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(72) HARBERD, NICHOLAS PAUL, GB
(72) RICHARDS, DONALD ERNEST, GB
(72) PENG, JINRONG, GB
(71) PLANT BIOSCIENCE LIMITED, GB

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(54) **REGULATION GENETIQUE DE LA CROISSANCE ET DU DEVELOPPEMENT VEGETAUX**

(54) **GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT**

(57) L'invention concerne le gène Rht du blé et ses homologues d'autres espèces, y compris le riz et le maïs (le gène D8), utiles pour modifier les caractéristiques de la croissance et/ou du développement des plantes. L'invention concerne également des plantes transgéniques, des procédés et des moyens de production de celles-ci.

(57) The wheat Rht gene and homologues from other species including rice and maize (the D8 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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(71) Applicant *(for all designated States except US)*: PLANT BIOSCIENCE LIMITED [GB/GB]; Norwich Research Park, Colney Lane, Norwich, Norfolk NR4 7UH (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HARBERD, Nicholas, Paul [GB/GB]; 33 Mount Pleasant, Norwich, Norfolk NR2 2DH (GB). RICHARDS, Donald, Ernest [GB/GB]; "Lynden", Long Street, Great Ellingham, Norfolk NR17 1LN (GB). PENG, Jinrong [CN/GB]; 12 Long View, Hethersett, Norfolk NR9 3JN (GB).

(74) Agents: WALTON, Seán, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

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(54) Title: GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

(57) Abstract

The wheat *Rht* gene and homologues from other species including rice and maize (the *D8* gene), useful for modification of growth and/or development characteristics of plants. Transgenic plants and methods and means for their production.

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.

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 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 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 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.

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 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 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 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 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.

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 "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 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 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 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.

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 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 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. 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.

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)).

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 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 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 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 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 GA_1, GA_3, GA_4 and GA_7) 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 GA_8, GA_{29}, GA_{34} and GA_{51}). *rht* mutants do not respond to GA treatment, e.g. treatment with GA_1, GA_3 or GA_4.

Treatment with GA is preferably by spraying with aqueous solution, for example spraying with 10^{-4}M GA_3 or GA_4 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 24ηl of 0.6, 4.0 or 300mM GA_3 or GA_4 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.

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 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 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 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 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 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 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 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 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 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 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 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 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. 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 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.

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 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, 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 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, 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 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 close to the N- or C- terminus of the protein and so may be used to purifiy 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.

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-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) 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 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 specificty 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.

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

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 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 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 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 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, 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.

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 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 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 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.

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.

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.

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 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 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 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 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 *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 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 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 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 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 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 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 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) 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.

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.

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 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 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 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 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 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 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 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 with any one or more of the specific sequences disclosed herein under stringent conditions.

Hybridisation may be be determined by probing with nucleic acid and identifying positive hybridisation under suitably 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.

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 examination of restriction fragment length polymorphisms, amplification using PCR, RNAase cleavage and allele specific oligonucleotide probing.

Probing may employ the standard Southern blotting technique. 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 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 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.

Suitable conditions include, e.g. for detection of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulphate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

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, 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 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, stringencycan be increased by increasing the temperature of the washes, and/or reducing or even omitting altogether, the SSC ini 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.)

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 aicds 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 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 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, 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, 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 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 aicds 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.

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

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.

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 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 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 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 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, 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, 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.

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 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 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 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 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.

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 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, 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.

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.

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).

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, 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 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 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 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.

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. 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 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, 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 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 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 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).

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 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 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, 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 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, *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.* (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; 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).

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).

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 reviewd in Vasil et al., *Cell Culture and Somatic Cel 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.

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 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.

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 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". 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 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 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).

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, 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 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 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 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.

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 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 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:

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).

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

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

Figure 3: Rht sequences.

Figure 3a shows a composite DNA sequence of wheat *Rht* gene 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 *Arabidopsis* (Gai). Regions of sequence identity are highlighted in black.

Figure 4: D39460 sequence.

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.

Figure 6: Rice EST sequence

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

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

Figure 7: Wheat C15-1 cDNA

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.

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.

Figure 9: Maize 1a1 genomic clone

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

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

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

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

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

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

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 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 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 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* 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; 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 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 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 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.

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.

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; 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 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; 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 *D*8-1, *D*8-2023 and *Rht-D1c*, using the following primers for amplification: for *D*8-1, primers ZM-15 and ZM-24; for *D*8-2023, 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 *D*8-1 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. *D*8-2023 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 netween 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

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

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

Name	Sequence	Sense
ZM-8	GGCGATGACACGGATGACG	Forward
ZM-9	CTTGCGCATGGCACCGCCCTGCGACGAAG	Reverse
ZM-10	CCAGCTAATAATGGCTTGCGCGCCTCG	Reverse
ZM-11	TATCCCAGAACCGAAACCGAG	Forward
ZM-12	CGGCGTCTTGGTACTCGCGCTTCATG	Reverse
ZM-13	TGGGCTCCCGCGCCGAGTCCGTGGAC	Reverse
ZM-14	CTCCAAGCCTCTTGCGCTGACCGAGATCGAG	Forward
ZM-15	TCCACAGGCTCACCAGTCACCAACATCAATC	Forward
ZM-16	ACGGTACTGGAAGTCCACGCGGATGGTGTG	Reverse
ZM-17	CGCACACCATCCGCGTGGACTTCCAGTAC	Forward
ZM-18	CTCGGCCGGCAGATCTGCAACGTGGTG	Forward
ZM-19	TTGTGACGGTGGACGATGTGGACGCGAGCCTTG	Reverse
ZM-20	GGACGCTGCGACAAACCGTCCATCGATCCAAC	Forward
ZM-21	TCCGAAATCATGAAGCGCGAGTACCAAGAC	Forward
ZM-22	TCGGGTACAAGGTGCGTTCGTCGGATATG	Forward
ZM-23	ATGAAGCGCGAGTACCAAGAC	Forward
ZM-24	GTGTGCCTTGATGCGGTCCAGAAG	Reverse
ZM-25	AACCACCCCTCCCTGATCACGGAG	Reverse
ZM-27	CACTAGGAGCTCCGTGGTCGAAGCTG	Forward
ZM-28	GCTGCGCAAGAAGCCGGTGCAGCTC	Reverse
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 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* 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 nucleotide sequence including GACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCG.

4. An isolated polynucleotide encoding a polypeptide which comprises the amino acid sequence shown in Figure 8b.

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 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.

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

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.

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 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.

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.

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 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 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.

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.

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 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 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 DELLAALGYKVRSSDMA are deleted.

21. An isolated polynucleotide according to claim 18 wherein the amino acid sequence VAQK is deleted.

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 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.

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

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.

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 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 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.

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

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.

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.

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, 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 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 cells.

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

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 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 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 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 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.

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 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.

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  ....MKRD HHHQDMKKT MMNEEDDGN GMDELLAVLG YKVRSSEMAD LAETVHHY NMEAELYTWLD
0803 EAGGSSGGGS SAHHQDMKDK VMAGAXGEEE XVDELLALLG YKVRSSDMAD RMGGSCRT WMRTKFI...

