

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
25 November 2004 (25.11.2004)

PCT

(10) International Publication Number
WO 2004/101792 A1

(51) International Patent Classification⁷: **C12N 15/29**,
A01H 5/00

John [NZ/NZ]; 44 Florence Avenue, Palmerston North
(NZ).

(21) International Application Number:
PCT/AU2004/000634

(74) Agent: **FREEHILLS CARTER SMITH BEADLE**;
Level 43, 101 Collins Street, Melbourne, Victoria 3000
(AU).

(22) International Filing Date: 14 May 2004 (14.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2003902412 16 May 2003 (16.05.2003) AU

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(71) Applicants (*for all designated States except US*):
AGRICULTURE VICTORIA SERVICES PTY LTD
[AU/AU]; 475 Mickleham Road, Attwood, Victoria 3049
(AU). **AGRESEARCH LIMITED** [NZ/NZ]; 5th Floor,
Tower Block, Ruakura Research Centre, East Street,
Hamilton (NZ).

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **KARDAILSKY**,
Igor [RU/NZ]; 296 Old West Road, Palmerston North
(NZ). **VEIT, Bruce, Edward** [US/NZ]; Pohangina Valley
East Road, Ashhurst, RD14 (NZ). **FORESTER, Natasha**,
Talei [NZ/NZ]; 2 Rata Street, Tokomaru (NZ). **GAGIC**,
Milan [NZ/NZ]; 5 Mokau Place, Parklands, Palmerston
North (NZ). **RICHARDSON, Kim, Archer** [NZ/NZ]; 18
Hardie Street, Palmerston North (NZ). **FAVILLE, Martin**,

Published:

— with international search report

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: FLOWERING INHIBITION

(57) Abstract: The present invention relates to nucleic acids or nucleic acid fragments encoding amino acid sequences for proteins involved in the control of flowering in plants, and the use thereof for the modification of flowering, particularly inhibiting flowering. In particular, the present invention relates to nucleic acids or nucleic acid fragments encoding amino acid sequences of FLOWERING LOCUS T (FT), TERMINAL FLOWER (TFL), GIGANTEA (GI) and SHORT VEGETATIVE PHASE (SVP) polypeptides.

WO 2004/101792 A1

FLOWERING INHIBITION

The present invention relates to nucleic acid fragments encoding amino acid sequences for proteins involved in the control of flowering in plants, and the use thereof for the modification of flowering, particularly inhibiting flowering.

5 Genetic pathways that control flowering time in the model plant *Arabidopsis thaliana* have been studied recently, and progress in identification of genes that control key steps in these pathways has been made, see ¹⁻⁴ for a review. It was found that increased expression of the FLOWERING LOCUS T (FT) gene leads to accelerated floral development, and almost complete loss of
10 a normal photoperiodic response ⁵⁻⁷. This flowering promoting action of FT is counteracted in part by the TERMINAL FLOWER (TFL) gene product, which acts as a repressor, but has significant sequence similarity to FT ^{8,9}. The FT family is comprised of 6 genes in arabidopsis, and of 9 genes in rice. With regard to this family, there is data to support validity of the arabidopsis model
15 of the genetic control of flowering time when applied to other plant species. Rice heading date (flowering time) QTL mapping ¹⁰ and identification of the genes underlying natural variation revealed the same genetic pathway, at least for the photoperiodic control of flowering ¹¹⁻¹³, despite the fact that rice has the short day photoperiod as opposed to the long day one of arabidopsis.
20 Increased expression of the putative rice orthologue of FT called Hd3a seems to correlate with transition to flowering ¹⁴. The LpTFL gene *Lolium perenne* described previously has been shown to function in arabidopsis in the expected way ^{15,16}, so these genes appear to be attractive targets for attenuating photoperiodic signalling.

25 In addition to FT, which is believed to be at the final stage of the photoperiodic signal transduction chain, the GIGANTEA (GI) gene has been shown to affect flowering dramatically, and it is positioned early in the interface of the circadian clock and the photoperiod perception mechanisms ¹⁷⁻²⁰. The gene is present as a single copy in arabidopsis and in rice, and its manipulation
30 in rice has similar consequences as in arabidopsis, further supporting validity of

the arabidopsis model for at least the photoperiodic control ¹⁴, both in short day and in long day plants.

Another inductive pathway in arabidopsis combines autonomous cues and vernalization signalling. While the flowering locus C (FLC) MADS box gene
5 appears to be a central integrator for much of this signalling in arabidopsis ^{21,22}, no corresponding gene in the monocots has been reported to day that would match the FLC either by sequence homology or by the functional assay. In arabidopsis, additional MADS box genes apart from FLC has been implicated in both the autonomous and vernalization control, often found in pairs that
10 seem to have opposite effects on the trait ²³⁻²⁸. Orthologues of these genes and combinations thereof could perform the FLC function in monocots, including cereals and forage grasses.

While there are a great number of mutants that display a delay in flowering in arabidopsis, there is no single mutation that would lead to a
15 complete loss of flowering. This perhaps reflects redundancy of the many genetic elements, and parallelism in signalling via different pathways. However, it was shown that combining mutations in the three major pathways that control flowering (autonomous, photoperiodic, and GA-dependent) leads to a complete loss of flowering in the absence of vernalization treatment²⁹.

20 Therefore, while nucleic acid sequences encoding some of the proteins involved in the control of flowering have been isolated for certain species of plants, there remains a need for materials useful in the modification of flowering in a wide range of plants, and for methods for their use.

Accordingly, there is a need for a system that enables flowering to be
25 reduced or delayed. In particular, such a system would be useful in forage plants.

It is an object of the present invention to overcome, or at least alleviate, one or more of these needs in light of the prior art.

In one aspect, the present invention provides substantially purified or isolated nucleic acids encoding amino acid sequences of FLOWERING LOCUS T (FT) and TERMINAL FLOWER (TFL) proteins, and functionally active fragments and variants thereof, the presence of which inhibits flowering.

5 The present invention also provides substantially purified or isolated nucleic acid fragments encoding amino acid sequences for a class of proteins, which are related to FT and TFL, the presence of which inhibits flowering. Such polypeptides are referred to herein as FT-like and TFL-like respectively. The genes which encode these polypeptides are expressed in a similar manner
10 to FT and TFL, respectively. The invention also encompasses functionally active fragments and variants of nucleic acids encoding such polypeptides.

As used herein the term FT-like relates to polypeptides that are produced in the plant in substantially the same organs and at substantially the same developmental stages as FT.

15 As used herein the term TFL-like relates to polypeptides that are produced in the plant in substantially the same organs and at substantially the same developmental stages as TFL.

In a further aspect, the present invention provides substantially purified or isolated nucleic acids encoding amino acid sequences of GIGANTEA (GI)
20 and SHORT VEGETATIVE PHASE (SVP) proteins, and functionally active fragments and variants thereof.

The present invention also provides substantially purified or isolated nucleic acid fragments encoding amino acid sequences for a class of proteins, which are related to GI and SVP, and functionally active fragments and
25 variants thereof. Such polypeptides are referred to herein as GI-like and SVP-like, respectively. The genes which encode these polypeptides are expressed in a similar manner to GI and SVP, respectively. The invention also encompasses functionally active fragments and variants of nucleic acids encoding such polypeptides.

As used herein the term GI-like relates to polypeptides that are produced in the plant in substantially the same organs and at substantially the same developmental stages as GI.

As used herein the term SVP-like relates to polypeptides that are
5 produced in the plant in substantially the same organs and at substantially the same developmental stages as SVP.

The nucleic acid fragments are obtained from ryegrass (*Lolium*) or fescue (*Festuca*) species. These species may be of any suitable type, including Italian or annual ryegrass, perennial ryegrass, tall fescue, meadow fescue and
10 red fescue. Preferably the species is a ryegrass, more preferably perennial ryegrass (*L. perenne*).

Nucleic acids according to the invention may be full-length genes or part thereof, and are also referred to as "nucleic acid fragments" and "nucleotide sequences" on this specification.

15 The nucleic acid fragment may be of any suitable type and includes DNA (such as cDNA or genomic DNA) and RNA (such as mRNA) that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases, and combinations thereof.

The term "isolated" means that the material is removed from its original
20 environment (eg. the natural environment if it is naturally occurring). For example, a naturally occurring nucleic acid fragment or polypeptide present in a living plant is not isolated, but the same nucleic acid fragment or polypeptide separated from some or all of the coexisting materials in the natural system, is isolated. Such an isolated nucleic acid fragment could be part of a vector
25 and/or such nucleic acid fragments could be part of a composition, and still be isolated in that such a vector or composition is not part of its natural environment.

By "functionally active" in respect of a nucleotide sequence is meant that the fragment or variant (such as an analogue, derivative or mutant) is capable of modifying flowering in a plant. Such variants include naturally occurring allelic variants and non-naturally occurring variants. Additions, deletions, substitutions and derivatizations of one or more of the nucleotides are contemplated so long as the modifications do not result in loss of functional activity of the fragment or variant. Preferably the functionally active fragment or variant has at least approximately 80% identity to the relevant part of the above mentioned sequence, more preferably at least approximately 90% identity, most preferably at least approximately 95% identity. Such functionally active variants and fragments include, for example, those having nucleic acid changes which result in conservative amino acid substitutions of one or more residues in the corresponding amino acid sequence. Preferably the fragment has a size of at least 30 nucleotides, more preferably at least 45 nucleotides, most preferably at least 60 nucleotides.

By "functionally active" in the context of a polypeptide is meant that the fragment or variant has one or more of the biological properties of the FT, FT-like, TFL, TFL-like, GI, GI-like, SVP or SVP-like proteins. Additions, deletions, substitutions and derivatizations of one or more of the amino acids are contemplated so long as the modifications do not result in loss of functional activity of the fragment or variant. Preferably the functionally active fragment or variant has at least approximately 60% identity to the relevant part of the above mentioned sequence, more preferably at least approximately 80% identity, most preferably at least approximately 90% identity. Such functionally active variants and fragments include, for example, those having conservative amino acid substitutions of one or more residues in the corresponding amino acid sequence. Preferably the fragment has a size of at least 10 amino acids, more preferably at least 15 amino acids, most preferably at least 20 amino acids.

By "operatively linked" is meant that a regulatory element is capable of causing expression of said nucleic acid in a plant cell and said terminator is capable of terminating expression of said nucleic acid in a plant cell.

Preferably, said regulatory element is upstream of said nucleic acid and said terminator is downstream of said nucleic acid.

By "an effective amount" is meant an amount sufficient to result in an identifiable phenotypic trait in said plant, or a plant, plant seed or other plant part derived therefrom. Such amounts can be readily determined by an appropriately skilled person, taking into account the type of plant, the route of administration and other relevant factors. Such a person will readily be able to determine a suitable amount and method of administration. See, for example, Maniatis et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, the entire disclosure of which is incorporated herein by reference.

It will also be understood that the term "comprises" (or its grammatical variants) as used in this specification is equivalent to the term "includes" and should not be taken as excluding the presence of other elements or features.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction.

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid fragment encoding a FT or TFL protein includes a nucleotide sequence selected from the group consisting of (a) sequences shown in Figure 1 hereto (Sequence ID Nos: 1-5); (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid fragment encoding a GI or SVP protein includes a nucleotide sequence selected from the group consisting of (a) sequences shown in Figures 3 and 4 hereto (Sequence ID Nos. 6-12); (b)

complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

- 5 The nucleic acid fragments of the present invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species.

 Additionally, genes encoding other flowering control proteins, either as cDNAs or genomic DNAs, may be isolated directly by using all or a portion of
10 the nucleic acid fragments of the present invention as hybridisation probes to screen libraries from the desired plant employing the methodology known to those skilled in the art. Specific oligonucleotide probes based upon the nucleic acid sequences of the present invention can be designed and synthesized by methods known in the art. Moreover, the entire sequences can be used directly
15 to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labelling, nick translation, or end-labelling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the sequences of the present invention. The resulting amplification products can be labelled
20 directly during amplification reactions or labelled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

 In addition, two short segments of the nucleic acid fragments of the present invention may be used in polymerase chain reaction protocols to
25 amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the nucleic acid fragments of the present invention, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid
30 tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the

cloning vector. For example, those skilled in the art can follow the RACE protocol ³⁰ (the entire disclosure of which is incorporated herein by reference) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Using commercially available
5 3' RACE and 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated ^{31,32}. Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs.

In a further aspect of the present invention there is provided a substantially purified or isolated polypeptide from a ryegrass (*Lolium*) or fescue
10 (*Festuca*) species, selected from the group consisting of FT, FT-like, TFL and TFL-like proteins, and functionally active fragments and variants thereof.

The ryegrass (*Lolium*) or fescue (*Festuca*) species may be of any suitable type, including Italian or annual ryegrass, perennial ryegrass, tall fescue, meadow fescue and red fescue. Preferably the species is a ryegrass,
15 more preferably perennial ryegrass (*L. perenne*).

In a preferred embodiment of this aspect of the invention, there is provided a substantially purified or isolated FT, FT-like, TFL or TFL-like polypeptide including an amino acid sequence selected from the group of sequences translated from nucleotide sequences shown in Figure 1 hereto
20 (Sequence ID Nos. 1-5); and functionally active fragments and variants thereof.

In a further embodiment of this aspect of the invention, there is provided a polypeptide recombinantly produced from a nucleic acid according to the present invention. Techniques for recombinantly producing polypeptides are known to those skilled in the art.

25 In a further aspect of the present invention there is provided a substantially purified or isolated polypeptide from a ryegrass (*Lolium*) or fescue (*Festuca*) species, selected from the group consisting of GI, GI-like, SVP and SVP-like proteins, and functionally active fragments and variants thereof.

In a preferred embodiment of this aspect of the invention, there is provided a substantially purified or isolated GI, GI-like, SVP and SVP-like polypeptide including an amino acid sequence selected from the group consisting of (a) sequences translated from nucleotide sequences shown in
5 Figures 3 and 4 hereto (Sequence ID Nos. 8, 10 and 11); (b) sequences shown in Figures 3 and 4 hereto (Sequence ID Nos. 9 and 12); and (c) functionally active fragments and variants of (a) and (b).

In a further embodiment of this aspect of the invention, there is provided a polypeptide recombinantly produced from a nucleic acid according to the
10 present invention. Techniques for recombinantly producing polypeptides are known to those skilled in the art.

Availability of the nucleotide sequences of the present invention and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant
15 amino acid sequences may be synthesized. These peptides can be used to immunise animals to produce polyclonal or monoclonal antibodies with specificity for peptides and/or proteins comprising the amino acid sequences. These antibodies can be then used to screen cDNA expression libraries to isolate full-length cDNA clones of interest.

20 A genotype is the genetic constitution of an individual or group. Variations in genotype are essential in commercial breeding programs, in determining parentage, in diagnostics and fingerprinting, and the like. Genotypes can be readily described in terms of genetic markers. A genetic marker identifies a specific region or locus in the genome. The more genetic
25 markers, the finer defined is the genotype. A genetic marker becomes particularly useful when it is allelic between organisms because it then may serve to unambiguously identify an individual. Furthermore, a genetic marker becomes particularly useful when it is based on nucleic acid sequence information that can unambiguously establish a genotype of an individual and
30 when the function encoded by such nucleic acid is known and is associated with a specific trait. Such nucleic acids and/or nucleotide sequence information

including single nucleotide polymorphisms (SNPs), variations in single nucleotides between allelic forms of such nucleotide sequence, can be used as perfect markers or candidate genes for the given trait.

In a further aspect of the present invention there is provided a method of
5 isolating a nucleic acid of the present invention including a single nucleotide polymorphism (SNP), said method including sequencing nucleic acid fragments from a nucleic acid library.

The nucleic acid library may be of any suitable type and is preferably a cDNA library. The nucleic acid fragments may be isolated from recombinant
10 plasmids or may be amplified, for example using polymerase chain reaction. The sequencing may be performed by techniques known to those skilled in the art.

In a further aspect of the present invention, there is provided use of nucleic acids of the present invention including SNP's, and/or nucleotide
15 sequence information thereof, as molecular genetic markers.

