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(54) Title: GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT		
(57) Abstract The wheat <i>Rht</i> gene and homologues from other species including rice and maize (the <i>D8</i> gene), useful for modification of growth and/or development characteristics of plants. Transgenic plants and methods and means for their production.		

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GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

This invention relates to the genetic control of growth and/or development of plants and the cloning and expression of genes involved therein. More particularly, the invention relates to the cloning and expression of the *Rht* gene of *Triticum Aestivum*, and homologues from other species, and use of the genes in plants.

10 An understanding of the genetic mechanisms which influence growth and development of plants, including flowering, provides a means for altering the characteristics of a target plant. Species for which manipulation of growth and/or development characteristics may be advantageous includes all 15 crops, with important examples being the cereals, rice and maize, probably the most agronomically important in warmer climatic zones, and wheat, barley, oats and rye in more temperate climates. Important crops for seed products are oil seed rape and canola, maize, sunflower, soyabean and 20 sorghum. Many crops which are harvested for their roots are, of course, grown annually from seed and the production of seed of any kind is very dependent upon the ability of the plant to flower, to be pollinated and to set seed. In horticulture, control of the timing of growth and 25 development, including flowering, is important.

Horticultural plants whose flowering may be controlled include lettuce, endive and vegetable brassicas including cabbage, broccoli and cauliflower, and carnations and

geraniums. Dwarf plants on the one hand and over-size, taller plants on the other may be advantageous and/or desirable in various horticultural and agricultural contexts, further including trees, plantation crops and grasses.

5

Recent decades have seen huge increases in wheat grain yields due to the incorporation of semi-dwarfing *Rht* homeoalleles into breeding programmes. These increases have enabled wheat productivity to keep pace with the demands of the rising
10 world population. Previously, we described the cloning of the *Arabidopsis gai* alleles (International patent application PCT/GB97/00390 filed 12 February 1997 and published as WO97/29123 on 14 August 1998, John Innes Centre Innovations Limited, the full contents of which are incorporated herein
15 by reference) which, like *Rht* mutant alleles in wheat (a monocot), confers a semi-dominant dwarf phenotype in *Arabidopsis* (a dicot) and a reduction in responsiveness to the plant growth hormone gibberellin (GA). *gai* encodes a mutant protein (*gai*) which lacks a 17 amino acid residue
20 segment found near the N-terminus of the wild-type (GAI) protein. The sequence of this segment is highly conserved in a rice cDNA sequence (EST). Here we show that this cDNA maps to a short section of the overlapping cereal genome maps known to contain the *Rht* loci, and that we have used the cDNA
25 to isolate the *Rht* genes of wheat. That genomes as widely diverged as those of *Arabidopsis* and *Triticum* should carry a conserved sequence which, when mutated, affects GA responsiveness, indicates a role for that sequence in GA

signalling that is conserved throughout the plant kingdom. Furthermore, cloning of *Rht* permits its transfer to many different crop species, with the aim of yield enhancement as great as that obtained previously with wheat.

5

The introduction of semi-dwarfing *Rht* homeoalleles (originally known as Norin 10 genes, derived from a Japanese variety, Norin 10) into elite bread-wheat breeding lines was one of the most significant contributors to the so-called
10 "green revolution" (Gale *et al*, 1985. Dwarfing genes in wheat. In: Progress in Plant Breeding, G.E. Russell (ed) Butterworths, London pp 1-35). Wheat containing these homeoalleles consistently out-yield wheats lacking them, and now comprise around 80% of the world's wheat crop. The
15 biological basis of this yield-enhancement appears to be two-fold. Firstly, the semi-dwarf phenotype conferred by the *Rht* alleles causes an increased resistance to lodging (flattening of plants by wind/rain with consequent loss of yield). Secondly, these alleles cause a reallocation of
20 photoassimilate, with more being directed towards the grain, and less towards the stem (Gale *et al*, 1985). These properties have major effects on wheat yields, as demonstrated by the fact that UK wheat yields increased by over 20% during the years that *Rht*-containing lines were
25 taken up by farmers.

The *rht* mutants are dwarfed because they contain a genetically dominant, mutant *rht* allele which compromises

their responses to gibberellin (GA, an endogenous plant growth regulator) (Gale *et al*, 1976. *Heredity* 37; 283-289). Thus the coleoptiles of *rht* mutants, unlike those of wild-type wheat plants, do not respond to GA applications. In addition, *rht* mutants accumulate biologically active GAs to higher levels than found in wild-type controls (Lenton *et al*, 1987. *Gibberellin insensitivity and depletion in wheat - consequences for development*. In: *Hormone action in Plant Development - a critical appraisal*. GV Haod, JR Lenton, MB Jackson and RK Atkin (eds) Butterworths, London pp 145-160). These properties (genetic dominance, reduced GA-responses, and high endogenous GA levels) are common to the phenotypes conferred by mutations in other species (*D8/D9* in maize; *gai* in *Arabidopsis*), indicating that these mutant alleles define orthologous genes in these different species, supported further by the observation that *D8/D9* and *Rht* are syntenous loci in the genomes of maize and wheat.

According to a first aspect of the present invention there is provided a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide with *Rht* function. The term "Rht function" indicates ability to influence the phenotype of a plant like the *Rht* gene of *Triticum*. "Rht function" may be observed phenotypically in a plant as inhibition, suppression, repression or reduction of plant growth which inhibition, suppression, repression or reduction is antagonised by GA. *Rht* expression tends to confer a dwarf phenotype on a plant which is antagonised by GA.

Overexpression in a plant from a nucleotide sequence encoding a polypeptide with *Rht* function may be used to confer a dwarf phenotype on a plant which is correctable by treatment with GA.

5

Also according to an aspect of the present invention there is provided a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide with ability to confer a *rht* mutant phenotype upon expression. *rht* mutant plants are
10 dwarfed compared with wild-type, the dwarfing being GA-insensitive.

Herein, "Rht" (capitalised) is used to refer to the wild-type function, while "rht" (uncapitalised) is used to refer to
15 mutant function.

Many plant growth and developmental processes are regulated by specific members of a family of tetracyclic diterpenoid growth factors known as gibberellins (GA) (Hooley, *Plant Mol.*
20 *Biol.* 26, 1529-1555 (1994)). By gibberellin or GA is meant a diterpenoid molecule with the basic carbon-ring structure shown in Figure 5 and possessing biological activity, i.e. we refer to biologically active gibberellins.

25 Biological activity may be defined by one or more of stimulation of cell elongation, leaf senescence or elicitation of the cereal aleurone α -amylase response. There are many standard assays available in the art, a positive

result in any one or more of which signals a test gibberellin as biologically active (Hoad et al., *Phytochemistry* 20, 703-713 (1981); Serebryakov et al., *Phytochemistry* 23, 1847-1854 (1984); Smith et al., *Phytochemistry* 33, 17-20 (1993)).

5

Assays available in the art include the lettuce hypocotyl assay, cucumber hypocotyl assay, and oat first leaf assay, all of which determine biological activity on the basis of ability of an applied gibberellin to cause elongation of the
10 respective tissue. Preferred assays are those in which the test composition is applied to a gibberellin-deficient plant. Such preferred assays include treatment of dwarf GA-deficient *Arabidopsis* to determine growth, the dwarf pea assay, in which internode elongation is determined, the Tan-ginbozu
15 dwarf rice assay, in which elongation of leaf sheath is determined, and the d5-maize assay, also in which elongation of leaf sheath is determined. The elongation bioassays measure the effects of general cell elongation in the respective organs and are not restricted to particular cell
20 types.

Further available assays include the dock (*Rumex*) leaf senescence assay and the cereal aleurone α -amylase assay. Aleurone cells which surround the endosperm in grain secrete
25 α -amylase on germination, which digests starch to produce sugars then used by the growing plant. The enzyme production is controlled by GA. Isolated aleurone cells given biologically active GA secrete α -amylase whose activity can

then be assayed, for example by measurement of degradation of starch.

Structural features important for high biological activity (exhibited by GA₁, GA₃, GA₄ and GA₇) are a carboxyl group on C-6 of B-ring; C-19, C-10 lactone; and β -hydroxylation at C-3. β -hydroxylation at C-2 causes inactivity (exhibited by GA₈, GA₂₉, GA₃₄ and GA₅₁). *rht* mutants do not respond to GA treatment, e.g. treatment with GA₁, GA₃ or GA₄.

10

Treatment with GA is preferably by spraying with aqueous solution, for example spraying with 10⁻⁴M GA₃ or GA₄ in aqueous solution, perhaps weekly or more frequently, and may be by placing droplets on plants rather than spraying. GA may be applied dissolved in an organic solvent such as ethanol or acetone, because it is more soluble in these than in water, but this is not preferred because these solvents have a tendency to damage plants. If an organic solvent is to be used, suitable formulations include 247l of 0.6, 4.0 or 300mM GA₃ or GA₄ dissolved in 80% ethanol. Plants, e.g. *Arabidopsis*, may be grown on a medium containing GA, such as tissue culture medium (GM) solidified with agar and containing supplementary GA.

25 Nucleic acid according to the present invention may have the sequence of a wild-type *Rht* gene of *Triticum* or be a mutant, derivative, variant or allele of the sequence provided. Preferred mutants, derivatives, variants and alleles are

those which encode a protein which retains a functional characteristic of the protein encoded by the wild-type gene, especially the ability for plant growth inhibition, which inhibition is antagonised by GA, or ability to confer on a
5 plant one or more other characteristics responsive to GA treatment of the plant. Other preferred mutants, derivatives, variants and alleles encode a protein which confers a *rht* mutant phenotype, that is to say reduced plant growth which reduction is insensitive to GA, i.e. not
10 overcome by GA treatment. Changes to a sequence, to produce a mutant, variant or derivative, may be by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion or substitution of one or more amino
15 acids in the encoded polypeptide. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence are included.

A preferred nucleotide sequence for a *Rht* gene is one which
20 encodes the RHT amino acid sequence shown in Figure 3b, especially a *Rht* coding sequence shown in Figure 3a. A preferred *rht* mutant lacks part or all of the 17 amino acid sequence underlined in Figure 3b, and/or part or the sequence DVAQKLEQLE, which immediately follows the 17 amino acid
25 sequence underlined in Figure 3b.

Further preferred nucleotide sequences encode the amino acid sequence shown in any other figure herein, especially a

coding sequence shown in a Figure. Further embodiments of the present invention, in all aspects, employ a nucleotide sequence encoding the amino acid sequence shown in Figure 6b, 7b, 8b, 9b, 11b, 11d or 12b. Such a coding sequence may be
5 as shown in Figure 6a, 7a, 8a, 9a, 11a, 11c or 12a.

The present invention also provides a nucleic acid construct or vector which comprises nucleic acid with any one of the provided sequences, preferably a construct or vector from
10 which polypeptide encoded by the nucleic acid sequence can be expressed. The construct or vector is preferably suitable for transformation into a plant cell. The invention further encompasses a host cell transformed with such a construct or vector, especially a plant cell. Thus, a host cell, such as
15 a plant cell, comprising nucleic acid according to the present invention is provided. Within the cell, the nucleic acid may be incorporated within the chromosome. There may be more than one heterologous nucleotide sequence per haploid genome. This, for example, enables increased expression of
20 the gene product compared with endogenous levels, as discussed below.

A construct or vector comprising nucleic acid according to the present invention need not include a promoter or other
25 regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome. However, in one aspect the present invention provides a nucleic acid construct comprising a *Rht*

or *rht* coding sequence (which includes homologues from other than *Triticum*) joined to a regulatory sequence for control of expression, the regulatory sequence being other than that naturally fused to the coding sequence and preferably of or
5 derived from another gene.

Nucleic acid molecules and vectors according to the present invention may be as an isolate, provided isolated from their natural environment, in substantially pure or homogeneous
10 form, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the sequence encoding a polypeptide able to influence growth and/or development, which may include flowering, eg in *Triticum Aestivum* nucleic acid other than the *Rht* coding sequence.
15 The term "nucleic acid isolate" encompasses wholly or partially synthetic nucleic acid.

Nucleic acid may of course be double- or single-stranded, cDNA or genomic DNA, RNA, wholly or partially synthetic, as
20 appropriate. Of course, where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as encompassing the RNA equivalent, with U substituted for T.

25 The present invention also encompasses the expression product of any of the nucleic acid sequences disclosed and methods of making the expression product by expression from encoding nucleic acid therefor under suitable conditions in suitable

host cells. Those skilled in the art are well able to construct vectors and design protocols for expression and recovery of products of recombinant gene expression. Suitable vectors can be chosen or constructed, containing
5 appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al,
10 1989, Cold Spring Harbor Laboratory Press. Transformation procedures depend on the host used, but are well known. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and
15 gene expression, and analysis of proteins, are described in detail in *Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. Specific procedures and vectors previously used with wide success upon plants are described by Bevan, *Nucl. Acids Res.* (1984) 12,
20 8711-8721), and Guerineau and Mullineaux, (1993) *Plant transformation and expression vectors*. In: *Plant Molecular Biology Labfax* (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148. The disclosures of Sambrook et al. and Ausubel et al. and all other documents mentioned herein
25 are incorporated herein by reference.

Expression as a fusion with a polyhistidine tag allows purification of Rht or rht to be achieved using Ni-NTA resin

available from QIAGEN Inc. (USA) and DIAGEN GmbH (Germany).
See Janknecht et al., *Proc. Natl. Acad. Sci. USA* 88, 8972-
8976 (1991) and EP-A-0253303 and EP-A-0282042. Ni-NTA resin
has high affinity for proteins with consecutive histidines
5 close to the N- or C- terminus of the protein and so may be
used to purify histidine-tagged Rht or rht proteins from
plants, plant parts or extracts or from recombinant organisms
such as yeast or bacteria, e.g. *E. coli*, expressing the
protein.

10

Purified Rht protein, e.g. produced recombinantly by
expression from encoding nucleic acid therefor, may be used
to raise antibodies employing techniques which are standard
in the art. Antibodies and polypeptides comprising antigen-
15 binding fragments of antibodies may be used in identifying
homologues from other species as discussed further below.

Methods of producing antibodies include immunising a mammal
(eg human, mouse, rat, rabbit, horse, goat, sheep or monkey)
20 with the protein or a fragment thereof. Antibodies may be
obtained from immunised animals using any of a variety of
techniques known in the art, and might be screened,
preferably using binding of antibody to antigen of interest.
For instance, Western blotting techniques or
25 immunoprecipitation may be used (Armitage et al, 1992, *Nature*
357: 80-82). Antibodies may be polyclonal or monoclonal.

As an alternative or supplement to immunising a mammal,

antibodies with appropriate binding specificity may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, eg using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047.

Antibodies raised to a Rht, or rht, polypeptide can be used in the identification and/or isolation of homologous polypeptides, and then the encoding genes. Thus, the present invention provides a method of identifying or isolating a polypeptide with Rht function or ability to confer a rht mutant phenotype, comprising screening candidate polypeptides with a polypeptide comprising the antigen-binding domain of an antibody (for example whole antibody or a fragment thereof) which is able to bind an *Triticum Aestivum* Rht or rht polypeptide, or preferably has binding specificity for such a polypeptide, such as having the amino acid sequence shown in Figure 3b.

20

Candidate polypeptides for screening may for instance be the products of an expression library created using nucleic acid derived from a plant of interest, or may be the product of a purification process from a natural source.

25

A polypeptide found to bind the antibody may be isolated and then may be subject to amino acid sequencing. Any suitable technique may be used to sequence the polypeptide either

wholly or partially (for instance a fragment of the polypeptide may be sequenced). Amino acid sequence information may be used in obtaining nucleic acid encoding the polypeptide, for instance by designing one or more
5 oligonucleotides (e.g. a degenerate pool of oligonucleotides) for use as probes or primers in hybridisation to candidate nucleic acid, as discussed further below.

A further aspect of the present invention provides a method
10 of identifying and cloning *Rht* homologues from plant species other than *Triticum* which method employs a nucleotide sequence derived from any shown in Figure 2 or Figure 3a, or other figure herein. Sequences derived from these may themselves be used in identifying and in cloning other
15 sequences. The nucleotide sequence information provided herein, or any part thereof, may be used in a data-base search to find homologous sequences, expression products of which can be tested for *Rht* function. Alternatively, nucleic acid libraries may be screened using techniques well known to
20 those skilled in the art and homologous sequences thereby identified then tested.