Gai  MMSNVQEDDL SQQLAEQLEQ LKKVAQKLEQ LVVAQKLEQM
0803 AMGMGGVTPP AQQLAEQLEQ LKKVAQKLEQ LMVAQKLEQM
```

Figure 2a

CCCCGACGGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGG
TCGACACGCAGGAGGCCGGGATTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGG
AGGCCGTGCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTGGTGAAGCAGATAC
CCTTGCTGGCCGCGTCCCAGGGCGGCGCGATGCGCAAGGTCGCCGCCTACTTCGG
CGAGGCCCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTC
CTCGACGCCGCCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGCCCCTA
CCTCAAGTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCGCCGGC
TGCCGCCGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGTGGCCC
GCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTCAC
CGGCGTCGGCCCCCCGCAGCCGGACGAGACCGACGCCCTGCAGCAGGTGGGCTG
GAAGCTCGCCCAGTTCGCGCACACCATCCGCGTCGACTTCCAGTACCGCGGCC
TCGTCGCCGCCACGCTCGCGGACCTGGAGCCGTTCATGCTGCAGCCGGAGGGCG
AGGAGGACCCGAACGAAGANCCCGANGTAATCGCCGTCAACTCAGTCTTCGAGA
TGCACCGGCTGCTCGCGCAGCCCGGCGCCCTGGAAAAGGTTCTTGGGCACCGTGC
GCCCCCGTGCGGCCCAGAATTCNTCACCGTGGTGGAAACAGGAGGCAAATCACA
ACTCCGGCACATTCCTGGACCGCTTCACCGAGTCTCTGCACTACTACTCCACCAT
GTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCATCG
GGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAGGTCATNTCCGAGGTGT
ACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAACGCACAG
ANCGCCACGAGACGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCG
AGACCGTCCACCTGGGCTCCAATGCCTACAAGCAGGCGANCACGCTGCTGGCGC
TCTTCGCCGGCGGCGAACGGCTACANGTGGAAGAAAAGGAAGGCTGCCTGACGC
TGGGGTTGCACACNCCCCCCTGATTGCCACCTCGGCATGGCGCCTGGCCGGGCCG
TGATCTCGCGAGTTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACA
CAGCCCCGGCGGCCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGA
AGAAGAAGAAGCTAAATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTA
CGATCGATCGGGCTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGA
CGACGAACTCCGAGCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGT
TCCCAGTTCTCCACCGCCTCCATGATCACCCGTAAAACTCCTAAGCCCTATTATTA
CTACTATTATGTTTAAATGTCTATTATTGCTATGTGTAATTCCTCCAACCGCTCAT
ATCAAAATAAGCACGGGCCGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAA

Figure 2b(1)

CGCGCAATGCTTAAGGTCNCCGCCTACTTCGGNGCAGGCCCTCGCCCGCCGCGTC
TTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCT
CCTCCACGCGCACTTCTACNAGTCCTGCCCCTACCTCAAGTTCGCGCACTTCACCG
CCAATTAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGA
CTTCGGCATCAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTC
CGTCCCGGCGGCCCTCCCTCGTTCCGCCTCACCGGCGTCGGCCCCCCGCAGCCGG

Figure 2b(2)

ACCTCCTTCGTCGTCTNTNNGGTGGGGGCGCCAGGAGCTTATGTGGTGGAGGNTG
GCCCCNCCGGTCGCGACCGCGNCCTACGNGACGCCCGCGCTGCCGGTCGTCGTGG
TCGACACGCAGGAGGCCGGGATTCGGNTGGTNCACGCGCTGCTGGNGTGCGNGG
AGNCCGTGCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTNGTGAAGNAGATAC
CCNTGCTGGCCGAGTCCCAGGGCGGCGAGATGNGCAAGGTNGCAGCTTACTTNG
NAGANGCCCTCGCCCGCNGAGTGATTCCACTTANCGCCTGCAGCCGGANAGCTCC
GTCCTCGAANCCGCNTTNGCCGACCTCCTCCACGNGCACNTNTACGAGTC

Figure 2b(3)

TANTAGTCTCTCGGTGGGGGCGCCAGGAGCTCTNTGGTGGAGGCNGCCCCGCCG
GTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACACG
CAGGAGGCCGGGATTCGGATGGTGCACGCGCTGNTGGCGTGCGCGGAGGCCGTG
AAACAGTTGAAGGNCCNCGCCTNNNNNCNCACAANNTGAAAGCCCCGNG

Figure 2b(4)

GGCTNCCNCCNCGTGCACGTCGTCGACTTCGGCATCAAGCATGGGATGCANTGGC
NCGNACTTCTCCANGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTC
ACCGGCGTCGGCCCCCCCGCAGCCGGACGAGACCGACGCCCTGCANCAGGTGGGC
TGGAAGCTCGCCCAGTTCGCGCACACCATCCGCGTCGACTTCCANTACCGTGGCC
TCGTCGCCGCCACGCTCGCGGACCTGGAGCCGTTCATGCTGCANCCGGAGGGCGA
GGAGGACCCGAACGACGGAGCCCGAGGTAATCGCCGTCAACTCAGTCTTCGAGA
TGCACCGGGCTGCTCNCGCANCCCGGCGACNCTGGAANAA

Figure 2b Continued

Figure 2b(5)

CAAGANGCTAATCACAACTCCGGCACATTCCTGGACCGCTTCACCGAGTCTCTGC
ANTACTACTCCACCATGTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCC
ATCCGAAGTCTCATCGGGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAT
GTCATGTCCGAXGTGTACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGG
GGGCGGAGCGCACANTANCGCCACGCAGACNCTGGGCCAGTGGCGTGAACCGGC
TGGGCAACGCCNGGTTCANNNNCCGTCCACCTGGGCTCCAATGCCTACAATCAN
GCNNNCACGCTGCTGGCGCCTCTTCGCCC

Figure 2b(6)

TCGCCANTCGGCATGGNGCCTGGCCGGGCCGTGATCTCGCGAGTTTTGAACGCTG
TAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCGGCCGCCCCGGCT
CTCCGGCGAACGCACGCACGCACGCACTTGGAAGAAGAANAAGCTAAATGTCAT
GTCAGTGAGCGCTGAATTGCAACGACCGGCTACGATCGATCGGGCTACGGGTGG
TTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCGANCCGACCAC
CACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTCTCCACCGCCTCCAT
GGATCACCCGTAAAACTCCTAAGCCCTAATTATNNACTAACTAATTATGTTTTAA
AATGTTCTAATTAATTGGCTATGTTGTAATNCCTCCAAACCGGCTCATTTTCAAA
NATTAAGCCACGGGCCCGGAACTTTGGTTTAACAACCTCCCNATTGNAAAATTNA
AATNGAAATTTTTGGTTNC

Figure 2b(7)

GTTGGTGGNGGCGATTTGGGTACAAGGTGCGCGCCTCCGACATGGNGGANGTGG
GGCAGAAGCTGGAGCAGNTCGAGATGGCCATGGGGATGGGNGGCGTGGGCGCT
GGCGCCGCCCCTGACGACAGGTTNGCCACCCGCNGGCCGCGGACACNGTGCANT
ACAACCCCACNGACNTGTCGTCTTGGGTCGAGAGCATGCTGTCGGAGCTAAANG
AGCCGCNGCCGCCCCTCCCGCCCGCCCCGCAGCTCAACGCCTCCACCTCCTCCAC
CGTCACGGGCAGCGGCGGCTACTTCGATAACCCTCCCTG

Figure 2b(8)

TGATGGNGGGAGNTTANGGGTTANAAATGTGGGGGANTTCCGAANNGGTGAGG
ANATATNNTCAGAAGTTGGAGCAGATGAGAGATNGCTGATGGGGATAGGGTAGG
NGTGGGTGCCGGTGCNGCCCCCNAGGANAGATTGGCCACCCACTTAGCAAGTGG
ANACCGTGGATTACNACCCCACAGACCTGTCGTGGTTGGGTTTGAGAGCGTGGTG
TGGGAGCTGAACGGGCNGCGGCGTGCCCCTCCCGCCCGCCCCGCAGCTCAACGCC
TCCACCTCCTCCACCGTACACGGGCAGCGGCGGCTAGTTCGATCTCCCGCCCTCC
GTCGACTCCTCCAGCAGCATNTANGCGCTGCGGCCGATCCCCTNCCCAAGCNNGC
GNGGNCCGAGCCGTGTAN

Figure 2b Continued

Figure 2b(9)

TTTCANTTTCNTCCTTTTTTCTTCTTTTTCCAACCCCCGGCCCCCNGACCCTTGGAT
CCAAATCCCGAACCCGCCCCCAGAACCNGGAACCGAGGCCAAGCAAAAGNTTTG
CGCCAATTATTGGCCAGAGATAGATAGAGAGGCGAGGTAGCTCGCGGATCATGA
AGCGGGAGTACCAGGACGCCGGAGGGAGCGGCGGCGGCGGTGGCGGCATGGGT
TCGTCCGAGGACAAGATGATGGTGTCGGCGGCGGCGGGGGAGGGGGAGGAGGT
GGACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGA
CGTGGCGCAGAAGCTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGG
CGCCGGCGCCGCCCCCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGTG
CAGTACAACCNCCCNGACC

Figure 2b(10)

GGACGACGACCTCCGAGCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTT
CNTTCCCAGTNCTCCACCGCCTCCATGATCACCCGTAAAACTCCTAAGCCCTATT
ATTACTACTATTATGTNTAANTGTCTATTATTGCTANGTGTAATTCCTCCAACCGC
TCATATCAAAATAAGCACGGGCCGGACTTTGTTANCAGCTCCAATGAGAATGAA
ATGAATTTTGTACGCAAGGCACGTCCAAAACTGGGCTGAGCTTTGTTCTGTTCTG
TTATGTTCATGGTGCTCACTGCTCTGATGAACATGATGGTGCCTCCAATGGTGGC
TTTGCAATTGTTGAAACGTTTGGCTTGGGGGACTTGNGTGGGTGGGTGCATGGGG
ATGAATATTCACATCNCCGGATTAAAATTAAGCCATCCCGTTGGCCGTCCTTTGA
ATANCTTGCCCNAAACGAAATTTCCCCCNATC

Figure 2b(11)

AAANCCTANAANATATAGAGGCGATGTNGCNCCCCNATCANNAACNGGATTACN
GNAACNCCNGAAGGAGCGGCGGCGGCGGTGGCAGCATNGGCTCGTCCGATGACA
AATATCATGGTGTCGGCGGCGGCGGGGGACGGGGAGGAGGTGCACAACNTTTNG
