and having elements (in addition to the foreign gene) that facilitate transformation of a particular host cell. "Expression cassette" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that allow for enhanced expression of that gene in a foreign host.

"Marker" refers to a gene encoding a trait or a phenotype which permits the selection of, or the screening for, a plant or plant cell containing the marker.

The term "conserved domain" means a set of amino acids conserved at specific positions along an aligned sequence of evolutionarily related proteins. While amino acids at other positions can vary between homologous proteins, amino acids that are highly conserved at specific positions indicate amino acids that are essential in the structure, the stability, or the activity of a protein. Because they are identified by their high degree of conservation in aligned sequences of a family of protein homologues, they can be used as identifiers, or "signatures", to determine if a protein with a newly determined sequence belongs to a previously identified protein family. Conserved domains are specifically described for the family of *cis*-prenyltransferases, according to the work of Apfel, C.M. et al. (*J. Bact.* 181(2): 483-492 (1999)) and Hallahan and Keiper-Hrynko (PCT/US03/36164).

The term "non-conserved domain" means a set of amino acids, present between conserved domains, which whilst the individual amino acids are not conserved at specific positions along an aligned sequence of evolutionarily related proteins, is recognizable by its presence or absence in aligned sequences of evolutionary related proteins. The presence of such a domain, despite positional non-conservation among its constituent amino acids, indicates that the domain plays a role essential in the structure, the stability, or the activity of a protein, e.g., by increasing the distance between other (conserved) domains. Because they are identified

by their presence in aligned sequences of a family of protein homologues, they can be used as identifiers, or "signatures", to determine if a protein with a newly determined sequence belongs to a previously identified protein family or subfamily.

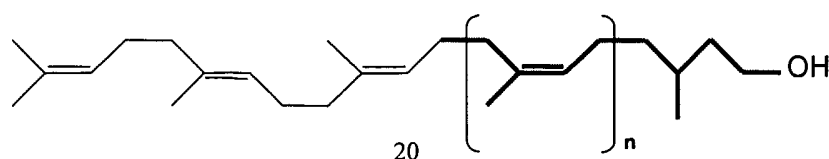
5 The term "sequence analysis software" refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence analysis software will include, but is not limited to: 1.) the GCG suite of
10 programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI); 2.) BLASTP, BLASTN, BLASTX (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990); 3.) Vector NTI (InforMax Inc., North Bethesda, MD); and 4.) DNASTAR (DNASTAR Inc., Madison, WI). Within the context of this application it will be understood that where sequence
15 analysis software is used for analysis, that the results of the analysis will be based on the "default values" of the program referenced, unless otherwise specified. As used herein "default vales" will mean any set of values or parameters which originally load with the software when first initialized.

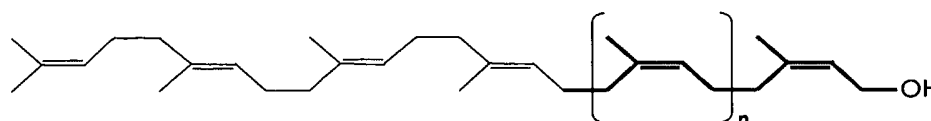
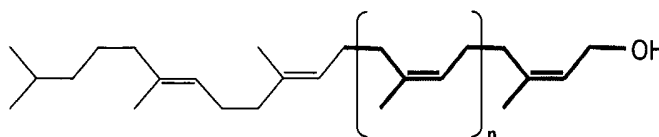
20 Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described by: Sambrook, J., Fritsch, E. F. and Maniatis, T., Molecular Cloning: A Laboratory Manual; 2nd ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989) (hereinafter "Maniatis"); and by Silhavy, T. J., Bennis, M. L. and Enquist,
25 L. W., Experiments with Gene Fusions; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1984); and by Ausubel, F. M. et al., Current Protocols in Molecular Biology; Greene Publishing Assoc. and Wiley-Interscience (1987).

Polyisoprenoids

30 Plants synthesize a variety of hydrocarbons built up of isoprene units (C_5H_8), termed polyisoprenoids (Tanaka, Y. In *Rubber and Related Polyprenols. Methods in Plant Biochemistry*; Dey, P. M. and Harborne, J. B., Eds., Academic: San Diego, CA (1991); Vol. 7, pp 519-536). Representative polyisoprenoid structures are shown below for:

35 1. Dolichols ($n = 12-18$):



2. 3-*trans*, poly-*cis* polyprenols (e.g., ficaprenols, n = 10-12):3. 2-*trans*, poly-*cis* polyprenols (e.g., betulaprenols, n = 6-9)

- 5 The left-handed portion of each of the molecules above are formed from allylic terpenoid diphosphate initiators (e.g., dimethylallyldiphosphate (DMAPP; C₅), geranyl diphosphate (GPP; C₁₀), farnesyl diphosphate (FPP; C₁₅), and geranylgeranyl diphosphate (GGPP; C₂₀)). The remaining portion of the molecules shown with heavy-set lines are formed
- 10 by the activity of *cis*-prenyltransferases catalyzing sequential additions of isopentenyl diphosphate (IPP; C₅)). In general, those polyisoprenoids with 45 to 115 carbon atoms, and varying numbers of *cis*- and *trans*- (Z- and E-) double bonds, are termed polyprenols, while those of longer chain length are termed rubbers (Tanaka, Y. In *Minor Classes of Terpenoids*. Methods in Plant Biochemistry, Dey, P. M. and Harborne, J. B., Eds., Academic: San Diego, CA (1991); Vol. 7, pp 537-542).

There are several suggested functions for plant polyisoprenoids. Terpenoid quinones are most likely involved in photophosphorylation and respiratory chain phosphorylation. Rubbers have been implicated in plant

20 defense against herbivory, possibly serving to repel and entrap insects and seal wounds in a manner analogous to plant resins. The specific roles of the C₄₅-C₁₁₅ polyprenols remain unidentified; although, as with most secondary metabolites, they too are thought most likely to function in plant defense. Short-chain polyprenols may also be involved in protein

25 glycosylation in plants, by analogy with the role of dolichols in animal metabolism. In no case has a role for these secondary metabolites in modulating plant development been proposed.

Cis-Prenyltransferases

30 *Cis*-prenyltransferases are a family of enzymes that are responsible for synthesizing plant polyisoprenoids (specifically polyprenols and natural rubbers), by catalyzing the sequential addition of IPP to an initiator

molecule in head-to-tail condensation reactions. The initiator molecules themselves are derived from isoprene units through the action of distinct prenyltransferases.

Cis-prenyltransferases are ~30 kD proteins. The expression of full-length plant *cis*-prenyltransferase cDNAs yields a mature protein capable of the synthesis of *cis*-polyisoprenoids from IPP as the substrate. *Cis*-prenyltransferases were previously known to play a vital role in cellular activity, the biosynthesis of plant cell walls, and postranslational glycosylation of proteins. In the present invention, the roles of *cis*-prenyltransferases have been expanded to further include their ability to affect plant growth and development.

The identification of genes encoding the bacterial *cis*-prenyltransferase undecaprenyl diphosphate synthase (di-*trans*,poly-*cis*-decaprenylcistransferase, or Upp synthetase; EC 2.5.1.31) (Shimizu et al., *J. Biol. Chem.* 273:19476-19481 (1998); Apfel et al., *J. Bacteriol.* 181:483-492 (1999)) and yeast dehydrodolichyl diphosphate (Dedol-PP) synthase (Sato et al., *Mol. Cell. Biol.* 19:471-483 (1999)) have facilitated the identification of prenyltransferases that condense isoprene units in a *cis*-configuration in other organisms. This was, in part, enabled by the publication of Apfel et al. (*supra*) of an alignment of the deduced amino acid sequence of the *E. coli* Upp synthase gene with a number (28) of other publicly-available sequences from bacteria, yeast (*Saccharomyces cerevisiae*) and one eukaryote (*Caenorhabditis elegans*), which revealed five conserved domains. Four of these domains are included herein as SEQ ID Nos 7-10.

The authors predicted that these conserved domains, as well as a few single conserved amino acids outside of the conserved domains, likely represented the active site of the protein.

Since the work of Apfel et al. (*supra*), US 6,645,747 taught the identification and characterization of *cis*-prenyltransferase proteins from wheat, grape, soybean, rice, African daisy, rubber tree (*Hevea brasiliensis*) and pot marigold (see also GenBank Accession Numbers: AY124934, AY124474, AY124473, AY124472, AY124471, AY124470, AY124469, AY124468, AY124467, AY124466, AY124465, AY124464). Additional *cis*-prenyltransferases have been isolated from *H. brasiliensis* by Asawatreratanakul, K. et al. (AB061236) and Sando, T. et al. (AB074307) and from *Arabidopsis* by Oh, S.K. et al. (AF162441).

Furthermore, available knowledge concerning *cis*-prenyltransferases was further advanced by Hallahan and Keiper-Hrynko (PCT/US03/36164), with: 1.) the isolation of *cis*-prenyltransferase cDNAs from the natural rubber-producing plants russian dandelion (*Taraxacum*
5 *kok-saghyz*) and sunflower (*Helianthus annus*); and 2.) the description of modified sequences of conserved Domains I (SEQ ID NO:11), Domain IV (SEQ ID NO:12), and Domain V (SEQ ID NO:13), with respect to Apfel et al. (*supra*), that are indicative of the subfamily of *cis*-prenyltransferases associated with rubber-producing plants and the presence of a unique
10 non-conserved domain between conserved domain IV and V, that is present in *cis*-prenyltransferases from rubber-producing plants and that is absent in *cis*-prenyltransferases from other plants.

In one embodiment of the invention, most preferred *cis*-prenyltransferase proteins are those from rubber (*Hevea brasiliensis*),
15 rice, and soybean, (SEQ ID NOs:2, 4, and 6 respectively) and a newly identified *cis*-prenyltransferase homolog from *Arabidopsis*, Apt5 (SEQ ID NO:21). However, it will be obvious to one of skill in the art that a variety of *cis*-prenyltransferase genes and their homologs would likely be suitable for the purposes of the invention herein. The source from which these *cis*-
20 prenyltransferase genes are derived (e.g., microbial, plant, animal etc.) is not limiting to the invention herein. Thus, those nucleic acids containing significant homology to Domain I (SEQ ID NO:7), Domain II (SEQ ID NO:8), Domain III (SEQ ID NO:9), and Domain V (SEQ ID NO:10), as described by Apfel *et al.* (*supra*) or the modified domains described by
25 Hallahan and Keiper-Hrynko (PCT/US03/36164) (SEQ ID NOs: 11-13), would be expected to convey a similar phenotype of modified growth to maturity and/or yield and/or architecture for plants transformed with these sequences.

In an alternative embodiment of the invention, suitable nucleic
30 acids useful in the methods described herein encode polypeptides having *cis*-prenyltransferase activity, wherein the polypeptide is capable of catalyzing the sequential addition of IPP units to polyprenols and rubbers in *cis* 1-4 orientation. In a preferred embodiment, suitable nucleic acids useful for the purposes described herein are at least about 70% identical,
35 preferably at least about 80% identical to the Hpt2, Spt1, Rpt1, and/or Apt5 amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid

fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein.

- 5 Suitable nucleic acid fragments not only have the above homologies but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids.

10 Recombinant Expression in Plants

- It was unexpected that introduction of chimeric genes encoding the instant *cis*-prenyltransferase enzymes, under the control of the appropriate promoters, would produce a phenotype of modified growth to maturity. While not intending to suggest a mechanism for the present invention, it may be that, given the involvement of IPP as a precursor of many classes of plant hormones (i.e., gibberellins, brassinosteroids, cytokinins and abscissic acid), perturbations in IPP flux may result in altered hormone biosynthesis in the transgenic plants. And yet, although the phenomenon is not clearly understood, it is contemplated that it will be useful to overexpress *cis*-prenyltransferase genes both in natural host cells as well as heterologous plant hosts.

- In some applications, it might be desirable to express the *cis*-prenyltransferases in specific plant tissues and/or cell types (e.g., to modify fruit or seed production, manipulate the strength/thickness of a stem, etc.), or during developmental stages in which they would normally not be encountered.

- Alternatively, the constitutive over-expression of a *cis*-prenyltransferase in a transformed plant could modify the plant's overall growth rate to maturity. When the result of such over-expression was to produce a phenotype characterized with an accelerated growth rate to maturity, this would be desirable when modifying the plant such that it would be capable of maturing in shorter growing seasons, thus permitting expansion of the geographic range in which these plants grew. Additionally, an overall increase in growth rate to maturity could provide significant economic advantages to the grower (e.g., in silviculture, cell culture, etc.).

Genetically modified plants of the present invention are produced by overexpression of the instant *cis*-prenyltransferases. Generally, this

may be accomplished by first constructing chimeric genes in which the *cis*-prenyltransferase coding region is operably-linked to control sequences capable of directing expression of the gene in the desired tissues at the desired stage of development. These control sequences may comprise a promoter, enhancer, silencer, intron sequences, 3'UTR and/or 5'UTR regions, protein and/or RNA stabilizing elements. For reasons of convenience, the chimeric genes may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals must also be provided. It is preferred that the chimeric gene be introduced via a vector and that the vector harboring the *cis*-prenyltransferase sequence also contain one or more selectable marker genes so that cells transformed with the chimeric gene can be selected from non-transformed cells.

Promoters

The present invention makes use of a variety of plant promoters to drive the expression of the chimeric genetic sequence comprising a *cis*-prenyltransferase gene or functional fragment thereof.

Regulated expression of *cis*-prenyltransferase expression is possible by placing the *cis*-prenyltransferase under the control of promoters that may be conditionally regulated. Any promoter functional in a plant will be suitable, including (but not limited to): constitutive plant promoters, plant tissue-specific promoters, plant development-stage specific promoters, inducible plant promoters, viral promoters, male germline-specific promoters, female germline-specific promoters, flower-specific promoters, and vegetative shoot apical meristem-specific promoters.

Some suitable examples of constitutive promoters include those from nopaline synthase (*nos*), octopine synthase (*ocs*), cauliflower mosaic virus (*CaMV*) (35S [Odell et al., *Nature*, 313: 810-812 (1985)] and 19S [Nilsson et al., *Physiol. Plant.* 100:456-462 (1997)]), actin (McElroy et al., *Plant Cell*, 2:163-171 (1990)), actin 2 (An et al., *Plant J.* 10(1):107-121 (1996)) and ubiquitin (Christensen et al., *Plant Mol. Biol.* 18: 675-689 (1992)) genes.