In a further aspect of the present invention there is provided use of a nucleic acid according to the present invention, and/or nucleotide sequence information thereof, as a molecular genetic marker. More particularly, nucleic acids according to the present invention and/or nucleotide sequence
20 information thereof may be used as a molecular genetic marker for quantitative trait loci (QTL) tagging, QTL mapping, DNA fingerprinting and in marker assisted selection, particularly in ryegrasses and fescues. Even more particularly, nucleic acids according to the present invention and/or nucleotide sequence information thereof may be used as molecular genetic markers in
25 forage and turf grass improvement, e.g. tagging QTLs for herbage quality traits, dry matter digestibility, mechanical stress tolerance, disease resistance, insect pest resistance, plant stature, leaf and stem colour. Even more particularly, sequence information revealing SNPs in allelic variants of the nucleic acids of the present invention and/or nucleotide sequence information thereof may be

used as molecular genetic markers for QTL tagging and mapping and in marker assisted selection, particularly in ryegrasses and fescues.

In a still further aspect of the present invention there is provided a construct including a nucleic acid according to the present invention. The
5 construct may be a vector. In a preferred embodiment of this aspect of the invention, the vector may include a regulatory element such as a promoter, a nucleic acid according to the present invention and a terminator; said regulatory element, nucleic acid and terminator being operatively linked.

The vector may be of any suitable type and may be viral or non-viral.
10 The vector may be an expression vector. Such vectors include chromosomal, non-chromosomal and synthetic nucleic acid sequences, eg. derivatives of plant viruses; bacterial plasmids; derivatives of the Ti plasmid from *Agrobacterium tumefaciens*, derivatives of the Ri plasmid from *Agrobacterium rhizogenes*; phage DNA; yeast artificial chromosomes; bacterial artificial
15 chromosomes; binary bacterial artificial chromosomes; vectors derived from combinations of plasmids and phage DNA. However, any other vector may be used as long as it is replicable, or integrative or viable in the plant cell.

The regulatory element and terminator may be of any suitable type and may be endogenous to the target plant cell or may be exogenous, provided
20 that they are functional in the target plant cell.

In another embodiment, the construct or vector may include more than one nucleic acid. The nucleic acids within the same construct or vector may have identical or differing sequences. In one preferred embodiment, the construct or vector has at least two nucleic acids encoding proteins involved in
25 the control of flowering.

In another preferred embodiment the construct or vector may include one or more FT or FT-like nucleic acids and one or more TFL or TFL-like nucleic acids according to the present invention, or functionally active fragments or variants thereof, in combination with other genes involved in the

control of flowering timing. The genes involved in the control of flowering timing may be selected from a group consisting of GIGANTEA (GI), GI-like, SVP and SVP-like, and fragments and variants thereof.

Preferably one of the regulatory element is a promoter. A variety of
5 promoters which may be employed in the vectors of the present invention are well known to those skilled in the art. Factors influencing the choice of promoter include the desired tissue specificity of the vector, and whether constitutive or inducible expression is desired and the nature of the plant cell to be transformed (eg. monocotyledon or dicotyledon). Particularly suitable
10 constitutive promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) promoter, the maize Ubiquitin promoter, and the rice Actin promoter.

A variety of terminators which may be employed in the vectors of the present invention are also well known to those skilled in the art. It may be from the same gene as the promoter sequence or a different gene. Particularly
15 suitable terminators are polyadenylation signals, such as the CaMV 35S polyA and other terminators from the nopaline synthase (*nos*) and the octopine synthase (*ocs*) genes.

The vector, in addition to the regulatory element, the nucleic acid of the present invention and the terminator, may include further elements necessary
20 for expression of the nucleic acid, in different combinations, for example vector backbone, origin of replication (*ori*), multiple cloning sites, spacer sequences, enhancers, introns (such as the maize Ubiquitin Ubi intron), antibiotic resistance genes and other selectable marker genes (such as the neomycin phosphotransferase (*npt2*) gene, the hygromycin phosphotransferase (*hph*)
25 gene, the phosphinothricin acetyltransferase (*bar* or *pat*) gene), and reporter genes (such as beta-glucuronidase (GUS) gene (*gusA*). The vector may also contain a ribosome binding site for translation initiation. The vector may also include appropriate sequences for amplifying expression.

As an alternative to use of a selectable marker gene to provide a
30 phenotypic trait for selection of transformed host cells, the presence of the

construct or vector in transformed cells may be determined by other techniques well known in the art, such as PCR (polymerase chain reaction), Southern blot hybridisation analysis, histochemical GUS assays, northern and Western blot hybridisation analyses.

- 5 Those skilled in the art will appreciate that the various components of the construct or vector are operatively linked, so as to result in expression of said nucleic acid. Techniques for operatively linking the components of the construct or vector of the present invention are well known to those skilled in the art. Such techniques include the use of linkers, such as synthetic linkers,
10 for example including one or more restriction enzyme sites.

- The constructs and vectors of the present invention may be incorporated into a variety of plants, including monocotyledons (such as grasses from the genera *Lolium*, *Festuca*, *Paspalum*, *Pennisetum*, *Panicum* and other forage and turfgrasses, corn, rice, sugarcane, oat, wheat and barley, dicotyledons,
15 such as arabidopsis, tobacco, soybean, canola, cotton, potato, chickpea, medics, white clover, red clover, subterranean clover, alfalfa, eucalyptus, poplar, hybrid aspen, and gymnosperms (pine tree). In a preferred embodiment, the constructs and vectors are used to transform monocotyledons, preferably grass species such as ryegrasses (*Lolium* species)
20 and fescues (*Festuca* species), even more preferably a ryegrass, most preferably perennial ryegrass, including forage- and turf-type cultivars.

- Techniques for incorporating the constructs and vectors of the present invention into plant cells (for example by transduction, transfection or transformation) are well known to those skilled in the art. Such techniques
25 include *Agrobacterium* mediated introduction, electroporation to tissues, cells and protoplasts, protoplast fusion, injection into reproductive organs, injection into immature embryos and high velocity projectile introduction to cells, tissues, calli, immature and mature embryos. The choice of technique will depend largely on the type of plant to be transformed.

Cells incorporating the constructs and vectors of the present invention may be selected, as described above, and then cultured in an appropriate medium to regenerate transformed plants, using techniques well known in the art. The culture conditions, such as temperature, pH and the like, will be
5 apparent to the person skilled in the art. The resulting plants may be reproduced, either sexually or asexually, using methods well known in the art, to produce successive generations of transformed plants.

In a further aspect of the present invention there is provided a plant cell, plant, plant seed or other plant part, including, e.g. transformed with, a
10 construct or vector of the present invention.

The plant cell, plant, plant seed or other plant part may be from any suitable species, including monocotyledons, dicotyledons and gymnosperms. In a preferred embodiment the plant cell, plant, plant seed or other plant part is from a monocotyledon, preferably a grass species, more preferably a ryegrass
15 (*Lolium* species) or fescue (*Festuca* species), even more preferably a ryegrass, most preferably perennial ryegrass, including both forage- and turf-type cultivars.

The present invention also provides a plant, plant seed or other plant part derived from a plant cell of the present invention. The present invention
20 also provides a plant, plant seed or other plant part derived from a plant of the present invention.

In a further aspect of the present invention there is provided a method of modifying flowering in a plant, said method including introducing into said plant an effective amount of a nucleic acid, construct and/or vector according to the
25 present invention. Preferably the method includes inhibiting flowering in said plant.

In a preferred embodiment more than one regulatory gene is manipulated, to inactivate multiple redundant and parallel genetic pathways that normally promote flowering in response to internal and external cues. The

manipulated genes can be the FT/TFL family members, including both promoters and repressors; orthologs of the Gigantea (GI) gene, to disrupt the circadian clock, and therefore to interfere with the photoperiodic response; and the AGL24/SVP MADS box transcription factors, to interfere with response to
5 autonomous and vernalization signals.

To inactivate a gene, either the RNA interference in transgenic plants can be used, or naturally or artificially induced hypomorphic alleles could be identified and bred into a production cultivar.

Using the methods and materials of the present invention, flowering may
10 be accelerated or delayed. It may be accelerated, for example, by incorporating additional copies of a sense nucleic acid of the present invention. It may be delayed, for example, by incorporating an antisense nucleic acid or dsRNA or small interfering RNA (siRNA) derived from the nucleotide sequences of the present invention. In addition, the number of copies of genes encoding for
15 different proteins involved in the timing of flowering may be simultaneously manipulated to modify flowering.

In a further aspect of the present invention there is provided a preparation for transforming a plant comprising at least one nucleic acid according to the present invention. The preparation may contain vectors or
20 other constructs to facilitate administration to and/or transformation of the plant with the nucleic acid.

The principle of elimination of flowering combined attenuation of expression of genes that control different genetic pathways that is described here can be applied to ryegrass to better control pasture production cycle, and
25 to improve persistence by allowing a more controlled heading. The technology to eliminate flowering can be used in combination with system for accelerating or initiating flowering to achieve complete artificial control over vegetative to floral transition that will be independent of weather and growth conditions. Consequently, its application should not be limited to just forage grasses, and

such system can be applied to any agricultural crop to facilitate more controlled production, and reduce dependence of yields on weather.

Additionally, ability to eliminate natural flowering may be a prerequisite for the release of transgenic plants into the field due to regulatory
5 requirements.

The present invention will now be more fully described with reference to the accompanying Examples and drawings. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

10 In the Figures:

Figure 1 shows the sequences of RgFT1 (SEQ ID No. 2), RgFT2 (SEQ ID No. 1), RgMFT (SEQ ID No. 3), RgTFL1 (SEQ ID No. 5) and RgTFL2 (SEQ ID No. 4).

Figure 2 shows the alignment of translated protein sequences for
15 RgFT2, RgFT1, RgTFL1 and RgTFL2 in comparison to the sequences from other species.

Figure 3 shows the sequence of the putative ryegrass ortholog of the Gl gene (SEQ ID Nos. 6 and 7).

Figure 4a shows the complete cDNA sequence of the RgSVP gene
20 (SEQ ID No. 8).

Figure 4b shows a translation of the RgSVP coding region (SEQ ID No. 9).

Figure 4c shows the gene structure of ryegrass SVP (SEQ ID Nos. 10-12).

Figure 5 shows the alignment of RgSVP predicted protein sequence with SVP proteins for other species.

Figure 6 shows the RNA interference construct to inactivate the RgSVP gene.

- 5 Figure 7 shows the alignment of protein translations of the ryegrass members of the FT/TFC family with those from other plant species (SEQ ID Nos. 13-38).

Figure 8 is a genetic linkage map of perennial ryegrass linkage group LG3, showing the position of the Gigantea gene in the ryegrass map of the linkage group 3. Marker locus names are indicated on the right side of the bar, with centimorgan (cM) distances on the left. The RgGI SNP marker is indicated in bold. The remaining loci, prefixed 'pps', are EST-SSR loci.

Figure 9 shows heading dates for LpGI RNAi transgenics. The line FN5001 flowered substantially later than the others.

- 15 Figure 10 shows delayed flowering phenotypes of a GI-RNAi ryegrass transformant FN5001 (right).

Fig. 11 Circadian expression of the RgGI gene as measured by quantitative RT-PCR CL2 represents samples collected in long day conditions, CS2 samples were harvested in short days.

20 **EXAMPLES**

1. FT/TFL

Gene isolation

Partial sequences of the other two FT-like genes of ryegrass, called RgFT1 and 2, and 2 members of the TFL-like subfamily RgTFL1 and 2 were isolated. These share significant sequence similarity with FT, but perform the opposite function of floral repression in arabidopsis^{8,9,33} rather than accelerating flowering. cDNAs for these genes were isolated using degenerate

primers design to amplify conserved regions of the family. Complete cDNA sequences, where available, were obtained by 3' and 5'RACE. Sequences of these additional members of the ryegrass family are shown in Figure 1.

For the purposes of inactivation of normal transition, these genes can be
5 manipulated as follows:

RgFT1, 2 and 3 are inactivated via RNAi in transgenics, TILLING, or identification and selective breeding to fix hypomorphic natural alleles.

RgTFL1, 2 and LpTFL are constitutively overexpressed, using appropriate transgenic constructs.

10 *Polymorphism analysis*

We have identified the following polymorphisms within the ryegrass FT/TFL family of genes, which can be used in mapping and allele association studies for identification of hypomorphic alleles:

gene	pos	translation	comment
Rgft1	190	silent	
Rgft1	325	polyA	50 bp extra
Rgft1	99	Q-R	observed variant, more common in tfls
Rgft1	325	polyA	90 bp extra
Rgft1	156	L-P	
Rgft1	190	silent	
Rgft1	325	polyA	32 bp extra
Rgft1	133	silent	
Rgft1	-	wt	wt sample
Rgft1	270	3'utr	
Rgft2	337	3'utr	
Rgft2	356	polyA	21 bp extra
Rgft2	356	polyA	28 bp extra
Rgft2	106	silent	the same as the one that defines entry clones as rgft1
Rgft2	184	silent	
Rgft2	-	wt	wt sample
Rgft2	283	3'utr	
Rgft2	356	polyA	19 bp extra
rgtfl1-1			
rgtfl1-1	53	5' utr	
rgtfl1-1	160	silent	

rgtfl1-1	395	Trp->Arg	
rgtfl1-1	235	silent	
rgtfl1-1	250	silent	
rgtfl1-1	255	silent	
rgtfl1-1	259	silent	
rgtfl1-1	268	silent	and some other silent ones not catalogued
rgtfl1-1	275	Val->Ile	
rgtfl1-1	279	Val-Ala	
rgtfl1-1	281	Ser->Ala	
rgtfl1-1	293	Ile->Val	
rgtfl1-1	314	met->leu	
rgtfl1-1	405	Asn->Thr	
rgtfl1-1	407	Asn->Asp	
rgtfl1-2	12	5' utr	
rgtfl1-2	280	Agr->Ile	
rgtfl1-2			
rgtfl1-2	247	Lys->Arg	

2. Gigantea

Gene isolation

Partial cDNA sequence of the putative ryegrass orthologue was identified in the
 5 cDNA library. Complete cDNA sequence was obtained using 5'-RACE.

The cDNA sequence and corresponding translation are shown in Figure 3.

Sequence analysis

shows that the cDNA sequence of the putative ryegrass GI gene has been
 isolated, based on comparison with GI-like genes from other grasses. The
 10 sequence contains uninterrupted open reading frame representing the full-size
 ryegrass GI protein.

Polymorphism analysis

By analysing diverse population of plants within a cultivar, and by
 comparison of the cultivars, we have identified the following genetic variation
 15 within the genomic sequence:

trace name	wt	mut	pos	translation	comment
h6	c	t	209	silent	
b2	c/g het	c	477	silent	
e2	c/g het	c	477	silent	in some others
est	c/g het	c	477	silent	
g6	c/g het	c	477	silent	
a2	T	a/t het	607	intron	
b4	T	a/t het	607	intron	
d4	T	a/t het	607	intron	
d6	T	a/t het	607	intron	
e2	T	a/t het	607	intron	
e4	T	a/t het	607	intron	
f2	T	a/t het	607	intron	
f4	T	a/t het	607	intron	
multi	t	g	637	intron	both types and
multi	a	g	644	intron	both types and
b4	t	t/c	689	intron	
b4	t	t/c	699	intron	
a2	ttaat	wt/agaca	589-593	intron	
b4	ttaat	wt/agaca	589-593	intron	
d4	ttaat	wt/agaca	589-593	intron	
d6	ttaat	wt/agaca	589-593	intron	
e4	ttaat	wt/agaca	589-593	intron	
f2	ttaat	wt/agaca	589-593	intron	
f4	ttaat	wt/agaca	589-593	intron	

These variants are used to create markers, and to use in allele association studies.

Additionally, we have identified the following sequence polymorphisms
5 between the parental lines of the mapping population:

name	before	after	A8830/1030	A10622/2	pos	oligo
T87	TGATGCATGTCATG AAGGCAATGAAG (SEQ ID No. 39)	CAATGTCTGTTCCTC (SEQ ID No. 40)	C	Y	87	GIK17
A125	CGTGAGGAAATGA ACAACAGA (SEQ ID No. 41)	ACAAAGCAAAAAT (SEQ ID No. 41)	T	W	125	GIK17

These polymorphisms were used to establish the map position of the ryegrass *Gigantea* gene as follows:

Methods:

10 Plant material and DNA isolation

The perennial ryegrass population used for the genetic mapping of the rgFT3 SNP (single nucleotide polymorphism) marker and SSR (simple sequence repeat) markers was an F₁ progeny set derived from a pair cross between the heterozygous parental genotypes A8830/1030 (from the cultivar 'Grasslands Samson') and A10622/2 (from the cultivar 'Grasslands Impact'). Ninety-four individual progeny from the population were used for genetic linkage analysis. Genomic DNA was extracted by the 1 x CTAB method of Fulton et al. (1995).