For instance, the present invention also provides a method of identifying and/or isolating a *Rht* or *rht* homologue gene,
25 comprising probing candidate (or "target") nucleic acid with nucleic acid which encodes a polypeptide with *Rht* function or a fragment or mutant, derivative or allele thereof. The candidate nucleic acid (which may be, for instance, cDNA or

genomic DNA) may be derived from any cell or organism which may contain or is suspected of containing nucleic acid encoding such a homologue.

5 In a preferred embodiment of this aspect of the present invention, the nucleic acid used for probing of candidate nucleic acid encodes an amino acid sequence shown in Figure 3b, a sequence complementary to a coding sequence, or a fragment of any of these, most preferably comprising a
10 nucleotide sequence shown in Figure 3a.

Alternatively, as discussed, a probe may be designed using amino acid sequence information obtained by sequencing a polypeptide identified as being able to be bound by an
15 antigen-binding domain of an antibody which is able to bind a Rht or rht polypeptide such as one with the Rht amino acid sequence shown in Figure 3b.

Preferred conditions for probing are those which are
20 stringent enough for there to be a simple pattern with a small number of hybridizations identified as positive which can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

25

As an alternative to probing, though still employing nucleic acid hybridisation, oligonucleotides designed to amplify DNA sequences from *Rht* genes may be used in PCR or other methods

involving amplification of nucleic acid, using routine procedures. See for instance "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, 1990, Academic Press, New York.

5

Preferred amino acid sequences suitable for use in the design of probes or PCR primers are sequences conserved (completely, substantially or partly) between *Rht* genes.

10 On the basis of amino acid sequence information, oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where appropriate, codon usage of the organism from which the candidate nucleic acid is derived. In particular, primers
15 and probes may be designed using information on conserved sequences apparent from, for example, Figure 3 and/or Figure 4, also Figure 10.

Where a full-length encoding nucleic acid molecule has not
20 been obtained, a smaller molecule representing part of the full molecule, may be used to obtain full-length clones. Inserts may be prepared for example from partial cDNA clones and used to screen cDNA libraries. The full-length clones isolated may be subcloned into vectors such as expression
25 vectors or vectors suitable for transformation into plants. Overlapping clones may be used to provide a full-length sequence.

The present invention also extends to nucleic acid encoding *Rht* or a homologue obtainable using a nucleotide sequence derived from Figure 2 or Figure 3a, and such nucleic acid obtainable using one or more, e.g. a pair, of primers
5 including a sequence shown in Table 1.

Also included within the scope of the present invention are nucleic acid molecules which encode amino acid sequences which are homologues of the polypeptide encoded by *Rht* of
10 *Triticum*. A homologue may be from a species other than *Triticum*.

Homology may be at the nucleotide sequence and/or amino acid sequence level. Preferably, the nucleic acid and/or amino
15 acid sequence shares homology with the sequence encoded by the nucleotide sequence of Figure 3a, preferably at least about 50%, or 60%, or 70%, or 80% or 85% homology, most preferably at least 90%, 92%, 95% or 97% homology. Nucleic acid encoding such a polypeptide may preferably share with
20 the *Triticum Rht* gene the ability to confer a particular phenotype on expression in a plant, preferably a phenotype which is GA responsive (i.e. there is a change in a characteristic of the plant on treatment with GA), such as the ability to inhibit plant growth where the inhibition is
25 antagonised by GA. As noted, *Rht* expression in a plant may affect one or more other characteristics of the plant. A preferred characteristic that may be shared with the *Triticum Rht* gene is the ability to complement a *Rht* null mutant

phenotype in a plant such as *Triticum*, such phenotype being resistance to the dwarfing effect of paclobutrazol. The *slender* mutant of barley maps to a location in the barley genome equivalent to that of *Rht* in the wheat genome. Such
5 mutant plants are strongly paclobutrazol resistant. The present inventors believe that the *slender* barley mutant is a null mutant allele of the orthologous gene to wheat *Rht*, allowing for complementation of the barley mutant with the wheat gene. Ability to complement a *slender* mutant in barley
10 may be a characteristic of embodiments of the present invention.

Some preferred embodiments of polypeptides according to the present invention (encoded by nucleic acid embodiments
15 according to the present invention) include the 17 amino acid sequence which is underlined in Figure 3b, or a contiguous sequence of amino acids residues with at least about 10 residues with similarity or identity with the respective corresponding residue (in terms of position) in 17 amino
20 acids which are underlined in Figure 3b, more preferably 11, 12, 13, 14, 15, 16 or 17 such residues, and/or the sequence DVAQKLEQLE, or a contiguous sequence of amino acids with at least about 5 residues with similarity or identity with the respective corresponding residue (in terms of position)
25 within DVAQKLEQLE, more preferably 6, 7, 8 or 9 such residues. Further embodiments include the 27 amino acid sequence DELLAALGYKVRASDMADVAQKLEQLE, or a contiguous sequence of amino acids residues with at least about 15

residues with similarity or identity with the respective corresponding residue (in terms of position) within this sequence, more preferably 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or 26 such residues.

5

As is well-understood, homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Similarity may be as defined and determined by the TBLASTN program, of Altschul et al. (1990) *J. Mol. Biol.* 215: 403-10, which is in standard use in the art, or more preferably GAP (Program Manual for the Wisconsin Package, Version 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, USA), which uses the algorithm of Needleman and Wunsch to align sequences. Suitable parameters for GAP include the default parameters, a gap creation penalty = 12 and gap extension penalty = 4, or gap creation penalty 3.00 and gap extension penalty 0.1. Homology may be over the full-length of the *Rht* sequence of Figure 3b, or may more preferably be over a contiguous sequence of 10 amino acids compared with DVAQKLEQLE, and/or a contiguous sequence of 17 amino acids, compared with the 17 amino acids underlined in Figure 3b, and/or a contiguous sequence of 27 amino acids compared with DELLAALGYKVRASDMADVAQKLEQLE, or a

longer sequence, e.g. about 30, 40, 50 or more amino acids, compared with the amino acid sequence of Figure 3b and preferably including the underlined 17 amino acids and/or DVAQKLEQLE.

5

At the nucleic acid level, homology may be over the full-length or more preferably by comparison with the 30 nucleotide coding sequence within the sequence of Figure 3a and encoding the sequence DVAQKLEQLE and/or the 51 nucleotide
10 coding sequence within the sequence of Figure 3a and encoding the 17 amino acid sequence underlined in Figure 3b, or a longer sequence, e.g. about, 60, 70, 80, 90, 100, 120, 150 or more nucleotides and preferably including the 51 nucleotide of Figure 3 which encodes the underlined 17 amino acid
15 sequence of Figure 3b.

As noted, similarity may be as defined and determined by the TBLASTN program, of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-10, which is in standard use in the art, or the standard
20 program BestFit, which is part of the Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). BestFit makes an optimal alignment of the best segment of similarity between two sequences. Optimal alignments are
25 found by inserting gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman (*Adv. Appl. Math.* (1981) 2: 482-489). Other algorithms include GAP, which uses the Needleman and Wunsch algorithm to

align two complete sequences that maximizes the number of matches and minimizes the number of gaps. As with any algorithm, generally the default parameters are used, which for GAP are a gap creation penalty = 12 and gap extension
5 penalty = 4. The algorithm FASTA (which uses the method of Pearson and Lipman (1988) *PNAS USA* 85: 2444-2448) is a further alternative.

Use of either of the terms "homology" and "homologous" herein
10 does not imply any necessary evolutionary relationship between compared sequences, in keeping for example with standard use of terms such as "homologous recombination" which merely requires that two nucleotide sequences are sufficiently similar to recombine under the appropriate
15 conditions. Further discussion of polypeptides according to the present invention, which may be encoded by nucleic acid according to the present invention, is found below.

The present invention extends to nucleic acid that hybridizes
20 with any one or more of the specific sequences disclosed herein under stringent conditions.

Hybridisation may be determined by probing with nucleic acid and identifying positive hybridisation under suitably
25 stringent conditions (in accordance with known techniques). For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which

can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

- 5 Binding of a probe to target nucleic acid (e.g. DNA) may be measured using any of a variety of techniques at the disposal of those skilled in the art. For instance, probes may be radioactively, fluorescently or enzymatically labelled. Other methods not employing labelling of probe include
- 10 examination of restriction fragment length polymorphisms, amplification using PCR, RNAase cleavage and allele specific oligonucleotide probing.

- Probing may employ the standard Southern blotting technique.
- 15 For instance DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the
- 20 filter and binding determined. DNA for probing may be prepared from RNA preparations from cells by techniques such as reverse-transcriptase- PRC.

- Preliminary experiments may be performed by hybridising under
- 25 low stringency conditions various probes to Southern blots of DNA digested with restriction enzymes. For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of

hybridisations identified as positive which can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain. Suitable conditions would be achieved when a large number of hybridising fragments were obtained while the background hybridisation was low. Using these conditions nucleic acid libraries, e.g. cDNA libraries representative of expressed sequences, may be searched. Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on.

For instance, screening may initially be carried out under conditions, which comprise a temperature of about 37°C or more, a formamide concentration of less than about 50%, and a moderate to low salt (e.g. Standard Saline Citrate ('SSC') = 0.15 M sodium chloride; 0.15 M sodium citrate; pH 7) concentration.

Alternatively, a temperature of about 50°C or more and a high salt (e.g. 'SSPE' = 0.180 mM sodium chloride; 9 mM disodium hydrogen phosphate; 9 mM sodium dihydrogen phosphate; 1 mM sodium EDTA; pH 7.4). Preferably the screening is carried out at about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5 X SSC, or a temperature of about 50°C and a salt concentration of about 2 X SSPE. These

conditions will allow the identification of sequences which have a substantial degree of homology (similarity, identity) with the probe sequence, without requiring the perfect homology for the identification of a stable hybrid.

5

Suitable conditions include, e.g. for detection of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na₂HPO₄, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For

10 detection of sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na₂HPO₄, pH 7.2, 6.5% SDS, 10% dextran sulphate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

15

Conditions that may be used to differentiate *Rht* genes and homologues from others may include the following procedure:

First and second DNA molecules are run on an agarose gel,
20 blotted onto a membrane filter (Sambrook et al, 1989). The filters are incubated in prehybridization solution [6xSSC, 5x Denhart's solution, 20 mM Tris-HCl, 0.1% SDS, 2mM EDTA, 20 µg/ml Salmon sperm DNA] at 65°C for 5 hours, with constant shaking. Then, the solution is replaced with 30 ml of the
25 same, containing the radioactively-labelled second DNA (prepared according to standard techniques; see Sambrook et al, 1989), and incubated overnight at 65°C, with constant shaking. The following morning the filters are rinsed (one

rinse with 3xSSC-0.1% SDS solution); and then washed: one wash at 65°C, for 25 minutes, with 3x SSC-0.1% SDS solution; and a second wash, at the same temperature and for the same time, with 0.1xSSC-0.1% SDS. Then the radioactive pattern on the filter is recorded using standard techniques (see Sambrook *et al*, 1989).

If need be, stringency can be increased by increasing the temperature of the washes, and/or reducing or even omitting altogether, the SSC in the wash solution.

(SSC is 150 mM NaCl, 15 mM sodium citrate. 50x Denhart's solution is 1% (w/v) ficoll, 1% polyvinylpyrrolidone, 1% (w/v) bovine serum albumin.)

15

Homologues to *rht* mutants are also provided by the present invention. These may be mutants where the wild-type includes the 17 amino acids underlined in Figure 3b, or a contiguous sequence of 17 amino acids with at least about 10 (more preferably 11, 12, 13, 14, 15, 16 or 17) which have similarity or identity with the corresponding residue in the 17 amino acid sequence underlined in Figure 3, but the mutant does not. Similarly, such mutants may be where the wild-type includes DVAQKLEQLE or a contiguous sequence of 10 amino acids with at least about 5 (more preferably 6, 7, 8 or 9) which have similarity or identity with the corresponding residue in the sequence DVAQKLEQLE, but the mutant does not. Nucleic acid encoding such mutant polypeptides may on

expression in a plant confer a phenotype which is insensitive or unresponsive to treatment of the plant with GA, that is a mutant phenotype which is not overcome or there is no reversion to wild-type phenotype on treatment of the plant
5 with GA (though there may be some response in the plant on provision or depletion of GA).

A further aspect of the present invention provides a nucleic acid isolate having a nucleotide sequence encoding a
10 polypeptide which includes an amino acid sequence which is a mutant, allele, derivative or variant sequence of the *Rht* amino acid sequence of the species *Triticum Aestivum* shown in Figure 3b, or is a homologue of another species or a mutant, allele, derivative or variant thereof, wherein said mutant,
15 allele, derivative, variant or homologue differs from the amino acid sequence shown in Figure 3b by way of insertion, deletion, addition and/or substitution of one or more amino acids, as obtainable by producing transgenic plants by transforming plants which have a *Rht* null mutant phenotype,
20 which phenotype is resistance to the dwarfing effect of paclobutrazol, with test nucleic acid, causing or allowing expression from test nucleic acid within the transgenic plants, screening the transgenic plants for those exhibiting complementation of the *Rht* null mutant phenotype to identify
25 test nucleic acid able to complement the *Rht* null mutant, deleting from nucleic acid so identified as being able to complement the *Rht* null mutant a nucleotide sequence encoding the 17 amino acid sequence underlined in Figure 3b or a

contiguous 17 amino acid sequence in which at least 10 residues have similarity or identity with the respective amino acid in the corresponding position in the 17 amino acid sequence underlined in Figure 3b, more preferably 11, 12, 13, 14, 15, 16 or 17, and/or a nucleotide sequence encoding DVAQKLEQLE or a contiguous sequence of 10 amino acids with at least about 5 (more preferably 6, 7, 8 or 9) which have similarity or identity with the corresponding residue in the sequence DVAQKLEQLE.

10

A cell containing nucleic acid of the present invention represents a further aspect of the invention, particularly a plant cell, or a bacterial cell.

15 The cell may comprise the nucleic acid encoding the protein by virtue of introduction into the cell or an ancestor thereof of the nucleic acid, e.g. by transformation using any suitable technique available to those skilled in the art.

20 Also according to the invention there is provided a plant cell having incorporated into its genome nucleic acid as disclosed.

Where a complete naturally occurring sequence is employed the 25 plant cell may be of a plant other than the natural host of the sequence.

The present invention also provides a plant comprising such a

plant cell.

Also according to the invention there is provided a plant cell having incorporated into its genome a sequence of
5 nucleotides as provided by the present invention, under operative control of a regulatory sequence for control of expression. A further aspect of the present invention provides a method of making such a plant cell involving introduction of a vector comprising the sequence of
10 nucleotides into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the sequence of nucleotides into the genome.

A plant according to the present invention may be one which
15 does not breed true in one or more properties. Plant varieties may be excluded, particularly registrable plant varieties according to Plant Breeders' Rights. It is noted that a plant need not be considered a "plant variety" simply because it contains stably within its genome a transgene,
20 introduced into a cell of the plant or an ancestor thereof.

In addition to a plant, the present invention provides any clone of such a plant, seed, selfed or hybrid progeny and descendants, and any part of any of these, such as cuttings,
25 seed. The invention provides any plant propagule, that is any part which may be used in reproduction or propagation, sexual or asexual, including cuttings, seed and so on. Also encompassed by the invention is a plant which is a sexually

or asexually propagated off-spring, clone or descendant of such a plant, or any part or propagule of said plant, off-spring, clone or descendant.

5 The invention further provides a method of influencing the characteristics of a plant comprising expression of a heterologous *Rht* or *rht* gene sequence (or mutant, allele, derivative or homologue thereof, as discussed) within cells of the plant. The term "heterologous" indicates that the
10 gene/sequence of nucleotides in question have been introduced into said cells of the plant, or an ancestor thereof, using genetic engineering, that is to say by human intervention, which may comprise transformation. The gene may be on an extra-genomic vector or incorporated, preferably stably, into
15 the genome. The heterologous gene may replace an endogenous equivalent gene, ie one which normally performs the same or a similar function in control of growth and/or development, or the inserted sequence may be additional to an endogenous gene. An advantage of introduction of a heterologous gene is
20 the ability to place expression of the gene under the control of a promoter of choice, in order to be able to influence gene expression, and therefore growth and/or development of the plant according to preference. Furthermore, mutants and derivatives of the wild-type gene may be used in place of the
25 endogenous gene. The inserted gene may be foreign or exogenous to the host cell, e.g. of another plant species.