Several tissue-specific regulated genes and/or promoters have been reported in plants. These include genes encoding: 1.) the seed storage proteins (e.g., napin, cruciferin, α -conglycinin, and phaseolin); zein or oil body proteins (e.g., oleosin); genes involved in fatty acid biosynthesis (e.g., acyl carrier protein, stearyl-ACP desaturase, and fatty

acid desaturases (fad 2-1)); and 4.) other genes expressed during embryo development (e.g., Bce4 [see, for example, EP 255378 and Kridl et al., *Seed Science Research* 1:209-219 (1991)]). Particularly useful for seed-specific expression is the pea vicilin promoter (Czako et al., *Mol. Gen.*

5 *Genet.* 235(1): 33-40 (1992)). Other useful promoters for expression in mature leaves are those that are switched on at the onset of senescence, such as the SAG promoter from *Arabidopsis* (Gan et al., *Science* (Washington, D.C.) 270(5244):1986-8 (1995)).

A class of fruit-specific promoters expressed at or during anthesis
10 through fruit development, at least until the beginning of ripening, is discussed in U.S. 4,943,674, the disclosure of which is hereby incorporated by reference. cDNA clones that are preferentially expressed in cotton fiber have been isolated (John et al., *Proc. Natl. Acad. Sci. U.S.A.* 89(13): 5769-73 (1992)). cDNA clones from tomato displaying
15 differential expression during fruit development have been isolated and characterized (Mansson et al., *Mol. Gen. Genet.* 200:356-361 (1985); Slater et al., *Plant Mol. Biol.* 5:137-147 (1985)). The promoter for polygalacturonase gene is active in fruit ripening. The polygalacturonase gene is described in the following U.S. Patents, which disclosures are
20 incorporated herein by reference: U.S. 4,535,060, U.S. 4,769,061, U.S. 4,801,590, and U.S. 5,107,065.

Mature plastid mRNA for psbA (one of the components of photosystem II) reaches its highest level late in fruit development, in contrast to plastid mRNAs for other components of photosystem I and II
25 which decline to nondetectable levels in chromoplasts after the onset of ripening (Piechulla et al., *Plant Mol. Biol.* 7:367-376 (1986)). Recently, cDNA clones representing genes apparently involved in tomato pollen (McCormick et al., *Tomato Biotechnology*, Alan R. Liss: New York (1987)) and pistil (Gasser et al., *Plant Cell* 1:15-24 (1989)) interactions have also
30 been isolated and characterized.

Other examples of tissue-specific promoters include those that direct expression in leaf cells following damage to the leaf (e.g., from chewing insects), in tubers (e.g., patatin gene promoter), and in fiber cells. One example of a developmentally-regulated fiber cell protein is E6 (John
35 et al., *Proc. Natl. Acad. Sci. U.S.A.* (89(13): 5769-73 1992)); the E6 gene is most active in fiber, although low levels of transcripts are found in leaf, ovule and flower.

Although the promoters described above are provided for the purposes of exemplification only, the present invention is not to be limited by those provided therein. Those skilled in the art will readily be in a position to provide additional tissue-specific promoters that are useful in performing the present invention (see, for example U.S. 5,589,379) which are:

1. stem-specific (e.g., to modify strength and thickness of a plant stem [wherein increased strength and thickness can confer improved stability and wind-resistance]);
2. meristem-specific (e.g., to modify apical dominance or the "bushiness" of a plant);
3. tuber-specific (e.g., to modify tuber production);
4. seed-specific (e.g., to modify seed production in plants [wherein increased seed production can be quantitated as increased seed set and/or seed production and/or seed yield]);
5. endosperm-specific (e.g., to modify grain yield, since grain yield in crop plants is largely a function of the amount of starch produced in the endosperm of the seed);
6. root-specific (e.g., to modify the production of roots or storage organs derived from roots);
7. nodule-specific (e.g., to modify the nitrogen-fixing capability of a plant);
8. embryo-specific (e.g., to modify embryo size, which is important for growth after germination); and
9. leaf-specific, flower-specific, or fruit-specific.

The tissue-specificity of some "tissue-specific" promoters may not be absolute and may be tested by one skilled in the art using the *diphtheria* toxin sequence. One can also achieve tissue-specific expression with "leaky" expression by a combination of different tissue-specific promoters (Beals et al., *Plant Cell*, 9:1527-1545 (1997)).

Germline specific promoters, responsive to male, female, or both male-female specific cell lineages are also useful in the present invention. For instance, transgenes can be expressed or removed from pollen by site-specific recombinase expression under the control of male germline-specific genes in anther primordia genes (e.g., *Arabidopsis* *Apetalla* 3 and *Pistilata* (PI) or their orthologs from other plant species), in sporophytic anther tissue (e.g., Bcp I and TA29 promoters) or gametophytic pollen. Similarly, transgenes can be expressed or removed from ovules by site-

specific recombinase expression under the control of female germline-specific genes in ovule primordia. Transgenes can be expressed or removed from both male- and female-specific germ lines by expression of an active site-specific recombinase gene under the control of a promoter
5 for genes common to both male and female lineages in flower (e.g., *Arabidopsis agamous* gene or its orthologs in other species), in floral meristem (e.g., *Arabidopsis Apetala 1*, *Leafy*, and *Erecta* or their orthologs from other species), and in vegetative shoot apical meristem (such as *Arabidopsis WUSCHEL* (WUS) and *SHOOT MERISTEMLESS* (STM) or
10 their orthologs from other species). Promoters of shoot apical meristem are especially useful for removing or expressing transformation marker genes early in tissue-culture following selection or *in planta* following a transformation phenotype.

Similarly, several inducible promoters ("gene switches") have been
15 reported. Many are described in the reviews by Gatz (*Current Opinion in Biotechnology*, 7:168-172 (1996); *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 89-108 (1997)). These include tetracycline repressor systems, *Lac* repressor systems, copper-inducible systems, salicylate-inducible systems (e.g., the PR1a system), and glucocorticoid- (Aoyama T. et al.,
20 *N-H Plant Journal* 11:605-612 (1997)) and ecdysone-inducible systems. Also included are the benzene sulphonamide- (U.S. 5,364,780) and alcohol- (WO 97/06269 and WO 97/06268)-inducible systems and glutathione S-transferase promoters. Other studies have focused on
25 genes inducibly regulated in response to environmental stress or stimuli such as increased salinity, drought, pathogen, and wounding (Graham et al., *J. Biol. Chem.* 260:6555-6560 (1985); Graham et al., *J. Biol. Chem.* 260:6561-6554 (1985); Smith et al., *Planta* 168:94-100 (1986)). Accumulation of a metallocarboxypeptidase-inhibitor protein has been reported in leaves of wounded potato plants (Graham et al., *Biochem*
30 *Biophys Res Comm* 101:1164-1170 (1981)). Other plant genes that have been reported to be induced include: methyl jasmonate, elicitors, heat-shock, anerobic stress, or herbicide safeners

Expression Vectors
Plasmid vectors comprising the chimeric *cis*-prenyltransferase genes can then be constructed. The choice of a plasmid vector depends
35 upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene.

Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1989 (hereinafter "Maniatus"); and by Ausubel et al., Current Protocols in Molecular Biology, published by Greene Publishing Assoc. and Wiley-Interscience, 1987.

A preferred vector of the invention is an expression vector that provides for expression of a *cis*-prenyltransferase coding sequence in the selected host. Expression vectors can for instance be cloning vectors, binary vectors or integrating vectors. Expression comprises transcription of the nucleic acid molecule preferably into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells are well known to those skilled in the art. In the case of eukaryotic cells, they normally comprise promoters ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript (for example, those of the 35S RNA from Cauliflower Mosaic Virus (CaMV)). The termination signals usually employed are from the Nopaline Synthase promoter or from the CAMV 35S promoter. A plant translational enhancer often used is the tobacco mosaic virus (TMV) omega sequences; additionally, the inclusion of an intron (e.g., Intron-1 from the Shrunken gene of maize) has been shown to increase expression levels by up to 100-fold (Mait, *Transgenic Res.* 6:143-156 (1997); Ni, *Plant Journal* 7:661-676 (1995)). Additional regulatory elements may include transcriptional as well as translational enhancers.

In addition to the elements described above for a preferred expression vector, it is also useful for the vector to comprise a selectable and/or scorable marker. Preferably, the marker gene is an antibiotic resistance gene whereby the appropriate antibiotic can be used to select for transformed cells from among cells that are not transformed. Selectable marker genes useful for the selection of transformed plant cells, callus, plant tissue and plants are well known to those skilled in the art. Examples include, but are not limited to: *npt*, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin; *hygro*, which confers resistance to hygromycin; *trpB*, which allows cells to utilize indole in place of tryptophan; *hisD*, which allows cells to utilize histinol in place of histidine (Hartman, *Proc. Natl. Acad. Sci. USA* 85:8047 (1988)); mannose-6- phosphate isomerase, which allows cells to utilize mannose

- (WO 94/20627); ODC (ornithine decarboxylase), which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, In: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1987)); and
- 5 deaminase from *Aspergillus terreus*, which confers resistance to Blasticidin S (Tamura, *Biosci. Biotechnol. Biochem.* 59 2336-2338 (1995)).

Useful scorable markers are also known to those skilled in the art and are commercially available, such as the genes encoding luciferase (Giacomin, *Pl. Sci.* 116:59-72 (1996); Scikantha, *J. Bact.* 178:121 (1996)),

10 green fluorescent protein (Gerdes, *FEBS Lett.* 389:44- 47 (1996)) or R-glucuronidase (Jefferson, *EMBO J.* 6:3901-3907 (1987)). This embodiment is particularly useful for simple and rapid screening of cells, tissues and organisms containing a vector comprising a *cis*-prenyltransferase.

- 15 For some applications it may be useful to direct the *cis*-prenyltransferase proteins to different cellular compartments. It is thus envisioned that the chimeric genes described above may be further modified by the addition of appropriate intracellular targeting sequences to their coding regions (and/or with targeting sequences that are already
- 20 present removed). These additional targeting sequences include chloroplast transit peptides (Keegstra et al., *Cell* 56:247-253 (1989)), signal sequences that direct proteins to the endoplasmic reticulum (Chrispeels et al., *Ann. Rev. Plant Phys. Plant Mol.* 42:21-53 (1991)), and nuclear localization signals (Raikhel et al., *Plant Phys.* 100:1627-1632
- 25 (1992)). While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future which are useful in the invention.

Transformation Methods

- A variety of techniques are available and known to those skilled in
- 30 the art for introduction of constructs into a plant cell host. These techniques include transformation with DNA employing *Agrobacterium tumefaciens* or *A. rhizogenes* as the transforming agent. It is particularly preferred to use the binary type vectors of Ti and Ri plasmids of *Agrobacterium spp.* Ti-derived vectors transform a wide
- 35 variety of higher plants, including monocotyledonous and dicotyledonous plants such as soybean, cotton, rape, tobacco, and rice (Pacciotti et al., *Bio/Technology* 3:241 (1985); Byrne et al., *Plant Cell, Tissue and Organ Culture* 8:3 (1987); Sukhapinda et al., *Plant Mol. Biol.* 8:209-216 (1987);

Lorz et al., *Mol. Gen. Genet.* 199:178 (1985); Potrykus, *Mol. Gen. Genet.* 199:183 (1985); Park et al., *J. Plant Biol.* 38(4):365-71 (1995); Hiei et al., *Plant J.* 6:271-282 (1994)). The use of T-DNA to transform plant cells has received extensive study and is amply described (EP 120516; Hoekema, In: The Binary Plant Vector System, Offset-drukkerij Kanters B.V.; Alblasterdam (1985), Chapter V; Knauf et al., *Genetic Analysis of Host Range Expression by Agrobacterium*, In: Molecular Genetics of the Bacteria-Plant Interaction, Puhler, A. Ed.; Springer-Verlag: New York, 1983, p 245; and An et al., *EMBO J.* 4:277-284 (1985)). For introduction into plants, the chimeric genes of the invention can be inserted into binary vectors as described in the examples.

Other transformation methods are available to those skilled in the art, such as: 1.) direct uptake of foreign DNA constructs (see EP 295959); 2.) techniques of electroporation (see Fromm et al., *Nature* (London) 319:791 (1986)); 3.) high-velocity ballistic bombardment with metal particles coated with the nucleic acid constructs (see Kline et al., *Nature* (London) 327:70 (1987), and see U.S. Patent No. 4,945,050); or 4.) microinjection (see Gene Transfer To Plants, Potrykus and Spangenberg, Eds., Springer Verlag: Berlin, NY (1995)). The transformation of most dicotyledonous plants is possible with the methods described above; however, additional transformation techniques have been developed for the successful transformation of monocotyledonous plants. These include protoplast transformation and transformation by an *in planta* method using *Agrobacterium tumefaciens*. This *in planta* method (Bechtold and Pelletier, *C. R. Acad. Sci. Paris*, 316:1194 (1993); or Clough S. J., Bent A. F.; *Plant Journal* 16(6): 735-43 (1998)) involves the application of *A. tumefaciens* to the outside of the developing flower bud and then introduction of the binary vector DNA to the developing microspore and/or macrospore and/or developing seed, so as to produce a transformed seed without the exogenous application of cytokinin and/or gibberellin. Those skilled in the art will be aware that the selection of tissue for use in such a procedure may vary; however, it is preferable generally to use plant material at the zygote formation stage for *in planta* transformation procedures.

Once transformed, the plant cells can be regenerated by those skilled in the art. Of particular relevance are the recently described methods to transform foreign genes into commercially important crops, such as rapeseed (see De Block et al., *Plant Physiol.* 91:694-701 (1989)),

sunflower (Everett et al., *Bio/Technology* 5:1201 (1987)), soybean (McCabe et al., *Bio/Technology* 6:923 (1988); Hinchee et al., *Bio/Technology* 6:915 (1988); Chee et al., *Plant Physiol.* 91:1212-1218 (1989); Christou et al., *Proc. Natl. Acad. Sci USA* 86:7500-7504 (1989); EP 301749), rice (Hiei et al., *supra*), corn (Gordon-Kamm et al., *Plant Cell* 2:603-618 (1990); Fromm et al., *Biotechnology* 8:833-839 (1990)), and *Hevea* (Yeang, H.Y., et al., Rubber Latex as an Expression System for High-value Proteins. In, *Engineering Crop Plants for Industrial End Uses*. Shewry, P.R., Napier, J.A., David, P.J., Eds.; Portland: London, 1998; pp 55-64).