EST-SSR and SNP analysis

Genotypic data for 94 mapping population progeny was generated using 157 EST-SSRs and the RgGI SNP markers T87 and A125. EST-SSR PCR was conducted using the three primer protocol described by Schuelke (2000). An 8 µL reaction volume was used, containing 10 ng of genomic DNA, 2.5 mM magnesium chloride, 1x PCR buffer (Invitrogen, Carlsbad, California, USA), 0.05 mM of each dNTP, 0.0375 µM forward primer, 0.15 µM reverse primer, 0.15 µM of fluorescent-labelled M13 primer and 0.3 U of Platinum Taq DNA polymerase (Invitrogen). Fluorophores used were 6-FAM™, NED™, VIC™ and PET™ (Applied Biosystems, Foster City, California, USA). EST-SSR primers were synthesised and supplied by either Invitrogen or Integrated DNA Technologies (Coralville, Iowa, USA). PCR reactions were run in iCyclers (BioRad, Hercules, California, USA), employing the following profile: (1) 94°C for 4:00 minutes, (2) 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, (3) 8 cycles of: 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 30 seconds, (4) 72°C for 30 minutes.

PCR products were analysed on an ABI 3100 Genetic Analyser using a 22 cm capillary array with POP-7™ polymer (Applied Biosystems). Electropherograms were analysed using ABI Prism GeneScan (v 3.7, Applied Biosystems), and genotype data was scored using ABI Prism Genotyper (v 3.7, Applied Biosystems). The allelic status of the RgGI SNPs was determined by direct sequencing of amplification products produced with oligonucleotides

GIK16 5'CATGAAATGTGCAGAACAGG3' (SEQ ID No. 43) and GIK17 5'TGGTTGACTCTCCACATCTTC3' (SEQ ID No. 44).

Genetic linkage analysis

The A8830/1030 x A10622 population was analysed as a two-way pseudo-testcross (Grattapaglia and Sederoff 1994). Genetic linkage analysis was conducted using the CP module of JoinMap[®] 3.0 software (www.kyazma.nl). Map distances in centimorgans (cM) were calculated using the Kosambi mapping function (Kosambi 1944). Genetic linkage maps were first established separately for A8830/1030 and A10622 using segregation data from EST-SSR and SNP markers that could be derived as dominant features. Polymorphic loci detected by the same EST-SSR primer pair at similar locations on the maps of both parents were used to identify and align homologous linkage groups in the two parental maps, and to check for consistency of recombination frequency between the parental genotypes. Parental datasets were then combined and a consensus genetic linkage map was constructed, using a maximum recombination frequency of 0.4 and minimum LOD threshold of 2.0.

Results

Genetic linkage analysis enabled the location of 126 EST-SSR loci and the RgGI SNP on a consensus map covering 354 cM across seven linkage groups (LG1 – LG7). The RgGI SNP mapped to a location at position 9 cM on LG3 (Figure 8).

We have identified partial synteny between the ryegrass LG3 and the rice chromosome 1 around the Gl gene, as shown in the following table:

rg map position	marker	rice BLAST hit acc no.	assigned to rice csome	BAC/PAC	Contig position on csome
0cM	pps0133b	AP003215	1	OSJNBa0089K24	3724261bp – 3878397bp
1.1 cM	pps0502a	AC084763	10	OSJNBa0027P10	
9.0 cM	gi_T87	OsGl	1	AP003047_70329_141	4326527bp - 4333377

23

				983	bp
	pps0710				
14.1 cM	a	no hits			
	pps0639				4031060bp –
17.6 cM	z	AP002903	1	P0509B06	4135574bp
	pps0488				6715722bp –
18.5 cM	c	AP002835	1	P0417G05	6863524bp
	pps0558				
19.3 cM	a	AC091749	10	OSJNBb0008A05	
	pps0488				6715722bp –
24.3 cM	a	AP002835	1	P0417G05	6863524bp
	pps0642				7336755bp –
24.8 cM	b	AP002481	1	P0702F03	7477865bp

Strong sequence similarity, and sythenic position of the ryegrass gene on the map support our claim that the ryegrass Gl gene we identified functions in controlling photoperiodic flowering in ryegrass, and can be used to

5 manipulate flowering behaviour of the plant.

Transgenic plants

We have generated transgenic ryegrass plants in which expression of the Gl gene is reduced by RNA interference, using the 3' end part of the cDNA sequence shown above. Of >20 independent transgenic plants obtained using

10 this construct, one displayed significant delay in flowering compared to the others and to the non-transformed control, as shown in Fig. 9 and 10.

(a) Expression analysis of the RgGl gene

Methods:

Ryegrass plants were grown outside and subjected to cold over the winter to

15 achieve natural vernalization. They were then transferred to a glass house, and grown either in natural short day (SD) conditions (<11 hrs daylight), or with supplementary light to create artificial long day (LD) conditions (18 hr day). End of day in both conditions was at ~18:00. Plant samples were harvested as entire above-ground tillers 10 days after the long day treatment started,

20 samples were taken every 2 hrs for 24 hours from both LD and SD treated plants.

Total RNA was extracted from samples using the Trizol protocol as follows:

Extraction of RNA from 1g (nett) of *L. perenne* plant tissue using the Trizol[®] Reagent (Invitrogen)

- Plant tissue pre-homogenisation treatment

- 5 Remove relevant bags of plant tissue from -80°C freezer. Keep frozen in liquid N₂ or on dry ice until ready for treatment. Prechill coffee grinder by processing two dry ice pellets (7g pellets).

NB: In between samples, wipe out the coffee grinder with 75% ethanol soaked kim wipes and repeat prechill step

- 10
 - Weigh bag of tissue (approx 7-10g). Empty contents into coffee grinder and blend until tissue is the consistency of salt granules (no larger). Pour coarsely ground tissue into a fresh prechilled bag and keep frozen until required for next stage. Reweigh just before taking sample for fine grinding (allowing for difference in bag weight).

- 15 Homogenisation

- Remove 1.1-1.2g of coarsely ground tissue (taking into account the increase of weight due to dry ice contribution) and [⊗]homogenise further in a prechilled mortar containing liquid N₂ until consistency of icing sugar. Add powder to 10ml of Trizol[®] in mortar (room temperature) and homogenise
- 20 further as quickly as possible. Pour soup into 14ml disposable falcon tube.
- [⊗]Incubate samples at least 5 min at RT with gentle inversion.

- Phase separation

- Add 2ml chloroform per sample. Cap and mix vigorously 15s by hand. Incubate RT 2-3min. Spin in swinging bucket rotor at 3200 x g for 30 min[⊗].
- 25 Remove 4 ml to fresh tube (with modified cap*). There will be ~1ml aqueous phase left, purposely done to reduce/avoid DNA contamination.

- RNA precipitation

Add 5ml Isopropanol[®]. Mix by inversion ~6 times. Incubate for 10 min at RT. Centrifuge at $\leq 2,000 \times g$ for 10 mins. Decant and discard supernatant.

5

- RNA wash

Add 10 ml 75% ethanol. Vortex and mix by inversion to wash lid.
[®]Centrifuge $\leq 7,500 \times g$, 5 mins, 4°C. Decant and discard supernatant. To reduce drying time, centrifuge again to collect excess wash solution to bottom of the tube at $\leq 7,500 \times g$, 2 mins,
10 4°C. Remove excess liquid with RNase-free pipette tip and air-dry pellet approx 10-15 min.

- RNA resuspension

Add 0.8ml of DEPC treated water. Gently resuspend pellet with pipette tip. Transfer liquid to eppendorf tube. An incubation at 55°C
15 for 10 min may be required if resuspension difficult.

- RNA storage

Store samples labelled well at -80°C with bulk sample ~700ul and a 80ul working aliquot.

Total RNA was converted to cDNA using standard protocols using 1-5ug of
20 RNA as measured by OD260.

Quantitative RT-PCR was performed in the BioRad iCycler instrument using standard protocols and SyberGreen as reporting dye

Actin gene levels were assayed using oligonucleotides GTF037
5'GCTGTTTTCCCTAGCATTGTTGG3' (SEQ ID No. 45) and GTF038
25 5'ATAAGAGAATCCGTGAGATCCCG3'(SEQ ID No. 46), and served as standards to normalize measurements of other genes.

RgGl mRNA levels were measured using oligonucleotides GIK16 and GIK17 described in the mapping example.

To create a copy number calibration curve for individual genes, standards were made up from corresponding PCR products, measuring their concentration
5 using gel serial dilution methods and spectrophotometry. Standard copy numbers were varied from 1 to 10^8 with 10x increments.

Amplifications were performed in triplicates, error rates were estimated by adding the average variance between triplicate samples, and average error of the standard curve fit of the standards of both the gene of interest and of the
10 actin standard.

Results

The mRNA levels of the RgGl gene change significantly over the 24-hour cycle. The periodicity of expression suggests that the ryegrass gene, like its arabidopsis orthologue, is under the control of the circadian clock. The
15 expression levels do not change significantly in photo inductive conditions, however, there may be a phase shift in long days to earlier increase, coincident with light exposure. Such circadian regulation of expression, as well as phase shift in long days, strongly support the role of the RgGl gene in mediating and controlling the photoperiodic floral response of ryegrass, as postulated in the
20 external coincidence hypothesis for arabidopsis

3. RgSVP

Gene isolation

The gene was identified in the EST collection, and the EST clone was completely sequenced. Diagram of the gene structure is shown in Figure 4.

25 *Sequence analysis*

Alignment of the RgSVP predicted protein translation has shown that it clusters with the arabidopsis SVP and AGL24 genes of arabidopsis, as shown in Figure 5.

While SVP seems to be a repressor of floral development, ^{26,34} acting largely in the autonomous pathway, AGL24 seems to be involved in some promotive vernalization signalling that bypasses the FLC ^{23,35}, providing an attractive target for inactivation of vernalization pathway.

- 5 The constructs for RNA interference to inactivate the RgSVP gene were created as shown in the Figure 6.

Other RNAi-type constructs described here for FT/TFL and GI genes involved similar vector system and experimental approach.

Polymorphism analysis

- 10 The following SNPs were identified upon sequencing cDNA and genomic clones derived from the genetically diverse stocks:

allele	wt	mut	seq before	seq after	pos cDNA	translation	wt aa	conservation
SVP1	T	G	CGCCATCGCCGCTCCGA	TCTCGCGCTCTCCC	7	G	W	high
SVP2	G	T	acggagagctctc	cgacgtcggcgtgcacatg	111	silent		high
SVP3	G	T	gtgctgtactgtcgatga	ccccagggtcttagaatg	213	silent		medium
SVP4	A	G	ttcaagtcaagagaaggctggt	catactactatgttctaa	261	silent		medium
SVP5	G	A	agactagcttccgcaagc	gcctctcatctgtctaaagtcg	315	silent		medium
SVP6	G	C	tcaacccctcaagcccctcg	actgctggagttcatcaaca	356	S	T	medium
SVP7	GA	CC	gcttcccggagtgcacgcccgtc	gaaccatcatcgttatcctgt	619	E	P	
SVP8	A	G	tctgaaccatcatcggttattc	taatcccaatttcagggtatc	645	silent		medium

These SNPs can be used for mapping of the gene, and for allele association studies and identification of hypomorphic alleles.

4. Ryegrass transformation system

This invention can be applied to ryegrass *Lolium perenne*, for which we have developed efficient stable transformation system as follows:

Materials

5

florally induced tillers of <i>Lolium perenne</i>
Na-hypochlorite (5% available chlorine)
sterile ddH ₂ O
100mm Petri plates containing LP5 medium*
100mm Petri plates containing LP3-OS medium
100mm Petri plates containing LP3 medium
100mm Petri plates containing LP3 medium + 200 mg/L Hygromycin (Hm)
100mm Petri plates containing MSK medium + 200 mg/L Hm
250 ml culture vessels containing MSO medium + 200mg/L
Hygromycin stock solution (50 mg/ml in PDS, sterile)

Procedure

- 1) Harvest and surface sterilise floral tillers of *Lolium perenne* in 5% available chlorine Na-hypochlorite for 15 minutes using a Mason jar (or equivalent) under constant agitation.
- 2) Rinse tillers with autoclaved ddH₂O.
- 3) Aseptically dissect floral meristems.
- 4) Culture meristems on callus induction medium LP5 (16-20 explants per plate) and incubate in the dark for four to six weeks.

10

- 5) On the day of transformation transfer embryogenic callus material to high osmotic medium LP3-OS. Arrange approximately 4 cm² of calli in the centre of the Petri dish.
- 6) Incubate calli for 4-6 hours at room temperature.
- 5 7) Prepare particles and perform biolistic transformation following the protocol: "Biolistic Transformation of *Lolium perenne* with the Bio-Rad Particle Delivery System (PDS)". Plasmids are co-transformed. One plasmid (pAcH1) contains the hygromycin phosphotransferase gene conferring resistance to the antibiotic hygromycin expressed from the rice actin promoter and the second plasmid contains the genetic
10 construct of interest for transformation. Plasmids are mixed in a one to one ratio at 1 µg/µL and simultaneously coated onto the microcarriers.
- 8) Incubate bombarded calli on high osmotic medium LP3-OS for an additional 12-16 hours (overnight) at 25°C in the dark.
- 15 9) Transfer bombarded calli to LP3 medium and incubate for 48 hours at 25°C in the dark
- 10) Plate calli on selection medium (LP3 + 200 mg/l Hygromycin (Hm)). Incubate at 25°C in the dark on selection medium for two weeks.
- 20 11) Transfer all Hm-resistant callus material to regeneration medium MSK + 200 mg/l Hm and incubate for four weeks at 25°C under a 16hour photoperiod.
- 12) Transfer developed shoots to MS0 + 200 mg/l Hm and incubate for another two to four weeks at 25°C under 16hour photoperiod.
- 13) Screen by PCR Hm-resistant plants growing on MS0 + 200 mg/L Hm.

Microprojectile bombardment of *Lolium perenne* with the Bio-Rad Particle Delivery System (PDS-1000/He)

Taken from the PDS-100/He manual. These procedures were developed by Sanford *et al.* (1992).

5 Materials and Solutions

Bio-Rad Biolistic® PDS-1000/He Particle Delivery System
Rupture disks (900 PSI)
Macrocarriers
Macrocarrier holders
Microcarriers (1.0 µm)
Stopping screens
Autoclaved 1.5 ml eppendorf tubes
Micropipette tips
Vortex and microfuge
Torque wrench tool
Pen vac
70% Ethanol
Absolute Ethanol
2.5 M CaCl ₂
100 mM Spermidine

(A) Microcarrier preparation

For 120 bombardments using 500 µg per bombardment.

1. In a 1.5 ml microfuge tube, weigh out 60 mg of microparticles.
- 10 2. Add 1 ml of 70% ethanol, freshly prepared.
3. Vortex on a platform vortexer for 3-5 minutes.
4. Incubate for 15 minutes.
5. Pellet the microparticles by spinning for 5 seconds in a microfuge.

6. Remove the liquid and discard.
7. Repeat the following steps three times:
 - a. Add 1 ml of sterile water
 - b. Vortex for 1 minute
 - 5 c. Allow the particles to settle for 1 minute
 - d. Pellet the microparticles by spinning for 2 seconds in a microfuge.
 - e. Remove the liquid and discard.
8. Add sterile 50% glycerol to bring the microparticle concentration to 60
10 mg/ml (assume no loss during preparation).
9. Store the microparticles at room temperature for up to 2 weeks.

(B) Coating DNA onto microcarriers

The following procedure is sufficient for six bombardments; if fewer bombardments are needed, prepare enough microcarriers for three
15 bombardments by reducing all volumes by one half. When removing aliquots of microcarriers, it is important to vortex the tube containing the microcarriers continuously in order to maximise uniform sampling.