The principal characteristic which may be altered using the

present invention is growth.

According to the model of the *Rht* gene as a growth repressor, under-expression of the gene may be used to promote growth, at least in plants which have only one endogenous gene conferring *Rht* function (not for example *Arabidopsis* which has endogenous homologues which would compensate). This may involve use of anti-sense or sense regulation. Taller plants may be made by knocking out *Rht* or the relevant homologous gene in the plant of interest. Plants may be made which are resistant to compounds which inhibit GA biosynthesis, such as paclobutrazol, for instance to allow use of a GA biosynthesis inhibitor to keep weeds dwarf but let crop plants grow tall.

Over-expression of a *Rht* gene may lead to a dwarf plant which is correctable by treatment with GA, as predicted by the *Rht* repression model.

Since *rht* mutant genes are dominant on phenotype, they may be used to make GA-insensitive dwarf plants. This may be applied for example to any transformable crop-plant, tree or fruit-tree species. It may provide higher yield/reduced lodging like *Rht* wheat. In rice this may provide GA-insensitive rice resistant to the Bakane disease, which is a problem in Japan and elsewhere. Dwarf ornamentals may be of value for the horticulture and cut-flower markets. Sequence manipulation may provide for varying degrees of severity of dwarfing, GA-insensitive phenotype, allowing tailoring of the

degree of severity to the needs of each crop-plant or the wishes of the manipulator. Over-expression of *rht*-mutant sequences is potentially the most useful.

5 A second characteristic that may be altered is plant development, for instance flowering. In some plants, and in certain environmental conditions, a GA signal is required for floral induction. For example, GA-deficient mutant *Arabidopsis* plants grown under short day conditions will not
10 flower unless treated with GA: these plants do flower normally when grown under long day conditions. *Arabidopsis gai* mutant plants show delayed flowering under short day conditions: severe mutants may not flower at all. Thus, for instance by *Rht* or *rht* gene expression or over-expression,
15 plants may be produced which remain vegetative until given GA treatment to induce flowering. This may be useful in horticultural contexts or for spinach, lettuce and other crops where suppression of bolting is desirable.

20 The nucleic acid according to the invention may be placed under the control of an externally inducible gene promoter to place the *Rht* or *rht* coding sequence under the control of the user.

25 The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied

stimulus. The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression increases upon application of the relevant stimulus by an amount effective to alter a phenotypic characteristic. Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about a desired phenotype (and may in fact be zero). Upon application of the stimulus, expression is increased (or switched on) to a level which brings about the desired phenotype.

Suitable promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is expressed at a high level in virtually all plant tissues (Benfey et al, 1990a and 1990b); the maize glutathione-S-transferase isoform II (GST-II-27) gene promoter which is activated in response to application of exogenous safener (WO93/01294, ICI Ltd); the cauliflower meri 5 promoter that is expressed in the vegetative apical meristem as well as several well localised positions in the plant body, eg inner phloem, flower primordia, branching

points in root and shoot (Medford, 1992; Medford et al, 1991) and the *Arabidopsis thaliana* *LEAFY* promoter that is expressed very early in flower development (Weigel et al, 1992).

5 The GST-II-27 gene promoter has been shown to be induced by certain chemical compounds which can be applied to growing plants. The promoter is functional in both monocotyledons and dicotyledons. It can therefore be used to control gene expression in a variety of genetically modified plants,
10 including field crops such as canola, sunflower, tobacco, sugarbeet, cotton; cereals such as wheat, barley, rice, maize, sorghum; fruit such as tomatoes, mangoes, peaches, apples, pears, strawberries, bananas, and melons; and vegetables such as carrot, lettuce, cabbage and onion. The
15 GST-II-27 promoter is also suitable for use in a variety of tissues, including roots, leaves, stems and reproductive tissues.

Accordingly, the present invention provides in a further
20 aspect a gene construct comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention, such as the *Rht* gene of *Triticum* a homologue from another plant species or any mutant, derivative or allele thereof. This enables control of
25 expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a construct into a plant cell and/or induction of expression of a construct within a plant cell,

by application of a suitable stimulus, an effective exogenous inducer. The promoter may be the GST-II-27 gene promoter or any other inducible plant promoter.

- 5 When introducing a chosen gene construct into a cell, certain considerations must be taken into account, well known to those skilled in the art. The nucleic acid to be inserted should be assembled within a construct which contains effective regulatory elements which will drive transcription.
- 10 There must be available a method of transporting the construct into the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material either will or will not occur. Finally, as far as plants are concerned the target cell type must be such that
- 15 cells can be regenerated into whole plants.

Selectable genetic markers may be used consisting of chimaeric genes that confer selectable phenotypes such as resistance to antibiotics such as kanamycin, hygromycin,

20 phosphinotricin, chlorsulfuron, methotrexate, gentamycin, spectinomycin, imidazolinones and glyphosate.

An aspect of the present invention is the use of nucleic acid according to the invention in the production of a transgenic

25 plant.

A further aspect provides a method including introducing the nucleic acid into a plant cell and causing or allowing

incorporation of the nucleic acid into the genome of the cell.

Any appropriate method of plant transformation may be used to
5 generate plant cells comprising nucleic acid in accordance with the present invention. Following transformation, plants may be regenerated from transformed plant cells and tissue.

Successfully transformed cells and/or plants, i.e. with the
10 construct incorporated into their genome, may be selected following introduction of the nucleic acid into plant cells, optionally followed by regeneration into a plant, e.g. using one or more marker genes such as antibiotic resistance (see above).

15

Plants transformed with the DNA segment containing the sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells using any suitable
20 technology, such as a disarmed Ti-plasmid vector carried by *Agrobacterium* exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616) microinjection (WO 92/09696, WO
25 94/00583, EP 331083, EP 175966, Green et al. (1987) *Plant Tissue and Cell Culture*, Academic Press), electroporation (EP 290395, WO 8706614 Gelvin Debeyser - see attached) other forms of direct DNA uptake (DE 4005152, WO 9012096, US

4684611), liposome mediated DNA uptake (e.g. Freeman et al. *Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g. Kindle, *PNAS U.S.A.* 87: 1228 (1990d). Physical methods for the transformation of plant cells are reviewed in Oard, 5 1991, *Biotech. Adv.* 9: 1-11.

Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine 10 production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, et al. (1988) *Plant Cell Rep.* 7, 379-384; Zhang, et al. (1988) *Theor Appl Genet* 76, 835-840; Shimamoto, et al. (1989) *Nature* 338, 274-276; Datta, 15 et al. (1990) *Bio/Technology* 8, 736-740; Christou, et al. (1991) *Bio/Technology* 9, 957-962; Peng, et al. (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, et al. (1992) *Plant Cell Rep.* 11, 585-591; Li, et al. (1993) *Plant Cell Rep.* 12, 250-255; Rathore, et al. 20 (1993) *Plant Molecular Biology* 21, 871-884; Fromm, et al. (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, et al. (1990) *Plant Cell* 2, 603-618; D'Halluin, et al. (1992) *Plant Cell* 4, 1495-1505; Walters, et al. (1992) *Plant Molecular Biology* 18, 189-200; Koziel, et al. (1993) *Biotechnology* 11, 194-200; 25 Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, et al. (1993) *Plant Physiology* 102, 1077-1084; Somers, et al. (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In particular, *Agrobacterium* mediated transformation is now

emerging also as an highly efficient transformation method in monocots (Hiei et al. (1994) *The Plant Journal* 6, 271-282).

The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, et al. (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).

10

Microprojectile bombardment, electroporation and direct DNA uptake are preferred where *Agrobacterium* is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, eg bombardment with *Agrobacterium* coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* (EP-A-486233).

20 *Brassica napus* transformation is described in Moloney et al. (1989) *Plant Cell Reports* 8: 238-242.

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewed in Vasil et al., *Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and*

III, Laboratory Procedures and Their Applications, Academic Press, 1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

- 5 The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the
- 10 particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.
- 15 In the present invention, over-expression may be achieved by introduction of the nucleotide sequence in a sense orientation. Thus, the present invention provides a method of influencing a characteristic of a plant, the method comprising causing or allowing expression of nucleic acid
- 20 according to the invention from that nucleic acid within cells of the plant.

Under-expression of the gene product polypeptide may be achieved using anti-sense technology or "sense regulation".

- 25 The use of anti-sense genes or partial gene sequences to down-regulate gene expression is now well-established. DNA is placed under the control of a promoter such that transcription of the "anti-sense" strand of the DNA yields

RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene. For double-stranded DNA this is achieved by placing a coding sequence or a fragment thereof in a "reverse orientation" under the control of a promoter. The complementary anti-sense RNA sequence is thought then to bind with mRNA to form a duplex, inhibiting translation of the endogenous mRNA from the target gene into protein. Whether or not this is the actual mode of action is still uncertain. However, it is established fact that the technique works. See, for example, Rothstein et al, 1987; Smith et al, (1988) *Nature* 334, 724-726; Zhang et al, (1992) *The Plant Cell* 4, 1575-1588, English et al., (1996) *The Plant Cell* 8, 179-188. Antisense technology is also reviewed in Bourque, (1995), *Plant Science* 105, 125-149, and Flavell, (1994) *PNAS USA* 91, 3490-3496.

The complete sequence corresponding to the coding sequence in reverse orientation need not be used. For example fragments of sufficient length may be used. It is a routine matter for the person skilled in the art to screen fragments of various sizes and from various parts of the coding sequence to optimise the level of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG codon, and perhaps one or more nucleotides upstream of the initiating codon. A further possibility is to target a regulatory sequence of a gene, e.g. a sequence that is characteristic of one or more genes in one or more pathogens against which resistance is desired. A suitable fragment

may have at least about 14-23 nucleotides, e.g. about 15, 16 or 17, or more, at least about 25, at least about 30, at least about 40, at least about 50, or more. Other fragments may be at least about 300 nucleotides, at least about 400
5 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides or more. Such fragments in the sense orientation may be used in co-suppression (see below).

10 Total complementarity of sequence is not essential, though may be preferred. One or more nucleotides may differ in the anti-sense construct from the target gene. It may be preferred for there to be sufficient homology for the respective anti-sense and sense RNA molecules to hybridise,
15 particularly under the conditions existing in a plant cell.

Thus, the present invention also provides a method of influencing a characteristic of a plant, the method comprising causing or allowing anti-sense transcription from
20 nucleic acid according to the invention within cells of the plant.

When additional copies of the target gene are inserted in sense, that is the same, orientation as the target gene, a
25 range of phenotypes is produced which includes individuals where over-expression occurs and some where under-expression of protein from the target gene occurs. When the inserted gene is only part of the endogenous gene the number of

under-expressing individuals in the transgenic population increases. The mechanism by which sense regulation occurs, particularly down-regulation, is not well-understood.

However, this technique is also well-reported in scientific
5 and patent literature and is used routinely for gene control.
See, for example, See, for example, van der Krol *et al.*,
(1990) *The Plant Cell* 2, 291-299; Napoli *et al.*, (1990) *The
Plant Cell* 2, 279-289; Zhang *et al.*, (1992) *The Plant Cell* 4,
1575-1588, and US-A-5,231,020.

10

Thus, the present invention also provides a method of
influencing a characteristic of a plant, the method
comprising causing or allowing expression from nucleic acid
according to the invention within cells of the plant. This
15 may be used to influence growth.

Aspects and embodiments of the present invention will now be
illustrated, by way of example, with reference to the
accompanying figures. Further aspects and embodiments will
20 be apparent to those skilled in the art. All documents
mentioned in this text are incorporated herein by reference.

The following Figures are included herein:

25 **Figure 1:** Alignment of N-terminus predicted GAI amino acid
sequence (Gai) with rice EST D39460 (0830), with a region of
homology outlined in black.

Figure 2: DNA sequences from C15-1, 14a1 and 5a1.

Figure 2a shows a consensus DNA sequence cDNA C15-1 (obtained via single-pass sequencing).

5

Figure 2b shows data from original DNA sequencing runs from 14a1 (single-pass).

Figure 2c shows data from original DNA sequencing runs from 10 5a1 (single-pass).

Figure 3: Rht sequences.

Figure 3a shows a composite DNA sequence of wheat *Rht* gene 15 derived from data in Figure 2, including coding sequence.

Figure 3b shows an alignment of the entire predicted Rht protein sequence encoded by the coding sequence of Figure 2 (rht) with the entire predicted GAI protein sequence of 20 *Arabidopsis* (Gai). Regions of sequence identity are highlighted in black.

Figure 4: D39460 sequence.

25 Figure 4a shows DNA sequence (single-pass) of rice cDNA D39460. This cDNA is an incomplete, partial clone, missing the 3' end of the mRNA from which it is derived.

Figure 4b shows alignment of the entire predicted Rht protein sequence (wheat - encoded by the coding sequence of Figure 2) with that of GAI (Gai) and rice protein sequence predicted from DNA sequence in Figure 4a (Rice). Regions of amino acid identity are highlighted in black; some conservative substitutions are shaded.

Figure 5: The basic carbon-ring structure of gibberellins.

10 **Figure 6:** Rice EST sequence

Figure 6a shows the nucleotide sequence of rice EST D39460, as determined by the present inventors.

15 **Figure 6b** shows the predicted amino acid sequence encoded by the rice EST sequence of Figure 6a.

Figure 7: Wheat C15-1 cDNA

20 **Figure 7a** shows the nucleotide sequence of the wheat C15-1 cDNA.

Figure 7b shows the predicted amino acid sequence of the wheat C15-1 cDNA of Figure 7a.

25

Figure 8: Wheat 5a1 genomic clone

Figure 8a shows the nucleotide sequence of the 5a1 wheat

genomic clone.

Figure 8b shows the predicted amino acid sequence of the 5a1 wheat genomic clone of Figure 8a.

5

Figure 9: Maize 1a1 genomic clone

Figure 9a shows the nucleotide sequence of the 1a1 maize genomic clone, i.e. *D8*.

10

Figure 9b shows the amino acid sequence of the maize 1a1 genomic clone of Figure 9a.

Figure 10 shows a PRETTYBOX alignment of amino acid sequences of the maize *D8* polypeptide with, the wheat *Rht* polypeptide the rice EST sequence determined by the present inventors and the *Arabidopsis thaliana* *Gai* polypeptide.

Figure 11: Sequences of maize *D8* alleles

20

Figure 11a shows a partial nucleotide sequence of the maize *D8-1* allele.

Figure 11b shows a partial amino acid sequence of the maize *D8-1* allele.

Figure 11c shows a partial nucleotide sequence of the maize *D8-2023* allele.

Figure 11d shows a partial amino acid sequence of the maize D8-2023 allele.

Figure 12: Wheat *rht-10* allele

5

Figure 12a shows a partial nucleotide sequence of the wheat *rht-10* allele.

Figure 12b shows a partial amino acid sequence of the wheat
10 *rht-10* allele.

Previously, we cloned the *GAI* gene of *Arabidopsis*
(PCT/GB97/00390 - WO97/29123 published 14 August 1997).

15 Comparison of the DNA sequences of the wild-type (*GAI*) and
mutant (*gai*) alleles showed that *gai* encodes a mutant
predicted protein product (*gai*) which lacks a segment of 17
amino acids from close to the N-terminus of the protein.
Screening of the DNA sequence databases with the *GAI* sequence
20 revealed the existence of a rice EST (D39460) which contains
a region of sequence very closely related to that of the
segment that is deleted from *GAI* in the *gai* protein. A
comparison of the predicted amino acid sequences from the
region DELLA to EQLE are identical in both sequences. The
25 two differences (V/A; E/D) are conservative substitutions, in
which one amino acid residue is replaced by another having
very similar chemical properties. In addition, the region of
identity extends beyond the boundary of the deletion region

in the *gai* protein. The sequence DVAQKLEQLE is not affected by the deletion in *gai*, and yet is perfectly conserved between the *GAI* and D39460 sequences (Figure 1).