Transgenic plant cells are then placed in an appropriate selective medium for selection of transgenic cells that are then grown to callus. Shoots are grown from callus and plantlets generated from the shoot by growing in rooting medium. The various constructs normally will be joined to a marker for selection in plant cells. Conveniently, the marker may be resistance to a biocide (particularly an antibiotic such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, herbicide, or the like). The particular marker used will allow for selection of transformed cells as compared to cells lacking the DNA that has been introduced. Components of DNA constructs including transcription cassettes of this invention may be prepared from sequences which are native (endogenous) or foreign (exogenous) to the host. By "foreign" it is meant that the sequence is not found in the wild-type host into which the construct is introduced. Heterologous constructs will contain at least one region that is not native to the gene from which the transcription-initiation-region is derived.

One skilled in the art recognizes that the expression level and regulation of a transgene in a plant can vary significantly from line to line. Thus, one has to test several lines to find one with the desired expression level and regulation. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., *EMBO J.* 4:2411-2418 (1985); De Almeida et al., *Mol. Gen. Genetics* 218:78-86 (1989)), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA blots (Southern, *J. Mol. Biol.* 98: 503 (1975)), Northern analysis of mRNA expression (Kroczeck, *J. Chromatogr. Biomed. Appl.*, 618(1-2): 133-145 (1993)), Western analysis

of protein expression, or phenotypic analysis. One particularly useful way to quantitate protein expression and to detect replication in different plant tissues is to use a reporter gene, such as GUS. Once transgenic plants have been obtained, they may be grown to produce plant tissues or parts having the desired phenotype. The plant tissue or plant parts may be harvested, and/or the seed collected. The seed may serve as a source for growing additional plants with tissues or parts having the desired characteristics.

Preferred Plant Hosts

In general, plants that can be manipulated according to the invention to display a modified growth phenotype (in terms of rate of growth to maturity, and/or yield and/or architecture) can be derived from any desired plant species that will support expression of a *cis*-prenyltransferase. Plants so transformed can be monocotyledonous plants or dicotyledonous plants, and preferably they belong to plant species of interest in agriculture, silviculture or horticulture (e.g., a crop plant, root plant, oil-producing plant, wood producing plant, agricultural plant, fodder or forage legume, companion plant, or horticultural plant).

Suitable plant species include, but are not limited to: those plant species which produce natural rubber (e.g., *Hevea brasiliensis*, *Taraxacum* spp., *Parthenium argentatum*), tobacco (*Nicotiana* spp.), tomato (*Lycopersicon* spp.), potato (*Solanum* spp.), hemp (*Cannabis* spp.), sunflower (*Helianthus* spp.), sorghum (*Sorghum vulgare*), wheat (*Triticum* spp.), maize (*Zea mays*), rice (*Oryza sativa*), rye (*Secale cereale*), oats (*Avena* spp.), barley (*Hordeum vulgare*), rapeseed (*Brassica* spp.), broad bean (*Vicia faba*), french bean (*Phaseolus vulgaris*), other bean species (*Vigna* spp.), lentil (*Lens culinaris*), soybean (*Glycine max*), arabidopsis (*Arabidopsis thaliana*), cotton (*Gossypium hirsutum*), petunia (*Petunia hybrida*), flax (*Linum usitatissimum*), carrot (*Daucus carota sativa*), tea, celery, brussel sprout, artichoke, okra, squash, kale, asparagus (*Asparagus*), banana (*Musa*), blueberry (*Vaccinium*), cacao (*Theobroma*), capsicum pepper (*Capsicum*), cassava (*Manihot*), cucumber (*Cucumis*), eggplant (*Solanum*), lettuce (*Lactuca*), mango (*Mangifera*), oilseed rape, canola, cabbage, broccoli, cauliflower (*Brassica*), onions (*Allium*), papaya (*Carica*), peas (*Pisum*), peanut (*Arachis*), pineapple (*Ananas*), pinto bean, mung bean, pumpkin, zucchini (*Cucurbita*), radish (*Raphanus*), sesame (*Sesame*), spinach (*Spinaceae*), sorghum (*Sorghum*), strawberry (*Fragaria*), sugarcane (*Saccharum*),

sugar beet (*Beta*), sweet potato (*Ipomoea*), watermelon (*Citrullus*), yam (*Dioscorea*), alfalfa (*Medicago*), amaranth (*Amaranthus*), angelica (*Agelica*), castorbean (*Ricinus*), colewort (*Crambe*), jojoba (*Simmondsia*), jute (*Corchorus*), kenaf (*Hibiscus*), lupine (*Lupinus*), plantain (*Plantago*),
 5 sisal (*Agave*), snapdragon (*Antirrhinum*), switch grass (*Panicum*), apple (*Malus*), acacia (*Acacia*), chestnut (*Castanea*), citrus (*Citrus*), coconut (*Cocos*), coffee (*Coffea*), cypress (*Cupressus*), eucalypti (*Eucalyptus*), grape (*Vitis*), hemlock (*Tsuga*), hickory (*Carya*), maple (*Acer*), oak (*Quercus*), pear (*Pyrus*), peach, plum, cherry (*Prunus*), pine (*Pinus*),
 10 poplar (*Populus*), rose (*Rosa*), spruce (*Picea*), and walnut (*Juglans*).

Phenotype of Plants Expressing *cis*-Prenyltransferases

The present invention provides a method for manipulating the rate of growth to maturity and/or yield and/or architecture of a genetically modified plant, as compared to a plant of the same species not genetically
 15 modified (i.e., a 'wild-type' plant). This method relies on elevating the expression of *cis*-prenyltransferase gene(s) in the plant. Thus, the method comprises:

- a) transforming a plant cell with a an isolated nucleic acid molecule encoding a *cis*-prenyltransferase under the control of suitable regulatory sequences;
 20
- b) recovering a transformed plant cell produced in step (a);
- c) regenerating a plant from the transformed plant cell of step (b); and
- d) growing the transformed plant produced in step (c) under
 25 conditions wherein the isolated nucleic acid molecule encoding a *cis*-prenyltransferase is expressed and the growth phenotype of the transformed plant is altered.

"Plant architectural trait" refers to the general morphology or trait of a plant including (but not limited) to any one of the structural features
 30 provided as examples below: shape, size, number, colour, texture, arrangement and patternation of any cell, tissue or organ or groups of cells, tissues, or organs of plants including the root, leaf, shoot, fruit, petiole, trichome, flower, sepals, petals, hypocotyl, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre
 35 nodule, cambium, wood, heartwood, parenchyma, sclerenchyma, seive element, phloem, or vascular tissue, amongst others.

In a preferred embodiment, over-expression of a *cis*-prenyltransferase protein or functional fragment thereof, operably

associated with a DNA sequence regulating its expression, will result in genetically transformed plant cells having an altered growth phenotype wherein the growth rate to maturity is accelerated and/or yield is increased. An "increased" or "accelerated growth rate" will refer to either
5 the total plant or the growth rate of specific tissues/organs of the plant (e.g., the rate of root or shoot growth, or the timing associated with commencement of flowering, seed set, or ripening of fruits). "Increased yield" refers to an increased or enhanced biomass of any harvestable material of the transgenic plant, (either the total plant or specific
10 tissues/organs of the plant [e.g., root, leaf, shoot, fruit, petiole, trichome, flower, sepals, petal, hypocotyl, stigma, style, stamen, pollen, ovule, seed, bulb, embryo, endosperm, seed coat, aleurone, fibre, nodule, cambium, wood, heartwood, parenchyma, sclerenchyma, sieve element, phloem, or vascular tissue]). Thus, increased yield includes, but is not limited to:
15 increased or enhanced biomass of the root or shoot, seed production, grain yield, fruit size, nitrogen fixing capacity, nodule size, tuber formation, stem thickness, endosperm size, and number of fruit per plant, etc. Increased yield may also refer to accumulation of metabolites and/or the sink/source relationships in the total plant or specific portions of the plant.
20 Increased growth rate is another measure of the effect of the expression of the present *cis*-prenyltransferase in plants. As used herein "increased growth rate" will include but not be limited to characteristics selected from group consisting of: decreased time to germination, increased root growth rate, increased shoot growth rate, decreased time
25 to flowering, decreased time for fruit maturation, and decreased time of seed setting.

As an example of the accelerated or increased growth rate to maturity and/or increased yield that can be observed by following the methodology of the present invention, in a preferred embodiment, the
30 model organism *Arabidopsis* was transformed with a *cis*-prenyltransferase gene. The phenotype of the transformant plants included the following: decreased time to bolting and flowering, and increased seed yield at maturity. Additionally, significant modifications in plant architecture were observed, based on an increased leaf size and total leaf
35 area, and increased plant height prior to maturity.

In view of the teachings herein, it will be appreciated by one of skill in the art that any desired plant species that supports production of a *cis*-prenyltransferase could be modified to exhibit a modified growth rate

to maturity and/or yield and/or modifications in plant architecture. These broad modifications to overall plant growth phenotypes include--but are not limited to-- the initiation, promotion, stimulation or enhancement of, or inhibition or diminishment of: cell division, seed development, tuber
5 formation, shoot initiation, leaf initiation, root growth, properties of apical dominance, etc. Any transformed plant obtained according to the invention can be used in a conventional breeding scheme or in *in vitro* plant propagation to produce more transformed plants with the same characteristics and/or can be used to introduce the same characteristic in
10 other varieties of the same or related species. The plants of this invention are fertile (i.e., capable of self- or cross-pollination with other plants of the same species to produce seed) and such plants are included as a part of the invention. Seeds obtained from the transformed plants genetically also contain the same characteristics and are capable of germination and
15 growth. These seeds are also part of the invention herein.

In yet another aspect, the invention also relates to harvestable parts and to propagation material of the transgenic plants according to the invention containing transgenic plant cells over-expressing a *cis*-prenyltransferase. Harvestable parts can be in principle any useful parts of
20 a plant (e.g., flowers, pollen, seedlings, tubers, leaves, stems, fruit, seeds, roots, etc.). Propagation material includes, but is not limited to: seeds, fruits, cuttings, seedlings, tubers, and rootstocks, etc.

Most advantageously to the purposes of the present invention, overexpression of *cis*-prenyltransferases for the purposes of generating
25 transformant plants (exhibiting modified characteristics of growth rate and/or yield and/or modifications in plant architecture) does not affect other plant properties in ways deleterious to agriculture, silviculture, horticulture, floriculture or cell culture.

Pathway Engineering

30 As one skilled in the art will appreciate, it may be useful to manipulate the polyisoprenoid biosynthetic pathway of a plant as a mechanism for modifying the level of *cis*-prenyltransferase expression. Methods of manipulating genetic pathways are common and well known in the art. Selected genes in a particularly pathway may be up-regulated or
35 down-regulated by variety of methods. Additionally, competing pathways in an organism may be eliminated or sublimated by gene disruption and similar techniques.

Once a key genetic pathway has been identified and sequenced, specific genes may be up-regulated to increase the output of the pathway. For example, additional copies of the targeted genes may be introduced into the host cell on multicopy plasmids such as pBR322. Alternatively the
5 target genes may be modified so as to be under the control of non-native promoters. Where it is desired that a pathway operate at a particular point in a cell cycle, regulated or inducible promoters may be used to replace the native promoter of the target gene. Similarly, in some cases the native or endogenous promoter may be modified to increase gene expression. For
10 example, endogenous promoters can be altered *in vivo* by mutation, deletion, and/or substitution (see, Kmiec, U.S. Patent 5,565,350; Zarling *et al.*, PCT/US93/03868).

Alternatively, it may be necessary to reduce or eliminate the expression of certain genes in the target pathway or in competing
15 pathways that may serve as competing sinks for energy or carbon. Methods of down-regulating genes for this purpose have been explored.

For example, where sequence of the gene to be disrupted is known, one of the most effective methods for gene down-regulation is targeted gene disruption where foreign DNA is inserted into a structural
20 gene so as to disrupt transcription. This can be effected by the creation of genetic cassettes comprising the DNA to be inserted (often a genetic marker) flanked by sequences having a high degree of homology to a portion of the gene to be disrupted. Introduction of the cassette into the host cell results in insertion of the foreign DNA into the structural gene via
25 the native DNA replication mechanisms of the cell (see for example Hamilton *et al.* *J. Bacteriol.* 171:4617-4622 (1989); Balbas *et al.* *Gene* 136:211-213 (1993); Gueldener *et al.* *Nucleic Acids Res.* 24:2519-2524 (1996); and Smith *et al.* *Methods Mol. Cell. Biol.* 5:270-277(1996)).

Alternative methods are available to reduce or eliminate expression
30 of a specific gene of interest encoding a polypeptide, if desirable in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Antisense technology requires that a nucleic acid
35 segment from the desired gene is cloned and operably linked to a promoter such that the anti-sense strand of RNA will be transcribed. This construct is then introduced into the host cell and the antisense strand of RNA is produced. Antisense RNA inhibits gene expression by preventing

the accumulation of mRNA which encodes the protein of interest. The person skilled in the art will know that special considerations are associated with the use of antisense technologies in order to reduce expression of particular genes. For example, the proper level of
5 expression of antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Nonetheless, either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes is reduced or eliminated.

10 Finally, one recent variation upon "classical" antisense and cosuppression methodologies is embodied in WO 02/00904, published on January 3, 2002. Specifically, it was found that suitable nucleic acid sequences and their reverse complement can be used to alter the expression of any mRNA encoding a protein of interest which is in
15 proximity to the suitable nucleic acid sequence and its reverse complement. Surprisingly, the suitable nucleic acid sequence and its reverse complement can be either unrelated to any endogenous RNA in the host or can be encoded by any nucleic acid sequence in the genome of the host provided that the nucleic acid sequence does not encode any
20 target mRNA or any sequence that is substantially similar to the target mRNA. A preferred artificial and non-naturally occurring, sequence is that encoded by the peptide "ELVISLIVES" (SEQ ID NO:3433). This approach permits a very efficient and robust approach to achieving single, or multiple, gene co-suppression using single plasmid transformation.

25 Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression or similar methodologies thereto (U.S.
30 5,190,931; U.S. 5,107,065; U.S. 5,283,323; WO 02/00904). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity, these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be
35 advantageous from a breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to the reproductive tissues of the plant by the use of tissue-specific promoters may confer agronomic

advantages relative to conventional mutations that may have an effect in all tissues in which a mutant gene is ordinarily expressed.

5 A person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual
10 transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression
15 by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one that allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired
20 phenotype.