GCGGGACTCGNGTACCACGTGNACGGTGCCGCNCTNGNGGATNTGGCCCTNGAA
GATGGGCCACCTCCAAA

Figure 2b Continued

Figure 2b(12)

CGGCGGCCCCCGTGGCGGCATGGGCTCGTCCGAGGACNAGATGATGGTGTCGGCG
GCGGCGGGGGANGGGGATGATGTGGACTATCTGCTGGCGGCGCTCGGGTACAAG
GTGCGCGCCTCCGACAGGCGGAGCCCGCGCATAACTGGAGCCGCTCGAGATGGC
CNTGGGGATNGGCGGCNTGGGCNCCNGCGCCTCCCCCG

Figure 2b(13)

TGGNGCTCGGGTGNCCCGTGCGCGCCTCCGACATGGCGGGACGTGGCGCAGAAC
TGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCCC
CCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGGCACACAACCCCACCG
ACCTGTCGTCTTGGGTCGAGAGCATGCTGTCGGATCTCNACGCGCCNCCGNCGCC
CCTCCCGCCCGC

Figure 2c(1)

ANNTTGTNCNNNNTACATCCCATGNGCCGCGCNATGCTNAAGGTCGCCGCCTACT
TCGGCGCAGGCCCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCT
CCCTCCTCGACGCCGCCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGC
CCCTACCTCAAGTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCG
CCGGCTGCCGCCGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGT
GGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGC
CTCACCGGCGTTCGGCCCCCCGCAGCCGGACGANAACGACGCCCTG

Figure 2c(2)

NTTCCCCGGCAGTTAAAAGCNTCCACTTCTTCCACCGTCACGGGCAGCGGCGGNT
ACTTNGATCTCCCGCCCTCAGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCC
GATCCCCTCCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTG
CGGGATCCCAAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCT
CATANTCGTCTCTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCNGCCCCGCC
GGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACAC
GCAGGAGGCCGGGATTCGGATGGTGCACGCGCTGNTGGCGTGCGCGGAGGCCGT
GNAAGCAGTTNGAAGGGCCTNCGCCGTGNATNNCGCAACAANNNGGAAGNCCN

Figure 2c(3)

CANCCCGCTGNTCGCCACCTCGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGAG
TTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCG
GCCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAG
CTAAATGTCATGTCAGTGAGCGCTGAATTGCANCGACCGGCTACGATCGATCGG
GCTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCC
GANCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTTCTC
CACCGCCTCCATGATCACCCCGTAAAACTCCTAAGCCCTATNNNTTACTACNATT
AATGTTTTAAANTGTTCTANTAATTGCTATGNTGTTTATTNCC

Figure 2c(4)

TATCGAAGTAGCCGCCGCTGCCCNTGCACGGTGGAGGAGGTGGAGGCGTTGAGC
TGCGGGGCGGGCGGGAGGGGCGGCGGCGGCACGTTNAGCTCCGACAGCATGCTC
TCGACCCAAAACNACAGGTCGGTGGGGTTGTAGTGCACGGTGTCCGTGGCGAGG
GGGTGGCNAANCTGTCGTCAGGGGCGGCGCCNGCGCCCACNCCGCCCATCCCCA
TGGCCATCTCGANCTGCTCCAGCTTCTGCGCCACTTCCNCCATGTCNGATGCGCG
CNCCTTGTACCCGA

Figure 2c Continued

Figure 2c(5)

ACGGCGCGGNCCNCGCNNGCTTGGGAGGGGATCGGCCGCAGCGCNTANATGCTG
CTGGAGGAGTCGACGGAGGGCGGGAGATCGAACTAGCCGCCGCTGCCCGTGTAC
GGTGGAGGAGGTGGAGGCGTTGAGCTGCGGGGCGGGCGGGAGGGGCAGCNGCT
GCACGTTNAGCTCCCACACCACGTCTCTCAACCCAACCACGACNCGTCTGTGGGG
TNGTAATNCACGGTNTCCCTNGCTANGTGGGTGGCCAATCTNT

Figure 2c(6)

CACGGTGTCCGTGGCGAGGTGGGTGGCGAAGCTGTCGTCGGGGGCGGCGCCGGC
GCCCACGCCGCCCATCCCCATGGCCATCTCGAGCTGCTCCAGCTTCTGCGCCACG
TCCGCCATGTCGGAGGCGCGCACCTTGTACCCGAGCGCCGCCAGCAGCNCGNCC
ACCTCCTCCCCCTCCCCCCGCCGCCGCCGACACCATCATCTTGTCCTCGGACGANCC
CATGCCGCCACCGCCGCCGCCGCTCCCTCCGGCGTCCTGGTACTCCCGCTTCATG
ATCCGCGAGCTACCTCGCCTCTCTATCTATCTCTGGCCAATAATTGCGCA

Figure 2c(7)

GACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCNTTCCCAGTTCTCCACCGC
CTCCATGATCACCCGTAAAACTCCTAAGCCCTATTATTACTACTATTATGTNTAA
ATGTCTATTATTGCTANGTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACG
GGCCGGACTTTGTTAGCAGCTCCAATGAGAATGAAATGAATTTTGTACGCAAGGC
ACGTCCAAAACTGGGCTGAGCTTTGTTCTGTTCTGTTATGTTCATGGTGCTCACTG
CTCTGATGAACATGATGGTGCCTCCAATGGGTGGCTTTGCAATTGTTGAACGTTT
TGGCTTGGGGGACTTGGTGNNTGGTGCATGGGAATGAANATTCCACATCCNCNG
GAATTAAAATTAGCCCATCCCG

Figure 3a

TTTCANTTTCNTCCTTTTTTCTTCTTTTTCCAACCCCCGGCCCCCNGACCCTTGGATCC
AAATCCCGAACCCGCCCCCAGAACCNGGAACCGAGGCCAAGCAAAAGNTTTGCGCC
AATTATTGGCCAGAGATAGATAGAGAGGCGAGGTAGCTCGCGGATCATGAAGCGGG
AGTACCAGGACGCCGGAGGGAGCGGCGGCGGCGGTGGCGGCATGGGTTCGTCCGAG
GACAAGATGATGGTGTCGGCGGCGGCGGGGGAGGGGGAGGAGGTGGACGAGCTGC
TGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAG
CTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCTGGCGCCGCCCC
TGACGACAGGTTNGCCACCCGCNGGCCGCGGACACNGTGCANTACAACCCCACNGA
CNTGTCGTCTTGGGTCGAGAGCATGCTGTCGGAGCTAAANGAGCCGCNGCCGCCCC
TCCCGCCCGCCCCGCAGCTCAACGCCTCCACCGTCACGGGCAGCGGCGGNTACTTNG
ATCTCCCGCCCTCAGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCCGATCCCCT
CCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTGCGGGATCCC
AAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCTCATANTCGTCT
CTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCNGCCCCGCCGGTCGCGGCCGC
GGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACACGCAGGAGGCCGGGA
TTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGGAGGCCGTGCAGCAGGAGAACCTC
TCCGCCGCGGAGGCGCTGGTGAAGCAGATACCCTTGCTGGCCGCGTCCCAGGGCGG
CGCGATGCGCAAGGTCGCCGCCTACTTCGGCGAGGCCCTCGCCCGCCGCGTCTTCCG
CTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCTCCTCCA
CGCGCACTTCTACGAGTCCTGCCCCTACCTCAAGTTCGCGCACTTCACCGCCAACCA
GGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGACTTCGGCAT
CAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGG
CCCTCCCTCGTTCCGCCTCACCGGCGTCGGCCCCCCGCAGCCGGACGAGACCGACGC
CCTGCAGCAGGTGGGCTGGAAGCTCGCCCAGTTCGCGCACACCATCCGCGTCGACTT
CCAGTACCGCGGCCTCGTCGCCGCCACGCTCGCGGACCTGGAGCCGTTCATGCTGCA
GCCGGAGGGCGAGGAGGACCCGAACGAAGANCCCGANGTAATCGCCGTCAACTCA
GTCTTCGAGATGCACCGGCTGCTCGCGCAGCCCGGCGCCCTGGAAAAGGTTCTTGGG
CACCGTGCGCCCCCGTGCGGCCCAGAATTCNTCACCGTGGTGGAAACAGGAGGCAA
ATCACAACTCCGGCACATTCCTGGACCGCTTCACCGAGTCTCTGCACTACTACTCCA
CCATGTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCAT
CGGGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAGGTCATNTCCGAGGTGT
ACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAACGCACAGAN
CGCCACGAGACGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCGAGAC
CGTCCACCTGGGCTCCAATGCCTACAAGCAGGCGANCACGCTGCTGGCGCTCTTCGC
CGGCGGCGAACGGCTACANGTGGAAGAAAAGGAAGGCTGCCTGACGCTGGGGTTGC
ACACNCCCCCCTGATTGCCACCTCGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGA
GTTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCGG
CCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAGCTA
AATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTACGATCGATCGGGCTAC
GGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCGAGCCGA
CCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTCTCCACCGCCTCC
ATGATCACCCGTAAAACTCCTAAGCCCTATTATTACTACTATTATGTTTAAATGTCTA
TTATTGCTATGTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACGGGCCGGACT
TTGTTANCAGCTCCAATGAGAATGAAATGAATTTTGTACGCAAGGCACGTCCAAAA
CTGGGCTGAGCTTTGTTCTGTTCTGTTATGTTCATGGTGCTCACTGCTCTGATGAACA
TGATGGTGCCTCCAATGGTGGCTTTGCAATTGTTGAAACGTTTGGCTTGGGGGACTT
GNGTGGGTGGGTGCATGGGGATGAATATTCACATCNCCGGATTAAAATTAAGCCAT
CCCGTTGGCCGTCCTTTGAATANCTTGCCCNAAACGAAATTTCCCCCNATC

Figure 3b

PRETTYBOX of: My.Msf(*) August 7, 1997 13:06:42.76