5 An approximately 700 bp *SalI*-*NotI* subfragment of D39460 was used in low-stringency hybridization experiments to isolate hybridizing clones from wheat cDNA and genomic libraries (made from DNA from the variety Chinese Spring) and from a maize genomic library (made from line B73N). Several wheat
10 clones were isolated, including C15-1 and C15-10 (cDNAs), and 5a1 and 14a1 (genomic clones). Clone C15-1 has been used in gene mapping experiments. Nullisomic-tetrasomic analysis showed that clone C15-1 hybridizes to genomic DNA fragments derived from wheat chromosomes 4A, 4B and 4D. This is
15 consistent with clone C15-1 containing *Rht* sequence, since the *Rht* loci map to the group 4 chromosomes. Furthermore, recombinant analysis using a population segregating for the *Rht-D1b* (formerly *Rht2*) allele identified a hybridizing fragment that displayed perfect co-segregation with the
20 mutant allele. This placed the genomic location of the gene encoding the mRNA sequence in cDNA C15-1 within a 2 cM segment (that was already known to contain *Rht*) of the group 4 chromosomes, and provides strong evidence that the cDNA and genomic clones do indeed contain the *Rht* gene. The maize *D8*
25 DNA sequence disclosed herein is from subcloned contiguous 1.8 kb and 3.0 kb *SalI* fragments (cloned into Bluescript™ SK⁺) from 1a1. The wheat *Rht* sequence disclosed herein is from a 5.7 kb *DraI* subfragment cloned into Bluescript™ SK⁺)

from clone 5a1.

Figure 2a gives the complete (single-pass) DNA sequence of cDNA C15-1. We have also obtained DNA sequence for C15-10; 5 it is identical with that of C15-1, and is therefore not shown. Figures 2b and 2c show original data from individual sequencing runs from clones 14a1 and 5a1. The sequences shown in Figure 2 can be overlapped to make a composite DNA sequence, shown in Figure 3a. This sequence displays strong 10 homology with that of *Arabidopsis GAI*, as revealed by a comparison of the amino acid sequence of a predicted translational product of the wheat sequence (*Rht*) with that of *GAI* (*GAI*), shown in Figure 3b. In particular, the predicted amino acid sequence of the presumptive *Rht* reveals 15 a region of near-identity with *GAI* over the region that is missing in *gai* (Figure 4). Figure 4 reveals that the homology that extends beyond the *gai* deletion region in the rice EST is also conserved in *Rht* (DVAQKLEQLE), thus indicating that this region, in addition to that found in the 20 *gai* deletion, is involved in GA signal-transduction. This region is not found in *SCR*, another protein that is related in sequence to *GAI* but which is not involved in GA signalling. The primers used in the above sequencing experiments are shown in Table 1.

25

Further confirmation that these sequences are indeed the wheat *Rht* and maize *D8* loci has been obtained by analysis of gene sequences from various mutant alleles, as follows.

The present inventors obtained and sequenced the clone identified on the database as the rice EST D39460, and the nucleotide and predicted amino acid sequences resulting from that work are shown in Figure 6a and Figure 6b respectively.

5

Previous work on the *GAI* gene of *Arabidopsis* showed that the *GAI* protein consists of two sections, an N-terminal half displaying no homology with any protein of known function, and a C-terminal half displaying extensive homology with the *Arabidopsis* SCR candidate transcription factor (Peng et al. (1997) *Genes and Development* 11: 3194-3205; PCT/GB97/00390). As described above, deletion of a portion of the N-terminal half of the protein causes the reduced GA-responses characteristic of the *gai* mutant allele (Peng et al., 1997; 15 PCT/GB97/00390). The inventors therefore predicted that if *D8* and *Rht* are respectively maize and wheat functional homologues (orthologues) of *Arabidopsis GAI*, then dominant mutant alleles of *D8* and *Rht* should also contain mutations affecting the N-terminal sections of the proteins that they 20 encode.

Previous reports describe a number of dominant mutant alleles at *D8* and at *Rht*, in particular *D8-1*, *D8-2023* and *Rht-D1c* (formerly *Rht10*) (Börner et al. (1996) *Euphytica* 89: 69-75; 25 Harberd and Freeling (1989) *Genetics* 121: 827-838; Winkler and Freeling (1994) *Planta* 193: 341-348). The present inventors therefore cloned the candidate *D8/Rht* genes from these mutants, and examined by DNA sequencing the portion of

the gene that encodes the N-terminal half of the protein.

A fragment of the candidate *D8* or *Rht* genes that encodes a portion of the N-terminal half of the D8/Rht protein was amplified via PCR from genomic DNA of plants containing *D8-1*, *D8-2023* and *Rht-D1c*, using the following primers for amplification: for *D8-1*, primers ZM-15 and ZM-24; for *D8-2023*, primers ZM-9 and ZM-11; for *Rht-D1c*, nested PCR was performed using Rht-15 and Rht-26 followed by Rht-16 and Rha-
2. PCR reactions were performed using a Perkin Elmer geneAmp XL PCR kit, using the following conditions: reactions were incubated at 94°C for 1 min, then subjected to 13 cycles of 94°C, 15 sec - x°C for 15 sec - 69°C 5 min (where x is reduced by 1°C per cycle starting at 64°C and finishing at 52°C), then 25 cycles of 94°C, 15 sec - 53°C, 15 sec - 65°C, 5 min, then 10 min at 70°C. These fragments were then cloned into the pGEM^R-T Easy vector (Promega, see Technical Manual), and their DNA sequences were determined.

Mutations were found in the candidate *D8* and *Rht* genes in each of the above mutants. The *D8-1* mutation is an in-frame deletion which removes amino acids VAQK (55-59) and adds a G (see sequence in Figure 11a and Figure 11b). This deletion overlaps with the conserved DVAQKLEQLE homology block described above. *D8-2023* is another in-frame deletion mutation that removes amino acids LATDTVHYNPSD (87-98) from the N-terminus of the D8 protein (see Figure 11c and Figure 11d). This deletion does not overlap with the deletion in

gai or D8-1, but covers another region that is highly conserved between GAI, D8 and Rht (see Figure 10). Finally, *Rht-D1c* contains another small in-frame deletion that removes amino acids LNAPPPPLPPAPQ (109-121) in the N-terminal region of the mutant Rht protein that it encodes (see Figure 12a and Figure 12b) (LN-P is conserved between GAI, D8 and Rht, see Figure 10).

Thus all of the above described mutant alleles are dominant, and confer dwarfism associated with reduced GA-response. All three of these alleles contain deletion mutations which remove a portion of the N-terminal half of the protein that they encode. These observations demonstrate that the *D8* and *Rht* genes of maize and wheat have been cloned.

TABLE 1 - Primers used in the sequencing of Rht

<u>Name</u>		<u>Sequence</u>	
		<u>Sense</u>	
5	15-L	TTTGCGCCAATTATTGGCCAGAGATAGATAGAGAG	Forward
	16-L	GTGGCGGCATGGGTTCGTCCGAGGACAAGATGATG	Forward
	23-L	CATGGAGGCGGTGGAGAACTGGGAACGAAGAAGGG	Reverse
	26-L	CCCGGCCAGGCGCCATGCCGAGGTGGCAATCAGGG	Reverse
10	3-L	GGTATCTGCTTCACCAGCGCCTCCGCGGCGGAGAG	Reverse
	9-L	ATCGGCCGCGAGCGCGTAGATGCTGCTGGAGGAGTC	Reverse
	RHA-1	CTGGTGAAGCAGATACCCTTGC	Forward
	RHA-2	CTGGTTGGCGGTGAAGTGCG	Reverse
	RHA-3	GCAAGGGTATCTGCTTCACCAGC	Reverse
15	RHA-5	CGCACTTCACCGCCAACCAG	Forward
	RHA-6	TTGTGATTTGCCTCCTGTTTTCC	Forward
	RHA-7	CCGTGCGCCCCCGTGCGGCCAG	Forward
	RHA-8	AGGCTGCCTGACGCTGGGGTTGC	Forward
	RHT-9	GATCGGCCGCGAGCGCGTAGATGC	Reverse
20	RHT-10	GATCCCGCACGGAGTCGGCGGACAG	Reverse
	RHT-12	TCCGACAGCATGCTCTCGACCCAAG	Reverse
	RHT-13	TTCCGTCCGTCTGGCGTGAAGAGG	Forward
	RHT-14	AAATCCCGAACCCGCCCCCAGAAC	Forward
	RHT-15	GCGCCAATTATTGGCCAGAGATAG	Forward
25	RHT-16	GGCATGGGTTCGTCCGAGGACAAG	Forward
	RHT-18	TTGTCCTCGGACGAACCCATGCCG	Reverse
	RHT-19	GATCCAAATCCCGAACCCGCCC	Forward
	RHT-20	GTAGATGCTGCTGGAGGAGTCG	Reverse
	RHT-21	GTCGTCCATCCACCTCTTCACG	Reverse
30	RHT-22	GCCAGAGATAGATAGAGAGGCG	Forward
	RHT-23	TAGGGCTTAGGAGTTTTACGGG	Reverse
	RHT-24	CGGAGTCGGCGGACAGGTCGGC	Reverse
	RHT-25	CGGAGAGGTTCTCCTGCTGCACGGC	Reverse
	RHT-26	TGTGCAACCCCAGCGTCAGGCAG	Reverse
35	RHT-27	GCGGCCTCGTCGCCGCCACGCTC	Forward
	RHT-28	TGGCGGCGACGAGGCCGCGGTAC	Reverse
	RHT-29	AAGAATAAGGAAGAGATGGAGATGGTTG	Reverse
	RHT-30	TCTGCAACGTGGTGGCCTGCGAG	Forward
	RHT-31	CCCCTCGCAGGCCACCACGTTGC	Reverse
40	RHT-32	TTGGGTCGAGAGCATGCTGTCGGAG	Forward

TABLE 2 - Primers used in the sequence of D-8 clones

	<u>Name</u>	<u>Sequence</u>	<u>Sense</u>
5	ZM-8	GGCGATGACACGGATGACG	Forward
	ZM-9	CTTGCGCATGGCACCGCCCTGCGACGAAG	Reverse
	ZM-10	CCAGCTAATAATGGCTTGCGCGCCTCG	Reverse
	ZM-11	TATCCCAGAACCGAAACCGAG	Forward
	ZM-12	CGGCGTCTTGGTACTCGCGCTTCATG	Reverse
10	ZM-13	TGGGCTCCCGCGCCGAGTCCGTGGAC	Reverse
	ZM-14	CTCCAAGCCTCTTGCGCTGACCGAGATCGAG	Forward
	ZM-15	TCCACAGGCTCACCAGTCACCAACATCAATC	Forward
	ZM-16	ACGGTACTGGAAGTCCACGCGGATGGTGTG	Reverse
	ZM-17	CGCACACCATCCGCGTGGACTTCCAGTAC	Forward
15	ZM-18	CTCGGCCGGCAGATCTGCAACGTGGTG	Forward
	ZM-19	TTGTGACGGTGGACGATGTGGACGCGAGCCTTG	Reverse
	ZM-20	GGACGCTGCGACAAACCGTCCATCGATCCAAC	Forward
	ZM-21	TCCGAAATCATGAAGCGCGAGTACCAAGAC	Forward
	ZM-22	TCGGGTACAAGGTGCGTTCGTCCGATATG	Forward
20	ZM-23	ATGAAGCGCGAGTACCAAGAC	Forward
	ZM-24	GTGTGCCTTGATGCGGTCCAGAAG	Reverse
	ZM-25	AACCACCCCTCCCTGATCACGGAG	Reverse
	ZM-27	CACTAGGAGCTCCGTGGTCGAAGCTG	Forward
	ZM-28	GCTGCGCAAGAAGCCGGTGCAGCTC	Reverse
25	ZM-29	AGTACACTTCCGACATGACTTG	Reverse

CLAIMS:

1. An isolated polynucleotide encoding a polypeptide which comprises the amino acid sequence DELLAALGYKVRASDMA and which on expression in a *Triticum Aestivum* plant provides
5 inhibition of growth of the plant, which inhibition is antagonised by gibberellin.
2. An isolated polynucleotide according to claim 1 wherein the polypeptide includes the amino acid sequence of a *Rht*
10 polypeptide obtainable from *Triticum Aestivum*.
3. An isolated polynucleotide according to claim 2 which includes the nucleotide sequence of nucleic acid obtainable from *Triticum Aestivum* encoding the *Rht* polypeptide, the
15 nucleotide sequence including
GACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCG.
4. An isolated polynucleotide encoding a polypeptide which comprises the amino acid sequence shown in Figure 8b.
20
5. An isolated polynucleotide according to claim 4 which has the coding nucleotide sequence shown in Figure 8a.
6. An isolated polynucleotide encoding a polypeptide which
25 on expression in a plant provides inhibition of growth of the plant, which inhibition is antagonised by gibberellin, wherein the polypeptide has an amino acid sequence which shows at least 80% similarity with the amino acid sequence of

the *Rht* polypeptide of *Triticum Aestivum* encoded by nucleic acid obtainable from *Triticum Aestivum* which includes the nucleotide sequence

GACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCG.

5

7. An isolated polynucleotide according to claim 6 wherein said polypeptide includes the amino acid sequence

DELLAALGYKVRASDMA.

10 8. An isolated polynucleotide according to claim 6 wherein said polypeptide includes a contiguous sequence of 17 amino acids in which at least 10 residues show amino acid similarity or identity with the residue in the corresponding position in the amino acid sequence DELLAALGYKVRASDMA.

15

9. An isolated polynucleotide according to claim 8 wherein said polypeptide includes a contiguous sequence of 17 amino acids in which 16 residues show amino acid identity with the residue in the corresponding position in the amino acid

20 sequence DELLAALGYKVRASDMA.

10. An isolated polynucleotide according to claim 9 wherein said polypeptide includes the amino acid sequence shown in Figure 9b for the maize D8 polypeptide.

25

11. An isolated polynucleotide according to claim 10 which has the coding nucleotide sequence shown in Figure 9a.

12. An isolated polynucleotide according to claim 9 wherein said polypeptide includes the amino acid sequence shown in Figure 6b.

5 13. An isolated polynucleotide according to claim 12 which has the coding nucleotide sequence shown in Figure 6a.

14. An isolated polynucleotide encoding a polypeptide which on expression in a plant confers a phenotype on the plant
10 which is gibberellin-unresponsive dwarfism or which on expression in a *rht* null mutant phenotype plant complements the *rht* null mutant phenotype, such *rht* null mutant phenotype being resistance to the dwarfing effect of paclobutrazol, wherein the polypeptide has an amino acid sequence which
15 shows at least 80% similarity with the amino acid sequence of the *Rht* polypeptide of *Triticum Aestivum* encoded by nucleic acid obtainable from *Triticum Aestivum* which includes the nucleotide sequence

GACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCG.

20

15. An isolated polynucleotide according to claim 14 wherein the polypeptide includes the amino acid sequence of a *Rht* polypeptide obtainable from *Triticum Aestivum*, with one or more amino acids deleted.

25

16. An isolated polynucleotide according to claim 15 wherein the amino acid sequence DELLAALGYKVRASDMA is deleted.

17. An isolated polynucleotide according to claim 15 wherein the amino acid sequence LNAPPPPLPPAPQ is deleted.
18. An isolated polynucleotide according to claim 14 wherein
5 the polypeptide includes the amino acid sequence shown in Figure 9b for the maize D8 polypeptide, with one or more amino acids deleted.
19. An isolated polynucleotide according to claim 18 wherein
10 the amino acid sequence DELLAALGYKVRSSDMA is deleted.
20. An isolated polynucleotide according to claim 19 which has the coding nucleotide sequence shown in Figure 9a, wherein the nucleotides encoding the amino acid sequence
15 DELLAALGYKVRSSDMA are deleted.
21. An isolated polynucleotide according to claim 18 wherein the amino acid sequence VAQK is deleted.
- 20 22. An isolated polynucleotide according to claim 18 wherein the amino acid sequence LATDTVHYNPSD is deleted.
23. An isolated polynucleotide according to claim 14 wherein the polypeptide includes the amino acid sequence shown in
25 Figure 6b, with one or more amino acids deleted.
24. An isolated polynucleotide according to claim 23 wherein the amino acid sequence DELLAALGYKVRSSDMA deleted.