Although targeted gene disruption and antisense technology offer effective means of down-regulating genes where the sequence is known, other less specific methodologies have been developed that are not sequence based. For example, cells may be exposed to UV radiation and
25 then screened for the desired phenotype. Mutagenesis with chemical agents is also effective for generating mutants and commonly used substances include chemicals that affect nonreplicating DNA such as HNO₂ and NH₂OH, as well as agents that affect replicating DNA such as acridine dyes, notable for causing frameshift mutations. Specific methods
30 for creating mutants using radiation or chemical agents are well documented in the art. See, for example: Thomas D. Brock in *Biotechnology: A Textbook of Industrial Microbiology*, 2nd ed., Brock, T. D., Ed.; Sinauer Associates: Sunderland, MA, 1989; or Deshpande, Mukund V., *Appl. Biochem. Biotechnol.*, 36: 227 (1992).

35 Another non-specific method of gene disruption is the use of transposable elements or transposons. Transposons are genetic elements that insert randomly in DNA but can be later retrieved on the basis of sequence to determine where the insertion has occurred. Both *in vivo* and

15 EXAMPLES

GENERAL METHODS

Nucleotide and amino acid percent identity and similarity comparisons were made using the BLAST (Basic Local Alignment Search Tool; Altschul et al., *J. Mol. Biol.* 215:403-410 (1993); see also www.ncbi.nlm.nih.gov/BLAST/) algorithms and also the Vector NTI suite of programs, applying default parameters unless indicated otherwise.

The meaning of abbreviations is as follows: "sec" means second(s), "min" means minute(s), "hr" means hour(s), "d" means day(s), "μL" means microliter(s), "mL" means milliliter(s), "L" means liter(s), "μM" means micromolar, "mM" means millimolar, "M" means molar, "mmol" means millimole(s), "μmole" mean micromole, "g" means gram(s), "μg" means microgram(s), "ng" means nanogram(s), "U" means unit(s), "bp" means base pair(s), and "kB" means kilobase(s).

Example 1

Bioinformatic Analysis of Publically Available *Cis*-Prenyltransferase

10

Homologs

Arabidopsis is well-known as a "model organism" in plant science for investigations in a wide range of processes involved in controlling growth and development of flowering plants. This is largely based on: 1.) its small genome (five chromosomes and approximately 25,000 genes); 2.) rapid life cycle (about 6 weeks from seed to seed); 3.) prolific seed production; 4.) small size, thereby allowing a large number of plants to be grown in limited space; and 5.) ability to extrapolate results obtained in *Arabidopsis* to other agriculturally important crops. For these reasons, *Arabidopsis* was selected as a model organism in the present study, in which it would be desirable to express various *cis*-prenyltransferase genes.

Example 1 describes the selection of three exogenous *cis*-prenyltransferase homologs from rubber tree, soybean, and rice for expression in *Arabidopsis*. Additionally, an endogenous gene (Apt5) of *Arabidopsis* having significant homology to other known *cis*-prenyltransferase genes was identified and selected for over-expression.

Identification of Exogenous *Cis*-Prenyltransferase Homologs

Previous work (US 6,645,747) has described the preparation of cDNA libraries from *Hevea brasiliensis*, *Oryza sativa*, and *Glycine max* and the identification of *cis*-prenyltransferase homologs from these libraries. Three of these homologs are summarized below in Table 2, according to their original clone name and designated gene name.

35

TABLE 2
cDNAs Identified as *cis*-Prenyltransferase Homologs

Clone	Source	SEQ ID NO	Gene name
ehb2c.pk001.d17	<i>Hevea brasiliensis</i>	1	Hpt2
rr1.pk0050.h8	<i>Oryza sativa</i>	3	Rpt1
sl1.pk0128.h7	<i>Glycine max</i>	5	Spt1

- 5 Comparison of the nucleotide sequences of the rubber, soybean, and rice ESTs (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5) with those of representative bacterial, yeast, and *Arabidopsis cis*-prenyltransferases confirmed that each of these homologs exhibited significant homology with known examples of the *cis*-prenyltransferase gene family.
- 10 Specifically, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 were examined against *Micrococcus luteus* UPPS (Shimizu, N., et al., *J. Biol. Chem.* 273:19476-19481 (1998); GenBank Accession No. AB004319), yeast *rer2* (Sato, M., et al., *Mol. Cell. Biol.* 19, 471-483 (1999); AB013497), yeast *srt1* (AB013498), and *Arabidopsis* Apt1 (Oh, S.K., et al., *J. Biol. Chem.* 275:18482-18488 (2000); Cunillera, N., et al., *FEBS Letts.* 477:170-174 (2000); AF162441).

Identification of Endogenous *Cis*-Prenyltransferase Homologs

- In addition to the *cis*-prenyltransferase genes identified above, several *Arabidopsis thaliana* genomic DNA fragments containing putative
- 20 *cis*-prenyl transferase gene sequences were identified in public databases. Specifically, publically available sequences of bacterial and yeast *cis*-prenyl transferases were used to conduct BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for
- 25 similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL and DDBJ databases) using the BLASTN algorithm provided by the National
- 30 Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the

BLASTX algorithm (Gish, W. and States, D. J. *Nature Genetics* 3:266-272 (1993)) provided by the NCBI.

Putative *cis*-prenyl transferase gene sequences from *Arabidopsis thaliana* were additionally identified by a number of methods, including the following: 1) keyword searches (e.g., "undecaprenyl"), 2) searches of the database using the TBLASTN algorithm provided by the National Center for Biotechnology Information (NCBI) and short fragments of conserved sequence present in known *cis*-prenyltransferases (conserved domains I-V, as described by Apfel et al., *J. Bacteriol.* 81:483-492 (1999)). These sections of conserved sequence were expected to be diagnostic for the *cis*-prenyltransferase family of enzymes.

One gene, designated Apt5, from *Arabidopsis thaliana* chromosome 5 genomic DNA (GenBank Accession Number AB011483), contains an 813 bp open reading frame (SEQ ID NO:20) with no intron sequences, and encodes a protein with 271 amino acids (SEQ ID NO:21). This protein has extensive homology to known microbial and plant *cis*-prenyltransferase sequences. It was decided to include this gene in the present *Arabidopsis* transformation experiments to determine the effect of overexpression of an endogenous gene.

20 Comparison of Cis-Prenyltransferase Homologs

A more informative comparison of deduced amino acid sequences of the ORFs encoded by these cDNAs was carried out using the Vector NTI AlignX program, which uses the ClustalW algorithm to align and compare sequences for similarity and identity. A comparison of the Hpt2, Rpt1, Spt1, and Apt5 deduced amino acid sequences with that of *Arabidopsis* Apt1 (SEQ ID NO:32; GenBank Accession Number AF162441) is presented in Table 3.

TABLE 3

Identity Comparison Using the ClustalW Program of the Deduced Amino Acid Sequences from Plant *cis*-Prenyltransferases with the *Arabidopsis*

Apt1 *cis*-Prenyltransferase

Gene	SEQ ID NO	% similarity with <i>Arabidopsis</i> homolog Apt1	% identity with <i>Arabidopsis</i> homolog Apt1
Hpt2	2	36.9	22.9
Rpt1	4	36.8	23.4
Spt1	6	54.8	40.9

It is clear from this analysis that these sequences (exogenous to *Arabidopsis*) encode polypeptides with significant similarity to a known *cis*-prenyltransferase.

Furthermore, alignment of the deduced amino acid sequence of these cDNAs encoding Hpt2, Rpt1, and Spt1 (exogenous to *Arabidopsis*) and Apt5 (endogenous to *Arabidopsis*) with a known plant *cis*-prenyltransferase Apt1 (Figure 1) using the CLUSTALW program within the VECTOR NTI suite of programs reveals the presence of the conserved domains characteristic of this gene family (see, Apfel et al., *J. Bacteriol.* 81:483-492 (1999)).

EXAMPLE 2

Construction of *cis*-prenyltransferases Expression Vectors

The present Example describes construction of a binary vector for expression of the *cis*-prenyltransferase genes identified in Example 1. Each *cis*-prenyltransferase was amplified by PCR and cloned into an appropriate vector for subsequent expression in *Arabidopsis*.

Construction of pBI-35S

A binary vector, pBI-35S, was constructed for expression of several *cis*-prenyltransferase genes by ligating an 800 bp *Hind III*-*Xba I* CaMV35 promoter DNA fragment (Guilley H, et al., *Cell* 30(3):763-73 (1982)) into the corresponding sites of the vector pBIB/NPT (Detlef Becker, *Nucleic Acids Research* 18(1):203 (1990)) to yield the binary vector pBI-35S.

Construction of pGV827

Plasmid pGV827 contains the GFP gene under the control of the 35S cauliflower mosaic virus promoter and the nopaline synthase 3' translation termination sequence. It is derived from the commercially purchased vector pBIN19 (CloneTech; Frisch, R.A. et al., *Plant Molecular Biology* 27:405-409 (1995)) and from psmGFP (GenBank Accession Number U70495; Davis S.J., and R.D. Vierstra. *Plant Mol Biol* 36(4):521-8 (1998)). Specifically, psmGFP was digested with *EcoRI* and *HindIII*, to release the fragment containing 35S::GFP::nos. This was then ligated into *EcoRI*- and *HindIII*-digested pBIN19 to create pGV827.

Amplification and Cloning of *cis*-prenyltransferases

Chimeric genes comprising Hevea, rice and soybean *cis*-prenyltransferases (SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5) in sense orientation were constructed by polymerase chain reaction (PCR) from plasmids containing the Hevea, rice or soybean *cis*-prenyltransferase homologs, for expression in *Arabidopsis thaliana*. In contrast, the Apt5 gene (SEQ ID NO:20) was cloned by PCR amplification using *Arabidopsis thaliana* genomic DNA as a template.

More specifically, Hpt2 was amplified from clone ehb2c.pk001.d17, using oligonucleotide primers HW8 (SEQ ID NO:14) and HW12 (SEQ ID NO:15). The amplified Hpt2 cDNAs were digested with *XbaI* and *KpnI* and separated on an agarose gel. The DNA fragment was isolated and purified using a QIAquick Gel Extraction Kit, according to the manufacturer's instructions (Qiagen Inc., Chatsworth, CA). The purified DNA fragment was cloned into the corresponding sites of the binary vector pBI-35S (*supra*) to yield 35S::Hpt2.

Apt5 was isolated from *A. thaliana* genomic DNA, using primers Apt5/*XbaI* (SEQ ID NO:22) and Apt5/*KpnI* (SEQ ID NO:23). These primers were designed to include specific restriction sites at each end to facilitate in cloning. The amplified Apt5 gene was digested with *XbaI* and *KpnI* and separated on an agarose gel. The DNA fragment, ca. 850 bp in length, was isolated and purified using a QIAquick Gel Extraction Kit (Qiagen Inc., Chatsworth, CA); subsequently, the purified DNA fragments were cloned into a pBluescript vector according to manufacturer's instructions (Stratagene, LaJolla, CA). The *XbaI*-*KpnI* DNA fragment encoding the Apt5 gene (SEQ ID NO:20) was then cloned into the pBI-35S vector, yielding the construct 35S::Apt5.

Rpt1 and Spt1 were isolated in a manner similar to that for Hpt2; however, *Bam*HI and *Sac*I cloning sites were incorporated into the oligonucleotide primers to provide proper orientation of the DNA fragment upon insertion into the binary vector pGV827. Specifically:

- 5 • Rpt1 was amplified from clone rr1.pk0050.h8 using primers JK1 (SEQ ID NO:16) and JK7 (SEQ ID NO:20); and
- Spt1 was amplified from clone sl1.pk0128.h7 using primers JK3 (SEQ ID NO:18) and JK4 (SEQ ID NO:19).

10 PCR products were cloned into the pGEM T-easy vector using a TA-cloning kit (Promega Corporation, Madison, WI). Subsequently, the plasmids were digested with *Bam*HI and *Sac*I and the cDNA fragments encoding Rpt1 and Spt1 were isolated by agarose gel purification. The fragments were ligated into pGV827, to yield the resulting rice and soybean gene expression constructs 35S::rr1 and 35S::sl1, respectively.

15 Expression vectors 35S::Hpt2, 35S::rr1, 35S::sl1, and 35S::Apt5 were each individually transformed into *E. coli*. To verify integrity of the amplified DNAs, plasmids were isolated and purified using QIAFilter cartridges (Qiagen Inc., Chatsworth, CA) according to the manufacturer's instructions. Sequence was generated on an ABI Automatic sequencer using dye terminator technology (U.S. 5,366,860; EP 272007) and using a combination of vector-specific primers. Sequence editing was performed in Vector NTI.

Example 3

25 Transformation of Expression Vectors containing *cis*-prenyl transferases into *Arabidopsis thaliana*

The present Example describes the transformation of plasmids 35S::Hpt2, 35S::Apt5, 35S::rr1 and 35S::sl1 (from Example 2) into *Arabidopsis*.

30 Specifically, plasmids 35S::Hpt2 and 35S::Apt5 were transformed into *Agrobacterium tumefaciens* strain C58 using a freeze-thaw method (Holsters *et al.*, *Mol. Gen. Genet.* 163:181-187 (1978)). *Arabidopsis thaliana* plants were transformed via *Agrobacterium*-mediated transformation (Clough S. J., Bent A. F.; *Plant Journal* 16(6): 735-43 (1998)).

35 Plasmids 35S::rr1 and 35S::sl1 were also transformed into the *Agrobacterium tumefaciens* strain C58C1 by a freeze-thaw method (Holsters *et al.*, *supra*). However, *Agrobacterium* lines bearing the binary

vector constructs were selected using PCR and used to transform *Arabidopsis thaliana* using the floral dip method (Clough S. J., Bent A. F.; *supra*).

Example 4

5 Expression of *Cis*-prenyltransferase Genes in *Arabidopsis thaliana*

Example 4 describes the growth and identification of 4 lines (Rpt1 8-1, Spt1 10-4, Hpt2 16-2 and Apt5 1-4) of transgenic plants, carrying plasmids 35S::rr1, 35S::sl1, 35S::Hpt2, and 35S::Apt5, respectively. Reverse-transcriptase PCR was performed to confirm transgene
10 expression.