1. Vortex the microcarriers prepared in 50% glycerol (60 mg/ml) for 5
20 minutes on a platform vortexer to resuspend and disrupt agglomerated particles.
2. Remove 50 μ l (3 mg) of microcarriers to a 1.5 ml microfuge tube.
3. While vortexing vigorously, add in order:
 - 5 μ l DNA (1 μ g/ μ l)
 - 50 μ l CaCl_2 (2.5 M)
 - 25 20 μ l spermidine (0.1 M)
4. Continue vortexing for 2-3 minutes
5. Allow the microcarriers to settle for 1 minute
6. Pellet the microcarriers by spinning for 2 seconds in a microfuge
7. Remove the liquid and discard

8. Add 140 μ l of 70% ethanol without disturbing the pellet
9. Remove the liquid and discard
10. Add 140 μ l of 100% ethanol without disturbing the pellet
11. Remove the liquid and discard
- 5 12. Add 48 μ l of 100% ethanol
13. Gently resuspend the pellet by tapping the side of the tube several times, and then by vortexing at low speed for 2-3 seconds
14. Remove six 6 μ l aliquots of microcarriers and transfer them to the centre of a macrocarrier. An effort is made to remove equal amounts (500 μ g) of microcarriers each time and to spread them evenly over the central 1 cm of the macrocarrier using the pipette tip. Desiccate immediately.

C) Bombardment procedure

- 1) Open valve of helium cylinder
- 15 2) Adjust helium regulator by turning the helium pressure regulator to 200 PSI above chosen rupture disk (e.g. if a 900 PSI rupture disk will be used, the working pressure has to be adjusted to 1100 PSI)
- 3) Turn on vacuum pump
- 4) Place 900psi rupture disk in the rupture disk-retaining cap. Screw on and tighten retaining cap.
- 20 5) Place macrocarriers in sterile macrocarrier holder
- 6) Place stop screen and macrocarrier holder in the launch assembly, tighten screw lid and place below rupture disk-retaining cap. Launch assembly should be set to a Gap distance of 1/4 inch and macrocarrier travel distance of 11mm.
- 25 7) Place tissue sample at a target distance of 90mm.
- 8) Turn on main switch of PDS
- 9) Apply vacuum to 27 inches of Hg
- 10) Hold vacuum and press "fire" button until shot is performed (automatic)
- 11) Release "fire" button and vent chamber
- 30 12) After shooting close valve of helium cylinder and loosen pressure valve

Table 1. Compositions of the media used

Media component	LP3	LP5	LP3-OS	MSK	MS0
Macro elements (mg/l final concentration)	1900	1900	1900	1900	1900
KNO ₃	1650	1650	1650	1650	1650
NH ₄ NO ₃	440	440	440	440	440
CaCl ₂ x 2H ₂ O	370	370	370	370	370
MgSO ₄ x 2H ₂ O	170	170	170	170	170
KCl					
Micro elements (mg/l final concentration)	37.3	37.3	37.3	37.3	37.3
Na ₂ EDTA	27.8	27.8	27.8	27.8	27.8
FeSO ₄ x 7H ₂ O	6.2	6.2	6.2	6.2	6.2
H ₃ BO ₃	0.83	0.83	0.83	0.83	0.83
KI	16.9	16.9	16.9	16.9	16.9
MnSO ₄ x H ₂ O	8.6	8.6	8.6	8.6	8.6
ZnSO ₄ x 7H ₂ O	0.025	0.025	0.025	0.025	0.025
CuSO ₄ x 5H ₂ O	0.25	0.25	0.25	0.25	0.25
Na ₂ MoO ₄ x 2H ₂ O	0.025	0.025	0.025	0.025	0.025
CoCl ₂ x 6H ₂ O					
Carbohydrates (g/l final concentration)	30	30	30	30	30
Maltose			64		
D-Mannitol					
Hormones (mg/l final concentration)	3.0	5.0	3.0	0.2	
2,4-D					
Kinetin					
Vitamins (mg/l final concentration)	0.5	0.5	0.5	0.5	
Pyridoxine HCl	0.1	0.1	0.1	0.1	
Thiamine HCl	0.5	0.5	0.5	0.5	
Nicotinic acid	100	100	100	100	
Myo-Inositol					
Other organics (mg/l final concentration)	2	2	2	2	2
Glycine					

5 Culture Media

Weights and volumes required of each individual ingredient are specified in Table 1. Adjust media pH to 5.8 with KOH. The addition of a solidifying agent is required. Use agarose (for LP3, LP5 and LP3-OS) and 0.8% (w/v) Agar for MS0 and MSK prior to sterilising. Media LP3, LP5 and

10 MSK are modified from Murashige and Skoog (1962).

Those skilled in the art will appreciate that the invention described above is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and products referred to or indicated in this specification, individually or collectively, and any and all combinations of two or more of said steps or features.

REFERENCES

- 1 Davis, S.J. (2002) Photoperiodism: the coincidental perception of the
10 season. *Curr Biol* 12 (24), R841-843
- 2 Araki, T. (2001) Transition from vegetative to reproductive phase. *Curr Opin Plant Biol* 4 (1), 63-68.
- 3 Reeves, P.H. and Coupland, G. (2000) Response of plant development to environment: control of flowering by daylength and temperature. *Current*
15 *Opinion in Plant Biology* 3 (1), 37-42
- 4 Colasanti, J. and Sundaresan, V. (2000) 'Florigen' enters the molecular age: long-distance signals that cause plants to flower. *Trends Biochem Sci* 25 (5), 236-240.
- 5 Kardailsky, I. et al. (1999) Activation Tagging of the Floral Inducer FT.
20 *Science* 286 (5446), 1962-1965
- 6 Kobayashi, Y. et al. (1999) A Pair of Related Genes with Antagonistic Roles in Mediating Flowering Signals. *Science* 286 (5446), 1960-1962
- 7 Weigel, D. and Kardailsky, I. (2001) Flowering locus T (FT) and genetically modified plants having modulated flower development. Patent
25 6,225,530 The Salk Institute for Biological Studies (La Jolla, CA)

- 8 Bradley, D.J. et al. (1997) Inflorescence commitment and architecture in Arabidopsis. Science 275 (5296), 80-83
- 9 Bradley, D. et al. (1997) Flowering genes. Patent WO9710339
- 10 Yano, M. et al. (2001) Genetic control of flowering time in rice, a short-
5 day plant. Plant Physiol 127 (4), 1425-1429
- 11 Kojima, S. et al. (2002) Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. Plant Cell Physiol 43 (10), 1096-1105
- 12 Yano, M. et al. (2000) Hd1, a major photoperiod sensitivity quantitative
10 trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell 12 (12), 2473-2484.
- 13 Takahashi, Y. et al. (2001) Hd6, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. Proc Natl Acad Sci U S A 98 (14), 7922-7927
- 15 14 Hayama, R. et al. (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. Nature 422 (6933), 719-722
- 15 Jensen, C.S. et al. (2001) A Terminal Flower1-Like Gene from Perennial Ryegrass Involved in Floral Transition and Axillary Meristem Identity. Plant Physiol 125 (3), 1517-1528.
- 20 16 Emmerlin, M. et al. (2002) MANIPULATION OF FLOWERING AND PLANT ARCHITECTURE. Patent WO0233091 AGRICULTURE VICTORIA SERV PTY
- 17 Fowler, S. et al. (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein
25 with several possible membrane-spanning domains. Embo J 18 (17), 4679-4688.

- 18 Coupland, G. et al. (1999) PLANT CONTROL GENES. Patent WO9949064
- 19 Huq, E. et al. (2000) GIGANTEA is a nuclear protein involved in phytochrome signaling in Arabidopsis. Proc Natl Acad Sci U S A 97 (17),
5 9789-9794.
- 20 Park, D.H. et al. (1999) Control of circadian rhythms and photoperiodic flowering by the Arabidopsis GIGANTEA gene. Science 285 (5433), 1579-1582.
- 21 Michaels, S.D. and Amasino, R.M. (1999) FLOWERING LOCUS C
10 Encodes a Novel MADS Domain Protein That Acts as a Repressor of Flowering. Plant Cell 11 (5), 949-956
- 22 Sheldon, C.C. et al. (1999) The FLF MADS box gene. A repressor of flowering in arabidopsis regulated by vernalization and methylation. Plant Cell 11 (3), 445-458
- 15 23 Michaels, S.D. et al. (2003) AGL24 acts as a promoter of flowering in Arabidopsis and is positively regulated by vernalization. Plant J 33 (5), 867-874
- 24 Scortecci, K.C. et al. (2001) Identification of a MADS-box gene, FLOWERING LOCUS M, that represses flowering. Plant J 26 (2), 229-236
- 20 25 Michaels, S. et al. (2000) ALTERATION OF FLOWERING TIME IN PLANTS. Patent WO0050615 WISCONSIN ALUMNI RES FOUND (US)
- 26 Hartmann, U. et al. (2000) Molecular cloning of SVP: a negative regulator of the floral transition in arabidopsis. Plant J 21 (4), 351-360
- 27 Lee, H. et al. (2000) The AGAMOUS-LIKE 20 MADS domain protein
25 integrates floral inductive pathways in Arabidopsis. Genes Dev 14 (18), 2366-2376.

- 28 Michaels, S.D. and Amasino, R.M. (2001) Loss of flowering locus c activity eliminates the late-flowering phenotype of *frigida* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* 13 (4), 935-942.
- 5 29 Reeves, P.H. and Coupland, G. (2001) Analysis of flowering time control in *arabidopsis* by comparison of double and triple mutants. *Plant Physiol* 126 (3), 1085-1091
- 30 Frohman, M.A. et al. (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide
10 primer. *Proc Natl Acad Sci U S A* 85 (23), 8998-9002
- 31 Ohara, O. et al. (1989) One-sided polymerase chain reaction: the amplification of cDNA. *Proc Natl Acad Sci U S A* 86 (15), 5673-5677
- 32 Loh, E.Y. et al. (1989) Polymerase chain reaction with single-sided specificity: analysis of T cell receptor delta chain. *Science* 243 (4888), 217-
15 220
- 33 Ratcliffe, O.J. et al. (1998) A common mechanism controls the life cycle and architecture of plants. *Development* 125 (9), 1609-1615
- 34 Saedler, H. et al. (2000) TRANSGENIC PLANTS EXHIBITING AN ALTERED FLOWERING TIME. Patent EP1055729
- 20 35 Yu, H. et al. (2002) AGAMOUS-LIKE 24, a dosage-dependent mediator of the flowering signals. *Proc Natl Acad Sci U S A* 99 (25), 16336-16341

CLAIMS

1. A substantially purified or isolated nucleic acid or nucleic acid fragment from a ryegrass (*Lolium*) or fescue (*Festuca*) species encoding a polypeptide selected from the group consisting of (a) a FLOWERING LOCUS
5 T (FT) or TERMINAL FLOWER (TFL) polypeptide; (b) an FT-like or TFL-like polypeptide; or (c) a functionally active fragment or variant of (a) or (b), the presence of which inhibits flowering.
2. A substantially purified or isolated nucleic acid or nucleic acid fragment from a ryegrass (*Lolium*) or fescue (*Festuca*) species encoding a
10 polypeptide selected from the group consisting of (a) a GIGANTEA (GI) or SHORT VEGETATIVE PHASE (SVP) polypeptide; (b) a GI-like or SVP-like polypeptide; or (c) a functionally active fragment or variant of (a) or (b).
3. A nucleic acid or nucleic acid fragment according to claim 1 or 2 wherein said nucleic acid or nucleic acid fragment is from perennial ryegrass
15 (*Lolium perenne*).
4. A nucleic acid or nucleic acid fragment according to claim 1 including a nucleotide sequence selected from the group consisting of (a) sequences shown in Figure 1 hereto (SEQ ID Nos. 1 to 5); (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences
20 recited in (a) or (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).
5. A nucleic acid or nucleic acid fragment according to claim 2 including a nucleotide sequence selected from the group consisting of (a)
25 sequences shown in Figures 3 and 4 hereto (SEQ ID Nos. 6 to 12); (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).
- 30 6. A construct including one or more nucleic acids or nucleic acid fragments according to any one of claims 1 to 5.

7. A construct according to claim 6 wherein the one or more nucleic acids or nucleic acid fragments are operably linked to one or more regulatory elements, such that the one or more nucleic acids or nucleic acid fragments are each expressed.
- 5 8. A construct according to claim 7, wherein the one or more regulatory elements include a promoter and a terminator, said promoter, nucleic acid or nucleic acid fragment and terminator being operatively linked.
9. A construct according to claim 6 including one or more FT or FT-like nucleic acids and one or more TFL or TFL-like nucleic acids, or
10 functionally active fragments or variants thereof.
10. A construct according to claim 9, further including one or more nucleic acids according to claim 2.
11. A plant cell, plant, plant seed or other plant part, including a construct according to any one of claims 6 to 10.
- 15 12. A plant, plant seed or other plant part derived from a plant cell or plant according to claim 11.
13. A method of modifying flowering in a plant, said method including introducing into said plant an effective amount of a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5, or a construct
20 according to any one of claims 6 to 10.
14. A method according to claim 13 wherein said method includes inhibiting flowering in said plant.
15. Use of a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5, and/or nucleotide sequence information thereof, and/or
25 single nucleotide polymorphisms thereof as a molecular genetic marker.
16. A substantially purified or isolated nucleic acid or nucleic acid fragment including a single nucleotide polymorphism (SNP) from a nucleic acid fragment according to any one of claims 1 to 5.
17. A nucleic acid or nucleic acid fragment including an SNP
30 according to claim 16, wherein said nucleic acid or nucleic acid fragment is from perennial ryegrass (*Lolium perenne*).

18. A substantially purified or isolated polypeptide from a ryegrass (*Lolium*) or fescue (*Festuca*) species, selected from the group consisting of (a) FT and TFL polypeptides (b) FT-like and TFL-like polypeptides and (c) functionally active fragments and variants of (a) or (b).

5 19. A substantially purified or isolated polypeptide from a ryegrass (*Lolium*) or fescue (*Festuca*) species, selected from the group consisting of (a) GI and SVP polypeptides (b) GI-like and SVP-like polypeptides and (c) functionally active fragments and variants of (a) or (b).

20. A polypeptide according to claim 18 or 19, wherein said
10 polypeptide is from perennial ryegrass (*Lolium perenne*).

21. A polypeptide encoded by a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5.

22. A polypeptide according to claim 18, wherein said polypeptide includes an amino acid sequence selected from the group consisting of
15 sequences translated from the nucleotide sequences shown in Figure 1 hereto (SEQ ID Nos. 1-5) and functionally active fragments and variants thereof.

23. A polypeptide according to claim 19, wherein said polypeptide includes an amino acid sequence selected from the group consisting of (a)
20 sequences translated from the nucleotide sequences shown in Figures 3 and 4 hereto (SEQ ID Nos. 8, 10 and 11); (b) sequences shown in Figures 3 and 4 hereto (Sequence ID Nos. 9 and 12); and (c) functionally active fragments and variants of (a) and (b).

24. A preparation for transforming a plant comprising a nucleic acid
25 or nucleic acid fragment according to any one of claims 1 to 5, or a construct according to any one of claims 6 to 10.