25. An isolated polynucleotide according to claim 24 which has the coding nucleotide sequence shown in Figure 6a, wherein the nucleotides encoding the amino acid sequence DELLAALGYKVRSSDMA are deleted.

5

26. An isolated polynucleotide encoding a polypeptide which comprises the amino acid sequence shown in Figure 8b, with the amino acid sequence DELLAALGYKVRASDMA deleted.

10 27. An isolated polynucleotide according to claim 26 which has the coding nucleotide sequence shown in Figure 8a, wherein the nucleotides encoding the amino acid sequence DELLAALGYKVRASDMA are deleted.

15 28. An isolated polynucleotide wherein a polynucleotide according to any of claims 1 to 27 is operably linked to a regulatory sequence for expression.

29. An isolated polynucleotide according to claim 28 wherein
20 the regulatory sequence includes an inducible promoter.

30. An isolated polynucleotide of which the nucleotide sequence is complementary to a sequence of at least 50 contiguous nucleotides of the coding sequence or sequence
25 complementary to the coding sequence of nucleic acid according to any of claims 1 to 27 suitable for use in anti-sense or sense regulation ("co-suppression") of expression said coding sequence and under control of a regulatory

sequence for transcription.

31. A polynucleotide according to claim 30 wherein the regulatory sequence includes an inducible promoter.

5

32. A nucleic acid vector suitable for transformation of a plant cell and including a polynucleotide according to any preceding claim.

10 33. A host cell containing a heterologous polynucleotide or nucleic acid vector according to any preceding claim.

34. A host cell according to claim 33 which is microbial.

15 35. A host cell according to claim 33 which is a plant cell.

36. A plant cell according to claim 35 having heterologous said polynucleotide within its chromosome.

20 37. A plant cell according to claim 36 having more than one said polynucleotide per haploid genome.

38. A plant cell according to any of claims 35 to 37 which is comprised in a plant, a plant part or a plant propagule,
25 or an extract or derivative of a plant.

39. A method of producing a cell according to any of claims 33 to 37, the method including incorporating said

polynucleotide or nucleic acid vector into the cell by means of transformation.

40. A method according to claim 39 which includes
5 recombining the polynucleotide with the cell genome nucleic acid such that it is stably incorporated therein.

41. A method according to claim 39 or claim 40 which
includes regenerating a plant from one or more transformed
10 cells.

42. A plant comprising a plant cell according to any of claims 35 to 37.

15 43. A part or propagule of a plant comprising a plant cell according to any of claims 35 to 37.

44. A method of producing a plant, the method including incorporating a polynucleotide or nucleic acid vector
20 according to any of claims 1 to 32 into a plant cell and regenerating a plant from said plant cell.

45. A method according to claim 44 including sexually or asexually propagating or growing off-spring or a descendant
25 of the plant regenerated from said plant cell.

46. A method of influencing a characteristic of a plant, the method including causing or allowing expression from a

heterologous polynucleotide according to any of claims 1 to 31 within cells of the plant.

47. Use of a polynucleotide according to any of claims 1 to 5 32 in the production of a transgenic plant.

48. A method of identifying or obtaining a polynucleotide according to claim 6, the method including screening candidate nucleic acid using a nucleic acid molecule which 10 specifically hybridises with a polynucleotide according to any of claims 1 to 13.

49. A method according to claim 48 wherein oligonucleotide primers are employed in PCR.

15

50. A method according to claim 49 wherein said primers are selected from those shown in Tables 1 and 2.

51. An isolated polypeptide encoded by a polynucleotide 20 according to any of claims 1 to 27.

52. An antibody including an antigen-binding site with specific binding affinity for the polypeptide according to claim 51.

25

53. A polypeptide including the antigen-binding site of an antibody according to claim 52.

54. A method of identifying or obtaining a polypeptide according to claim 51, the method including screening candidate polypeptides with an antibody or polypeptide according to claim 52 or claim 53.

Figure 1

Gai	...	M	K	R	D	H	H	H	H	Q	D	K	K	T	M	M	N	E	E	D	D	G	N	G	M	D	E	L	L	A	V	L	G	T	K	V	R	S	S	E	M	A	D	V	A	Q	K	L	E	Q	L	E	V	54						
0803	E	A	G	G	S	S	G	G	G	S	S	A	D	M	G	S	C	K	D	K	V	M	A	G	A	X	G	E	E	E	X	V	D	E	L	L	A	L	G	T	K	V	R	S	S	D	M	A	D	V	A	Q	K	L	E	O	L	E	M	60

Gai	M	S	N	V	Q	E	D	D	L	S	Q	L	A	F	E	T	V	H	Y	N	P	A	E	L	Y	T	W	L	D	
0803	A	M	G	M	G	G	V	T	P	P	A	O	R	M	F	G	S	C	R	T	W	P	R	T	K	F	I	.	.	.

Figure 2a

CCCCGACGCGTCCGCGGCCGCGCCAAACGCGACGCCCGCGCTGCCGGTTCGTCTGG
TCGACACGCAGGAGGCCGGGATTCGGCTGGTGCACGCGCTGCTGGCGTGC GCGG
AGGCCGTGCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTGGTGAAGCAGATAC
CCTTGCTGGCCGCGTCCCAGGGCGGCGGATGCGCAAGGTCGCCGCCTACTTCGG
CGAGGCCCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTC
CTCGACGCCGCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGCCCTA
CCTCAAGTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCGCCGGC
TGCCGCCGCGTGCACGTCTGCTGACTTCGGCATCAAGCAGGGGATGCAGTGGCCC
GCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTCAC
CGGCGTCGGCCCCCGCAGCCGGACGAGACCGACGCCCTGCAGCAGGTGGGCTG
GAAGCTCGCCAGTTCGCGCACACCATCCGCGTCGACTTCCAGTACCGCGGCC
TCGTGCGCCGCCACGCTCGCGGACCTGGAGCCGTTTCATGCTGCAGCCGGAGGGCG
AGGAGGACCCGAACGAAGANCCCGANGTAATCGCCGTCAACTCAGTCTTCGAGA
TGCACCGGCTGCTCGCGCAGCCCGGCGCCCTGGAAAAGGTTCTTGGGCACCGTGC
GCCCCCGTGCGGCCCAGAATTCNTCACC GTGGTGAAACAGGAGGCAAATCACA
ACTCCGGCACATTCTTGACCGCTTCACCGAGTCTCTGCACTACTACTCCACCAT
GTTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGGCGGCCATCCGAAGTCTCATCG
GGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAGGTTCATNTCCGAGGTGT
ACCTCGGCCCGGCAGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAACGCACAG
ANCGCCACGAGACGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCG
AGACCGTCCACCTGGGCTCCAATGCCTACAAGCAGGCGANCACGCTGCTGGCGC
TCTTCGCCGGCGGCGAACGGCTACANGTGGAAGAAAAGGAAGGCTGCCTGACGC
TGGGGTTGCACACNCCCCCTGATTGCCACCTCGGCATGGCGCCTGGCCGGGCGG
TGATCTCGCGAGTTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACA
CAGCCCCGGCGGCCGCCGCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGA
AGAAGAAGAAGCTAAATGTCATGTGAGTGAGCGCTGAATTGCAGCGACCGGCTA
CGATCGATCGGGCTACGGGTGGTTCCGTCCGTCTGGCGTGAAAGAGGTGGATGGA
CGACGAACTCCGAGCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGT
TCCAGTTCTCCACCGCCTCCATGATCACCCGTAAAACTCCTAAGCCCTATTATTA
CTACTATTATGTTTAAATGTCTATTATTGCTATGTGTAATTCTCCAACCGCTCAT
ATCAAAATAAGCACGGGCCGGAAAAA
AAAAA
AAAAA

Figure 2b(1)

CGCGCAATGCTTAAGGTCNCCGCCTACTTCGGNGCAGGCCCTCGCCCCGCCGCGTC
TTCCGCTTCCGCCCCGACCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCT
CCTCCACGCGCACTTCTACNAGTCCTGCCCCCTACCTCAAGTTCGCGCACTTCACCG
CCAATTAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGA
CTTCGGCATCAAGCAGGGGATGCAGTGGCCCCGCACTTCTCCAGGCCCTCGCCCTC
CGTCCCGGCGGCCCTCCCTCGTTCCGCCTCACCGGCGTCGGCCCCCGCAGCCGG

Figure 2b(2)

ACCTCCTTCGTCGTCTNTNNGGTGGGGGCGCCAGGAGCTTATGTGGTGGAGGNTG
GCCCCNCCGGTCGCGACCGCGNCCTACGNGACGCCCGCGCTGCCGGTCGTCTGTGG
TCGACACGCAGGAGGCCGGGATTTCGGNTGGTNCACGCGCTGCTGGNGTGCNGGG
AGNCCGTGCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTNGTGAAGNAGATAC
CCNTGCTGGCCGAGTCCCAGGGCGGCGAGATGNGCAAGGTNGCAGCTTACTTNG
NAGANGCCCTCGCCCCGNGAGTGATTCCACTTANCGCCTGCAGCCGGANAGCTCC
GTCCTCGAANCCGCNTTNGCCGACCTCCTCCACGNGCACNTNTACGAGTC

Figure 2b(3)

TANTAGTCTCTCGGTGGGGGCGCCAGGAGCTCTNTGGTGGAGGCNGCCCCGCCG
GTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCTGTGGTGCACACG
CAGGAGGCCGGGATTTCGGATGGTGCACGCGCTGNTGGCGTGCGCGGAGGCCGTG
AAACAGTTGAAGGNCCNCGCCTNNNNNCNCACAAANTGAAAGCCCCGNG

Figure 2b(4)

GGCTNCCNCCNCGTGCACGTCGTCTCGACTTCGGCATCAAGCATGGGATGCANTGGC
NCGNACTTCTCCANGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTC
ACCGGCGTCGGCCCCCGCAGCCGGACGAGACCGACGCCCTGCANCAGGTGGGC
TGGAAGCTCGCCCAGTTCGCGCACACCATCCGCGTCGACTTCCANTACCGTGGCC
TCGTCTCGCCGCCACGCTCGCGGACCTGGAGCCGTTTCATGCTGCANCCGGAGGGCGA
GGAGGACCCGAACGACGGAGCCCGAGGTAATCGCCGTCAACTCAGTCTTCGAGA
TGCACCGGGCTGCTCNCGCANCCCGGCGACNCTGGAANAA

Figure 2b Continued

Figure 2b(5)

CAAGANGCTAATCACAACCTCCGGGCACATTCCTGGACCGCTTCACCGAGTCTCTGC
ANTACTACTCCACCATGTTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCC
ATCCGAAGTCTCATCGGGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAT
GTCATGTCCGAXGTGTACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGG
GGGCGGAGCGCACANTANCGCCACGCAGACNCTGGGCCAGTGGCGTGAACCGGC
TGGGCAACGCCNGGTTCANNNNCCGTCCACCTGGGCTCCAATGCCTACAATCAN
GCNNNCACGCTGCTGGCGCCTCTTCGCCC

Figure 2b(6)

TCGCCANTCGGCATGGNGCCTGGCCGGGGCCGTGATCTCGCGAGTTTTGAACGCTG
TAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCGGCCGCCCGGCT
CTCCGGCGAACGCACGCACGCACGCACTTGGAAGAAGANAAGCTAAATGTCAT
GTCAGTGAGCGCTGAATTGCAACGACCGGCTACGATCGATCGGGCTACGGGTGG
TTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCGANCCGACCAC
CACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTCTCCACCGCCTCCAT
GGATCACCCGTAAAACTCCTAAGCCCTAATTATNNACTAACTAATTATGTTTTAA
AATGTTCTAATTAATTGGCTATGTTGTAATNCCTCCAAACCGGCTCATTTTCAA
NATTAAGCCACGGGCCCCGGAACCTTTGGTTTAACAACCTCCCNATTGNAAAATTNA
AATNGAAATTTTTGGTTNC

Figure 2b(7)

GTTGGTGGNGGCGATTTGGGTACAAGGTGCGCGCCTCCGACATGGNGGANGTGG
GGCAGAAGCTGGAGCAGNTCGAGATGGCCATGGGGATGGGNGGCGTGGGCGCT
GGCGCCGCCCTGACGACAGGTTNGCCACCCGCNGGCCGCGGACACNGTGCANT
ACAACCCACNGACNTGTCGTCTTGGGTGAGAGCATGCTGTGCGGAGCTAAANG
AGCCGCNGCCGCCCTCCCGCCCCGCCCGCAGCTCAACGCCTCCACCTCCTCCAC
CGTCACGGGCAGCGGCGGCTACTTCGATAACCCTCCCTG

Figure 2b(8)

TGATGGNGGGAGNTTANGGGTTANAAATGTGGGGGANTTCCGAANNNGTGAGG
ANATATNNTCAGAAGTTGGAGCAGATGAGAGATNGCTGATGGGGATAGGGTAGG
NGTGGGTGCCGTGCNGCCCCCNAGGANAGATTGGCCACCCACTTAGCAAGTGG
ANACCGTGGATTACNACCCACAGACCTGTCGTGGTTGGGTTTGAGAGCGTGGTG
TGGGAGCTGAACGGGCGNGCGGCGTGCCCTCCCGCCCCGCCCGCAGCTCAACGCC
TCCACCTCCTCCACCGTACACGGGCAGCGGCGGCTAGTTCGATCTCCCGCCCTCC
GTCGACTCCTCCAGCAGCATNTANGCGCTGCGGCCGATCCCCTNCCCAAGCNGC
GNGGNCCGAGCCGTGTAN

Figure 2b Continued

Figure 2b(9)

TTTCANTTTCNTCCTTTTTTCTTCTTTTTTCCAACCCCCGGCCCCCNGACCCTTGGAT
CCAAATCCCGAACCCGCCCCAGAACCNGGAACCGAGGCCAAGCAAAAGNTTTG
CGCCAATTATTGGCCAGAGATAGATAGAGAGGCGAGGTAGCTCGCGGATCATGA
AGCGGGAGTACCAGGACGCCGGAGGGAGCGGCGGCGGCGGTGGCGGCATGGGT
TCGTCCGAGGACAAGATGATGGTGTCTGGCGGCGGCGGGGGAGGGGGAGGAGGT
GGACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGA
CGTGGCGCAGAAGCTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGG
CGCCGGCGCCGCCCCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGTG
CAGTACAACCNCCCNGACC

Figure 2b(10)

GGACGACGACCTCCGAGCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTT
CNTTCCCAGTNCTCCACCGCCTCCATGATCACCCGTAAACTCCTAAGCCCTATT
ATTACTACTATTATGTNTAANTGTCTATTATTGCTANGTGTAATTCCTCCAACCGC
TCATATCAAAATAAGCACGGGCCGGACTTTGTTANCAGCTCCAATGAGAATGAA
ATGAATTTTGTACGCAAGGCACGTCCAAAACCTGGGCTGAGCTTTGTTCTGTTCTG
TTATGTTTCATGGTGCTCACTGCTCTGATGAACATGATGGTGCCTCCAATGGTGGC
TTTGCAATTGTTGAAACGTTTGGCTTGGGGGACTTGNGTGGGTGGGTGCATGGGG
ATGAATATTCACATCNCCGATTAAAATTAAGCCATCCCGTTGGCCGTCCTTTGA
ATANCTTGCCCNAAACGAAATTTCCCCCNATC

Figure 2b(11)

AAANCCTANAANATATAGAGGCGATGTNGCNCCCCNATCANNAACNNGGATTACN
GNAACNCCNGAAGGAGCGGCGGCGGCGGTGGCAGCATNGGCTCGTCCGATGACA
AATATCATGGTGTCTGGCGGCGGCGGGGACGGGGAGGAGGTGCACAACNTTTNG
GCGGGACTCGNGTACCACGTGNACGGTGCCGCNCTNGNGGATNTGGCCCTNGAA
GATGGGCCACCTCCAAA

Figure 2b Continued

Figure 2b(12)

CGGCGGCCCCGTGGCGGCATGGGCTCGTCCGAGGACNAGATGATGGTGTCGGCG
GCGGCGGGGGANGGGGATGATGTGGACTATCTGCTGGCGGCGCTCGGGTACAAG
GTGCGCGCCTCCGACAGGCGGAGCCCGCGCATAACTGGAGCCGCTCGAGATGGC
CNTGGGGATNGGCGGCNTGGGCNCCNGCGCCTCCCCCG