```
Gai  .........M  KRDHHHHHHQ.  .........D  KKTMMMNEED  DGNGMDELLA  VLGYKVRSSE   41
Rht  IERRGSSRIM  KREYQDAGGS  GGGGGGMGSE  DKMMVSAAAG  EGEEVDELLA  ALGYKVRASD   60

Gai  MADVAQKLEQ  LEVMMS....  ....NVQEDD  LSQLATETVH  YNPAELYTWL  DSMLTDLNPP   93
Rht  MADVAQKLEQ  LEMAMGMGGV  GAGAAPDRQV  XHPXAADTVX  YNPTDXSSWV  ESMLSELXEP  120

Gai  ..........  ..........  .........S  SNAEYDLKAI  PGDAILNQFA  IDSASSSNQ.  123
Rht  XPPLPPAPQL  NASTVTGSGG  YXDLPPSVDS  SSSIYALRPI  PSPAGATAPA  DLSADSVRDP  180

Gai  ....GGGGDT  YTTNKRLKCS  NG.....VVE  .....TTTAT  AESTRHVVLV  DSQENGVRLV  169
Rht  KRMRTGGSST  SSSSSSXSSL  GGGARSSVVE  AAPPVAAAAN  ATPALPVVVV  DTQEAGIRLV  240

Gai  HALLACAEAV  QKENLTVAEA  LVKQIGFLAV  SQIGAMRKVA  TYFAEALARR  IYRLSPSQ..  227
Rht  HALLACAEAV  QQENLSAAEA  LVKQIPLLAA  SQGGAMRKVA  AYFGEALARR  VFRFRPQPDS  300

Gai  SPIDHSLSDT  LQMHFYETCP  YLKFAHFTAN  QAILEAFQGK  KRVHVIDFSM  SQGLQWPALM  287
Rht  SLLDAAFADL  LHAHFYESCP  YLKFAHFTAN  QAILEAFAGC  RRVHVVDFGI  KQGMQWPALL  360

Gai  QALALRPGGP  PVFRLTGIGP  PAPDNFDYLH  EVGCKLAHLA  EAIHVEFEYR  GFVANTLADL  347
Rht  QALALRPGGP  PSFRLTGVGP  PQPDETDALQ  QVGWKLAQFA  HTIRVDFQYR  GLVAATLADL  420

Gai  DASMLELRPS  EIES.....V  AVNSVFELHK  LLGRPGAIDK  VLG.VVNQIK  PEIFTVVE.Q  400
Rht  EPFMLQPEGE  EDPNEXPXVI  AVNSVFEMHR  LLAQPGALEK  VLGHRAPPCG  PEFXTVVETQ  480

Gai  ESNHNSPIFL  DRFTESLHYY  STLFDSLEGV  PSGQ......  ..........  ..DKVMSEVY  442
Rht  EANHNSGTFL  DRFTESLHYY  STMFDSLEGG  SSGGGPSEVS  SGAAAAPAAA  GTDQVXSEVY  540

Gai  LGKQICNVVA  CDGPDRVERH  ETLSQWRNRF  GSAGFAAAHI  GSNAFKQASM  LLALFNGGEG  502
Rht  LGRQICNVVA  CEGAERTXRH  ETLGQWRNRL  GNAGFETVHL  GSNAYKQAXT  LLALFAGGER  600