1/27

```
>rgft2.seq
TGTATCTTTTGGGACTGAGGTTGTGTGCTACGAGAGCCCTCGGCCGGTGCTCGGGATCCACCGGCTGGT
GTTTCTGCTCT
TCCAACAGCTCGGCCGTGAGACCGTYTACGCTCCGGGCTGGCGGCAGAATTTAGCACCCGCGACTTCG
CCGAGCTGTAC
AACCTCGGCTCGCCGGTCGCCGCCGTCTACTTCAACTGCCAAAGGGAGTCGGGAACCTGGTGGAAGGAGG
ATGTGAATTCT
CTGACACTAGTCGTCAACCCGTGCGCCTGCACAGGCTAGTAATTGGTGAAATCGAATCATGGAGCCAAA
AAGGAGTTGTT
TCCAAATTTAATTGTGAAAATGGTTTTGATTTTCATTatatatagaattatatttAAAAAAAAAAAAAA
Aaaa
```

```
>rgft1.seq
TGTATCTTTTGGGACTGAGGTTGTGTGCTACGAGAGCCCTCGGCCGGTGCTCGGGATCCACCGGCTGGT
GTTTCTGCTCT
TCCAACAGCTCGGCCGTGAGACCGTTTACGCTCCGGGCTGGCGGCAGAATTTAGCACCCGCGACTTCG
CCGAGCTGTAC
AACCTCGGCTCGCCGGTCGCCGCCGTCTACTTCAACTGCCAAAGGGAGTCGGGAACCTGGTGGAAGGAGG
ATGTGAATTCT
CTGACATATTATGACCATATAGGTTGAGCGTGATGATTGTATCGGGGGTTATACGTGGCTAAATGAAA
AAAGATCATTG TAATAAAAAAAAAAAAAAAa
```

```
>rgmft.seq
GGTAAATTCTACTCATTGTGTATGACTGATCCGGCCGCACCGAGCAGGAAAGAACCTAAATTCAGGGAA
TGGCATCATTG
GCTCGTAGGCAATGTCCCAGAATGTGATGTAGCCAAGGGCGAACTTTATCCGAATATATTGGCTCTGG
ACCTCCCCCAG
ATACTGGACTTCATCGTTATGTTTTCTCGTCTACCTTCAGCCGGGTAAAATCGATTTCAAAGATGTGC
CACGTTTGACC
AACAGATCTGGTGACAATCGTGCCAATTTTAAATTCAGCTTTTCGCCGATAAATATAATTTGGGCGAT
CCGGTGGCCTC
AGCTTATTATCGAGCCGAATACGACGACTACGTGCCAATTTTGTACAAACAATTGGGAGCA
```

FIGURE 1

2/27

```
>rgtfl1-2.seq
GCAGCAGCACATACACAATTACCTGAGCTTCCATTTCAGCAAAGAGGAACACACGCACACTGATCATCCC
TCGGGTTCCGA
TTTCAAGGCATCAACATGTCAAGGGCGTTGGAGCCTCTCGTTGTGGGGAAGGTGATTGGTGAGGTGCTG
GACAGCTTCAA
CCCCACCGTGAAGATGACGGCAACCTACAGCTCCAACAAGCAGGTGTTCAACGGCCATGAGTTCTTCCC
CTCGGCCATCG
CCGCTAAGCCGCGTGTGAGGTTTCAGGGGGGCGACCTTAGATCCTTCTTACATTGGTGATGACTGACC
CTGATGTGCCA
GGACCCAGTGATCCGTACCTGAGGGAGCATCTTCACTGGATTGTTACTGATATTCTTGGGACTACTGAT
GCTTCTTTTGG
GAAGGAGGTGGTGCACTACGAGAGCCCAAAGCCAAACATCGGCATCCACAGGTTTCATCCTCGTGCTGTT
CCAGCAGACGC
ACCGGGGCTCGGTAAAGAACACACCCGTGTCGAGGGACCGCTTCAGGACCCGCGAGTTCGCCAAGGATA
ACGAGCTCGGC
CTCCCTGTGCGYGCTGTCTACTTCAACGCGCAGCGGGAGACCGCCGCCCGCCGGCGATAGCTCAACGGC
AACCGAACCA
CCAACCAGCAACACCCCCCTACTATGTACCTGATCTAGCTACATGATAAAACGAACTGCGTACAATCAC
CTATTAGCTAG
CTTCGATGGCCTTTCTTGCTACATCCAAGCATGCACAATGTCTGAATAAAACACACCGGTAAATTAGCT
GTTTGCACGAG
AAAGCTGCTCCYTACTAGTACGTAGCCGTTGCCCATTTAGTTAATTTTTGTGAATGTGACAAGATCGAT
GATTGGGAAGA
GATTGCAGTGTTGACTGAGAAAAAAGTGCAAGATTTGAAGCAATAATAGTCGTCAGGGAGTA-----
AAAAAAAAAAAAA AAAAAAAAAAAAA
```

```
>rgtfl1-1.seq
AACAACCATGAGGTTATATTTCTCCTCATTCTCAAGACTGTTTACCTAGTGCTTCGACTTGCTAGTTACC
TGTCAGCTGTA
GATATTTGGCATCGATCATCACCACCTTTTCAGTTCAAGTTAGCTAGCTAGACATGGCTAGGGTGGTGG
AGCCTCTCATC
GTCGGAAAGGTGATCGGGGAGGTCATCGACAACTTCACCCCCACTGAGAAGATGACAGTAACCTACAGC
TCCAACAAGCA
GGTGTTCAATGGCCACGAATTCTTCCCGTCGGCAGTCGTCTCCAAGCCACGCATTGAGGTCCAGGGTGG
CGACATGAGAT
CCTTCTTTCACACTGGTCATGACGGACCCAGATGTGCCAGGGCCTAGCGACCCATACCTGAGGGAGCATC
TCCACTGGATC
GTCAATAATATTCCAGGCACCACCTGATGCTTCTTTTGGAAAGGAGGTGGTGAGCTACGAGAGCCCCAAG
CCCAACATCGG
CATTACAGGTTACCTTCGTGCTGTTCAAGCAGAAGCAGCGGCAGACAATGAGCCCTCCCTCCAGCAG
GGACTACTTCA
ACACTCGCCGCTTCGCTGCCGCGAACGATCTTGGCCTCCAGTCGCTGCCGTCTACTTCAACGCGCAGC
GGGAGACAGCT
GCGCGCCGCCGCTGATGGAAACAATCAGCGAAACCTTCTTCTCGCGCATGCATGGCGCATGCATGCAA
TCCTATGGATC
AACCAATAGCTCTACTACTAGCTACCCTTTATGTCTTTCATTCAAATAAGAGTTTGCTTGTGAGCgtct
tgtgtcAAAAA AAAAAAAAAAAAA
```

FIGURE 1 (cont.)

3/27

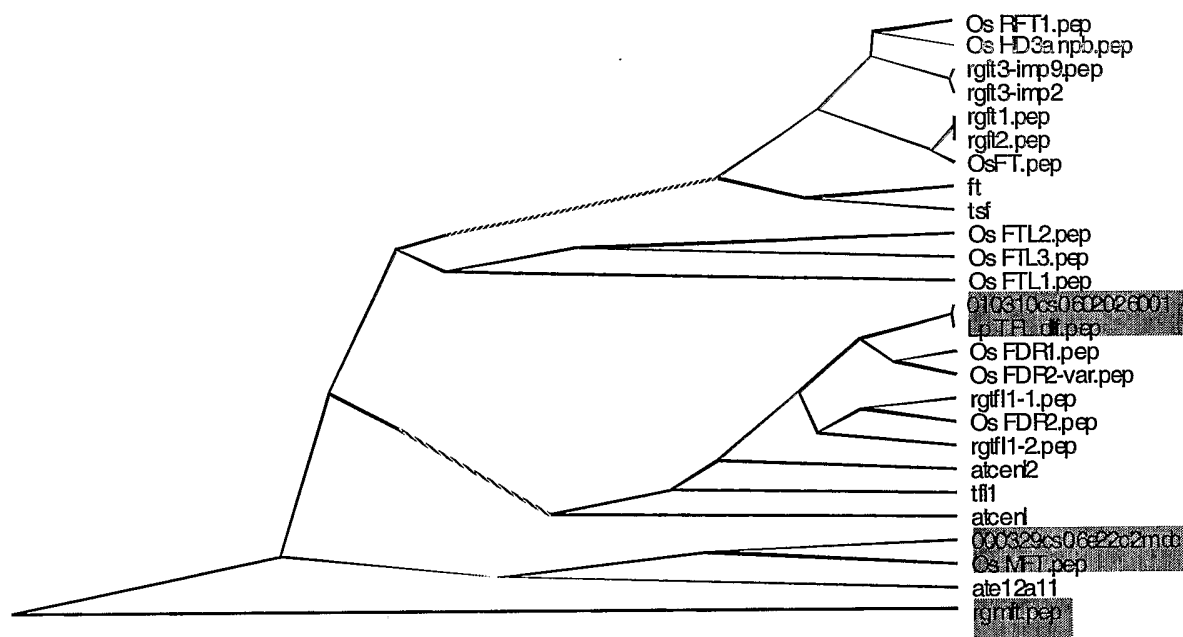


FIGURE 2

4/27

1 TCGACTGGAG CACGAGGACA CTGACATGGA CTGAAGGAGT AGAAACCCCA ACCACGTTAC GGATGAAAAA
71 ATCTTTTCT CGCCTCTCCT CGCCCCCTGC TCCAAGCTTC TCTCTCCTCG CCGTCTACCG CTCGCCGCCG
M X .
~~~~~  
141 CCGCTGATTG GCCGCCGGAG CCACGGAGTA GAGCGCGCCC AGTCTAGGAT CTTAAACTAA TAAGTATGTC  
. V S N G K W I D G L Q F S S L F W P P P H D A  
~~~~~  
211 TGTCTCAAAT GGAAGTGGA TCGACGGGCT CCAGTTCTCT TCACTATTCT GGCCCCCGCC ACACGATGCA
Q Q K Q A Q T L A Y V E Y F G Q F T S D S E Q F .
~~~~~  
281 CAGCAGAAAC AGGCACAAAC TTTGGCCTAC GTTGAGTACT TTGTCAGTT TACATCTGAC AGTGAGCAAT  
. P E D V A Q L I Q S Y Y P S K E K R L V D E V .  
~~~~~  
351 TCCCGGAGGA TGTAGCTCAG CTCATCCAAA GTTACTATCC ATCGAAAGAA AAACGCTTGG TAGATGAAAGT
. L A T F V L H H P E H G H A V V H P I L S R I
~~~~~  
421 ATTAGCAACC TTTGTTCTCC ATCACCCTCGA GCATGGTCAT GCAGTTGTAC ATCCAATTCT TTCACGCATC  
I D G S L S Y D R H G S P F N S F I S L F T Q T .  
~~~~~

FIGURE 3

5/27

491 ATAGATGGGT CCTGAGTTA TGATAGACAT GGTTCCCCAT TCAATTCTTT CATCTCTTTA TTACCCAAA
· A E K E Y S E Q W A L A C G E I L R V L T H Y ·
~~~~~  
561 CTGCTGAGAA AGAGTATTCA GAGCAGTGGG CTTTGGCGTG TGGAGAAATT CTTAGAGTTC TTACTCACTA  
· N R P I F K V A E C N D T S D Q A T T S Y S L  
~~~~~  
631 CAATAGGCCA ATCTTCAAAG TTGCAGAATG TAACGACACC TCCGACCAGG CCACAACAAG TTATTCCTTA
H D K A N S S P E N E P E R K P L R P L S P W I ·
~~~~~  
701 CATGACAAAG CTAATAGCTC TCCAGAAAAT GAACCTGAAC GGAAGCCATT GAGGCCATTA TCTCCTTGGA  
· T D I L L N A P L G I R S D Y F R W C G G V M ·  
~~~~~  
771 TCACAGACAT TTTGTTAAAT GCACCTTTGG GCATTAGAAG TGACTATTTT AGATGGGTGTG GTGGAGTCAT
· G K Y A A G G E L K P P T T A Y S R G A G K H
~~~~~  
841 GGGAAAATAC GCAGCTGGTG GAGAACTGAA GCCTCCAACA ACTGCTTACA GCCGGGGAGC TGGTAAGCAT  
P Q L M P S T P R W A V A N G A G V I X X V C D ·  
~~~~~

FIGURE 3 (cont.)

6/27

```

911 CCACAACTTA TGCCATCCAC CCCTAGATGG GCTGTTGCCA ATGGAGCTGG AGTCATCWTA WGTGTCTGTG
    . E E V A R Y E T A N L T A A A V P X L L L P P .
    ~~~~~

981 ACGAGGAAGT AGCTCGTTAC GAGACAGCAA ACTTAACCGC AGCAGCTGTT CCTGSGCTTC TGCTACCTCC
    . P T M P L D E H L V A G L P P L E P Y A R L F
    ~~~~~

1051 ACCGACAATG CCCTTGGATG AGCATTTGGT GGCAGGGTTG CCCCTCTCTG AACCATACGC TCGCTTGTTT
    H R Y Y A I A T P S A T Q R L L F G L L E A P P .
    ~~~~~

1121 CACAGATACT AYGCAATTGC TACTCCAAGT GCTACACARA GGTGCTCTTT TGGTCTTCTT GAAGCACCGC
    . S W A P D A L D A A V Q L V E L L R A A E D Y .
    ~~~~~

1191 CTTTCATGGC TCCAGATGCA CTTGATGCAG CAGTACAGCT TGTGAACTC CTTCGAGCAG CCGAAGATTA
    . A T G M R L P K N W L H L H F L R A I G T A M
    ~~~~~

1261 TGCTACTGGC ATGCGGCTTC CGAAAAATTG GCTGCATCTT CATTTCTTGC GTGCAATCGG AACTGCAATG
    S M R A G M A A D T A A A L L F R I L S Q P T L .
    ~~~~~

```

FIGURE 3 (cont.)

7/27

1331 TCTATGAGAG CTGGYATGGC TGCTGATACG GCCGCTGCCT TGCTATTTTCG TATACTATCC CAACCAACGT
· L F P P L R H A E G V V Q H E P L G G Y V S S ·
~~~~~  
1401 TGCTTTTTC TCCACTAAGA CATGCCGAAG GAGTTGTGCA GCATGAACCA CTAGGTGGCT ATGTATCATC  
· Y K R Q L E I P A S E T T I D A T A Q G I A S  
~~~~~  
1471 ATACAAAAGA CAGCTGGARA TTCCTGCATC TGAACCACT ATTGATGCTA CTGCACAAGG CATTCCTTCC
L L C A H G P D V X W R I C T I W E A A Y G L L ·
~~~~~  
1541 TTGCTGTGYG CTCATGGTCC TGATGTIGAK TGGAGAATAT GTACCATCTG GGAAGCTGCC TATGGTTTGT  
· P L N S S A V D L P E I V A A P L Q P P T L ·  
~~~~~  
1611 TACCTCTGAA TTCRTCAGCA GTCGATTTC CTGAAATTGT TGTAGCTGCT CCGCTTCAGC CACCTACTTT
· S W S L Y L P L L K V F E Y L P R G S P S E A
·
~~~~~  
1681 ATCATGGAGC CTATATTTC CACTGTTGAA AGTATTTGAG TATCTACCTC GTGGAAGTCC ATCTGAAGCA  
C L M R I F V A T V E A I L R R T F P S E T E P ·  
~~~~~

FIGURE 3 (cont.)

8/27

```

1751 TGCCTTATGA GAATATTGT GGCAACTGTT GAAGCTATAC TCAGGAGAAC TTTCCTTCG GAAACCGAAC
    . S K K P R S P S K S L A V A E L R T M I H S L .
    ~~~~~
1821 CATCCAAAA ACCAAGAAGT CCATCTAAGA GCCTTGCTGT TGCTGAAC TC GTACGATGA TACATTCAC
    . F V E S C A S M N L A S R L L F V V L T V S V
    ~~~~~
1891 CTTTGTGAA TCATGTGCCT CAATGAACCT TGCCTCGGG TTATTGTTG TAGTATTGAC TGTCCTCAGTC
    S H Q A L P G G S K R P T G S E N H S S E S T .
    ~~~~~
1961 AGTCATCAAG CTCGCGCGG GGGCAGCAA AGACCTACAG GCAGTGAGAA CCATTCTTCT GAGGAGTCCA
    . E D S K L T N G R N R C K K K Q G P V G T F D .
    ~~~~~
2031 CTGAGGACTC AAAATTAACC AATGGAAGAA ACAGATGCAA GAAGAAACAA GGGCCTGTG GTACCTTTGA
    . S Y V L A A V C A L S C E L Q L F P I L C K N
    ~~~~~
2101 CTCGTATGTG CTGGCTGCTG TTTGTGCTTT ATCTTGTGAG CTCAGCTGT TCCCTATACT TTGCAAGAAT
    V T K T N I K D S I K I T M P G K T N G I S N E .
    ~~~~~
2171 GTTACGAAGA CAAACATAAA AGACTCTATA AAGATTACCA TGCCTGGAAA AACCAATGGG ATCAGTAAATG
    . L H N S V N S A I L H T R R I L G I L E A L F .
    ~~~~~

```

FIGURE 3 (cont.)