Growth and Identification of Transformant Lines of *Arabidopsis*

The seeds produced from infected plants transformed with vectors 35S::Hpt2, 35S::rr1, 35S::sl1, and 35S::Apt5 were germinated on agar plates containing 100 µg/mL kanamycin. *Arabidopsis* plants resistant to
15 kanamycin were selected and planted into soil. Seed was collected from these plants, and germinated on agar plates containing 100 µg/mL kanamycin. Three 35S::Rpt1 lines, five 35S::Spt1 lines, five 35S::Hpt2 and three 35S::Apt5 lines were selected as segregating 3:1 for resistance after germination on agar plates containing 100 µg/mL kanamycin. Subsequent
20 selection for 100% resistance yielded three 35S::Rpt1 lines, three 35S::Spt1 lines, four 35S::Hpt2 and two 35S::Apt5 lines. Of these, four were selected for further study and designated as Rpt1 8-1, Spt1 10-4, Hpt2 16-2 and Apt5 1-4, respectively.

Analysis of Transgene Expression, by RT-PCR

25 Relative quantitative reverse-transcriptase PCR (RT-PCR) was performed to confirm transgene expression and to compare expression levels among the four lines, using primers specific to the *cis*-prenyltransferase sequences. RNA was prepared from *Arabidopsis* leaves of the following lines: Apt5 1-4, Hpt2 16-2, Rpt1 8-1-5-4 and Spt1 10-4-3-
30 3, using the RNAeasy Midi-Kit (Qiagen, Valencia, CA), according to the manufacturer's supplied protocol for samples from plant tissue. RNA was quantified on a fluorometer (Turner Designs, Sunnyvale, CA).

To control for variations in RNA quality, quantitation errors and random variation in RT-PCR, "multiplex" RT-PCR was performed.
35 Multiplex PCR utilizes two or more primer sets in one reaction: one set to amplify the cDNA of interest and one set to amplify an invariant endogenous control. Primers used for amplification of each of the specific cDNAs were:

- Apt5s (SEQ ID NO:24) and Apt5as (SEQ ID NO:25) for Apt5;
- H2s (SEQ ID NO:26) and H2as (SEQ ID NO:27) for Hpt2;
- NKH33 (SEQ ID NO:28) and NKH34 (SEQ ID NO:29) for Rpt1; and,
- NKH35 (SEQ ID NO:30) and NKH36 (SEQ ID NO:31) for Spt1.

The primer sets used to amplify the endogenous control were the 18S PCR primer set and 18S PCR competitor set, supplied in the QuantumRNA Plant 18S Internal Standard Kit (Ambion, Austin, TX). One-step RT-PCR was performed on 1 ng and 2 ng of each RNA sample, according to manufacturer's supplied protocol (Qiagen). Amplification was carried out as follows: initial incubation at 50°C for 30 min; initial denaturation at 95°C for 15 min, followed by 28 cycles of 94°C (1 min), 52°C (1 min) and 72°C (1.5 min). A final extension cycle of 72°C for 10 min was performed.

The RT-PCR results of *cis*-prenyltransferase transcripts from transgenic *Arabidopsis* plants are shown in Figure 2 and indicated the expression of the *cis*-prenyltransferase transgenes in each of the lines tested. Relative transgene expression levels from lowest to highest were: Rpt1 8-1-5-4, Apt5 1-4, Spt1 10-4-3-3, Hpt2 16-2. These transgenic lines were used in all subsequent experiments, where they are designated by the abbreviations Rpt1, Apt5, Spt1 and Hpt2.

EXAMPLE 5

Analysis of *Arabidopsis* Transgenic for *cis*-prenyltransferases

Example 5 shows that constitutive expression of individual *cis*-prenyltransferase genes (expressed from plasmids 35S::Hpt2, 35S::Apt5, 35S::rr1 and 35S::sl1) is sufficient to modify growth, architecture and yield in transgenic *Arabidopsis* plants. In the majority of cases, constitutive expression of individual *cis*-prenyltransferase genes resulted in an enhanced rate of growth to maturity and considerably increased seed yield, characteristics of central importance to the commercial uses of plants.

Growth Conditions

Seed obtained from lines homozygous for resistance to kanamycin (Rpt1 8-1-5, Spt1 10-4-3, Hpt2 16-2 and Apt5 1-4; Example 4) were sown in Metro-Mix and grown in either constant light or with a 12 hr photoperiod

(fluorescent supplemented with incandescent) at 22 °C, 50% relative humidity. Plants were watered with a nutrient solution containing 1 mM ammonium phosphate, 1 mM potassium nitrate, 1 mM calcium nitrate, 2 mM magnesium sulfate, 1 mM ammonium nitrate, 5 ppb Fe and the following trace elements: manganese, chloride, boric acid, zinc sulfate, cupric sulfate, and molybdcic acid. Plants (8–10 individual plants from each transgenic line) were observed during growth to determine whether the transgenes, overexpressed using the 35S promoter, affected growth rate. Overall, it was observed that most plants constitutively expressing *cis*-prenyltransferase transgenes exhibited accelerated growth to maturity as compared to untransformed (wild-type) plants.

Modified Growth Rate and/or Yield for Leaves

Marked effects of the transgenes on leaf dimensions were observed, with the transgenic plants exhibiting on average longer leaves (Tables 4 and 5) and broader leaves (Tables 6 and 7). Leaf length was measured as leaf rosette radii; leaf width was measured at widest point.

Table 4

Effect of *cis*-prenyltransferase transgene expression on leaf rosette radius
(grown under constant light)

Overexpressed transgene	Rosette radius (cm) at 30 dps ¹ (std. dev.)	Rosette radius (cm) at 36 dps ¹ (std. dev.)
None (wild-type)	2.09 (0.47)	2.4 (0.31)
Rpt1	2.93 (0.65)	3.13 (0.57)
Apt5	2.91 (0.4)	3.11 (0.35)
Spt1	3.23 (0.21)	3.33 (0.71)
Hpt2	3.17 (0.75)	3.23 (0.25)

¹Days post sowing

Table 5

Effect of *cis*-prenyltransferase transgene expression on leaf rosette radius
(grown with a 12 hr photoperiod)

Overexpressed transgene	Rosette radius (cm) at 28 dps ¹ (std. dev.)
None (wild type)	2.30 (0.94)
Rpt1	3.46 (1.23)
Apt5	4.89 (0.77)
Spt1	4.82 (0.62))
Hpt2	4.88 (1.39)

5 ¹ Days post sowing

Table 6

Effect of *cis*-prenyltransferase transgene expression on leaf width
(grown under constant light)

Overexpressed transgene	Leaf width (mm) at 21 dps ¹ (std. dev.)	Leaf width (mm) at 36 dps ¹ (std. dev.)
None (wild-type)	5.38 (1.38)	11.38 (1.86)
Rpt1	5.8 (1.54)	13.15 (3.62)
Apt5	7.45 (1.57)	18.35 (3.20)
Spt1	8.25 (1.58)	16.12 (2.99)
Hpt2	8.0 (0.75)	19.5 (4.36)

10 ¹ Days post sowing

Table 7

Effect of *cis*-prenyltransferase transgene expression on leaf width
(growth with a 12 hr photoperiod)

Overexpressed transgene	Leaf width (mm) at 28 dps ¹ (std. dev.)
None (wild type)	8.16 (2.72)
Rpt1	10.81 (3.00)
Apt5	17.23 (2.05)
Spt1	18.14 (4.50)
Hpt2	17.78 (4.75)

5 ¹ Days post sowing

Both of these parameters were increased ca.1.5-fold in plants expressing the soybean and Hevea *cis*-prenyltransferases, for example.

Overexpression of the rice Rpt1 gene also resulted in longer leaves, although in this case their average width was indistinguishable from wild-type plants.

Modified Growth Rate and/or Yield for Plant Height

Marked effects of transgene expression were also observed on plant height, when compared at fixed times after sowing (Table 8).

15 Table 8

Effect of *cis*-prenyltransferase transgene expression on plant height
(grown under constant light)

Overexpressed transgene	Height (cm) at 28 dps ¹ (std. dev.)	Height (cm) at 32 dps ¹ (std. dev.)
None (wild-type)	6.13 (5.47)	15.36 (8.07)
Rpt1	4.2 (4.69)	12.6 (13.3)
Apt5	16.56 (10.93)	29.98 (11.67)
Spt1	25.79 (6.09)	34.33 (6.28)
Hpt2	21.75 (9.9)	33.31 (8.3)

¹ Days post sowing

20

Data shows that bolting plants expressing Apt5, Spt1 or Hpt2 were (on average) 3-3.5 times taller than wild-type plants 28 days after sowing. This effect was not observed in plants expressing Rpt1, which exhibited considerable variability for this measurement and whose average height was statistically indistinguishable from wild-type plants at both times measurements were made.

Figures 3, 4, and 5 clearly illustrate that plants constitutively expressing *cis*-prenyltransferases displayed a modified growth rate, as compared to wild type plants. Specifically, Figure 3 is a comparison between transgenic *Arabidopsis* expressing the *Hevea brasiliensis* Hpt2 *cis*-prenyltransferase (line Hpt2 3-2) and a wild-type plant. Both plants were photographed 35 days after sowing and are representative examples of a population of plants. Clearly, overexpression of the Hpt2 *cis*-prenyltransferase resulted in increased plant growth, such that the genetically transformed plant reached maturity faster than the wild-type plant.

Figure 4A and 4B are comparisons between transgenic *Arabidopsis* expressing the *Glycine max* Spt1 *cis*-prenyltransferases (lines Spt2 3-5a-2 and Spt2 3-6-3, respectively) and wild-type plants. Again, plants were photographed 35 days after sowing and are representative examples of a population of plants. Expression of the Spt1 *cis*-prenyltransferase also resulted in accelerated growth of the transformed plant, relative to the wild-type, as determined by plant height. And, although not shown, overexpression of the endogenous Apt5 *cis*-prenyltransferase also led to a dramatic increase in the rate of growth to maturity of the transgenic plant, relative to the wild-type.

Figure 5 is a comparison between transgenic *Arabidopsis* expressing the *Oryza sativa* Rpt1 *cis*-prenyltransferase (line Rpt 8-1-1-6) and a wild-type plant. Both plants were photographed 35 days after sowing and are representative examples of a population of plants. Rpt1 *cis*-prenyltransferase over-expression appeared to modify growth of *Arabidopsis*, leading to a somewhat reduced growth rate as compared to wild type.

Modified Growth Rate and/or Yield for Flowering and Seeds

The effect of the transgenes on flowering time and seed yield were also monitored (Table 9). The data shows that plants overexpressing Apt5, Hpt2 and Spt1 on average bolted earlier than wild-type plants and

developed more numerous inflorescences. The most dramatic effect of transgene expression, however, was on final seed yield.

Seed yield was determined according to the average weight of seed per plant, since individual seed weight was not affected by expression of any of the transgenes. More specifically, 1000 seeds from either the wild-type or the transgenics plants (grown under constant light or with a 12 hr photoperiod) always weighed ca. 30 mg.

The data show that plants overexpressing *cis*-prenyltransferases produce more numerous seed than untransformed, wild-type plants. Those overexpressing Apt5, Hpt2 and Spt1 are capable of yielding 10-fold the number of seed that can be routinely obtained from wild-type *Arabidopsis*.

Table 9

Effect of *cis*-prenyltransferase transgene expression on bolting, flowering and seed yield (grown under constant light)

Overexpressed transgene	Bolting time in dps ¹ (std. dev.)	No. leaves at bolting (std. dev.)	No. inflorescences (std. dev.)	Seed yield (average weight of seed per plant) (mg)
None (wild-type)	28.88 (2.80)	14.11 (2.26)	4 (0.866)	23.8
Rpt1	30.37 (4.59)	14.9 (3.03)	5.8 (1.61)	68.9
Apt5	26.6 (3.53)	13 (2.30)	4.9 (0.99)	187.52
Spt1	25.25 (1.75)	12.5 (0.92)	4.5 (1.19)	241.09
Hpt2	25.75 (2.05)	13.75 (1.90)	4.38 (1.06)	238.8

¹ Days post sowing

Table 10

Effect of *cis*-prenyltransferase transgene expression on seed yield (growth with a 12 hr photoperiod)

Overexpressed transgene	Seed yield (average weight of seed per plant) (mg)
None (wild-type)	36.9
Rpt1	52.7
Apt5	231.9

Spt1	237.2
Hpt2	240.5

Example 6

Further Analysis of *Arabidopsis* Transgenic for *cis*-prenyltransferases

Example 6 describes a more detailed examination of various characteristics of the transgenic *Arabidopsis*, expressing the Apt5, Hpt2, Spt1, and Rpt1 *cis*-prenyltransferases. As demonstrated in Example 5, constitutive expression of the *cis*-prenyltransferase genes is sufficient to modify growth, architecture and yield in transgenic *Arabidopsis* plants.

Seed from the parental wild-type Columbia and from 4 T3 lines of homozygous transformed *Arabidopsis* (Spt1 (event 10-4-3-3), Rpt1 (event 8-1-5-4), Hpt2 (event 16-2), Apt5 (event 1-4)) were obtained.

Approximately 20 seed from each line were suspended in 1 mL of water and were put in the refrigerator for 2 d. Then, the seeds were planted on prewetted Metro Mix in 4-inch pots by pipetting the seed and water solution over 4 pots per genotype. The pots were then placed in a reach-in growth chamber and grown at 21 °C under continuous light until maturity.

After germination, the pots were thinned to 3 plants per pot, and thinned again to 1 per pot when a single healthy plant was observed in each pot. The plants were monitored for growth conditions daily and plant growth data and digital images were recorded at significant time points throughout each plant's life cycle (wherein plants were at their maximum size at 34 d after planting). Specifically, the following observations were made (all results represent those of the genotype average, unless specified to be otherwise):

- Days to germination (recorded as the day when 90% of the plants had exposed cotyledons);
- Days to first flowering;
- Number of leaves at 26 days;
- Average leaf size and total leaf area (wherein leaf measurements from 10 to 12 leaves from each genotype were taken by using digital image analysis (recorded as pixels) and averaged to get a genotype average);
- Stem diameter at 34 days (measured at the base of each plant and averaged for each genotype);
- Stem length or plant height at 34 days;
- Number of siliques on the mainstem at 34 days;
- Average silique length;
- Number of bolts or stems per plant at 34 days;
- Days until first mature seed;

- Average number of seeds per silique (determined by counting each seed from 3 average looking siliques from representative plants and averaged);
- Number of siliques per plant at plant maturity (recorded from a representative sample of plants from each genotype);
- Total number of seed per plant (generated from the number of siliques per plant and the number of seeds per silique); and
- Total seed weight (determined after harvesting all of the seed from each plant and reported as an average of all of the plants from each genotype).