Figure 2b(13)

TGGNGCTCGGGTGNCCCGTGCGCGCCTCCGACATGGCGGGACGTGGCGCAGAAC
TGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCCC
CCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGGCACACAACCCACCG
ACCTGTCGTCTTGGGTCGAGAGCATGCTGTCGGATCTCNACGCGCCNCCGNCGCC
CCTCCCGCCCCG

Figure 2c(1)

ANNTTGTNCNNNTACATCCCATGNGCCGCGCNATGCTNAAGGTCGCCGCCTACT
TCGGCGCAGGCCCTCGCCCGCCGCGTCTTCCGCTTCCGCCCCGAGCCGGACAGCT
CCCTCCTCGACGCCGCTTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGC
CCCTACCTCAAGTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTTCG
CCGGCTGCCGCCGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGT
GGCCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGC
CTCACCGGCGTTCGGCCCCCGCAGCCGGACGANAACGACGCCCTG

Figure 2c(2)

NTTCCCCGGCAGTTAAAAGCNTCCACTTCTTCCACCGTCACGGGCAGCGGCGGNT
ACTTNGATCTCCCGCCCTCAGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCC
GATCCCCTCCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTG
CGGGATCCCAAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCT
CATANTCGTCTCTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCNGCCCCGCC
GGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACAC
GCAGGAGGCCGGGATTCGGATGGTGCACGCGCTGNTGGCGTGCGCGGAGGCCGT
GNAAGCAGTTNGAAGGGCCTNCGCCGTGNATNNCGCAACAANNNGGAAGNCCN

Figure 2c(3)

CANCCCGCTGNTCGCCACCTCGGCATGGCGCCTGGCCGGGCGCGTGATCTCGCGAG
TTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCG
GCCGCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAG
CTAAATGTCATGTCAGTGAGCGCTGAATTGCANCGACCGGCTACGATCGATCGG
GCTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCC
GANCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCAGTTTCTC
CACCGCCTCCATGATCACCCCGTAAAACTCCTAAGCCCTATNNNTTACTACNATT
AATGTTTTAAANTGTTCTANTAATTGCTATGNTGTTTATTNCC

Figure 2c(4)

TATCGAAGTAGCCGCCGCTGCCCNTGCACGGTGGAGGAGGTGGAGGCGTTGAGC
TGCGGGGCGGGCGGGAGGGGCGGCGGCGGCACGTTNAGCTCCGACAGCATGCTC
TCGACCCAAAACNACAGGTCTGGTGGGGTTGTAGTGCACGGTGTCCGTGGCGAGG
GGGTGGCNAANCTGTCGTACGGGGCGGCGCCNGCGCCACNCCGCCCATCCCCA
TGGCCATCTCGANCTGCTCCAGCTTCTGCGCCACTTCNCCATGTCNGATGCGCG
CNCCTTGTAACCGA

Figure 2c Continued

Figure 2c(5)

ACGGCGCGGNCCNCGCNGCTTGGGAGGGGATCGGCCGCAGCGCNTANATGCTG
CTGGAGGAGTCGACGGAGGGCGGGAGATCGAACTAGCCGCCGCTGCCCCGTGTAC
GGTGGAGGAGGTGGAGGCGTTGAGCTGCGGGGCGGGCGGGAGGGGCAGCNGCT
GCACGTTNAGCTCCACACCACGTCTCTCAACCCAACCACGACNCGTCTGTGGGG
TNGTAATNCACGGTNTCCCTNGCTANGTGGGTGGCCAATCTNT

Figure 2c(6)

CACGGTGTCCGTGGCGAGGTGGGTGGCGAAGCTGTCGTCGGGGGCGGCGCCGGC
GCCACGCCGCCCATCCCCATGGCCATCTCGAGCTGCTCCAGCTTCTGCGCCACG
TCCGCCATGTTCGGAGGCGCGCACCTTGTACCCGAGCGCCGCCAGCAGCNCGNCC
ACCTCCTCCCCCTCCCCGCCGCCGCCGACACCATCATCTTGTCTCGGACGANCC
CATGCCGCCACCGCCGCCGCCGCTCCCTCCGGCGTCTCTGGTACTCCCGCTTCATG
ATCCGCGAGCTACCTCGCCTCTCTATCTATCTCTGGCCAATAATTGCGCA

Figure 2c(7)

GACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCNTTCCCAGTTCTCCACCGC
CTCCATGATCACCCGTAAACTCCTAAGCCCTATTATTACTACTATTATGTNTAA
ATGTCTATTATTGCTANGTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACG
GGCCGGACTTTGTTAGCAGCTCCAATGAGAATGAAATGAATTTTGTACGCAAGGC
ACGTCCAAAACTGGGCTGAGCTTTGTTCTGTTCTGTTATGTTTCATGGTGCTCACTG
CTCTGATGAACATGATGGTGCCTCCAATGGGTGGCTTTGCAATTGTTGAACGTTT
TGGCTTGGGGGACTTGGTGNNTGGTGCATGGGAATGAANATTCCACATCCNCNG
GAATTAAAATTAGCCCATCCCG

Figure 3a

TTTCANTTTCNTCCTTTTTTCTTCTTTTTTCCAACCCCCGGCCCCCNGACCCTTGGATCC
AAATCCCGAACCCGCCCCCAGAACCNGGAACCGAGGCCAAGCAAAAGNTTTGCGCC
AATTATTGGCCAGAGATAGATAGAGAGGCGAGGTAGCTCGCGGATCATGAAGCGGG
AGTACCAGGACGCCGGAGGGAGCGGCGGCGGGTGGCGGCATGGGTTCGTCCGAG
GACAAGATGATGGTGTGCGGCGGCGGCGGGGAGGGGGAGGAGGTGGACGAGCTGC
TGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAG
CTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCTGGCGCCGCCCC
TGACGACAGGTTNGCCACCCGCGGCGGACACNGTGCANTACAACCCACNGA
CNTGTCTGTTGGGTGAGAGCATGCTGTGCGAGCTAAANGAGCCGCGGCCGCCCC
TCCCGCCCGCCCCGAGCTCAACGCCTCCACCGTCACGGGCAGCGGCGGNTACTTNG
ATCTCCCGCCCTCAGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCGGATCCCCT
CCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCGACTCCGTGCGGGATCCC
AAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCTCATANTCGTCT
CTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCNGCCCCGCGGTGCGGGCCGC
GGCCAACGCGACGCCCGCGCTGCCGGTCGTGTCGACACGCGAGGAGGCCGGGA
TTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGGAGGCCGTGCAGCAGGAGAACCTC
TCCGCCGCGGAGGCGCTGGTGAAGCAGATACCCTTGCTGGCCGCGTCCCAGGGCGG
CGCGATGCGCAAGGTGCGCGCCTACTTCGGCGAGGCCCTCGCCCGCCGCGTCTTCCG
CTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCGCCTTCGCCGACCTCCTCCA
CGCGCACTTCTACGAGTCCTGCCCCACCTCAAGTTCGCGCACTTCAACGCCAACCA
GGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTGCTCGACTTCGGCAT
CAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGG
CCCTCCCTCGTTCGCGCTCACCGGCGTGGGCCCGCCGAGCCGGACGAGACCGACGC
CCTGCAGCAGGTGGGCTGGAAGCTCGCCAGTTCGCGCACACCATCCGCGTGCAGTTC
CCAGTACCGCGGCCTCGTCGCCGCCACGCTCGCGGACCTGGAGCCGTTTCATGCTGCA
GCCGGAGGGCGAGGAGGACCCGAACGAAGANCCCGANGTAATCGCCGTCAACTCA
GTCTTCGAGATGCACCGGCTGCTCGCGCAGCCCGGCGCCCTGGAAAAGGTTCTTGGG
CACCGTGCGCCCCCGTGCGGCCCAGAATTCNTCACCGTGGTGGAACAGGAGGCAA
ATCACAACCTCCGGCACATTCTTGACCGCTTCACCGAGTCTCTGCACTACTACTCCA
CCATGTTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCAT
CGGGGGCTGCTGCTGCTCCTGCCGCCCGCGGCACGGACCAGGTTCATNTCCGAGGTGT
ACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAACGCACAGAN
CGCCACGAGACGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCGAGAC
CGTCCACCTGGGCTCCAATGCCTACAAGCAGGCGANACGCTGCTGGCGCTCTTCGC
CGGCGGCGAACGGCTACANGTGGAAGAAAAGGAAGGCTGCCTGACGCTGGGGTTGC
ACACNCCCCCTGATTGCCACCTCGGCATGGCGCCTGGCCGGGGCCGTGATCTCGCGA
GTTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCGG
CCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAGCTA
AATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTACGATCGATCGGGCTAC
GGGTGGTTCGGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCGAGCCGA
CCACCACGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTCTCCACCGCCTCC
ATGATCACCCGTAAACTCCTAAGCCCTATTATTACTACTATTATGTTTAAATGTCTA
TTATTGCTATGTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACGGGCGGACT
TTGTTANCACTCCAATGAGAATGAAATGAATTTTGTACGCAAGGCACGTCCAAAA
CTGGGCTGAGCTTTGTTCTGTTCTGTTATGTTTCATGGTGCTCACTGCTCTGATGAACA
TGATGGTGCCTCCAATGGTGGCTTTGCAATTGTTGAAACGTTTGGCTTGGGGGACTT
GNGTGGGTGGGTGCATGGGGATGAATATTCACATCNCCGGATTAAATAAGCCAT
CCCGTTGGCCGTCTTTGAATANCTTGCCCNAAACGAAATTTCCCCCNATC

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Figure 3b

PRETTYBOX of: My.Msf{*} August 7, 1997 13:06:42.76

Gai	..RRGSSRIM	M	KRDH HHHQ	..GGGGG MGSE	D	KKTMM MNNEED	DGNGMDELLA	V	LG YKVRSS	41
Rht	IERRGSSRIM		KREYQDAGGS			DKMMV SAAAG	EGEEVDELLA	A	LG YKVRAS	60
Gai	MADV AQKLEQ		LEVMM S	..NVQEDD		LSQLA TE TVH	YNPAEL Y T ML	D	SMLT DD LNPP	93
Rht	MADV AQKLEQ		LEMAMGMGGV	GAGAAPDRQV		XHPXAADTVX	YNPTDXSSMV	E	SMLSELXEP	120
Gai	..PPAPQL		..STVTGSGG	..XDDLPPSVDS		SNAEYDLKAI	PGDAI LNQFA	I	DSAS SSNQ	123
Rht	XPPAPQL		NASTVTGSGG	YXDDLPPSVDS		SSSIYALRP	PSFAGATAPA	D	LSADS V RDP	180
Gai	..GGG GDT		YTTNKRLKCS	NG	..T TAT	AESTRH VLV	ATPALP VV V	D	SQENG VRLV	169
Rht	KRMRTGGSST		SSSSSSXSLS	GGGARS SVVE		AAPVAAAN		D	TOEAGIRLV	240
Gai	HALLACAEAV		QKENLTV AEA	LVKQIGFLAV		SOIGAMRKVA	TYFAEALARR	I	YR LSPSQ	227
Rht	HALLACAEAV		QQENLSA AEA	LVKQIPLLA		SOIGAMRKVA	AYFGEALARR	V	FRFRPQ PDS	300
Gai	SPI D HSLSDT		LQM HFYE TCP	YLKFAHFTAN		QAILLEAFQ GK	KRVHVIDFSM	S	QGLQMPALM	287
Rht	SLLDAAFA DL		LHAHFYE SCP	YLKFAHFTAN		QAILLEAFAGC	RRVHV VDFGI	K	QGMQMPAL	360
Gai	QALALRP GGP		PVFRLTGIGP	PA PDNFDY LH		EVGCKLAHLA	EAIHV E EYR	G	FVAN TLADL	347
Rht	QALALRP GGP		PSFRLTGVGP	PQPD ETDALQ		QVGMKLAQFA	HTLRVDFQYR	G	LVAA TLADL	420
Gai	DASMLELRPS		EIES	..V		AVNSVFE LHK	VLG	VVNQIK	PEI FT VVE	400
Rht	EPFMLQPEGE		EDPNEXX PVI	AVNSVFE MHR		LLGRPGAIDK	VLG	HRAPP CG	PEFXTVETQ	480
Gai	ESNHNSPIFL		DRFTESLHY	STLFDSLEGV		PSGQ	..S	SGAAA PAA	DKVMSEVY	442
Rht	EANHNSGTFL		DRFTESLHY	STMFD SLEGV		SSGGGPSEVS	..S	SGAAA PAA	GT DQVXSEVY	540
Gai	LGRQICNVVA		CDGPD RVERH	ETLSQWRNRF		GSAGFAAAHI	GSNAFKQAS	S	LLALFN GGE	502
Rht	LGRQICNVVA		CEGAERTXRH	ETLSQWRNRL		GNAGFETVHL	GSNAFKQAS	X	LLALFAGGER	600
Gai	YRVEESD GCL		MLGWHTRPLI	ATSAMK LSTN		532				
Rht	LXVEEKEGCL		TLGLHTXPLI	ATSAMRLAGP		630				

Figure 4a

ACGCGTCCGGAAGCCGGCGGGAGCAGCGGCGGCGGGAGCAGCGCCGATATGGG
GTCGTGCAAGGACAAGGTGATGGCGGGGGCGGCGGGGGAGGAGGAGGACGTCT
ACGAGCTGCTGGCGGGCGCTCGGGTACAAGGTGCGGTCGTCCGACATGGCCGACG
TCGCGCAGAANCTGGAGCAGCTGGAGATGGCCATGGGGATGGGCGGCGTGAGCG
CCCCCGGCGCCGCGGATGACGGGTTCGTGTGCGCACCTGGCCACGGACACCGTGC
ACTACAACCCCTCGGACCTCTCCTCCTGGGTTCNGAGAGCATGCTTTCGGAGTTA
AAGGCGCCCGTTGCCCCCTTATCCCGCCAGGCGCCGCGGGCTGCCCCGCCATGCTTT
CCAACTTCGTCCACTGTCACCGGCGGCGGTGGTAGCGGCTTCTTTGAANTCCCAG
CCGCTGCCGANTCGTCGAGTAGCACNTACGCCCTCAGGCCGATCTCCTTACCGGT
GGTGGCGACGGCTGACCCGTGCGGCTGCTGACTCGGCGAGGGACACCAAGCGGAT
GCGCACTGGCGGCGGCAGCACGTCGTCGTCCTCATCGTCGTCTTCCTCTCTGGGC
GGTGGGGCCTCGCGGGGCTCTGTGGTGGAGGCTGCTCCGCCGGCGACGCAAGGG
GCCGCGGCGGCGAATGCGCCCGCCGTGCCGGTTGTGGTGGTTGACACGCAGGAG
GCTGGNATCGGGCCTGGTGC