9/27

2241 AGCTACACAA TAGCGTTAAC TCAGCGATTG TCCTACTCTG TAGAATTCCT GGCACTCTGG AAGCTCTTTT
· S L K P S S V G T S W S Y S S N E I V A A A M
~
2311 CTCCTTGAAG CCATCATCAG TTGGTACCTC CTGGAGCTAT AGTTCAAATG AGATAGTTGC AGCAGCAAATG
V A A H V S E L F R R S R P C L N A L S A L K R ·
~
2381 GTTGCTGCTC ATGTTTCTGA GTTATTCGGT AGGTCGAGGC CATGCCCTAAA TGCACTATCT GCACTGAAGC
· C K W D A E I S T R A S S L Y H L I D L H G K ·
~
2451 GATGTAAGTG GGATGCTGAG ATTCTTACCA GGGCATCATC GCTTTACCAT CTGATCGACT TGCATGGTAA
· T V S S I V N K A E P L E A H L N L T A V K K
~
2521 AACTGTGTCA TCCATCGTGA ACAAAGCTGA GCCTTTGGAA GCTCACCTGA ACCTTACAGC AGTAAAGAAA
D D Q H H I E E S N T S S S D Y G N L E K K S K ·
~
2591 GATGATCAAC ACCACATTGA GGAAAGCAAT ACCAGCTCAT CGGATTATGG GAACTTGGAG AAGAAGAGTA
· K N G F S R P L M K C A E Q A R R N G N V A S ·
~
2661 AGAAAAATGG TTTTTCAGA CCACTCATGA AATGTGCAGA ACAGGCTAGG AGAAATGGTA ACGTTGCAAG
· T S G K A T A T L Q A E A S D L A N F L T M D
~

FIGURE 3 (cont.)