The results of these observations are summarized in Table 11 below.

Table 11

Effect of *cis*-prenyltransferase transgene expression in *Arabidopsis*

Over-expressed transgene	Days to germ-ination	Days to 1st flower	# Leaves	Avg. leaf size (pixels)	Total leaf area (pixels)	Stem dia-meter (mm)	Stem length (mm)	# Siliques per mainstem
None (wild-type)	7	27.5	8	263	2104	1	210	11
Rpt1	6	26	9.5	540	5130	1.8	292	15
Apt5	6	26	8.5	772	6562	1.8	400	20
Hpt2	5	23	8.5	1122	9537	1.5	432	30
Spt1	5	23	8.75	1079	9441.3	2	435	32

Over-expressed transgene	Silique length (mm)	# Bolts	Days to first mature seed	# Seed/silique	# Siliques at maturity	Avg. Total # seed	Avg. Total seed wgt (g)	Days to seed harvest
None (wild-type)	9	1	42	16	129	2,110	0.016	50
Rpt1	16	5	38	28	131	4,164	0.043	50
Apt5	18	5	38	53	124	6,919	0.102	50
Hpt2	19	5	37	64	383	24,319	0.266	50
Spt1	20	5	37	67	434	29,134	0.302	50

From the results presented above and the representative results shown in Figure 6 (taken at 26 d after planting), the increased plant growth rates in most of the transgenic lines were very obvious. The following general conclusions concerning the modified growth rates and/or yield and/or plant architectures observed can be summarized as shown below:

- Time to germination varied somewhat between transgenic lines, but all of the lines emerged 1 to 2 days (10-25%) earlier than the wild-type. The Spt1 and Hpt2 genotypes germinated most rapidly than the Apt5 and Rpt1 genotypes.
- The length of time until the first flower appeared on each transgenic genotype was approximately 15% earlier than the wild-type variety. Flowering was observed on the Rpt1 and Apt5 lines 1 to 2 days earlier than the wild-type, while the Hpt2 and Spt1 lines were 4 to 5 days earlier.
- The number of leaves present in the rosette was not significantly different from that of wild-type. However, the individual leaf size and the total leaf area of the plant was dramatically increased by up to 4 fold in the transgenic lines versus the wild-type. More specifically, expression of the Hpt2 and Spt1 *cis*-prenyltransferases had the greatest effect on leaf architecture, followed by Apt5 and Rpt1.
- The stem diameters for each of the transgenic lines were significantly greater than the wild-type.
- Concerning stem length, number of siliques per mainstem, average silique length, and the number of bold per plant, each of the 4 transgenic lines exhibited significantly increased growth and/or yield relative to the wild-type. Specifically, the transgenic lines were 2 times taller, the number and length of the siliques was 2-3 fold bigger, and the number of bolts was 5 times greater.
- The transgenic lines began producing mature seed about 12% faster than the wild-type. Seed yields from the *cis*-prenyltransferase-expressing plants were 5 to 10 times greater than wild-type yields. The increased number of siliques and increased size of the siliques contributed to the large increase in yields. As demonstrated in Table 11, the Spt1 and Hpt2 lines yielded the most seeds, followed by the Apt5 and Rpt1 lines.

- Despite the differences observed between the transgenic plants and the wild-type plants concerning rate of growth and/or yield and/or plant architecture, the time to seed harvest was still about the same for both transgenics and wild-type (50 days).

5 In summary, the data clearly shows that over-expression of the *cis*-prenyltransferase genes in *Arabidopsis* results in dramatic modification to the growth rates and/or yield and/or architecture of the transgenic plants, as compared to the wild-type. Seed yields are greatly increased due to the increased number and size of the siliques in the Hevea (Hpt2) and
10 Soy (Spt1) lines. Transgenic plant size and leaf area were also significantly increased, which could translate into more biomass per pot (or acre). This could be an especially beneficial result in any plant species that is grown commercially for biomass production (e.g., sugarcane, tobacco, cotton, etc.).

15

EXAMPLE 7

Analysis of *Arabidopsis* Transgenic for *cis*-prenyltransferases

Example 7 again shows that constitutive expression of individual *cis*-prenyltransferase genes (expressed from plasmids 35S::Hpt2,
20 35S::Apt5, 35S::rr1 and 35S::sl1, see above) is sufficient to modify growth, architecture and yield in transgenic *Arabidopsis* plants. In Examples 5 and 6, some measurements (in particular that of seed yield) made on plants grown as controls (untransformed wild-type plants) yielded values which appeared to be somewhat lower than might normally be
25 expected of wild-type *A. thaliana*. If this were the case, the net effect would be to exaggerate the differences between the transgenic lines and wild-type plants. In this Example, the results of additional experiments are described wherein the data from measurements made on wild-type control plants were more in line with expectations. The data presented in this
30 Example again shows that constitutive expression of several individual *cis*-prenyltransferase genes resulted in an enhanced rate of growth to maturity and considerably increased seed yield, characteristics of central importance to the commercial uses of plants.

35 Growth Conditions

Seed obtained from lines homozygous for resistance to kanamycin (Rpt1 8-1-5, Spt1 10-4-3, Hpt2 16-2 and Apt5 1-4; Example 4) were sown in Metro-Mix and grown in either constant light at 22 °C, with 50% relative

humidity, or with a 16 hr photoperiod (fluorescent supplemented with incandescent) at 22 °C (day) and 20 °C (night), with 60% relative humidity. Plants were watered with a nutrient solution containing 1 mM ammonium phosphate, 1 mM potassium nitrate, 1 mM calcium nitrate, 2 mM magnesium sulfate, 1 mM ammonium nitrate, 5 ppb Fe and the following trace elements: manganese, chloride, boric acid, zinc sulfate, cupric sulfate, and molybdic acid.

Plants were observed during growth to determine whether the transgenes, overexpressed using the 35S promoter, affected growth rate or seed yield. Overall, it was observed that most plants constitutively expressing *cis*-prenyltransferase transgenes exhibited accelerated growth to maturity and higher seed yield as compared to untransformed (wild-type) plants.

15 Leaf growth

An effect of transgene expression on leaf dimensions was observed, with the transgenic plants exhibiting on average longer leaves (Table 12) and broader leaves (Table 13). Leaf length was measured as leaf rosette radius; leaf width was measured at widest point.

20 Table 12

Effect of *cis*-prenyltransferase transgene expression on leaf rosette radius (16h photoperiod)

Overexpressed transgene	Rosette radius (mm) at 18 dps ¹ (std. dev.)	Rosette radius (mm) at 30 dps ¹ (std. dev.)
None (wild-type)	11.72 (4.22)	34.26 (8.01)
Rpt1	10.01 (3.91)	28.63 (8.17)
Apt5	19.29 (6.93)	44.47 (5.92)
Spt1	15.3 (4.52)	39.11 (6.43)
Hpt2	16.81 (4.88)	42.80 (7.42)

25 ¹Days post sowing

Table 13

Effect of *cis*-prenyltransferase transgene expression on leaf width
(growth with a 16 hr photoperiod)

Overexpressed transgene	Leaf width (mm) at 18 dps ¹ (std. dev.)	Leaf width (mm) at 30 dps ¹ (std. dev.)
None (wild type)	5.99 (1.69)	10.14 (1.6)
Rpt1	4.73 (1.54)	9.17 (3.24)
Apt5	8.13 (1.82)	12.62 (1.59)
Spt1	7.39 (1.76)	11.44 (1.58)
Hpt2	7.62 (1.32)	13.4 (1.66)

5 ¹ Days post sowing

Both of these parameters were increased ca. 1.5-fold in plants expressing the soybean and Hevea *cis*-prenyltransferases, for example.

10 Figure 7 illustrates the differences between a representative wild-type plant and representative transgenic 35S::Hpt2 plant, at 18 days (16h photoperiod) post sowing.

Plant Height

15 Marked effects of transgene expression were also observed on plant height, when compared at fixed times after sowing (Table 14).

Table 14

Effect of *cis*-prenyltransferase transgene expression on height of bolting
plants (16h photoperiod))

20

Overexpressed transgene	% plants bolting at 30dps ¹	Height (mm) at 30 dps ¹ (std. dev.)
None (wild-type)	50	66.49 (59.95)
Rpt1	61.9	40.0 (47.51)
Apt5	92	180.48 (93.68)
Spt1	100	141.06 (81.47)
Hpt2	96.42	106.08 (64.68)

¹ Days post sowing

The data shows that bolting plants expressing Apt5, Spt1 or Hpt2 were roughly 2x taller than wild-type plants 30 days after sowing. This effect was not observed in plants expressing Rpt1, which exhibited considerable variability for this measurement and whose average height was essentially indistinguishable from wild-type plants at the time measurements were made. Table 14 also shows that almost all Apt5, Spt1 and Hpt2 transgenic plants were producing bolts at 30 days post sowing, compared to only 50% of wild-type plants. Figure 8 illustrates the data shown in Tables 12 – 14, showing that representative plants constitutively expressing *cis*-prenyltransferases Apt5, Spt 1 and Hpt2 displayed a modified (enhanced) growth rate, as compared to a wild type plant. At 28 dps, any effect of the Rpt1 transgene is not obvious in this illustration.

15 Flowering and Seed Yield

The effect of the transgenes on flowering time and seed yield were also monitored (Tables 15,16). The data shows that plants overexpressing Apt5, Hpt2 and Spt1 on average produced inflorescences earlier than wild-type plants. The most dramatic effect of transgene expression, however, was on final seed yield.

Seed yield was determined according to the average weight of seed per plant, since individual seed weight was not affected by expression of any of the transgenes. More specifically, 1000 seeds from either the wild-type or the transgenics plants (grown under constant light or with a 12 hr photoperiod) always weighed ca. 30 mg.

The data (Table 16) show that plants overexpressing *cis*-prenyltransferases produce more numerous seed than untransformed, wild-type plants. Those overexpressing Apt5, Hpt2 and Spt1 are capable of yielding 1.5x the number of seed that can be routinely obtained from wild-type *Arabidopsis*.

Table 15
Effect of *cis*-prenyltransferase transgene expression on time to flowering
(16h photoperiod))

Overexpressed transgene	Days to flowering (std. dev.)
None (wild-type)	27.86 (2.58)
Rpt1	28.42 (2.33)
Apt5	23.04 (3.03)
Spt1	23.61 (3.26)
Hpt2	24.53 (2.02)

5

Table 16
Effect of *cis*-prenyltransferase transgene expression on seed yield
(growth with constant light)

10

Overexpressed transgene	Average weight of seed per plant in mg (std. dev.)
None (wild-type)	132.06 (29.41)
Rpt1	45.06 (12.57)
Apt5	204.08 (63.72)
Spt1	212.1 (65.03)
Hpt2	206.26 (68.35)

In summary, the data clearly shows that over-expression of *cis*-prenyltransferase genes in *Arabidopsis* results in dramatic modification to the growth rates and/or yield and/or architecture of the transgenic plants, as compared to the wild-type. With the majority of the exogenous transgenes, seed yields, plant size and leaf area were also increased, which could translate into more biomass per pot (or acre). This could be an especially beneficial result in any plant species that is grown commercially for biomass production (e.g., sugarcane, tobacco, cotton, etc.).

20

CLAIMS

What is claimed is:

1. A method for producing a transformed plant having an altered growth phenotype as compared with an untransformed plant comprising:
 - a) transforming a plant cell with a an isolated nucleic acid molecule encoding a *cis*-prenyltransferase under the control of suitable regulatory sequences;
 - b) recovering a transformed plant cell produced in step (a);
 - c) regenerating a plant from the transformed plant cell of step (b); and
 - d) growing the transformed plant produced in step (c) under conditions wherein the isolated nucleic acid molecule encoding a *cis*-prenyltransferase is expressed and the growth phenotype of the transformed plant is altered.
2. A method according to Claim 1 wherein the *cis*-prenyltransferase comprises a domain as defined by the amino acid sequence selected from the group consisting of SEQ ID NOs: 7-10.
3. A method according to Claim 1 wherein the *cis*-prenyltransferase comprises a domain as defined by the amino acid sequence selected from the group consisting of SEQ ID NOs: -11-13.
4. A method according to Claim 1 wherein the *cis*-prenyltransferase is a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:16.
5. A method according to Claim 1, wherein the isolated nucleic acid molecule encoding a *cis*-prenyltransferase is isolated from the group consisting of plants and microbes.
6. A method according to Claim 1 wherein the plant cell is selected from, but not limited to the group consisting of *Hevea brasiliensis*, *Taraxacum* spp., tobacco (*Nicotiana* spp.), tomato (*Lycopersicon* spp.), potato (*Solanum* spp.), hemp (*Cannabis* spp.),

sunflower (*Helianthus* spp.), sorghum (*Sorghum vulgare*), wheat (*Triticum* spp.), maize (*Zea mays*), rice (*Oryza sativa*), rye (*Secale cereale*), oats (*Avena* spp.), barley (*Hordeum vulgare*), rapeseed (*Brassica* spp.), broad bean (*Vicia faba*), french bean (*Phaseolus vulgaris*), other bean species (*Vigna* spp.), lentil (*Lens culinaris*), soybean (*Glycine max*), arabidopsis (*Arabidopsis thaliana*), guayule (*Parthenium argentatum*), cotton (*Gossypium hirsutum*), petunia (*Petunia hybrida*), flax (*Linum usitatissimum*), carrot (*Daucus carota sativa*), tea, celery, brussel sprout, artichoke, okra, squash, kale, asparagus (*Asparagus*), banana (*Musa*), blueberry (*Vaccinium*), cacao (*Theobroma*), capsicum pepper (*Capsicum*), cassava (*Manihot*), cucumber (*Cucumis*), eggplant (*Solanum*), lettuce (*Lactuca*), mango (*Mangifera*), oilseed rape, canola, cabbage, broccoli, cauliflower (*Brassica*), onions (*Allium*), papaya (*Carica*), peas (*Pisum*), peanut (*Arachis*), pineapple (*Ananas*), pinto bean, mung bean, pumpkin, zucchini (*Cucurbita*), radish (*Raphanus*), sesame (*Sesame*), spinach (*Spinaceae*), sorghum (*Sorghum*), strawberry (*Fragaria*), sugarcane (*Saccharum*), sugar beet (*Beta*), sweet potato (*Ipomoea*), watermelon (*Citrullus*), yam (*Dioscorea*), alfalfa (*Medicago*), amaranth (*Amaranthus*), angelica (*Agelica*), castorbean (*Ricinus*), colewort (*Crambe*), jojoba (*Simmondsia*), jute (*Corchorus*), kenaf (*Hibiscus*), lupine (*Lupinus*), plantain (*Plantago*), sisal (*Agave*), snapdragon (*Antirrhinum*), switch grass (*Panicum*), apple (*Malus*), acacia (*Acacia*), chestnut (*Castanea*), citrus (*Citrus*), coconut (*Cocos*), coffee (*Coffea*), cypress (*Cupressus*), eucalypti (*Eucalyptus*), grape (*Vitis*), hemlock (*Tsuga*), hickory (*Carya*), maple (*Acer*), oak (*Quercus*), pear (*Pyrus*), peach, plum, cherry (*Prunus*), pine (*Pinus*), poplar (*Populus*), rose (*Rosa*), spruce (*Picea*), and walnut (*Juglans*).