Figure 4b

Wheat Rice Gai	I E R R G S S R I M M	K R E Y Q D A G G S T R P E A G G S S G K R D H H H H H Q D	G G G G C M G S E G G S S A D M G S C	D K M M M S A A A G K K D K M M A G A A G K K T M M M N E E E E	E G E E V D E L L A E E E D V D E L L A E G A G M D E L L A	A L G Y K V R A S D A L G Y K V R S S D V L G Y K V R S S E	60 50 41
Wheat Rice Gai	M A D V A O K L E Q M A D V A O K L E Q M A D V A O K L E Q	L E M A N G M G G V L E M A N G M G G V L E M M M S	G A G A A P D R Q V S A P G A A D D G F N V Q E D D	X H P X A A A D T V X V S H L A T D T V H L S Q L A T E T V H	Y N P T D X S S W V Y N P S D L S S W V Y N P A L L Y T M M	E S M L S E L X E F E S M L S E L K A P E S M L T L N P P	120 110 93
Wheat Rice Gai	X P P L P P . A P Q L P L I P P G A A G	L N A S L P A M L S P T S S	T V T G S G . . G Y T V T G G G S G F	X D L P P S V D S S F E X P A A A X S S	S S I Y A L R P I P S S I T Y A L R P I S N A E Y D L L K A I L P	S P A G A T A P A D L P V V A T A D P S G D A I L N . . . Q	171 170 112
Wheat Rice Gai	L S A D S V R D P K A A D S A R D T K F A I D S A	R M R T G G S S T S R M R T G G C S T S	S S S S X S S L G S S S S S S S L G S S S N Q K G G G	G G A R S S V V E G G A S R G S V V E D T Y T T N K R K K	A A P P V . . A A A A A P P A T Q G A A C S N G V V E T T T	A N A T P A L P V V A A N A P A V P V V A T A E S T R H V V	228 229 157
Wheat Rice Gai	V V D T Q . . E A G V V D T Q E E E A G V V D S Q . . E N G	I R L V H A L L A C I R L V H A L L A C I R L V H A L L A C	A E A V Q Q E N L S X E A V Q Q E N L S A E A V Q K E N L T	A A E A L V K O I P V A E A L V K O I G	L L A A S Q G G A M F L A V S Q I G A M	R K V A A Y F C E A R K V A T Y F A E A	286 258 215
Wheat Rice Gai	L A R R V F R F R P L A R R I Y R L S P L A R R I Y R L S P	Q P D S S L L D A A S Q . . S P I D H S	F A D L L H A H F Y L S D T L Q M H F Y	E S C P Y L K F A H E T C P Y L K F A H	F T A N Q A I L E A F T A N Q A I L E A F Q G K K R V H V I	F A G C R R V H V V F Q G K K R V H V I	346 258 273
Wheat Rice Gai	D E G I K O G M Q M D E S M S O G L Q M	P A L L O A L A L R P A L M Q A L A L R	P G G P P S F R L T P G G P P V F R L T	G V G P P Q P D E T G I G P P A P D N F	D A L Q Q V G W K L D Y L H E V G C K L	A Q F A H T I R V D A H L A E A I H V E	406 258 333
Wheat Rice Gai	F Q Y R G L V A A T F E Y R G F V A N T	L A D L E P F M L Q L A D L D A S M L E	P E G E E D P N E X L R P S E I E S	P X V I A V N S V F V A V N S V F	E M H R L L A Q P G E L H K L L G R P G	A L E K V L G H R A A I D K V L G . V V	466 258 387
Wheat Rice Gai	P P C G P E F X T V N Q I K P E I F T V	V E T O E A N H N S V E . Q E S N H N S	G T F L D R F T E S P I F L D R F T E S	L H Y Y S T M F D S L H Y Y S T L F D S	L E G G S G G G P L E G V P S G Q	S E V S S G A A A A	526 258 434
Wheat Rice Gai	P A A A G T D Q V X	S E V Y L G R O I C S E V Y L G K O I C	N V V A C E G A E R N V V A C D G P D R	T X R H E T L G Q M V E R H E T L S Q M	R N R L G N A G F E R N R F G S A G E A	T V H L G S N A Y K A A H I G S N A F K	586 258 488
Wheat Rice Gai	O A X T L L A L F A O A S M L L A L F N	G G E R L X V E E K G G E G Y R V E E S	E G C L T L G L H T D G C L M L G W H T	X P L I A T S A M R R P L I A T S A M K	L A G P 630 258 L S T N 532		

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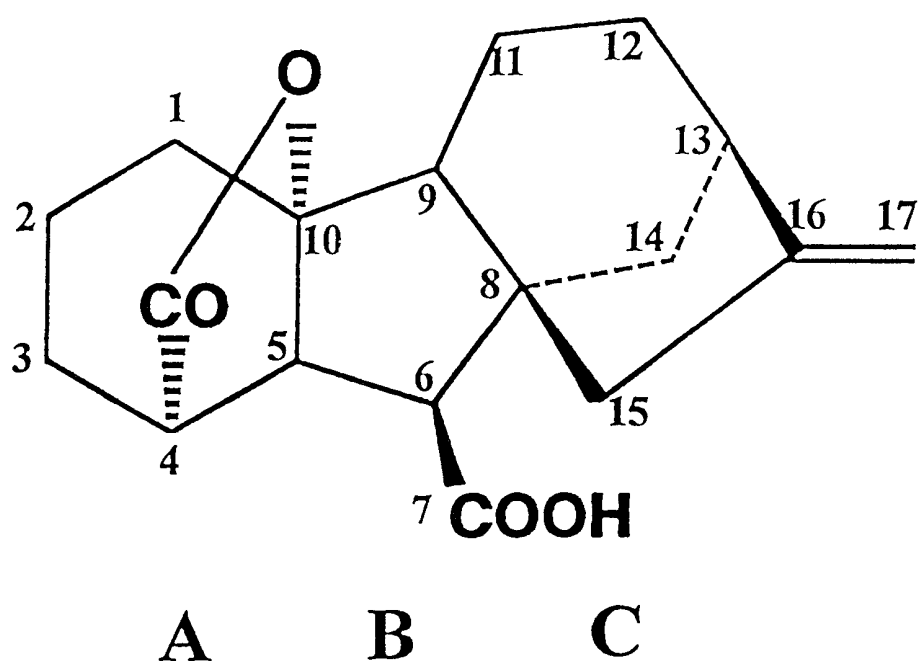
Figure 5

Figure 6a

GTCGACCCACGCGTCCGGAAGCCGGCGGGAGCAGCGGCGGGCGGGAGCAGCGCC
GATATGGGGTCGTGCAAGGACAAGGTGATGGCGGGGGCGGCGGGGGAGGAGGA
GGACGTCGACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGGTCTGCCGACAT
GGCCGACGTCGCGCAGAAGCTGGAGCAGCTGGAGATGGCCATGGGGATGGGCGG
CGTGAGCGCCCCCGGCGCCGCGGATGACGGGTTCGTGTCTGCACCTGGCCACGGA
CACCGTGCACTACAACCCCTCGGACCTCTCCTCCTGGGTCGAGAGCATGCTTTCC
GAGCTCAACGCGCCGCTGCCCCCTATCCCGCCAGCGCCGCGGCTGCCCCGCCATG
CTTCCACCTCGTCCACTGTACCCGGCGGCGGTGGTAGCGGCTTCTTTGAACTCCC
AGCCGCTGCCGACTCGTCGAGTAGCACCTACGCCCTCAGGCCGATCTCCTTACCG
GTGGTGGCGACGGCTGACCCGTCGGCTGCTGACTCGGCGAGGGACACCAAGCGG
ATGCGCACTGGCGGCGGCAGCACGTCGTCGTCCTCATCGTCGTCTTCTCTCTGG
GCGGTGGGGCCTCGCGGGGCTCTGTGGTGGAGGCTGCTCCGCCGGCGACGCAAG
GGGCCGCGGCGGCGAATGCGCCCGCCGTGCCGGTTGTGGTGGTTGACACGCAGG
AGGCTGGGATCCGGCTGGTGCACGCGTTGCTGGCGTGCGCGGAGGCCGTGCAGC
AGGAGAACTTC

Figure 6b

RPTRPEAGGSSGGGSSADMGSCKDKVMAGAAGEEEDVDELLAALGYKVRSSDMAD
VAQKLEQLEMAMGMGGVSAPGAADDGFVSHLATDTVHYNPSDLSSWVESMLSELN
APLPPIPPAPPAARHASTSSTVTGGGGSGFFELPAAADSSSSTYALRPISLPVVATADPS
AADSARDTKRMRTGGGSTSSSSSSSSSLGGGASRGSVVEAAPPATQGAAAANAPAVP
VVVVDTQEAGIRLVHALLACAEAVQQENF

Figure 7a

GCCAGGAGCTCTGTGGTGGAGGCTGCCCCGCCGGTCGCGGCCGCGGCCAACGCG
ACGCCCCGCGCTGCCGGTCGTCGTGGTCGACACGCAGGAGGCCGGGATTTCGGCTG
GTGCACGCGCTGCTGGCGTGC GCGGAGGCCGTGCAGCAGGAGAACCTCTCCGCC
GCGGAGGCGCTGGTGAAGCAGATACCCTTGCTGGCCGCGTCCCAGGGCGGCGCG
ATGCGCAAGGTCGCCGCCTACTTCGGCGAGGCCCTCGCCCCGCCGCTCTCCGCT
TCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCTCCTCCA
CGCGCACTTCTACGAGTCCTGCCCCCTACCTCAAGTTCGCGCACTTCACCGCCAAC
CAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGACTTCG
GCATCAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCC
CGGCGGCCCTCCCTCGTTCCGCCTACCGGCGCTCGGCCCGCCGAGCCGGACGAG
ACCGACGCCCTGCAGCAGGTGGGCTGGAAGCTCGCCCAGTTCGCGCACACCATC
CGCGTCGACTTCCAGTACCGCGGCCTCGTCGCCGCCACGCTCGCGGACCTGGAGC
CGTTCATGCTGCAGCCGGAGGGCGAGGAGGACCCGAACGAGGAGCCCGAGGTAA
TCGCCGTCAACTCAGTCTTCGAGATGCACCGGCTGCTCGCGCAGCCCGGCGCCCT
GGAGAAGGTCTCTGGGCACCGTGCGCGCCGTGCGGCCAGGATCGTCACCGTGGT
GGAGCAGGAGGCGAATCACAACCTCCGGCACATTCCTGGACCGCTTCACCGAGTC
TCTGCACTACTACTCCACCATGTTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGC
GGCCCATCCGAAGTCTCATCGGGGGCTGCTGCTGCTCCTGCCGCCCGCCGGCACGG
ACCAGGTTCATGTCCGAGGTGTACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTG
CGAGGGGGCGGAGCGCACAGAGCGCCACGAGACGCTGGGCCAGTGGCGGAACC
GGCTGGGCAACGCCGGGTTCGAGACCGTCCACCTGGGCTCCAATGCCTACAAGC
AGGCGAGCACGCTGCTGGCGCTCTTCGCCGGCGGCGACGGCTACAAGGTGGAGG
AGAAGGAAGGCTGCCTGACGCTGGGGTGGCACACGCGCCCGCTGATCGCCACCT
CGGCATGGCGCCTGGCCGGGGCGTGATCTCGCGAGTTTTGAACGCTGTAAGTACA
CATCGTGAGCATGGAGGACAACACAGCCCCGGCGGCCCGCCCGGCTCTCCGGCG
AACGCACGCACGCACGCACTTGAAGAAGAAGAAGCTAAATGTTCATGTTCAGTGAG
CGCTGAATTGCAGCGACCGGCTACGATCGATCGGGCTACGGGTGGTTCGGTCCGT
CTGGCGTGAAGAGGTGGATGGACGACGAACTCCGAGCCGACCACCACCGGCATG
TAGTAATGTAATCCCTTCTTCGTTCCAGTTCTCCACCGCCTCCATGATACCCGT
AAAACCTCCTAAGCCCTATTATTACTATTATGTTTAAATGTCTATTATTGCTAT
GTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACGGGCCGGAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 7b

ARSSVVEAAPPVAAAANATPALPVVVVDTEAGIRLVHALLACAEAVQQENLSAAE
ALVKQIPLLAASQGGAMRKVAA YFGEALARRVFRFRPQPDSSLLDAAFADLLHAHF
YESCPYLKFAHFTANQAILEAFAGCRRVHVVDGFIKQGMQWPALLQALALRPGGPS
FRLTGVPQPQDETDLQQVGWKLQFAHTIRVDFQYRGLVAATLADLEPFMLQPE
GEEDPNEEPEVIAVNSVFEMHRLLAQPGALEKVLGTVRAVRPRIVTVVEQEANHNSG
TFLDRFTESLHYYSTMFDSLEGGSSGGGPSEVSSGAAAAPAAAGTDQVMSEVYLGR
QICNVVACEGAERTERHETLGQWRNRLGNAGFETVHLGSNAYKQASTLLALFAGGD
GYKVEEKEGCLTLGWHTRPLIATSAWRLAGP

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Figure 8a

ATAGAGAGGCGAGGTAGCTCGCGGATCATGAAGCGGGAGTACCAGGACGCCGG
AGGGAGCGGCGGCGGCGGTGGCGGCATGGGCTCGTCCGAGGACAAGATGATGGT
GTCGGCGGCGGCGGGGGAGGGGGAGGAGGTGGACGAGCTGCTGGCGGCGCTCG
GGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAGCTGGAGCAGC
TCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCCGCCCCGACGACA
GCTTCGCCACCCACCTCGCCACGGACACCGTGCCTACAACCCACCGACCTGTC
GTCTTGGGTCGAGAGCATGCTGTGCGAGCTCAACGCGCCGCGCCGCCCCCTCCCG
CCCGCCCCGACGCTCAACGCCTCCACCTCCTCCACCGTCACGGGCAGCGGCGGCT
ACTTCGATCTCCCGCCCCCTCCGTGCGACTCCTCCAGCAGCATCTACGCGCTGCGGCC
GATCCCCCTCCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTG
CGGGATCCCAAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCT
CCTCGTCGTCCTCTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCTGCCCGGCC
GGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCTGTCGTGGTTCGACAC
GCAGGAGGCCGGGATTTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGGAGGCCGT
GCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTGGTGAAGCAGATAACCCTTGCT
GGCCGCGTCCCAGGGCGGCGCGATGCGCAAGGTCGCCGCTACTTCGCGCAGGC
CCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGAC
GCCGCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCTGCCCTACCTCAA
GTTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCCGCCGCTGCCGC
CGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGTGGCCCCGCACTTC
TCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCGCGCTACCGGCGTC
GGCCCCCGCAGCCGGACGAGACCGACGCCCTGCAGCAGGTGGGCTGGAAGCTC
GCCAGTTTCGCGCACACCATCCGCGTCGACTTCCAGTACCGCGGCCTCGTCGCCG
CCACGCTCGCGGACCTGGAGCCGTTTCATGCTGCAGCCGGAGGGCGAGGAAGACC
CGAACGAGGAGCCCGAGGTAATCGCCGTCAACTCAGTCTTCGAGATGCACCGGC
TGCTCGCGCAGCCCGGCGCCCTGGAGAAGGTCCTGGGCACCGTGCGCGCCGTGC
GGCCCAGGATCGTCACCGTGGTGGAGCAGGAGGCGAATCACAACCTCCGGCACAT
TCCTGGACCGCTTCACCGAGTCTCTGCACTACTACTCCACCATGTTTCGATTCCCTC
GAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCATCGGGGGCTGCTGCT
GCTCCTGCCGCCCGCCGGCACGGACCAAGGTCATGTCCGAGGTGTACCTCGGCCGGC
AGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAGCGCACAGAGCGCCACGAGA
CGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCGAGACCGTCCACC
TGGGCTCCAATGCCTACAAGCAGGCGAGCACGCTGCTGGCGCTCTTCGCCGGCGG
CGACGGCTACAAGGTGGAGGAGAAGGAAGGCTGCCTGACGCTGGGGTGGCACAC
GCGCCCGCTGATCGCCACCTCGGCATGGCGCCTGGCCGGGCGGTGATCTCGCGAG
TTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCG
GCCGCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAG
CTAAATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTACGATCGATCGGG
CTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACCTCCG

Figure 8b

MKREYQDAGGSGGGGGMGSSSEDKMMVSAAAGEGEEVDELLAALGYKVRASDM
ADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYNPTDLSSWVESMLS
ELNAPPPPLPPAPQLNASTSSTVTGSGGYFDLPPSVDSSSSIYALRPIPSAGATAPADL
SADSVRDPKRMRTGGSSTSSSSSSSSSLGGGARSSVVEAAPPVAAAANATPALPVVV
VDTQEAGIRLVHALLACAEAVQQENLSAAEALVKQIPLLAASQGGAMRKVAA YFGE
ALARRVFRFRPQPDSSLLDAAFADLLHAHFYESCPYLKFAHFTANQAILEAFAGCRR
VHVVDGFIKQGMQWPALLQALALRPGPPSFRLTGVGPPQPDDETALQQVGWKL
QFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVIAVNSVFEMHRLLAQ
PGALEKVLGTVRAVRPRIVTVVEQEANHNSGTFLDRFTESLHYYSTMFDSLEGGSSG
GGPSEVSSGAAAAPAAAGTDQVMSEVYLGRQICNVVACEGAERTERHETLGQWRN
RLGNAGFETVHLGSNAYKQASTLLALFAGGDGYKVEEKEGCLTLGWHTRPLIATSA
WRLAGP