10/27

2731 TACATCGGGG AAAGCTACTG CAACTTTTACA GCGGAAGCA TCTGATTGG CAAACTTCCT TACCATGGAC
R N G G Y G G S Q T L L R T V M S E K Q E L C F .
~~~~~  
2801 AGGAACGGGG GTTATGGAGG TTCTCAAACT CTCCTAAGAA CTGTAATGTC AGAAAAGCAG GAACTATGCT  
. S V V S L L W H K L I A S P E T Q M S A E S T .  
~~~~~  
2871 TTTCTGTTGT CTCGTTGCTG TGGCATAAGC TTATTGCATC TCCCGAAACA CAGATGTCTG CAGAGAGTAC
. S A H Q G W R K V A D A L C D V V S A S P A K
~~~~~  
2941 ATCAGCTCAT CAGGGTTGGA GAAAGGTTGC AGATGCGCTT TGTGATGTTG TTTCAGCTTC ACCGGCCAAG  
A X T A I V L Q A E K D L Q P W I A R D D E Q G .  
~~~~~  
3011 GCTHCAACTG CTATTGTCCT GCAGGCTGAG AAGGACTTGC AGCCCTGGAT TGCTCGAGAT GATGAGCAAG
. Q K M W R V N Q R I V K L I A E L M R N H D S .
~~~~~  
3081 GTCAGAAAGAT GTGGAGAGTC AACCAGCGAA TAGTGAAACT GATAGCTGAG CTTATGAGGA ACCATGATAG  
. P E A L I I L A S A S D L L L R A T D G M L V  
~~~~~  
3151 CCCAGAAGCA CTGATAATTC TTGCGAGCGC TTCAGACCTT CTGCTCCGTG CCACGGATGG GATGCTTGTT
D G E A C T L P Q L E L L E V T A R A I H L I V .
~~~~~

FIGURE 3 (cont.)

11/27

3221 GATGGTGAAG CTTGTACCTT GCCTCAATTG GAGCTTCTGG AAGTAACCGC CAGAGCCATT CATCTCATCG  
· E W G D P G V A V A D G L S N L L K C R L S P ·  
~~~~~  
3291 TTGAATGGGG AGATCCAGGT GTAGCAGTTG CTGATGGCCT CTCGAATCTG CTGAAGTGCC GGCTATCACC
· T I R C L S H P S A H V R A L S M S V L R D I
~~~~~  
3361 TACCATCCGA TGCCTTTCCC ACCCTAGTGC ACATGTACGG GCGCTCAGCA TGTCCGTCCT TCGCGACATC  
L N S G P I S S T K I I Q G E Q R N G I Q S P S ·  
~~~~~  
3431 TTGAACAGTG GACCAATAAG TTCCACCAAG ATAATTCAAG GAGAGCAGCG GAACGGCATC CAAAGCCCAA
· Y R C A A A S M T N W Q A D V E R C I E W E A ·
~~~~~  
3501 GTTACCGGTG CGCGGCAGCA AGTATGACCA ACTGGCAAGC GGACGTCGAG AGATGCATAG AGTGGGAAGC  
· H N R Q A T G M T L A F L T A A A N E L G C P  
~~~~~  
3571 CCACAACCGT CAGGCCACCG GGATGACGCT TGCCTTTCTC ACTGCAGCKG CTAACGAACT CGGATGCCCC
L P C *
~~~~~  
3641 CTTCCTTGCT GACACAGCCA TATTTGAAGC TGRCATCGGC GAYACTTGAC RGTAGCGCG AGCAGTTGCT  
3711 GCATGGTCAG CGAGCAGGAT GGCTAATCCY TKGCTCAAGG ATGACTTCCC AGTCTGAA

FIGURE 3 (cont.)

**12/27**

|     |                                        |                          |                           |                          |
|-----|----------------------------------------|--------------------------|---------------------------|--------------------------|
| 1   | AANTTGTACA<br>CTATATGCAT<br>TGCAGAATTC | CGATTTCACT<br>GCTCGAGCGG | ATAGTGGCGA<br>CCGCCAGTGT  | ATTGGGCCCT<br>GATGGATATC |
|     | TTNAACATGT<br>GATATACGTA<br>ACGTCTTAAG | GCTAAAGTGA<br>CGAGCTCGCC | TATCACCGCT<br>GGCGGTCACA  | TAACCCGGGA<br>CTACCTATAG |
| 91  | GGCCCTTTYM<br>GCACCACTGT<br>CTACGCCTCT | TMYYYCTCCT<br>GCCTCTCCGA | CTCCTTTTCGC<br>CCCCGGCCGG | ATCCCCAACC<br>CCGACGCGCG |
|     | CCGGGAAARK<br>CGTGGTGACA<br>GATGCGGAGA | AKRRRGAGGA<br>CGGAGAGGCT | GAGGAAAGCG<br>GGGGCCGGCC  | TAGGGGTTGG<br>GGCTGCGCGC |
| 181 | CGGCGTGDGG<br>RGTYGGGYYS<br>GATCAAGCGG | ACTGGGGRGG<br>GGAGGCGGCG | RRGGAGGAGA<br>ATGGCGCGGG  | KMGATCGATC<br>AGAGGCGCGA |
|     | GCCGCACHCC<br>YCARCCRRS<br>CTAGTTCGCC  | TGACCCCYCC<br>CCTCCGCGCG | YYCCTCCTCT<br>TACCGCGCCC  | MKCTAGCTAG<br>TCTCCGCGCT |
| 271 | ATAGAGAGCG<br>GCCGCAGGGG<br>CCTATGCGAC | CGGCGGCGCG<br>CCTCTTCAAG | CCAGGTCACC<br>AAGGCCGAGG  | TTCTCCAAGC<br>AGCTCTCCGT |

**FIGURE 4a**

**13/27**

|     |             |            |            |             |
|-----|-------------|------------|------------|-------------|
|     | TATCTCTCGC  | GCCGCCGCGC | GGTCCAGTGG | AAGAGGTTTCG |
|     | CGGCGTCCCC  | GGAGAAGTTC | TTCCGGCTCC | TCGAGAGGCA  |
|     | GGATACGCTG  |            |            |             |
| 361 | GCCGACGTCG  | CGCTCATCGT | CTTCTCGTCC | ACAGGGAAGC  |
|     | TCTCCCAGTT  | CGCAAGCTCC | AGTATGAATG | AGATCATCGA  |
|     | CAAGTACAGC  |            |            |             |
|     | CGGCTGCAGC  | GCGAGTAGCA | GAAGAGCAGG | TGTCCCTTCG  |
|     | AGAGGGTCAA  | GCGTTCGAGG | TCATACTTAC | TCTAGTAGCT  |
|     | GTTTCATGTCG |            |            |             |
| 451 | ACCCATTCTA  | AGAACCTGGG | GAAAGCAGAC | CAGCCTTCTC  |
|     | TTGACTTGAA  | TTTAGAACAT | AGTAAGTATG | CAAATCTGAA  |
|     | CGATCAGCTT  |            |            |             |
|     | TGGGTAAGAT  | TCTTGGACCC | CTTTCGTCTG | GTCGGAAGAG  |
|     | AACTGAACTT  | AAATCTTGTA | TCATTCATAC | GTTTAGACTT  |
|     | GCTAGTCGAA  |            |            |             |
| 541 | GCGGAAGCTA  | GTCTYCGACT | TAGACAGATG | AGAGGCGAGG  |
|     | GGCTTGAGGG  | GTTGAVTGTT | GATGAACTCC | AGCAGTTGGA  |
|     | GAAGAACCTT  |            |            |             |
|     | CGCCTTCGAT  | CAGARGCTGA | ATCTGTCTAC | TCTCCGCTCC  |
|     | CCGAACTCCC  | CAACTBACAA | CTACTTGAGG | TCGTCAACCT  |
|     | CTTCTTGAA   |            |            |             |

**FIGURE 4a (cont.)**

**14/27**

|     |                                        |                           |                          |                          |
|-----|----------------------------------------|---------------------------|--------------------------|--------------------------|
| 631 | GAAACTGGTC<br>AATTCTTGGA<br>ACAGCTGGCA | TGCACAGGGT<br>GCAGATCAAT  | GCTTCAGACG<br>GAATTGCAAC | AAAGATCAAC<br>GAAAGAGCTC |
|     | CTTTGACCAG<br>TTAAGAACCT<br>TGTCGACCGT | ACGTGTCCCA<br>CGTCTAGTTA  | CGAAGTCTGC<br>CTTAACGTTG | TTTCTAGTTG<br>CTTTCTCGAG |
| 721 | GAGGAGAACA<br>CAATAGCTGG<br>TATTGCTGAG | TGCAACTGAG<br>CAAGCCAGTA  | GAACCAAGTA<br>GTTGCTGATA | TCCCAGATAC<br>CCGAAAATGT |
|     | CTCCTCTTGT<br>GTTATCGACC<br>ATAACGACTC | ACGTTGACTC<br>GTTTCGGTCAT | CTTGGTTCAT<br>CAACGACTAT | AGGGTCTATG<br>GGCTTTTACA |
| 811 | GATGGGCAGT<br>CGGGAAGCTC<br>CCTGAAATTG | CCTCTGAATC<br>ACAGGATAAC  | TGTCATGACG<br>GATGATGGTT | GCGTTGCACT<br>CAGATGTATC |
|     | CTACCCGTCA<br>GCCCTTCGAG<br>GGACTTTAAC | GGAGACTTAG<br>TGTCCTATTG  | ACAGTACTGC<br>CTACTACCAA | CGCAACGTGA<br>GTCTACATAG |
| 901 | GGATTACCCT<br>TCRSTTCAGA<br>AGCTCTTGTG | GCAGTGCGTG<br>TCTTTATGGA  | GAAGTAACTA<br>ACTGCCACR  | TATAAAAYCG<br>TCAGTGGAGA |

**FIGURE 4a (cont.)**

**15/27**

|      |                                        |                          |                          |                          |
|------|----------------------------------------|--------------------------|--------------------------|--------------------------|
|      | CCTAATGGGA<br>AGYSAAGTCT<br>TCGAGAACAC | CGTCACGCAC<br>AGAAATACCT | CTTCATTGAT<br>TGACGGGTGY | ATATTTTRGC<br>AGTCACCTCT |
| 991  | TAATCSACAA<br>GAAGCGAGAT<br>GCATGACGTG | ACGTACCYGA<br>CAGTTAACCT | GCTGCAATAA<br>GATTTATCAT | TCTTGCRGCT<br>CCTTGTGGCT |
|      | ATTAGSTGTT<br>CTTCGCTCTA<br>CGTACTGCAC | TGCATGGRCT<br>GTCAATTGGA | CGACGTTATT<br>CTAAATAGTA | AGAACGYCGA<br>GGAACACCGA |
| 1081 | ATGTTCCCGT<br>TTTTAGATCG<br>TATTTGTTCA | TYTTACYGTT<br>ATCTGATGTC | TACTAGGATG<br>CATCTTATCC | TTAACTAAAC<br>CCGTTGGCAC |
|      | TACAAGGGCA<br>AAAATCTAGC<br>ATAACAAGT  | ARAATGRCAA<br>TAGACTACAG | ATGATCCTAC<br>GTAGAATAGG | AATTGATTTG<br>GGCAACCGTG |
| 1171 | TGGTATCCAT<br>ATGGTCTATA<br>RTCTRVTRRM | GTACCTTAAC<br>WWTKCWYYWG | TGTCAGTATA<br>CRABRYTARB | TCTTAAAMTT<br>YRRMVDTWYG |
|      | ACCATAGGTA<br>TACCAGATAT<br>YAGAYBAYYK | CATGGAATTG<br>WWAMGWRRWC | ACAGTCATAT<br>GYTVYRATYV | AGAATTTKAA<br>RYYKBHAWRC |

**FIGURE 4a (cont.)**

**16/27**

1261 DVMRMRMWMA      RWAVMAWRMA      WRDARAGGGG      CCAATTCGCC  
CTATAGTGAA TCGTGGTACA AANTTC

HBKYKYKWKT      YWTBKTWYKT      WYHTYTCCCC      GGTTAAGCGG  
GATATCACTT AGCACCATGT TTNAAG

**FIGURE 4a (cont.)**

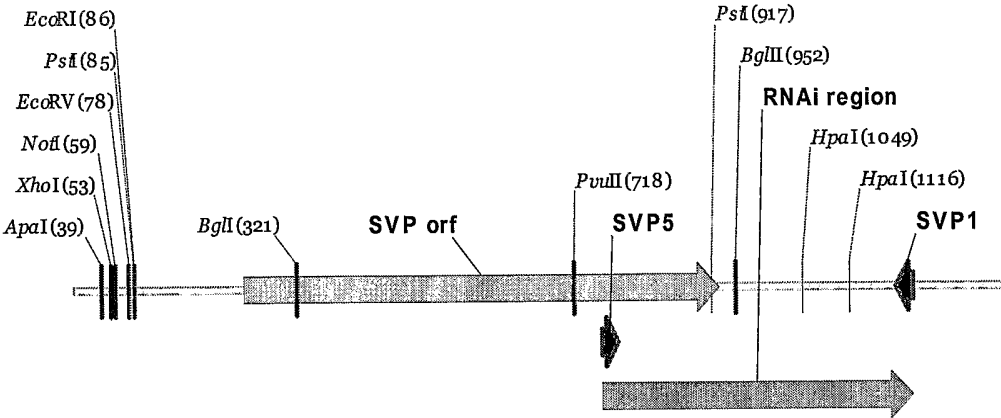
**17/27**

|     |                          |            |            |            |
|-----|--------------------------|------------|------------|------------|
| 1   | MARERREIKR<br>ADVALIVFSS | IESAAARQVT | FSKRRRGLFK | KAEELSVLCD |
| 51  | TGKLSQFASS<br>SKYANLNDQL | SMNEIIDKYS | THSKNLGKAD | QPSLDLNLEH |
| 101 | AEASLRLRQM<br>KDQQFLEQIN | RGEGLEGLXV | DELQQLEKNL | ETGLHRVLQT |
| 151 | ELQRKSSQLA<br>DGQSSESVMT | EENMQLRNQV | SQIPIAGKPV | VADTENVIAE |
| 201 | ALHSGSSQDN               | DDGSDVSLKL | GLPCSAWK   |            |

**FIGURE 4b**

18/27

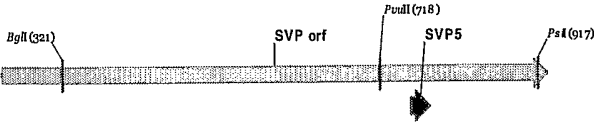
tgccgmggagaggcgagatcaagcgatagagcgccggcgccaggtaccttctcaagcgccgagggcctctcaagaaggcc  
gaggagctctccgtmctatgcgacgacgacgctcgcgctcatcgtctcgtccacaggaagctctccagttcgaagctccagatgaatgagatcat  
cgacaagtacagcacmacttaagaacctggggaagcagaccagccttctctgacttgaayttgaacatagtagtatgcaaatctgaacgacag  
cttgcgggaagctagtctcgtacttagacagatgagagggcgaggggcttgaggggtgavtgitgatgaactccagcagttggagaagaaccttgaaactg  
gtctgcacaggggtgcttcagacgaaagatcaacaattcttgagcagatcaatgaattgcaacgaaagagctcacagctggcagaggagaacatgcaac  
tgaggaaaccaagtatccagataccaatagctggcaagccagtagttgctgataccgaaaattgtattgctgaggatggcagtcctctgaatctgcatga  
cgcggttgctactcgggaagcksacaggataacgatgatggttcagaygtatccctgaaattgggattaccctgcagtgcgtggaag



SVP orf  
1329 bp

gataccaatagctggcaagccagtagttgctgataccgaaaattgtattgctgaggatgggcagtcctctgaatct  
gtcatgacggcggtgcactcgggaagcksacaggataacgatgatggttcagaygtatccctgaaattgggatt  
accctgcagtgcgtggaagtaactatataaaaycgtcrsttcagatcttatggaactgccacrtcagtgaggaa  
gtcttgtgtaatsacaaacgtaccygagctgcaataatcttgcrgctgaagcgagatcagttaacctgattatc  
atccttggctgcatgacgtgatgtmccgttytacgttactaggatgttaactaaacttttagatcgtatg  
ctcatctvatcccccttmmactattwttcatetawccatzatccttaactatcagtatc

MARERREIKR IESAAARQVT FSKRRRGLFK KAEELSVLCD ADVALIVFSS  