Gai  YRVEESDGCL  MLGWHTRPLI  ATSAWKLSTN  532
Rht  LXVEEKEGCL  TLGLHTXPLI  ATSAWRLAGP  630
```

Figure 4a

ACGCGTCCGGAAGCCGGCGGGAGCAGCGGCGGCGGGAGCAGCGCCGATATGGG
GTCGTGCAAGGACAAGGTGATGGCGGGGGCGGCGGGGGAGGAGGAGGACGTCT
ACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGGTCGTCCGACATGGCCGACG
TCGCGCAGAANCTGGAGCAGCTGGAGATGGCCATGGGGATGGGCGGCGTGAGCG
CCCCCGGCGCCGCGGATGACGGGTTCGTGTCGCACCTGGCCACGGACACCGTGC
ACTACAACCCCTCGGACCTCTCCTCCTGGGTTCNGAGAGCATGCTTTCGGAGTTA
AAGGCGCCGTTGCCCCTTATCCCGCCAGGCGCCGCCGGGCTGCCCGCCATGCTTT
CCAACTTCGTCCACTGTCACCGGCGGCGGTGGTAGCGGCTTCTTTGAANTCCCAG
CCGCTGCCGANTCGTCGAGTAGCACNTACGCCCTCAGGCCGATCTCCTTACCGGT
GGTGGCGACGGCTGACCCGTCGGCTGCTGACTCGGCGAGGGACACCAAGCGGAT
GCGCACTGGCGGCGGCAGCACGTCGTCGTCCTCATCGTCGTCTTCCTCTCTGGGC
GGTGGGGCCTCGCGGGGCTCTGTGGTGGAGGCTGCTCCGCCGGCGACGCAAGGG
GCCGCGGCGGCGAATGCGCCCGCCGTGCCGGTTGTGGTGGTTGACACGCAGGAG
GCTGGNATCGGGCCTGGTGC

Figure 4b

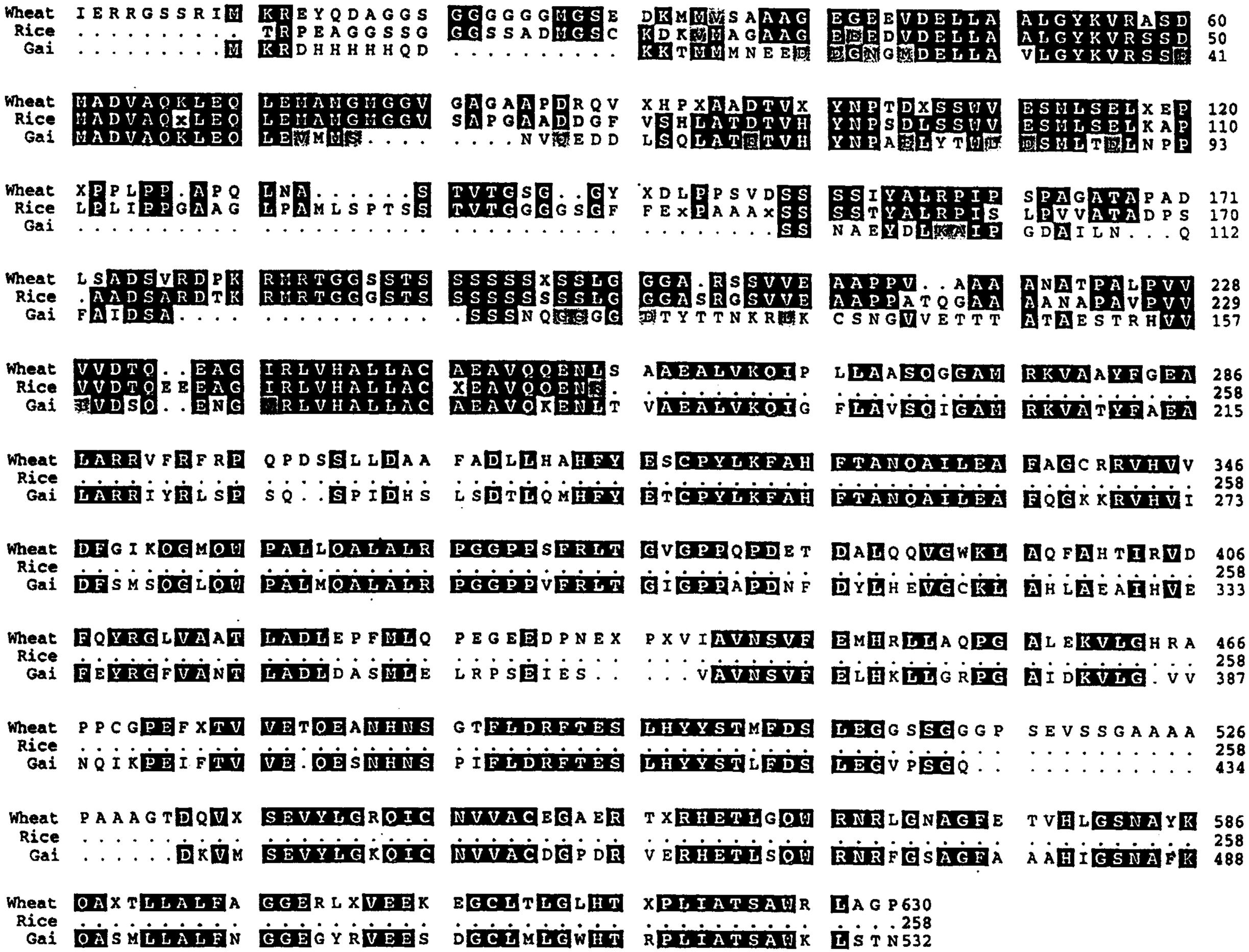

Wheat IERRGSSRIM KREYQDAGGS GGGGGGMGSE DKMMMSAAAG EGEEVDELLA ALGYKVRASD 60
Rice .....TRPEAGGSSG GGSSADMGSC KDKMMHAGAAG EEEDVDELLA ALGYKVRSSD 50
Gai .....MKRDHHHHHQD ..... KKTMMMNEED EGNGMDELLA VLGYKVRSSE 41
Wheat MADVAQKLEQ LEHAMGMGGV GAGAAPDRQV XHPXAADTVX YNPTDXSSWV ESMLSELXEP 120
Rice MADVAQxLEQ LEHAMGMGGV SAPGAADDGF VSHLATDTVH YNPSDLSSWV ESMLSELKAP 110
Gai MADVAQKLEQ LEMMMS.... ....NVMEDD LSQLATETVH YNPAELYTWE ESMLTELNPP 93
Wheat XPPLPP.APQ LNA.....S TVTGSG..GY XDLPPSVDSS SSIYALRPIP SPAGATAPAD 171
Rice LPLIPPGAAG LPAMLSPTSS TVTGGGGSGF FExPAAAxSS SSTYALRPIS LPVVATADPS 170
Gai ....SS NAEYDLKAIP GDAILN...Q 112
Wheat LSADSVRDPK RMRTGGSSTS SSSSSXSSLG GGA.RSSVVE AAPPV..AAA ANATPALPVV 228
Rice .AADSARDTK RMRTGGGSTS SSSSSSSSLG GGASRGSVVE AAPPATQGAA AANAPAVPVV 229
Gai FAIDSA.... .... .SSSNQGSGG ETYTTNKRMK CSNGVVETTT ATAESTRHVV 157
Wheat VVDTQ..EAG IRLVHALLAC AEAVQQENLS AAEALVKQIP LLAASQGGAM RKVAAYFGEA 286
Rice VVDTQEEEAG IRLVHALLAC XEAVQQENS. 258
Gai VVDSQ..ENG MRLVHALLAC AEAVQKENLT VAEALVKQIG FLAVSQIGAM RKVATYFAEA 215
Wheat LARRVFRFRP QPDSSLLDAA FADLLHAHFY ESCPYLKFAH FTANQAILEA FAGCRRVHVV 346
Rice ....I.... 258
Gai LARRIYRLSP SQ..SPIDHS LSDTLQMHFY ETCPYLKFAH FTANQAILEA FQGKKRVHVI 273
Wheat DFGIKQGMQW PALLQALALR PGGPPSFRLT GVGPPQPDET DALQQVGWKL AQFAHTIRVD 406
Rice 258
Gai DFSMSQGLQW PALMQALALR PGGPPVFRLT GIGPPAPDNF DYLHEVGCKL AHLAEAIHVE 333
Wheat FQYRGLVAAT LADLEPFMLQ PEGEEDPNEX PXVIAVNSVF EMHRLLAQPG ALEKVLGHRA 466
Rice ....I.... 258
Gai FEYRGFVANT LADLDASMLE LRPSEIES.. ...VAVNSVF ELHKLLGRPG AIDKVLG.VV 387
Wheat PPCGPEFXTV VETQEANHNS GTFLDRFTES LHYYSTMFDS LEGGSSGGGP SEVSSGAAAA 526
Rice ....I.... 258
Gai NQIKPEIFTV VE.QESNHNS PIFLDRFTES LHYYSTLFDS LEGVPSGQ.. 434
Wheat PAAAGTDQVX SEVYLGRQIC NVVACEGAER TXRHETLGQW RNRLGNAGFE TVHLGSNAYK 586
Rice ....I.... 258
Gai ......DKVM SEVYLGKQIC NVVACDGPDR VERHETLSQW RNRFGSAGFA AAHIGSNAFK 488
Wheat QAXTLLALFA GGERLXVEEK EGCLTLGLHT XPLIATSAWR LAGP630
Rice ....I.... 258
Gai QASMLLALFN GGEGYRVEES DGCLMLGWHT RPLIATSAWK LSTN532

Figure 5

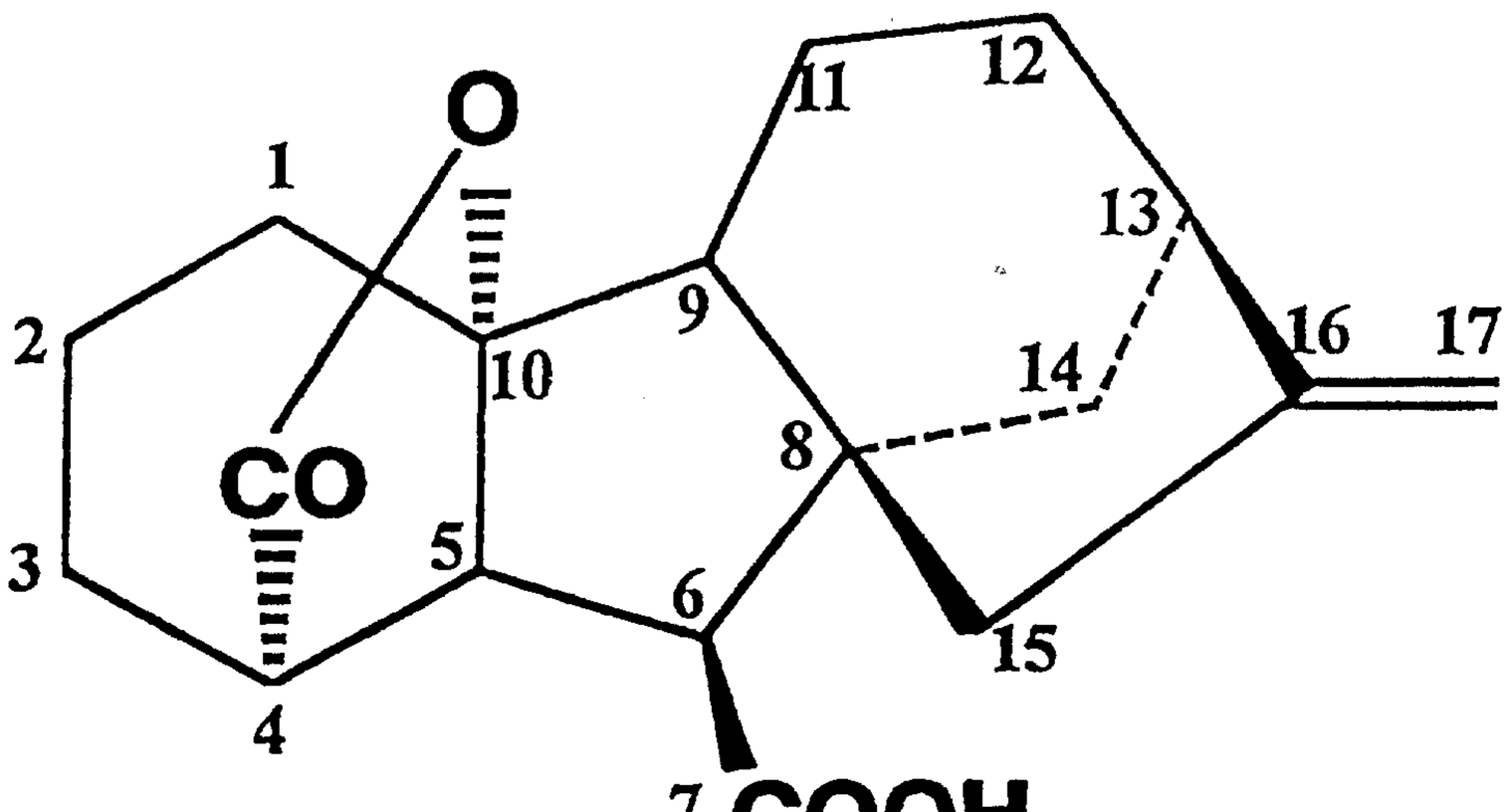


Figure 6a

GTCGACCCACGCGTCCGGAAGCCGGCGGGAGCAGCGGCGGCGGGAGCAGCGCC
GATATGGGGTCGTGCAAGGACAAGGTGATGGCGGGGGCGGCGGGGGAGGAGGA
GGACGTCGACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGGTCGTCCGACAT
GGCCGACGTCGCGCAGAAGCTGGAGCAGCTGGAGATGGCCATGGGGATGGGCGG
CGTGAGCGCCCCCGGCGCCGCGGATGACGGGTTCGTGTCGCACCTGGCCACGGA
CACCGTGCACTACAACCCCTCGGACCTCTCCTCCTGGGTCGAGAGCATGCTTTCC
GAGCTCAACGCGCCGCTGCCCCCTATCCCGCCAGCGCCGCCGGCTGCCCGCCATG
CTTCCACCTCGTCCACTGTCACCGGCGGCGGTGGTAGCGGCTTCTTTGAACTCCC
AGCCGCTGCCGACTCGTCGAGTAGCACCTACGCCCTCAGGCCGATCTCCTTACCG
GTGGTGGCGACGGCTGACCCGTCGGCTGCTGACTCGGCGAGGGACACCAAGCGG
ATGCGCACTGGCGGCGGCAGCACGTCGTCGTCCTCATCGTCGTCTTCCTCTCTGG