Figure 9a

TTTCGCCTGCCGCTGCTATTAATAATTGCCTTCTTGGTTTCCCCGTTTTTCGCCCCAG
CCGCTTCCCCCTCCCCTACCCTTTCCTTCCCCACTCGCACTTCCCAACCCTGGAT
CCAAATCCCAAGCTATCCCAGAACCGAAACCGAGGCGCGCAAGCCATTATTAGC
TGGCTAGCTAGGCCTGTAGCTCCGAAATCATGAAGCGCGAGTACCAAGACGCCG
GCGGGAGTGGCGGCGACATGGGCTCCTCCAAGGACAAGATGATGGCGGGCGGCGG
CGGGAGCAGGGGAACAGGAGGAGGAGGACGTGGATGAGCTGCTGGCCGCGCTC
GGGTACAAGGTGCGTTCGTCGGATATGGCGGACGTGCGCGCAGAAGCTGGAGCAG
CTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGGCGCCGGCGCTACCGCTGAT
GACGGGTTCGTGTCGCACCTCGCCACGGACACCGTGCCTACAATCCCTCCGACC
TGTCGTCTTGGGTGAGAGCATGCTGTCCGAGCTCAACGCGCCCCCAGCGCCGCT
CCCGCCCGCGACGCCGGCCCCAAGGCTCGCGTCCACATCGTCCACCGTCACAAGT
GGCGCCGCGCGCGGTGCTGGCTACTTCGATCTCCCGCCCGCCGTGGACTCGTCCA
GCAGTACCTACGCTCTGAAGCCGATCCCCTCGCCGGTGGCGGCGCCGTGCGCCGA
CCCGTCCACGGAATCGGCGCGGGAGCCCAAGCGGATGAGGACTGGCGGCGGCAG
CACGTCGTCCTCCTCTTCCTCGTCGTCATCCATGGATGGCGGTGCGCACTAGGAGCT
CCGTGGTTCGAAGCTGCGCCGCGGCGACGCAAGCATCCGCGGCGGCCAACGGGC
CCGCGGTGCCGTGGTGGTGGTGGACACGCAGGAGGCCGGGATCCGGCTCGTGC
ACGCGCTGCTGGCGTGC GCGGAGGCCGTGCAGCAGGAGAACTTCTCTGCGGCGG
AGGCGCTGGTCAAGCAGATCCCCATGCTGGCCTCGTTCGAGGGCGGTGCCATGC
GCAAGGTGCGCCGCTACTTCGGCGAGGCGCTTGCCCGCCGCGTGTATCGCTTCCG
CCCGCCACCGGACAGCTCCCTCCTCGACGCCGCTTCGCCGACCTCTTGACGCG
CACTTCTACGAGTCCTGCCCCCTACCTGAAGTTCGCCCCACTTCACCGCGAACCAGG
CCATCCTCGAGGCCTTCGCCGGCTGCCGCCGCGTCCACGTCGTCGACTTCGGCAT
CAAGCAGGGGATGCAGTGGCCGGCTCTTCTCCAGGCCCTCGCCCTCCGCCCTGGC
GGCCCCCGTCGTTCCGGCTCACCGGCGTCGGGCGCGCCGACGCCGAGAGACC
GACGCCTTGACAGCAGGTGGGCTGGAAACTTGCCCAGTTCGCGCACACCATCCGCG
TGGACTTCCAGTACCGTGGCCTCGTCGCGGCCACGCTCGCCGACCTGGAGCCGTT
CATGCTGCAACCGGAGGGCGATGACACGGATGACGAGCCCGAGGTGATCGCCGT
GAACTCCGTGTTTCGAGCTGCACCGGCTTCTTGCGCAGCCCGGTGCCCTCGAGAAG
GTCCTGGGCACGGTGC GCGCGGTGCGGCCGAGGATCGTGACCGTGGTTCGAGCAG
GAGGCCAACCACAACTCCGGCACGTTCTCGACCGCTTCACCGAGTCGCTGCACT
ACTACTCCACCATGTTTCGATTCTCTCGAGGGCGCCGGCGCCGGCTCCGGCCAGTC
CACCGACGCTCCCCGGCCGCGGCCGGCGGCACGGACCAGGTCATGTCGGAGGT
GTACCTCGGCCGGCAGATCTGCAACGTGGTGGCGTGCAGGGGCGCGGAGCGCAC
GGAGCGCCACGAGACGCTGGGCCAGTGGCGCAGCCGCTCGGCGGCTCCGGGTT
CGCGCCCGTGCACCTGGGCTCCAATGCCTACAAGCAGGCGAGCACGCTGCTGGC
GCTCTTCGCCGGCGGCGACGGGTACAGGGTGGAGGAGAAGGACGGGTGCCTGAC
CCTGGGGTGGCATAACGCGCCCGCTCATCGCCACCTCGGCGTGGCGCGTCGCCGCC
GCCGCCGCTCCGTGATCAGGGAGGGGTGGTTGGGGCTTCTGGACGCCGATCAAG
GCACACGTACGTCCCTGGCATGGCGCACCTCCCTCGAGCTCGCCGGCACGGGT
GAAGCTACCCGGGGGATCCACTAATTCTAAAACGGCCCCACCGCGGTGGAATC
CACCTTTTGTTCCCTTA

Figure 9b

MKREYQDAGGSGGDMGSSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDM
ADVAQKLEQLEMAMGMGGVGGAGATADDGFVSHLATDTVHYNPSDLSSWVESML
SELNAPPAPLPPATPAPRLASTSSTVTSGAAAGAGYFDLPPAVDSSSSTYALKPIPSV
AAPSADPSTDSAREPKRMRTGGGSTSSSSSSSSSSMDGGRTRSSVVEAAPPATQASAAA
NGPAVPVVVVDTQEAGIRLVHALLACAEAVQQENFSAAEALVKQIPMLASSQGGAM
RKVAAYFGEALARRVYRFRPPPDSSLLDAAFADLLHAHFYESCPYLKFAHFTANQAI
LEAFAGCRRVHVVDFFGIKQGMQWPALLQALALRPGGPPSFRLTGVGPPQPDETAL
QQVGWKLAAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGDDTDDEPEVIAVNSVF
ELHRLLAQPGALEKVLGTVRAVRPRIVTVVEQEANHNSGTFLDRFTESLHYYSTMFD
SLEGAGAGSGQSTDASPAAAGGTDQVMSEVYLGRQICNVVACEGAERTERHETLGQ
WRSRLGGSGFAPVHLGSNAYKQASTLLALFAGGDGYRVEEKDGCLTLGWHTRPLIA
TSAWRVAAAAAP

Figure 10

maiz-fin	MKREYQDAGG	S...G...GDM	GSSKDKMMMAA	AAGAGEQE	DVDELLAALG	YKVRSSDMAD	55
rht-fina	MKREYQDAGG	SGGGG...GGM	GSSKDKMMVS	AAAG...EGE	DVDELLAALG	YKVRSSDMAD	55
rice-fin	.RPTRPAGG	SSGGGSSADM	GSCKDKMMAG	AAG...EEE	DVDELLAALG	YKVRSSDMAD	55
gai	MKRPHHHHQ	D.....	...KKTMMMN	EED...DGN	GMDLLAALG	YKVRSSDMAD	44
maiz-fin	VAQKLEQLEM	AMGMGCVGGA	GATADDGFVS	HLATDTVHYN	PSDLSWVES	MLSELNAPPA	115
rht-fina	VAQKLEQLEM	AMGMGCV.GA	GAAADDGFAT	HLATDTVHYN	PSDLSWVES	MLSELNAPPA	114
rice-fin	VAQKLEQLEM	AMGMGCVSAP	GA.ADDGFVS	HLATDTVHYN	PSDLSWVES	MLSELNAPPA	114
gai	VAQKLEQLEV	MMS.....	...NVQEDDLS	QLATDTVHYN	PSDLSWVES	MLSELNAPPA	93
maiz-fin	PLPPATPAAR	LASTSSTVTS	GAAAGAGYFD	LPPAVDSSSS	TYALKPIPS	VAA..PSADPS	174
rht-fina	PLPPAPQLN	.ASTSSTVT.	...GSGGYFD	LPPSVDDSSS	TYALRRPIS	AGATAPADLS	168
rice-fin	PAPAPPAAR	HASTSSTVTG	G...GSGGYFD	LPPAAADSSS	TYALRRPIS	VVATADPS.A	171
gai				SSNA	EYDLKAI	A I L N Q F A . . .	114
maiz-fin	TDSARPKRM	RTGGGSGTSSS	SSSSSSSSMDGG	RTRSSVVEAA	PPATOASAAA	NGPAVPVVVV	234
rht-fina	ADSVRDPKRM	RTGGGSGTSSS	SSSSSSSLGGG	.ARSSVVEAA	PPV..AAAAA	ATPAVPVVVV	225
rice-fin	ADSARDTKRM	RTGGGSGTSSS	SSSSSSSLGGG	ASRGSVVEAA	PPATOGAAA	NAPAVPVVVV	231
gai		IDSSAS	SSNQGGGGDT	YTTNKRRLKCS	NGVVEETTAT	AESTRHVVVV	159
maiz-fin	DTQEAQIRLV	HALLACAEAV	QOENFSAAEA	LVKQIPMLAS	SQGGAMRKVA	AYFGEALARR	294
rht-fina	DTQEAQIRLV	HALLACAEAV	QOENLSAAEA	LVKQIPLLA	SQGGAMRKVA	AYFGEALARR	285
rice-fin	DTQEAQIRLV	HALLACAEAV	QOENF.....				256
gai	DSQENGRRLV	HALLACAEAV	QKENLTVAEA	LVKQIGFLAV	SQGGAMRKVA	TYFAEALARR	219
maiz-fin	VYRFRPPPPDS	SLLDAAAFADL	LHAHFYESCP	YLLKFAHFTAN	QAILAEAFAGC	RRVHVVDFFGI	354
rht-fina	VYRFRPPPPDS	SLLDAAAFADL	LHAHFYESCP	YLLKFAHFTAN	QAILAEAFAGC	RRVHVVDFFGI	345
rice-fin	YRSPSQ..	SPDHS.SDT	LQMHFYETCP	YLLKFAHFTAN	QAILAEAFAGC	RRVHVVDFFGI	256
gai						RRVHVVDFFGI	277
maiz-fin	KQGMQWPALL	QALALRPGGP	PSFRLTGVGP	POPDETALQ	QVGMKLAQFA	HTIRVDFQYR	414
rht-fina	KQGMQWPALL	QALALRPGGP	PSFRLTGVGP	POPDETALQ	QVGMKLAQFA	HTIRVDFQYR	405
rice-fin	SQGMQWPALL	QALALRPGGP	PVFRLTGVGP	PAPDNFDYLIH	EVGCKLAHHA	EA I H V F E Y R	256
gai							337
maiz-fin	GLVAATLADL	EPFMLQPEG.	EDTDDDEPEVI	AVNSVFEHLHR	LLAQPGALEK	VLGTVRAVRP	473
rht-fina	GLVAATLADL	EPFMLQPEG.	EDPNEEPEVI	AVNSVFEHLHR	LLAQPGALEK	VLGTVRAVRP	465
rice-fin	GLVAATLADL	EPFMLQPEG.	EDPNEEPEVI	AVNSVFEHLHR	LLAQPGALEK	VLGTVRAVRP	256
gai	GLVANTLADL	EPFMLQPEG.	ELRPSEIES	AVNSVFEHLHR	LLAQPGALEK	VLGTVRAVRP	392

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Figure 10 (Continued)

maiz-fin	RIVTVVEQEA	NHNSGTFFLDR	FTESLHYYST	MFDSEEGAGA	GSGQSTDA	A...	529
rht-fina	RIVTVVEQEA	NHNSGTFFLDR	FTESLHYYST	MFDSEEGGSS	GGGPS	AAAAPAAA	525
rice-fin	EFTTVVEQES	NHNSPIFFLDR	FTESLHYYST	FFDSEEGVPS	GQ	...	256
gai						...	434
maiz-fin	DQVMSEVYLG	RQICNVVACE	GAERTERHET	LGQWRSRLGG	SGFAPVHLGS	NAYKQASTLL	589
rht-fina	DQVMSEVYLG	RQICNVVACE	GAERTERHET	LGQWRNRLGN	AGFETVHLGS	NAYKQASTLL	585
rice-fin	DKVMSEVYLG	RQICNVVACD	GPDRVERHET	LISQWRNRHGS	AGFAAHIGS	NAKQASMLL	256
gai						...	494
maiz-fin	ALFAGGDGYR	VEEKDGCLTL	GWHTRP LIAT	SAWRVAAA	P	630	
rht-fina	ALFAGGDGYR	VEEKDGCLTL	GWHTRP LIAT	SAWRLAGP	...	623	
rice-fin	ALFNGGGLGYR	VEESDGCLML	GWHTRP LIAT	SAWKLS	STN	...	256
gai						...	532

Figure 11a

TACCAAGACGCCGGCGGGAGTGGCGGCGACATGGGCTCCTCCAAGGACAAGATG
ATGGCGGCGGCGGCGGGAGCAGGGGAACAGGAGGAGGAGGACGTGGATGAGCT
GCTGGCCGCGCTCGGGTACAAGGTGCGTTCGTTCGGATATGGCGGGGCTGGAGCA
GCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGGCGCCGGCGCTACCGCTGA
TGACGGGTTCGTGTTCGCACCTCGCCACGGACACCGTGCCTACAATCCCTCCGAC
CTGTCTCCTGGGTTCGAGAGCATGCTGTCCGA

Figure 11b

YQDAGGSGGDMGSSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDMAGLEQ
LEMAMGMGGVGGAGATADDGFVSHLATDTVHYNPSDLSSWVESMLS

Figure 11c

TCCTCCAAGGACAAGATGATGGCGGCGGCGGCGGGAGCAGGGGAACAGGAGGA
GGAGGACGTGGATGAGCTGCTGGCCGCGCTCGGGTACAAGGTGCGTTCGTTCGGA
TATGGCGGACGTTCGCGCAGAAGCTGGAGCAGCTCGAGATGGCCATGGGGATGGG
CGGCGTGGGCGGCGCCGGCGCTACCGCTGATGACGGGTTCGTGTTCGCACCTGTTCG
TCCTGGGTTCGAGAGCATGCTGTCCGAGCTCAACGCGCCCCCAGCGCCGCTCCCGC
CCGCGACGCGCGCCCCAAGGCTCGCGTCCACATCGTCCACCGTCACAAGTGGCGC
CGCCGCCGGTGCTGGCTACTTCGATCTCCCGCCCCGCCGTGGACTC

Figure 11d

SSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDMADVAQKLEQLEMAMGM
GGVGGAGATADDGFVSHLSSWVESMLSELNAPPAPLPPATPAPRLASTSSTVTSGAA
AGAGYFDLPPAVD

Figure 12a

GCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAG
CTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCC
CCCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGTGCACTACAACCCCA
CCGACCTGTTCGTCTTGGGTCGAGAGCATGCTGTTCGGAGCTCAACGCCTCCACCTC
CTCCACCGTTCACGGGCAGCGGCGGCTACTTCGATCTCCCGCCCTCCGTCGACTCC
TCCAGCAGCATCTACGCGCTGCGGCCGATCCCCTCCCCGGCCGGCGCGACGGCGC
CGGCCGACCTGTCCGCCGACTCCGTGCGGGATCCCAAGCGGATGCGCACTGGCG
GGAGCAGCACCTCGTCGTCATCCTCCTCCTCGTC

Figure 12b

AALGYKVRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYN
PTDLSSWVESMLSELNASTSSTVTGSGGYFDLPPSVDSSSSIYALRPIPSAGATAPAD
LSADSVRDPKRMRTGGSSTSSSSSSS

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02383

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/29 C12N15/82 C12Q1/68 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 6 C12N C12Q A01H

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	TROUNG, H.-N., ET AL.: "Sequence and characterization of two Arabidopsis thaliana cDNAs isolated by functional complementation of a yeast gln3 gdh1 mutant" FEBS LETTERS, vol. 410, June 1997, pages 213-218, XP002088384 see the whole document	6,8,9, 14,15, 18,23, 30,48, 51-54
X	SASAKI, T., ET AL.: "Rice cDNA, partial sequence (S0803_1A)" EMBL ACCESSION NO.D39460.13 November 1994, XP002088385 see the whole document	6,8,9, 12-15, 18,23,30



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

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Date of mailing of the international search report

12/01/1999

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02383

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Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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