7. A method according to Claim 1 wherein the suitable regulatory sequences comprise a promoter sequence.

8. A method according to Claim 7, wherein the promoter sequence is selected from the group consisting of:

- a) constitutive plant promoters;
- b) plant tissue-specific promoters;
- c) plant development-stage-specific promoters;
- d) inducible promoters; and
- e) viral promoters.

9. A method according to Claim 8, wherein the tissue-specific promoters are selected from the group consisting of:

- a) male germline promoters;
- b) female germline promoters;
- c) common germline promoters;
- d) flower promoters;
- e) vegetative shoot apical meristem promoters;
- f) floral shoot apical meristem promoters;
- g) stem promoters;
- h) meristem promoters;
- i) tuber promoters;
- j) seed promoters;
- k) endosperm promoters;
- l) root promoters;
- m) nodule promoters;
- n) embryo promoters;
- o) leaf promoters; and
- p) fruit promoters.

10. A method according to Claim 1 wherein the altered growth phenotype is increased growth rate of the transformed plant.

11. A method according to Claim 10 wherein the increased growth rate is defined by characteristics selected from group consisting of: decreased time to germination, increased root growth rate, increased shoot growth rate, decreased time to flowering, decreased time for fruit maturation, and decreased time of seed setting.

12. A method according to Claim 1 wherein the altered growth phenotype is increased yield of the transformed plant.

13. A method according to Claim 12 wherein the increased yield is defined by characteristics selected from group consisting of: increased total biomass, increased root growth, increased shoot growth, increased seed set, increased seed production, increased grain yield, increased fruit size, increased nitrogen fixing capacity, increased nodule size, increased

tuber formation, increased stem thickness, increased endosperm size, and an increased number of fruit per plant.

14. A method according to Claim 1 wherein the altered growth phenotype is a modified plant architectural trait.

15. A method according to Claim 14 wherein the modified architectural trait is selected from the group consisting of: modifications in the shape, size, number, color, texture, arrangement and patterning of the root, leaf, shoot, fruit, petiole, trichome, flower, sepals, petal, hypocotyl, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre nodule, cambium, wood, heartwood, parenchyma, sclerenchyma, seive element, phloem, or vascular tissue.

16. A method for altering the growth phenotype of a plant as compared with an untransformed plant comprising:

- a) providing a plant comprising a gene encoding a *cis*-prenyltransferase; and
- b) upregulating the gene of (a) wherein the growth phenotype of the plant is altered.

17. A method according to Claim 16 wherein the gene encoding a *cis*-prenyltransferase is endogenous to the plant.

18. A method according to Claim 16 wherein the gene encoding a *cis*-prenyltransferase is exogenous to the plant.

19. A method according to Claim 16 wherein the gene encoding a *cis*-prenyltransferase is under the control of an inducible promoter.

20. A method according to Claim 16 wherein the gene encoding a *cis*-prenyltransferase is expressed on a multicopy plasmid.

21. A plant produced by the method of Claim 1.

22. A plant expressing a foreign *cis*-prenyltransferase gene having an altered growth phenotype.

23. A plant having a growth phenotype altered by the method of Claim 16.
24. A plant expressing a foreign *cis*-prenyltransferase gene having an altered yield phenotype.
25. A plant having a yield phenotype altered by the method of Claim 16.

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Figure 1

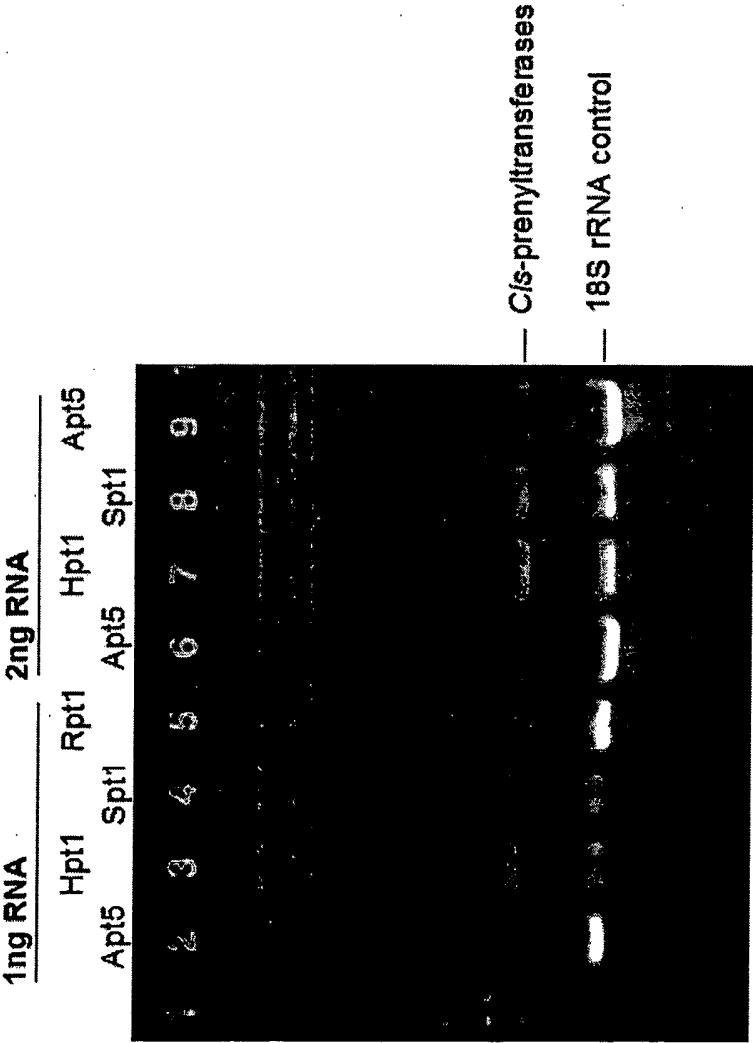


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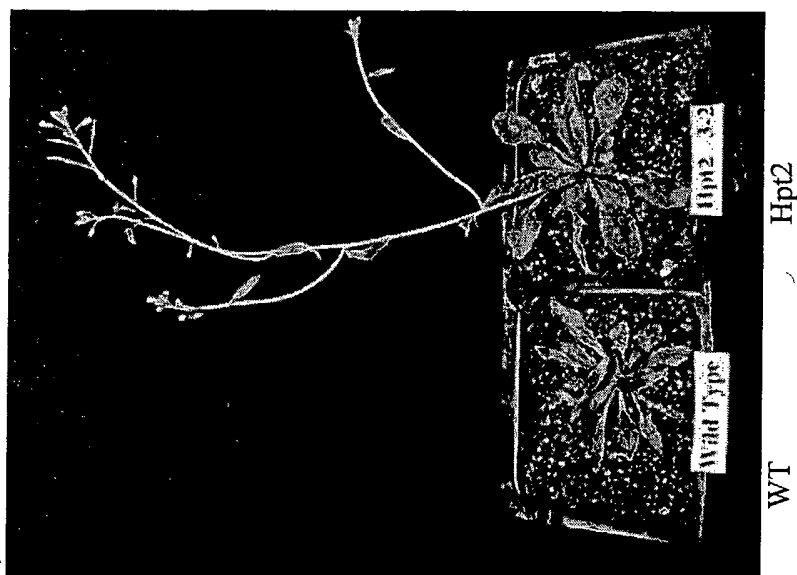


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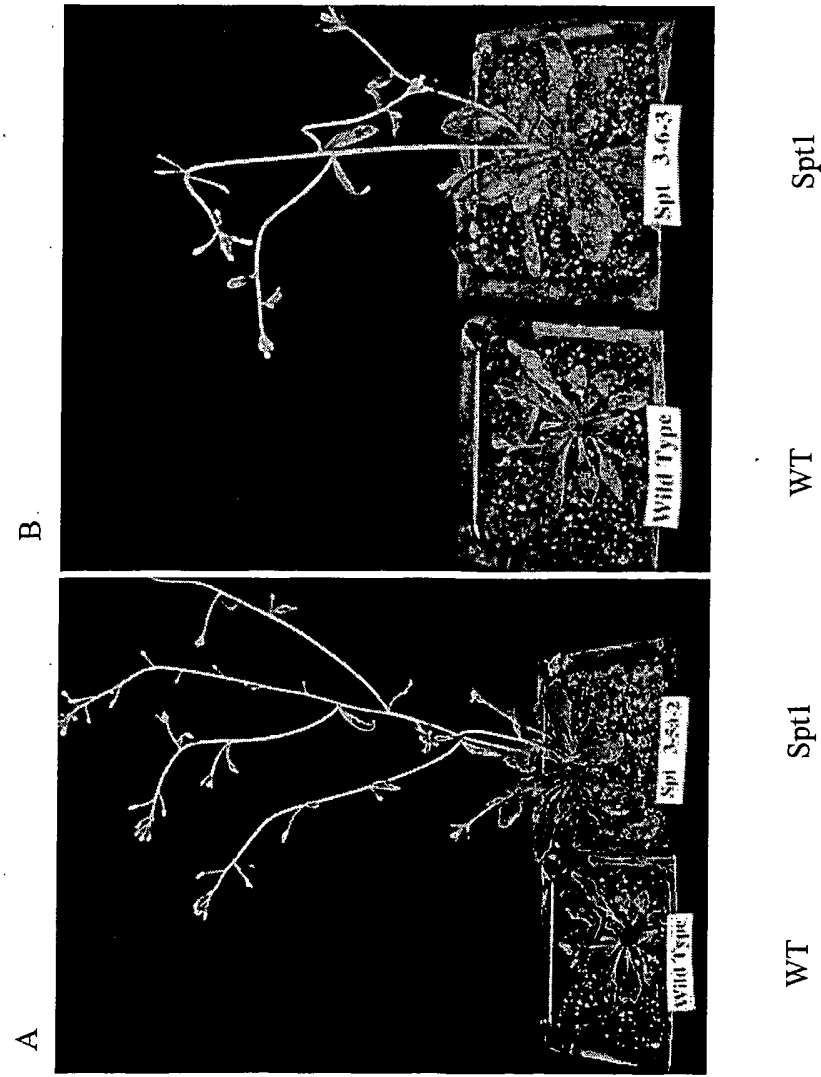
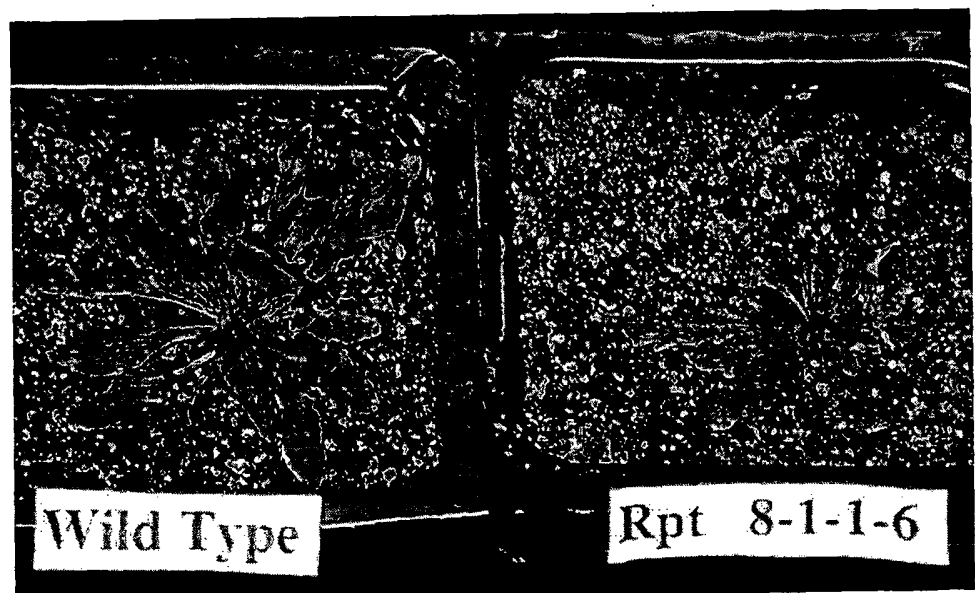