GCGGTGGGGCCTCGCGGGGCTCTGTGGTGGAGGCTGCTCCGCCGGCGACGCAAG
GGGCCGCGGCGGCGAATGCGCCCGCCGTGCCGGTTGTGGTGGTTGACACGCAGG
AGGCTGGGATCCGGCTGGTGCACGCGTTGCTGGCGTGCGCGGAGGCCGTGCAGC
AGGAGAACTTC

Figure 6b

RPTRPEAGGSSGGGSSADMGSCKDKVMAGAAGEEEDVDELLAALGYKVRSSDMAD
VAQKLEQLEMAMGMGGVSAPGAADDGFVSHLATDTVHYNPSDLSSWVESMLSELN
APLPPIPPAPPAARHASTSSTVTGGGGSGFFELPAAADSSSSTYALRPISLPVVATADPS
AADSARDTKRMRTGGGSTSSSSSSSSLGGGASRGSVVEAAPPATQGAAAANAPAVP
VVVVDTQEAGIRLVHALLACAEAVQQENF

Figure 7a

GCCAGGAGCTCTGTGGTGGAGGCTGCCCCGCCGGTCGCGGCCGCGGCCAACGCG
ACGCCCGCGCTGCCGGTCGTCGTGGTCGACACGCAGGAGGCCGGGATTCGGCTG
GTGCACGCGCTGCTGGCGTGCGCGGAGGCCGTGCAGCAGGAGAACCTCTCCGCC
GCGGAGGCGCTGGTGAAGCAGATACCCTTGCTGGCCGCGTCCCAGGGCGGCGCG
ATGCGCAAGGTCGCCGCCTACTTCGGCGAGGCCCTCGCCCGCCGCGTCTTCCGCT
TCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCTCCTCCA
CGCGCACTTCTACGAGTCCTGCCCCTACCTCAAGTTCGCGCACTTCACCGCCAAC
CAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGACTTCG
GCATCAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCC
CGGCGGCCCTCCCTCGTTCCGCCTCACCGGCGTCGGCCCCCCGCAGCCGGACGAG
ACCGACGCCCTGCAGCAGGTGGGCTGGAAGCTCGCCCAGTTCGCGCACACCATC
CGCGTCGACTTCCAGTACCGCGGCCTCGTCGCCGCCACGCTCGCGGACCTGGAGC
CGTTCATGCTGCAGCCGGAGGGCGAGGAGGACCCGAACGAGGAGCCCGAGGTAA
TCGCCGTCAACTCAGTCTTCGAGATGCACCGGCTGCTCGCGCAGCCCGGCGCCCT
GGAGAAGGTCCTGGGCACCGTGCGCGCCGTGCGGCCCAGGATCGTCACCGTGGT
GGAGCAGGAGGCGAATCACAACTCCGGCACATTCCTGGACCGCTTCACCGAGTC
TCTGCACTACTACTCCACCATGTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGC
GGCCCATCCGAAGTCTCATCGGGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGG
ACCAGGTCATGTCCGAGGTGTACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTG
CGAGGGGGCGGAGCGCACAGAGCGCCACGAGACGCTGGGCCAGTGGCGGAACC
GGCTGGGCAACGCCGGGTTCGAGACCGTCCACCTGGGCTCCAATGCCTACAAGC
AGGCGAGCACGCTGCTGGCGCTCTTCGCCGGCGGCGACGGCTACAAGGTGGAGG
AGAAGGAAGGCTGCCTGACGCTGGGGTGGCACACGCGCCCGCTGATCGCCACCT
CGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGAGTTTTGAACGCTGTAAGTACA
CATCGTGAGCATGGAGGACAACACAGCCCCGGCGGCCGCCCCGGCTCTCCGGCG
AACGCACGCACGCACGCACTTGAAGAAGAAGAAGCTAAATGTCATGTCAGTGAG
CGCTGAATTGCAGCGACCGGCTACGATCGATCGGGCTACGGGTGGTTCCGTCCGT
CTGGCGTGAAGAGGTGGATGGACGACGAACTCCGAGCCGACCACCACCGGCATG
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AAAACTCCTAAGCCCTATTATTACTACTATTATGTTTAAATGTCTATTATTGCTAT
GTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACGGGCCGGAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAA

Figure 7b

ARSSVVEAAPPVAAAANATPALPVVVVDTQEAGIRLVHALLACAEAVQQENLSAAE
ALVKQIPLLAASQGGAMRKVAAYFGEALARRVFRFRPQPDSSLLDAAFADLLHAHF
YESCPYLKFAHFTANQAILEAFAGCRRVHVVDFGIKQGMQWPALLQALALRPGGPPS
FRLTGVGPPQPDETDALQQVGWKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPE
GEEDPNEEPEVIAVNSVFEMHRLLAQPGALEKVLGTVRAVRPRIVTVVEQEANHNSG
TFLDRFTESLHYYSTMFDSLEGGSSGGGPSEVSSGAAAAPAAAGTDQVMSEVYLGR
QICNVVACEGAERTERHETLGQWRNRLGNAGFETVHLGSNAYKQASTLLALFAGGD
GYKVEEKEGCLTLGWHTRPLIATSAWRLAGP

Figure 8a

ATAGAGAGGCGAGGTAGCTCGCGGATCATGAAGCGGGAGTACCAGGACGCCGG
AGGGAGCGGCGGCGGCGGTGGCGGCATGGGCTCGTCCGAGGACAAGATGATGGT
GTCGGCGGCGGCGGGGGAGGGGGAGGAGGTGGACGAGCTGCTGGCGGCGCTCG
GGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAGCTGGAGCAGC
TCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCCCCCGACGACA
GCTTCGCCACCCACCTCGCCACGGACACCGTGCACTACAACCCCACCGACCTGTC
GTCTTGGGTCGAGAGCATGCTGTCGGAGCTCAACGCGCCGCCGCCGCCCCTCCCG
CCCGCCCCGCAGCTCAACGCCTCCACCTCCTCCACCGTCACGGGCAGCGGCGGCT
ACTTCGATCTCCCGCCCTCCGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCC
GATCCCCTCCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTG
CGGGATCCCAAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCT
CCTCGTCGTCTCTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCTGCCCCGCC
GGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACAC
GCAGGAGGCCGGGATTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGGAGGCCGT
GCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTGGTGAAGCAGATACCCTTGCT
GGCCGCGTCCCAGGGCGGCGCGATGCGCAAGGTCGCCGCCTACTTCGGCGAGGC
CCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGAC
GCCGCCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGCCCCTACCTCAA
GTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGC
CGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGTGGCCCGCACTTC
TCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTCACCGGCGTC
GGCCCCCCGCAGCCGGACGAGACCGACGCCCTGCAGCAGGTGGGCTGGAAGCTC
GCCCAGTTCGCGCACACCATCCGCGTCGACTTCCAGTACCGCGGCCTCGTCGCCG
CCACGCTCGCGGACCTGGAGCCGTTCATGCTGCAGCCGGAGGGCGAGGAAGACC
CGAACGAGGAGCCCGAGGTAATCGCCGTCAACTCAGTCTTCGAGATGCACCGGC
TGCTCGCGCAGCCCGGCGCCCTGGAGAAGGTCCTGGGCACCGTGCGCGCCGTGC
GGCCCAGGATCGTCACCGTGGTGGAGCAGGAGGCGAATCACAACTCCGGCACAT
TCCTGGACCGCTTCACCGAGTCTCTGCACTACTACTCCACCATGTTCGATTCCCTC
GAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCATCGGGGGCTGCTGCT
GCTCCTGCCGCCGCCGGCACGGACCAGGTCATGTCCGAGGTGTACCTCGGCCGGC
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CGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCGAGACCGTCCACC
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CGACGGCTACAAGGTGGAGGAGAAGGAAGGCTGCCTGACGCTGGGGTGGCACAC
GCGCCCGCTGATCGCCACCTCGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGAG
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GCCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAG
CTAAATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTACGATCGATCGGG
CTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCG

Figure 8b

MKREYQDAGGSGGGGGGMGSSEDKMMVSAAAGEGEEVDELLAALGYKVRASDM
ADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYNPTDLSSWVESMLS
ELNAPPPPLPPAPQLNASTSSTVTGSGGYFDLPPSVDSSSSIYALRPIPSPAGATAPADL
SADSVRDPKRMRTGGSSTSSSSSSSSLGGGARSSVVEAAPPVAAAANATPALPVVV
VDTQEAGIRLVHALLACAEAVQQENLSAAEALVKQIPLLAASQGGAMRKVAAYFGE
ALARRVFRFRPQPDSSLLDAAFADLLHAHFYESCPYLKFAHFTANQAILEAFAGCRR
VHVVDFGIKQGMQWPALLQALALRPGGPPSFRLTGVGPPQPDETDALQQVGWKLA
QFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVIAVNSVFEMHRLLAQ
PGALEKVLGTVRAVRPRIVTVVEQEANHNSGTFLDRFTESLHYYSTMFDSLEGGSSG
GGPSEVSSGAAAAPAAAGTDQVMSEVYLGRQICNVVACEGAERTERHETLGQWRN
RLGNAGFETVHLGSNAYKQASTLLALFAGGDGYKVEEKEGCLTLGWHTRPLIATSA
WRLAGP

Figure 9a

TTTCGCCTGCCGCTGCTATTAATAATTGCCTTCTTGGTTTCCCCGTTTTCGCCCCAG
CCGCTTCCCCCCTCCCCTACCCTTTCCTTCCCCACTCGCACTTCCCAACCCTGGAT
CCAAATCCCAAGCTATCCCAGAACCGAAACCGAGGCGCGCAAGCCATTATTAGC
TGGCTAGCTAGGCCTGTAGCTCCGAAATCATGAAGCGCGAGTACCAAGACGCCG
GCGGGAGTGGCGGCGACATGGGCTCCTCCAAGGACAAGATGATGGCGGCGGCGG
CGGGAGCAGGGGAACAGGAGGAGGAGGACGTGGATGAGCTGCTGGCCGCGCTC
GGGTACAAGGTGCGTTCGTCGGATATGGCGGACGTCGCGCAGAAGCTGGAGCAG
CTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGGCGCCGGCGCTACCGCTGAT
GACGGGTTCGTGTCGCACCTCGCCACGGACACCGTGCACTACAATCCCTCCGACC
TGTCGTCCTGGGTCGAGAGCATGCTGTCCGAGCTCAACGCGCCCCCAGCGCCGCT
CCCGCCCGCGACGCCGGCCCCAAGGCTCGCGTCCACATCGTCCACCGTCACAAGT
GGCGCCGCCGCCGGTGCTGGCTACTTCGATCTCCCGCCCGCCGTGGACTCGTCCA
GCAGTACCTACGCTCTGAAGCCGATCCCCTCGCCGGTGGCGGCGCCGTCGGCCGA
CCCGTCCACGGACTCGGCGCGGGAGCCCAAGCGGATGAGGACTGGCGGCGGCAG
CACGTCGTCCTCCTCTTCCTCGTCGTCATCCATGGATGGCGGTCGCACTAGGAGCT
CCGTGGTCGAAGCTGCGCCGCCGGCGACGCAAGCATCCGCGGCGGCCAACGGGC
CCGCGGTGCCGGTGGTGGTGGTGGACACGCAGGAGGCCGGGATCCGGCTCGTGC
ACGCGCTGCTGGCGTGCGCGGAGGCCGTGCAGCAGGAGAACTTCTCTGCGGCGG
AGGCGCTGGTCAAGCAGATCCCCATGCTGGCCTCGTCGCAGGGCGGTGCCATGC
GCAAGGTCGCCGCCTACTTCGGCGAGGCGCTTGCCCGCCGCGTGTATCGCTTCCG
CCCGCCACCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCTCTTGCACGCG
CACTTCTACGAGTCCTGCCCCTACCTGAAGTTCGCCCACTTCACCGCGAACCAGG
CCATCCTCGAGGCCTTCGCCGGCTGCCGCCGCGTCCACGTCGTCGACTTCGGCAT
CAAGCAGGGGATGCAGTGGCCGGCTCTTCTCCAGGCCCTCGCCCTCCGCCCTGGC
GGCCCCCCCGTCGTTCCGGCTCACCGGCGTCGGGCCGCCGCAGCCCGACGAGACC
GACGCCTTGCAGCAGGTGGGCTGGAAACTTGCCCAGTTCGCGCACACCATCCGCG
TGGACTTCCAGTACCGTGGCCTCGTCGCGGCCACGCTCGCCGACCTGGAGCCGTT
CATGCTGCAACCGGAGGGCGATGACACGGATGACGAGCCCGAGGTGATCGCCGT
GAACTCCGTGTTCGAGCTGCACCGGCTTCTTGCGCAGCCCGGTGCCCTCGAGAAG
GTCCTGGGCACGGTGCGCGCGGTGCGGCCGAGGATCGTGACCGTGGTCGAGCAG
GAGGCCAACCACAACTCCGGCACGTTCCTCGACCGCTTCACCGAGTCGCTGCACT
ACTACTCCACCATGTTCGATTCTCTCGAGGGCGCCGGCGCCGGCTCCGGCCAGTC
CACCGACGCCTCCCCGGCCGCGGCCGGCGGCACGGACCAGGTCATGTCGGAGGT
GTACCTCGGCCGGCAGATCTGCAACGTGGTGGCGTGCGAGGGCGCGGAGCGCAC
GGAGCGCCACGAGACGCTGGGCCAGTGGCGCAGCCGCCTCGGCGGCTCCGGGTT
CGCGCCCGTGCACCTGGGCTCCAATGCCTACAAGCAGGCGAGCACGCTGCTGGC
GCTCTTCGCCGGCGGCGACGGGTACAGGGTGGAGGAGAAGGACGGGTGCCTGAC
CCTGGGGTGGCATACGCGCCCGCTCATCGCCACCTCGGCGTGGCGCGTCGCCGCC
GCCGCCGCTCCGTGATCAGGGAGGGGTGGTTGGGGCTTCTGGACGCCGATCAAG
GCACACGTACGTCCCCTGGCATGGCGCACCCTCCCTCGAGCTCGCCGGCACGGGT
GAAGCTACCCGGGGGATCCACTAATTCTAAAACGGCCCCACCGCGGTGGAACTC
CACCTTTTGTTCCCTTTA

Figure 9b

MKREYQDAGGSGGDMGSSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDM
ADVAQKLEQLEMAMGMGGVGGAGATADDGFVSHLATDTVHYNPSDLSSWVESML
SELNAPPAPLPPATPAPRLASTSSTVTSGAAAGAGYFDLPPAVDSSSSTYALKPIPSPV
AAPSADPSTDSAREPKRMRTGGGSTSSSSSSSSSMDGGRTRSSVVEAAPPATQASAAA
NGPAVPVVVVDTQEAGIRLVHALLACAEAVQQENFSAAEALVKQIPMLASSQGGAM
RKVAAYFGEALARRVYRFRPPPDSSLLDAAFADLLHAHFYESCPYLKFAHFTANQAI
LEAFAGCRRVHVVDFGIKQGMQWPALLQALALRPGGPPSFRLTGVGPPQPDETDAL
QQVGWKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGDDTDDEPEVIAVNSVF
ELHRLLAQPGALEKVLGTVRAVRPRIVTVVEQEANHNSGTFLDRFTESLHYYSTMFD
SLEGAGAGSGQSTDASPAAAGGTDQVMSEVYLGRQICNVVACEGAERTERHETLGQ
WRSRLGGSGFAPVHLGSNAYKQASTLLALFAGGDGYRVEEKDGCLTLGWHTRPLIA
TSAWRVAAAAAP

Figure 10

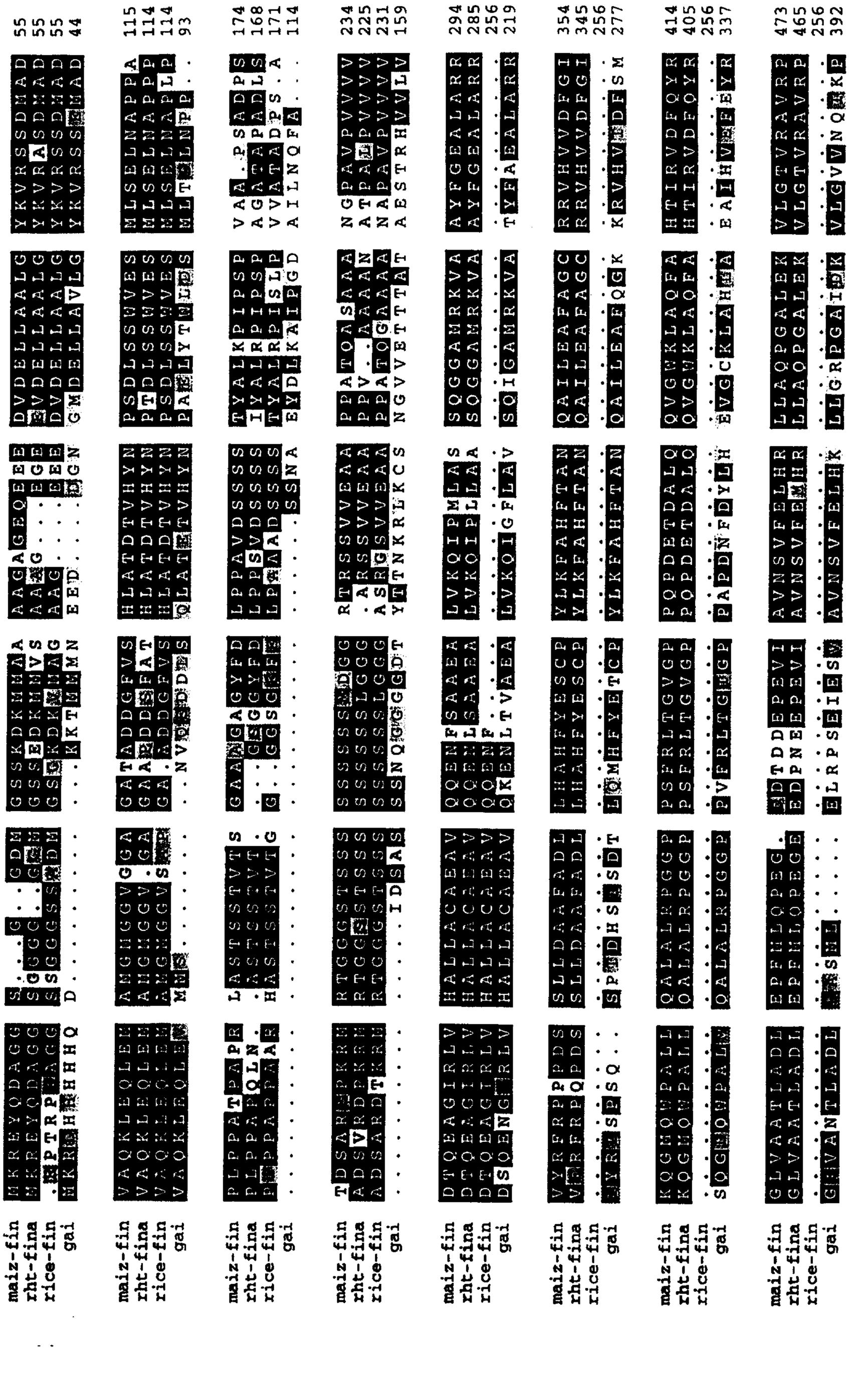


Figure 10 (Continued)

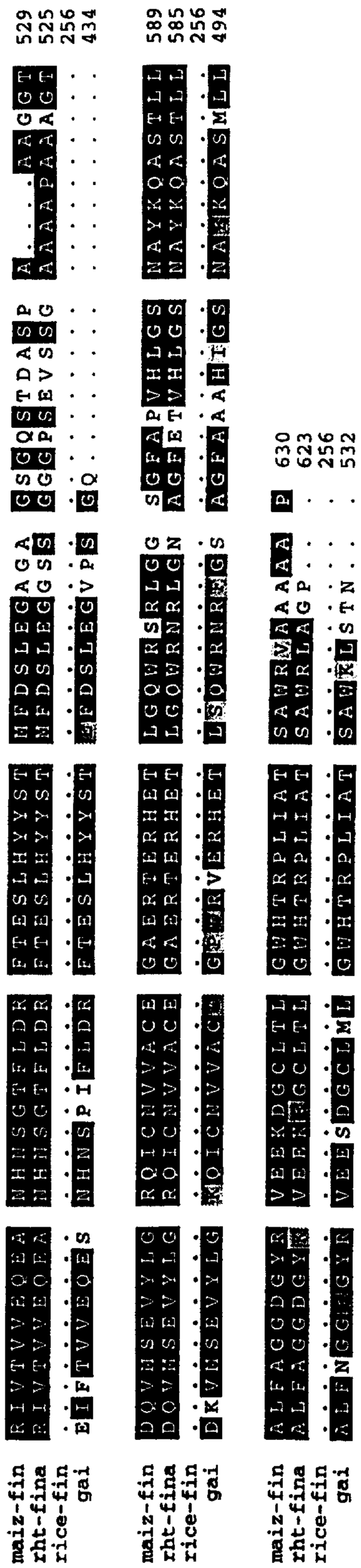


Figure 11a

TACCAAGACGCCGGCGGGAGTGGCGGCGACATGGGCTCCTCCAAGGACAAGATG
ATGGCGGCGGCGGCGGGAGCAGGGGAACAGGAGGAGGAGGACGTGGATGAGCT
GCTGGCCGCGCTCGGGTACAAGGTGCGTTCGTCGGATATGGCGGGGCTGGAGCA
GCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGGCGCCGGCGCTACCGCTGA
TGACGGGTTCGTGTCGCACCTCGCCACGGACACCGTGCACTACAATCCCTCCGAC
CTGTCGTCCTGGGTCGAGAGCATGCTGTCCGA

Figure 11b

YQDAGGSGGDMGSSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDMAGLEQ
LEMAMGMGGVGGAGATADDGFVSHLATDTVHYNPSDLSSWVESMLS

Figure 11c

TCCTCCAAGGACAAGATGATGGCGGCGGCGGCGGGAGCAGGGGAACAGGAGGA
GGAGGACGTGGATGAGCTGCTGGCCGCGCTCGGGTACAAGGTGCGTTCGTCGGA
TATGGCGGACGTCGCGCAGAAGCTGGAGCAGCTCGAGATGGCCATGGGGATGGG
CGGCGTGGGCGGCGCCGGCGCTACCGCTGATGACGGGTTCGTGTCGCACCTGTCG
TCCTGGGTCGAGAGCATGCTGTCCGAGCTCAACGCGCCCCCAGCGCCGCTCCCGC
CCGCGACGCCGGCCCCAAGGCTCGCGTCCACATCGTCCACCGTCACAAGTGGCGC
CGCCGCCGGTGCTGGCTACTTCGATCTCCCGCCCGCCGTGGACTC

Figure 11d

SSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDMADVAQKLEQLEMAMGM
GGVGGAGATADDGFVSHLSSWVESMLSELNAPPAPLPPATPAPRLASTSSTVTSGAA
AGAGYFDLPPAVD

Figure 12a

GCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAG
CTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCC
CCCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGTGCACTACAACCCCA
CCGACCTGTCGTCTTGGGTCGAGAGCATGCTGTCGGAGCTCAACGCCTCCACCTC
CTCCACCGTCACGGGCAGCGGCGGCTACTTCGATCTCCCGCCCTCCGTCGACTCC
TCCAGCAGCATCTACGCGCTGCGGCCGATCCCCTCCCCGGCCGGCGCGACGGCGC
CGGCCGACCTGTCCGCCGACTCCGTGCGGGATCCCAAGCGGATGCGCACTGGCG
GGAGCAGCACCTCGTCGTCATCCTCCTCCTCGTC

Figure 12b

AALGYKVRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYN
PTDLSSWVESMLSELNASTSSTVTGSGGYFDLPPSVDSSSSIYALRPIPSPAGATAPAD
LSADSVRDPKRMRTGGSSTSSSSSSS