Figure 4

WT

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Figure 5



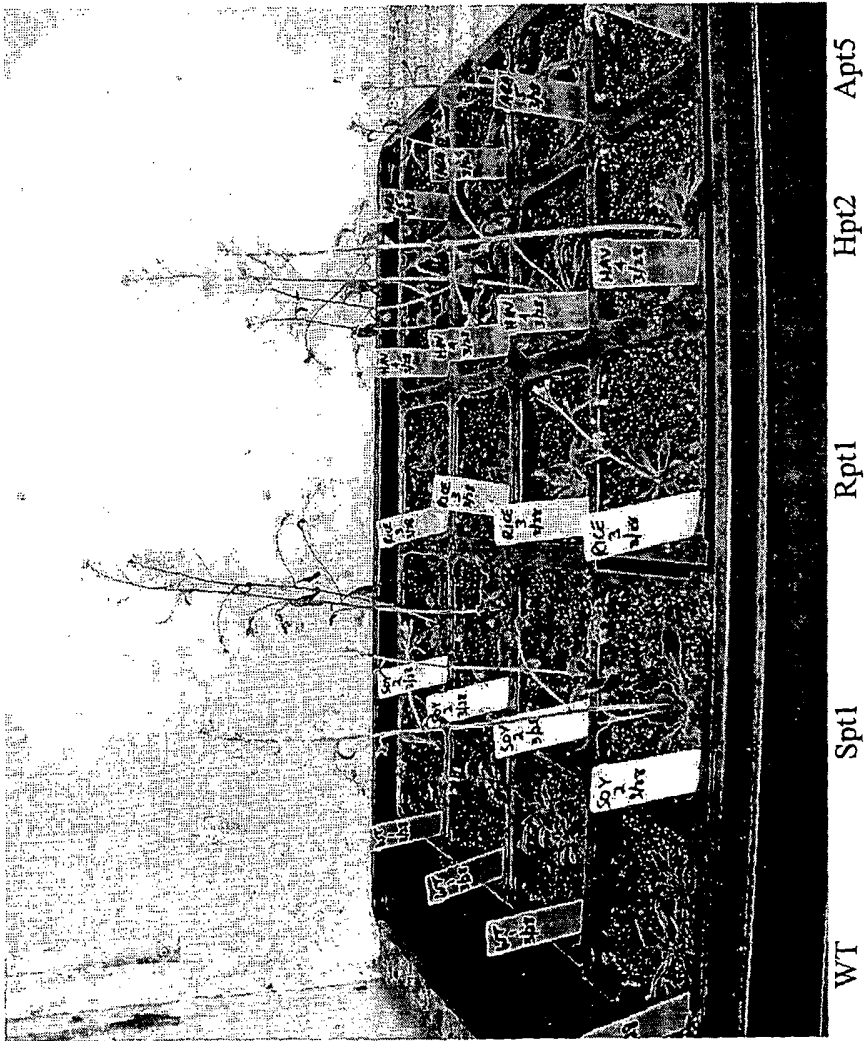


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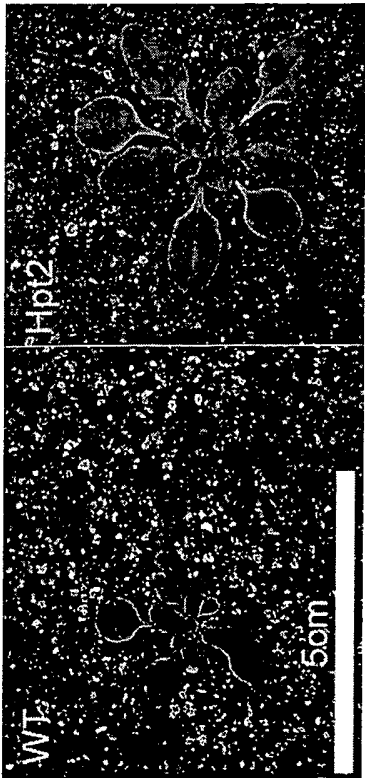


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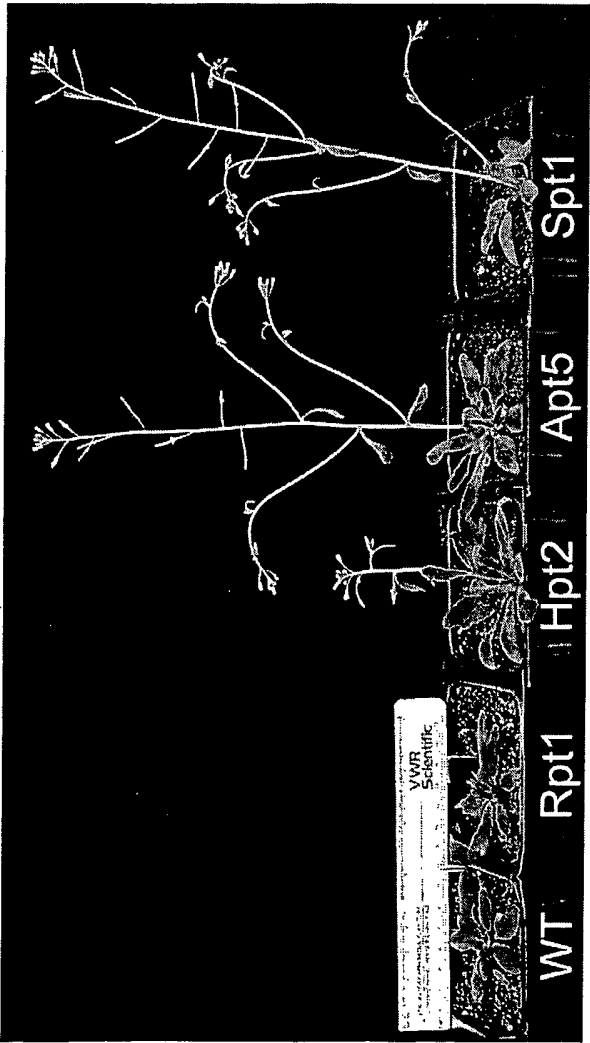


Figure 8

SEQUENCE LISTING

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Hallahan, David
Keiper-Hrynko, Natalie M.

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ARCHITECTURE IN PLANTS

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atgatgttgt gcaagcatgt aaaagtgtag ccaagaaagt caaagatggt cacattcact      780
tggtgacat aaacgaaaac attattgaac aagaattgga aactaattgt actgagtttc      840
cttatcctga tctactaata cgaactagtg gcgagcttag agtgagtaac ttcttgttgt      900
ggcaattagc ctacacagaa ctttatttta atcgggaact ctggccagat tttgggaagg      960
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Cys Tyr His Pro Phe His His Arg Ser Gln Thr Gln Ser Leu Ile Val
35 40 45

Ser Lys Arg Gly Ser Ala Ile Ala Lys Cys His Ala Asp Ser Val Thr
50 55 60

Leu Arg Asp Asp Gly Val Ser Leu Ala Gln Glu Ser Leu Glu Pro Leu
65 70 75 80

Pro Ala Glu Leu Ala Ala Glu Met Met Pro Lys His Val Ala Val Ile
85 90 95

Met Asp Gly Asn Gly Arg Trp Ala Lys Val Lys Gly Leu Pro Pro Ser
100 105 110

Ala Gly His Gln Ala Gly Val Gln Ser Leu Arg Lys Met Val Arg Leu
115 120 125

Cys Cys Ser Trp Gly Ile Lys Val Leu Thr Val Phe Ala Phe Ser Thr
130 135 140

Asp Asn Trp Val Arg Pro Lys Val Glu Val Asp Phe Leu Met Arg Leu
145 150 155 160

Phe Glu Arg Thr Ile Asn Ser Glu Val Gln Thr Phe Lys Arg Glu Gly
165 170 175

Ile Arg Ile Ser Val Ile Gly Asp Ser Ser Arg Leu Pro Glu Ser Leu
180 185 190

Lys Arg Met Ile Ala Ser Ala Glu Glu Asp Thr Lys Gln Asn Ser Arg
195 200 205

Phe Gln Leu Ile Val Ala Val Gly Tyr Ser Gly Lys Tyr Asp Val Val
210 215 220

Gln Ala Cys Lys Ser Val Ala Lys Lys Val Lys Asp Gly His Ile His
225 230 235 240

Leu Asp Asp Ile Asn Glu Asn Ile Ile Glu Gln Glu Leu Glu Thr Asn
245 250 255

Cys Thr Glu Phe Pro Tyr Pro Asp Leu Leu Ile Arg Thr Ser Gly Glu
260 265 270

Leu Arg Val Ser Asn Phe Leu Leu Trp Gln Leu Ala Tyr Thr Glu Leu
275 280 285

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1999 Jan;181(2):483-492)

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1999 Jan;181(2):483-492)

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1999 Jan;181(2):483-492)

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Xaa	Tyr	Xaa	Glu	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Xaa	Trp	Pro	Xaa	Phe					
1				5				10					15					20	
			25					30					35						

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gaggaaat tttgttcagag ttctatgcgt cggccaatc cctactaaca tttcattcat 180
catggatgga aaccgcaggt tcgctaagaa acacaatctt ataggcctag atgcaggaca 240

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gtgtgtgatg gatctaatac ttgagaaagt cgagctcgcg atcgatcaag ctgtatcagg 420
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tagagctgcg acaaagaaac tgatggagct tacggaggag aatagagatc tgattgtggt 540
ggtttgcggt gcttacagca caagtctcga gattgttcac gctgttcgaa aatcttgtgt 600
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agattacttg acgaagaaga aaaagctcga ttagatagtt tctaaagtta aaccctgcag 900
gaaagaactt ttaactcttt attacgttta atttacgtgt ttctatgact ggaaacgaga 960
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20          25          30

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Pro Ile Pro Thr Asn Ile Ser Phe Ile Met Asp Gly Asn Arg Arg Phe
35          40          45

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Ala Lys Lys His Asn Leu Ile Gly Leu Asp Ala Gly His Arg Ala Gly
50          55          60

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Phe Ile Ser Val Lys Tyr Ile Leu Gln Tyr Cys Lys Glu Ile Gly Val
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Pro Tyr Val Thr Leu His Ala Phe Gly Met Asp Asn Phe Lys Arg Gly
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Pro Glu Glu Val Lys Cys Val Met Asp Leu Met Leu Glu Lys Val Glu
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Leu Ala Ile Asp Gln Ala Val Ser Gly Asn Met Asn Gly Val Arg Ile
115          120          125

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15/18

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 130 135 140

Thr Lys Lys Leu Met Glu Leu Thr Glu Glu Asn Arg Asp Leu Ile Val
 145 150 155 160

Val Val Cys Val Ala Tyr Ser Thr Ser Leu Glu Ile Val His Ala Val
 165 170 175

Arg Lys Ser Cys Val Arg Lys Cys Thr Asn Gly Asp Asp Leu Val Leu
 180 185 190

Leu Glu Leu Ser Asp Val Glu Glu Cys Met Tyr Thr Ser Ile Val Pro
 195 200 205

Val Pro Asp Leu Val Ile Arg Thr Gly Gly Gly Asp Arg Leu Ser Asn
 210 215 220

Phe Met Thr Trp Gln Thr Ser Arg Ser Leu Leu His Arg Thr Glu Ala
 225 230 235 240

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Val Phe Leu Leu Lys Leu Ile Gly Leu Ile Lys Ile Lys Ala Ala Arg
35 40 45

Asp Asn Glu Lys Arg Asp Glu Gly Thr Tyr Val Val Arg Glu Asp Gly
50 55 60

Leu Gln Arg Glu Leu Met Pro Arg His Val Ala Phe Ile Leu Asp Gly
65 70 75 80

Asn Arg Arg Trp Ala Lys Arg Ala Gly Leu Thr Thr Ser Gln Gly His
85 90 95

Glu Ala Gly Ala Lys Arg Leu Ile Asp Ile Ala Glu Leu Cys Phe Glu
100 105 110

Leu Gly Val His Thr Val Ser Ala Phe Ala Phe Ser Thr Glu Asn Trp
115 120 125

Gly Arg Asp Lys Ile Glu Ile Asp Asn Leu Met Ser Leu Ile Gln His
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Tyr Arg Asn Lys Ser Asn Ile Lys Phe Phe His Arg Ser Glu Val Arg
145 150 155 160

Val Ser Val Ile Gly Asn Lys Thr Lys Ile Pro Glu Ser Leu Leu Lys
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 Leu Ile Met Ala Val Asp Tyr Ser Gly Lys Phe Asp Ile Met His Ala
 195 200 205
 Cys Lys Ser Leu Val Lys Lys Ser Glu Lys Gly Leu Ile Arg Glu Glu
 210 215 220
 Asp Val Asp Glu Ala Leu Ile Glu Arg Glu Leu Leu Thr Asn Cys Ser
 225 230 235 240
 Asp Phe Pro Ser Pro Asp Leu Met Ile Arg Thr Ser Gly Glu Gln Arg
 245 250 255
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INTERNATIONAL SEARCH REPORT

International Application No

P(2004/016432

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C12N9/10 A01H5/00

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)

IPC 7 C12N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/21650 A (COLDREN CHRIS ; DU PONT (US); WANG HONG (US); FLINT DENNIS (US); HALLA) 29 March 2001 (2001-03-29) page 16, line 25 - page 17, line 17; examples 5,7,8; sequences 8,16,18	1-9, 16-25
E	WO 2004/044173 A (KEIPER-HRYNKO NATALIE M ; DU PONT (US); HALLAHAN DAVID L (US)) 27 May 2004 (2004-05-27) abstract page 23, line 35 - page 25, line 9 page 27, line 8 - line 35 ----- -/--	1-3,5-9, 16-25

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in 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 document 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.

G document member of the same patent family

Date of the actual completion of the international search

7 October 2004

Date of mailing of the international search report

21/10/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Loubradou, G

INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP2004/016432

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KHAREL YUGESH ET AL: "Molecular analysis of cis-prenyl chain elongating enzymes." NATURAL PRODUCT REPORTS. FEB 2003, vol. 20, no. 1, February 2003 (2003-02), pages 111-118, XP009037794 ISSN: 0265-0568 abstract; figure 1 -----	
A	CUNILLERA N ET AL: "Characterization of dehydrolipichyl diphosphate synthase of Arabidopsis thaliana, a key enzyme in dolichol biosynthesis<1>" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 477, no. 3, 21 July 2000 (2000-07-21), pages 170-174, XP004337371 ISSN: 0014-5793 the whole document -----	
A	OH S K ET AL: "Molecular cloning, expression, and functional analysis of a cis-prenyltransferase from Arabidopsis thaliana. Implications in rubber biosynthesis" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 275, no. 24, 16 June 2000 (2000-06-16), pages 18482-18488, XP002223079 ISSN: 0021-9258 the whole document -----	
A	APFEL ET AL: "Use of Genomics To Identify Bacterial Undecaprenyl Pyrophosphate Synthetase" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 181, no. 2, January 1999 (1999-01), pages 483-492, XP002160874 ISSN: 0021-9193 cited in the application the whole document -----	
A	TANETOSHI KOYAMA: "MOLECULAR ANALYSIS OF PRENYL CHAIN ELONGATING ENZYMES" BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY, JAPAN SOC. FOR BIOSCIENCE, BIOTECHNOLOGY AND AGROCHEM. TOKYO, JP, vol. 63, no. 10, 1999, pages 1671-1676, XP002930842 ISSN: 0916-8451 the whole document ----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PC JS2004/016432

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	ASAWATRERATANAKUL KASEM ET AL: "Molecular cloning, expression and characterization of cDNA encoding cis-prenyltransferases from Hevea brasiliensis: A key factor participating in natural rubber biosynthesis." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 270, no. 23, December 2003 (2003-12), pages 4671-4680, XP002299740 ISSN: 0014-2956 the whole document -----	

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US2004/016432

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box II.2

Claims Nos.: -

Claim 4 has been searched incompletely. The subject-matter related to SEQ ID N°16 has not been searched because SEQ ID N°16 corresponds to a primer not to the sequence of a cis-prenyltransferase.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC JS2004/016432

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