TGKLSQFASS SMNEIIDKYS THSKNLGKAD QPSLDLNLH SKYANLNDQL  
AEASLRRLQM RGEGLLEGLXV DELQOLEKNL ETGLHRVLQT KDQQFLEQIN  
ELQRKSSQLA EENMQLRNQV SQIPTAGKPV VADTENVIAE DGQSSSESVMT  
ALHSGSXQDN DDGSDVSLKL GLPCSAWK



Fragment of SVP orf  
684 bp (molecule 1329 bp)

FIGURE 4c

19/27

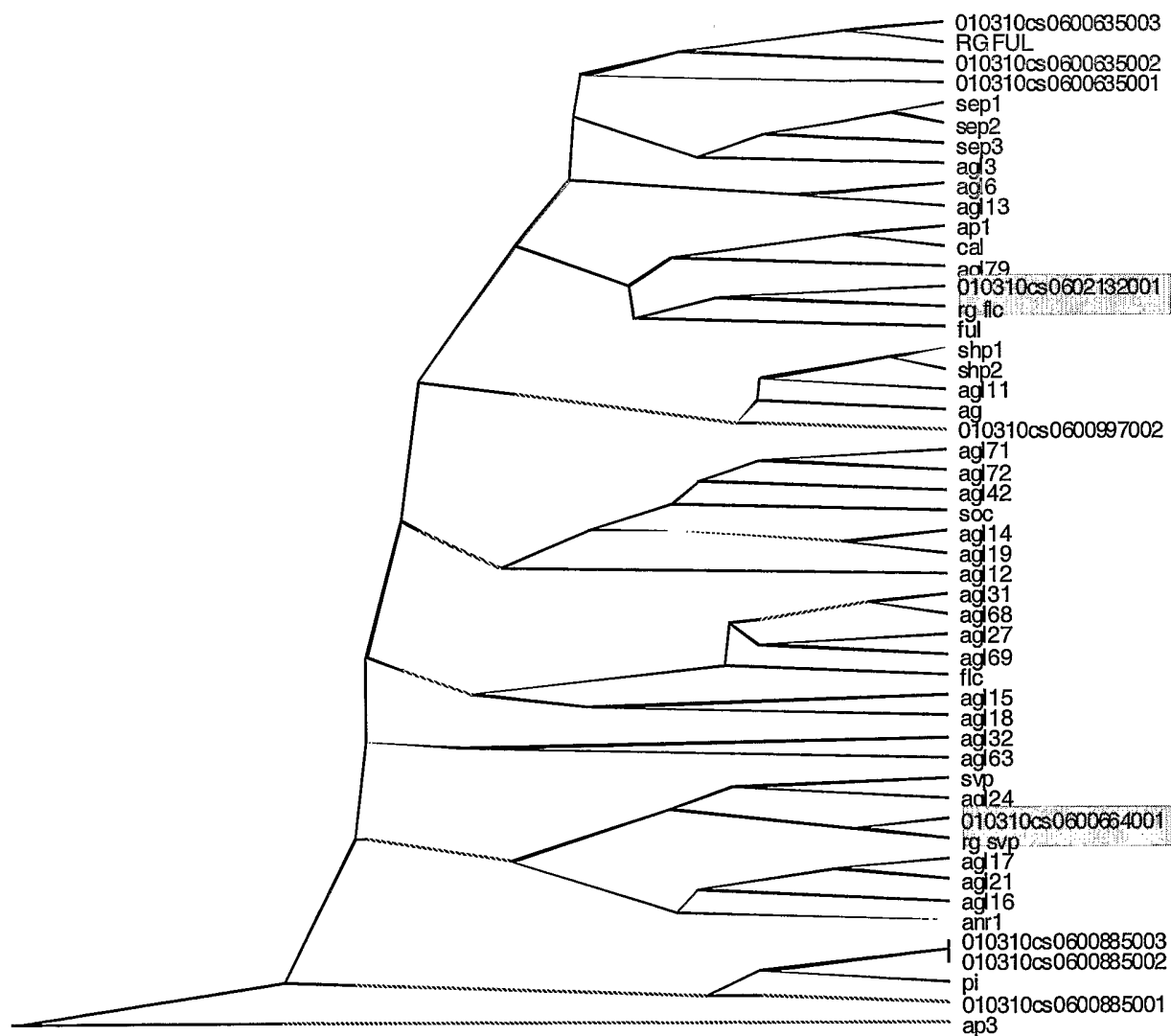


FIGURE 5

20/27



FIGURE 6

21/27

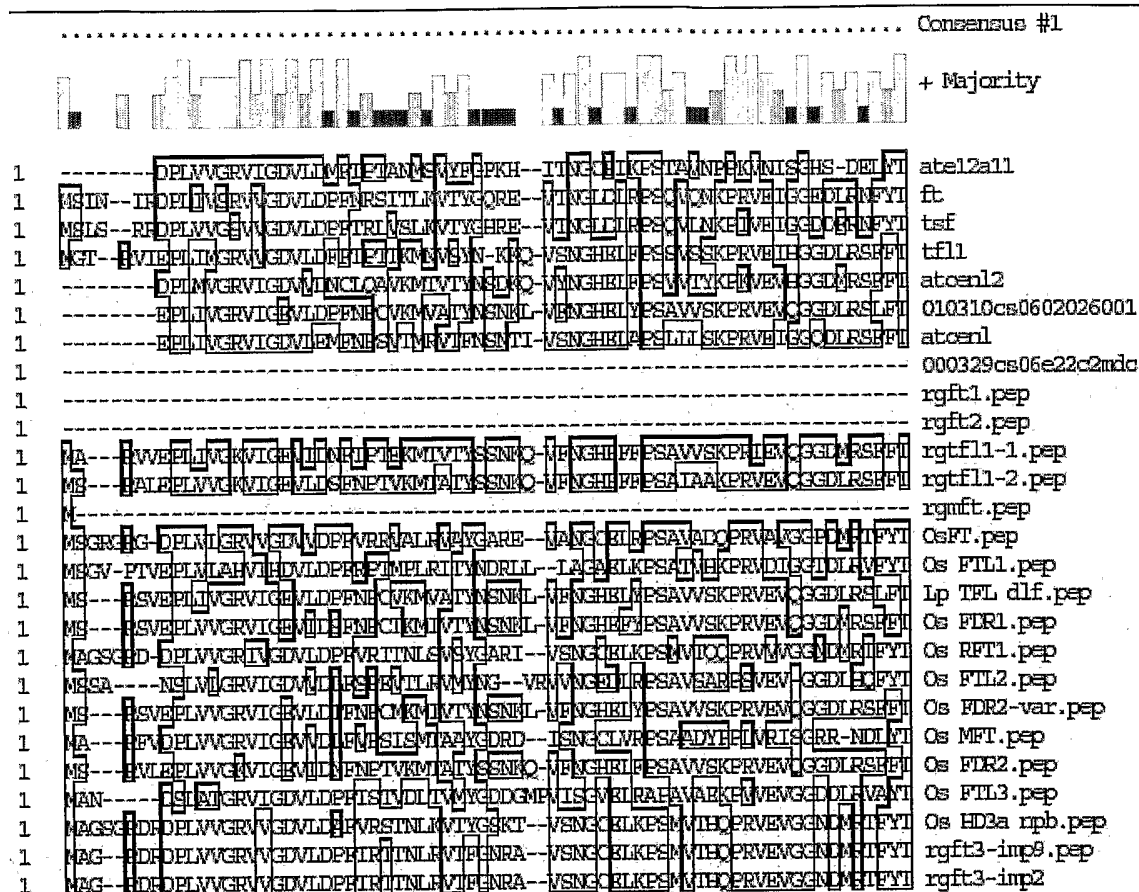


FIGURE 7

22/27

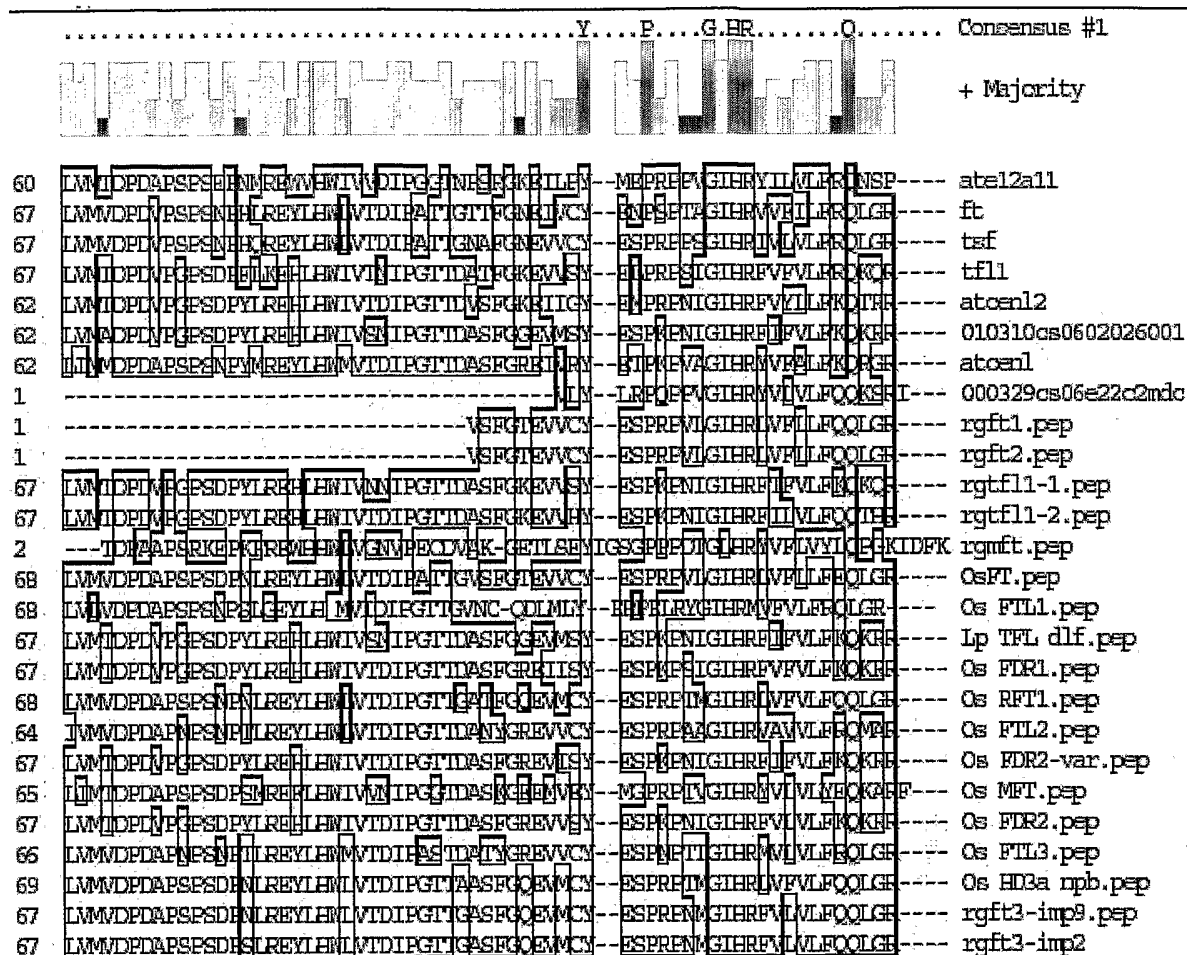


FIGURE 7 (cont.)

23/27

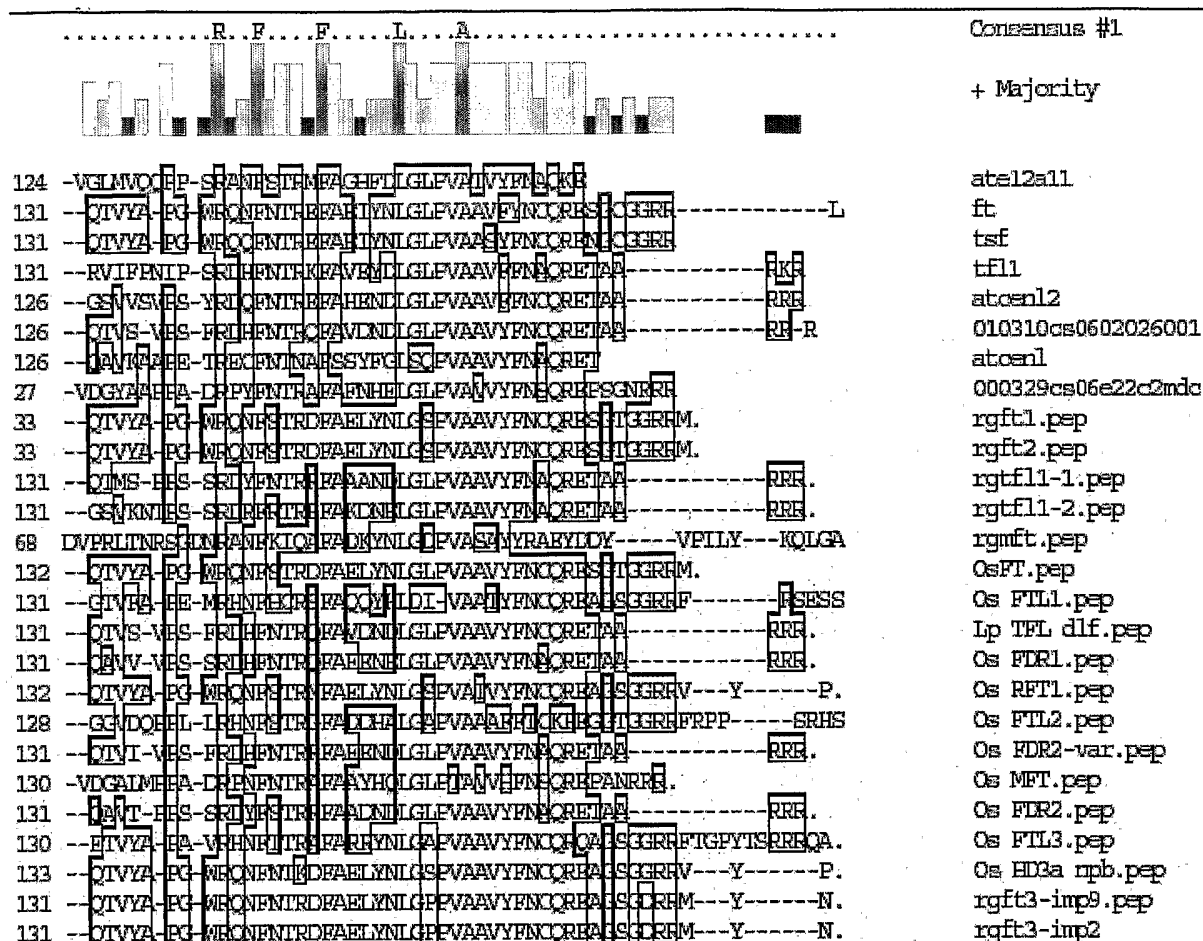


FIGURE 7 (cont.)

24/27

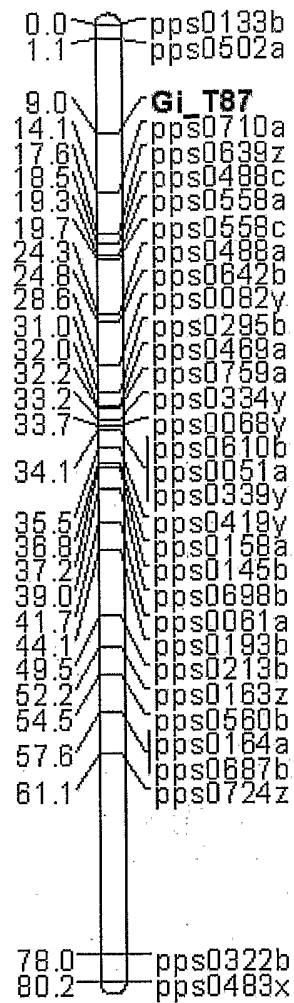
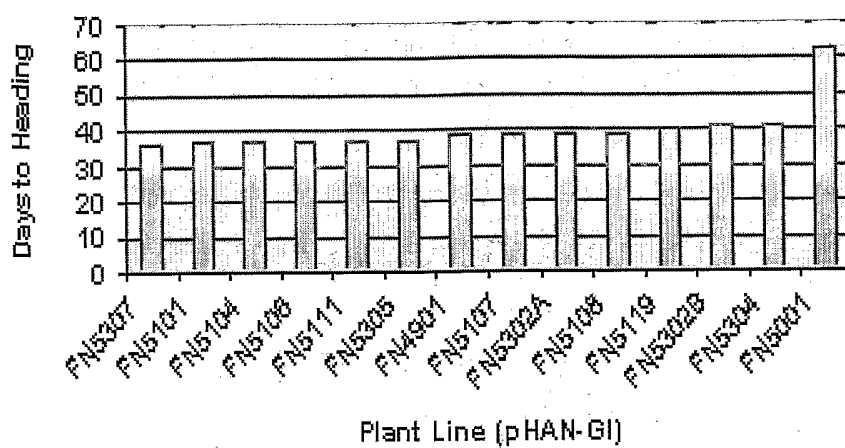


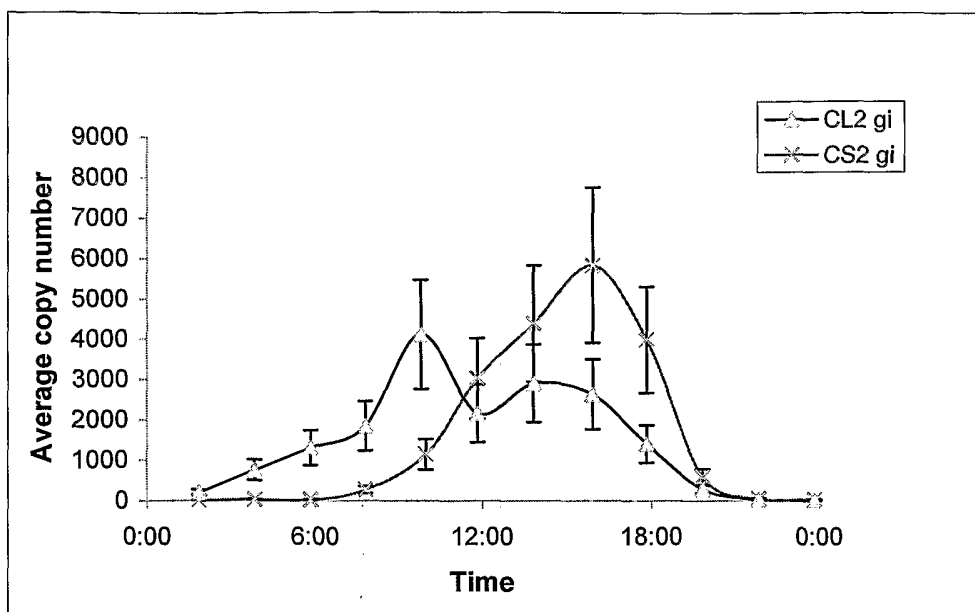
FIGURE 8

**25/27****FIGURE 9**

**26/27**



**FIGURE 10**

**27/27****FIGURE 11**

M80696400.ST25  
SEQUENCE LISTING

<110> Agriculture Victoria Services Pty Ltd  
AgResearch Limited

<120> Flowering Inhibition

<130> M80696400

<150> 2003902412

<151> 2003-05-16

<160> 46

<170> PatentIn version 3.2

<210> 1

<211> 393

<212> DNA

<213> Lolium perenne

<400> 1  
tgtatctttt gggactgagg ttgtgtgcta cgagagccct cggccggtgc tcgggatcca 60  
ccggctggtg tttctgctct tccaacagct cggccgtcag accgtytacg ctccgggctg 120  
gcggcagaat ttcagcaccg gcgacttcgc cgagctgtac aacctcggct cgccggtcgc 180  
cgccgtctac ttcaactgcc aaagggagtc gggaactggt ggaaggagga tgtgaattct 240  
ctgacactag tcgtcaaccg gtgcgcctgc acaggctagt aattggtgaa atcgaatcat 300  
ggagccaaaa aggagttggt tccaaattta attgtgaaaa tggttttgat tttcattata 360  
tatagaatta tatttaaaaa aaaaaaaaaa aaa 393

<210> 2

<211> 340

<212> DNA

<213> Lolium perenne

<400> 2  
tgtatctttt gggactgagg ttgtgtgcta cgagagccct cggccggtgc tcgggatcca 60  
ccggctggtg tttctgctct tccaacagct cggccgtcag accgtttacg ctccgggctg 120  
gcggcagaat ttcagcaccg gcgacttcgc cgagctgtac aacctcggct cgccggtcgc 180  
cgccgtctac ttcaactgcc aaagggagtc gggaactggt ggaaggagga tgtgaattct 240  
ctgacatatt atgaccatat aggttgagcg tggatgattg tatcgggggt tatactgggc 300  
taaatgaaaa aagatcattg taataaaaaa aaaaaaaaaa 340

<210> 3

<211> 381

<212> DNA

<213> Lolium perenne

<400> 3  
ggtaaattct actcattgtg tatgactgat ccggccgcac cgagcaggaa agaacctaaa 60  
ttcagggaat ggcattcatt gctcgtaggc aatgtcccag aatgtgatgt agccaagggc 120  
gaaactttat ccgaatatat tggctctgga cctccccag atactggact tcacgtttat 180

M80696400.ST25

|                                                                     |     |
|---------------------------------------------------------------------|-----|
| gttttcctcg tctaccttca gccgggtaaa atcgatttca aagatgtgcc acgtttgacc   | 240 |
| aacagatctg gtgacaatcg tgccaatttt aaaattcaag ctttcgccga taaatataat   | 300 |
| ttggg'gcgac cggtggcctc agcttattat cgagccgaat acgacgacta cgtgcccaatt | 360 |
| ttgtacaaac aattgggagc a                                             | 381 |

<210> 4  
 <211> 965  
 <212> DNA  
 <213> Lolium perenne

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| <400> 4                                                            |     |
| gcagcagcac atacacaatt acctgagctt ccattcagca aagaggaaca cacgcacact  | 60  |
| gatcatccct cgggttccga tttcaaggca tcaacatgtc aagggcgttg gagcctctcg  | 120 |
| ttgtggggaa ggtgattggt gaggtgctgg acagcttcaa cccacccgtg aagatgacgg  | 180 |
| caacctacag ctccaacaag caggtgttca acggccatga gttcttcccc tcggccatcg  | 240 |
| ccgctaagcc gcgtgtcgag gttcaggggg gcgaccttag atccttcttc acattggtga  | 300 |
| tgactgacct tgatgtgccca ggacccagtg atccgtacct gagggagcat cttcactgga | 360 |
| ttgttactga tattcctggg actactgatg cttcttttgg gaaggagggtg gtgcactacg | 420 |
| agagcccaaa gccaaacatc ggcatccaca gggtcatcct cgtgctgttc cagcagacgc  | 480 |
| accggggctc ggtaaagaac acaccgtcgt cgagggaccg cttcaggacc cgcgagttcg  | 540 |
| ccaaggataa cgagctcggc ctccctgtcg cygctgtcta cttcaacgcg cagcgggaga  | 600 |
| ccgccgcccc ccggcgatag ctcaacggca accgaaccaa ccaaccagca acacccccct  | 660 |
| actatgtacc tgatctagct acatgataaa acgaactgcg tacaatcacc tattagctag  | 720 |
| cttcgatggc ctttctgtct acatccaagc atgcacaatg tctgaataaa acacaccggt  | 780 |
| aaattagctg ttgacagag aaagctgctc cytactagta cgtagccgtt gcccatttag   | 840 |
| ttaatttttg tgaatgtgac aagatcgatg attgggaaga gattgcagtg ttgactgaga  | 900 |
| aaaaagtgca agatttgaag caataatagt cgtcagggag taaaaaaaaa aaaaaaaaaa  | 960 |
| aaaaa                                                              | 965 |

<210> 5  
 <211> 811  
 <212> DNA  
 <213> Lolium perenne

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| <400> 5                                                           |     |
| aacaaccatg aggttatatt tcctcattcc tcaagactgt ttacctagt cttcgacttg  | 60  |
| ctagttacct gtcagctgta gatatttggc atcgatcatc accacctttt cagttcaagt | 120 |
| tagctagcta gacatggcta ggggtgggga gcctctcatc gtcggaaagg tgatcgggga | 180 |
| ggtcatcgac aacttcaccc cactgagaa gatgacagta acctacagct ccaacaagca  | 240 |
| ggtgttcaat ggccacgaat tcttcccgtc ggcagtcgtc tccaagccac gcattgaggt | 300 |
| ccaggggtggc gacatgagat ctttcttcac actggtcagt acggaccag atgtgccagg | 360 |

M80696400.ST25

gcctagcgac ccataacctga gggagcatct ccactggatc gtcaataata ttccaggcac 420  
 cactgatgct tcttttggaa aggagggtggg gagctacgag agccccaagc ccaacatcgg 480  
 cattcacagg ttcaccttcg tgctgttcaa gcagaagcag cggcagacaa tgagccctcc 540  
 ctccagcagg gactacttca aactcgcgcg cttcgttgcc gcgaacgatc ttggcctccc 600  
 agtcgctgcc gtctacttca acgcgcagcg ggagacagct gcgcgccgcc gctgatggaa 660  
 acaatcagcg aaacccttct tctcgcgcgcat gcatggcgca tgcatgcaat cctatggatc 720  
 aaccaatagc tctactacta gctacccttt atgtctttca ttcaaataag agtttgcttg 780  
 tgagcgtctt gtgtcaaaaa aaaaaaaaaa a 811

&lt;210&gt; 6

&lt;211&gt; 1658

&lt;212&gt; DNA

<213> *Lolium perenne*

&lt;400&gt; 6

aacaccacat tgaggaaagc aataccagct catcggatta tgggaacttg gagaagaaga 60  
 gtaagaaaaa tggtttttca agaccactca tgaaatgtgc agaacaggct aggagaaatg 120  
 gtaacgttgc aagtacatcg gggaaagcta ctgcaacttt acaggcggaa gcatctgatt 180  
 tggcaaactt cttaccatg gacaggaacg ggggttatgg aggttctcaa actctcctaa 240  
 gaactgtaat gtcagaaaag caggaactat gcttttctgt tgtctcgttg ctgtggcata 300  
 agcttattgc atctcccga acacagatgt ctgcagagag tacatcagct catcagggtt 360  
 ggagaaaggt atgattacac atgctagtca taacacagta atcctatgga gatattttta 420  
 tttctggtag cattcttatt aactgtacat tttcacatct tttaatgtat aggttgacga 480  
 tgcgctttgt gatgttgttt cagcttcacc ggccaaggct tcaactgcta ttgtcctgca 540  
 ggtaaataatt tctgctgtat tcaaacattt ggtcataata attattttttt aayttttttg 600  
 ctttgtwtct gttgttcatt tctcacgttg ggaacakaca ttgrcttcat tgccttcatg 660  
 acatgcatca tatttcctgt catattttatg ggctcctgta tggcaggctg agaaggactt 720  
 gcagccctgg attgctcgag atgatgagca aggtcagaag atgtggagag tcaaccagcg 780  
 aatagtgaat ctgatagctg agcttatgag gaaccatgat agcccagaag cactgataat 840  
 tcttgcgagc gcttcagacc ttctgctccg tgccacggat gggatgcttg ttgatggatga 900  
 agcttgtacc ttgcctcaat tggagcttct ggaagtaacc gccagagcca ttcattctcat 960  
 cgttgaatgg ggagatccag gtgtagcagt tgctgatggc ctctcgaatc tgctgaagtg 1020  
 ccggctatca cctaccatcc gatgcctttc ccaccctagt gcacatgtac gggcgctcag 1080  
 catgtccgtc cttcgcgaca tcttgaacag tggaccaata agttccacca agataattca 1140  
 aggagagcag cggaacggca tccaaagccc aagttaccgg tgcgcggcag caagtatgac 1200  
 caactggcaa gcggacgtcg agagatgcat agagtgggaa gccacaacc gtcaggccac 1260  
 cgggatgacg cttgcctttc tactgcagc ggctaacgaa ctcggatgcc cccttccttg 1320  
 ctgacacagc catatttgaa gctgacatcg gcgacacttg acagttagcg cgagcagttg 1380

## M80696400.ST25

|                                                                    |      |
|--------------------------------------------------------------------|------|
| ctgcatggtc agcgagcagg atggctaadc ccttgctcaa ggatgacttc ccagtctgcc  | 1440 |
| cccattatgt gatttaaaac tgatgtatat tagttgaccc agtcatacgg agcttgctcc  | 1500 |
| cactgtgtga ttttaactttt aatctgacat tagatgttca agcatattga actgcttgtg | 1560 |
| ctgtaacttg tatttctgta gccgaaagat gtacactatg gtaaataag acatatcatt   | 1620 |
| tttcgtcatg gaaaaaaaaa aaaaaaaaaa aaaaaaaaaa                        | 1658 |

<210> 7  
 <211> 1389  
 <212> DNA  
 <213> *Lolium perenne*

|                                                                    |      |
|--------------------------------------------------------------------|------|
| <400> 7                                                            |      |
| aacaccacat tgaggaaagc aataccagct catcggatta tgggaacttg gagaagaaga  | 60   |
| gtaagaaaaa tgggtttttca agaccactca tgaaatgtgc agaacaggct aggagaaatg | 120  |
| gtaacgttgc aagtacatcg gggaaagcta ctgcaacttt acaggcggaa gcatctgatt  | 180  |
| tggcaacttt ccttaccatg gacaggaacg ggggttatgg aggttctcaa actctcctaa  | 240  |
| gaactgtaat gtcagaaaag caggaaactat gcttttctgt tgtctcgttg ctgtggcata | 300  |
| agcttattgc atctcccga acacagatgt ctgcagagag tacatcagct catcagggtt   | 360  |
| ggagaaagggt tgcagatgcg ctttgtgatg ttgtttcagc ttcaccggcc aaggcttcaa | 420  |
| ctgctattgt cctgcaggct gagaaggact tgcagccctg gattgctcga gatgatgagc  | 480  |
| aaggtcagaa gatgtggaga gtcaaccagc gaatagtga actgatagct gagcttatga   | 540  |
| ggaaccatga tagcccagaa gcaactgataa ttcttgcgag cgcttcagac cttctgctcc | 600  |
| gtgccacgga tgggatgctt gttgatggtg aagcttgtac cttgcctcaa ttggagcttc  | 660  |
| tggaaagtaac cgccagagcc attcatctca tcgttgaatg gggagatcca ggtgtagcag | 720  |
| ttgctgatgg cctctcgaat ctgctgaagt gccggctatc acctaccatc cgatgccttt  | 780  |
| cccaccctag tgcacatgta cgggcgctca gcatgtccgt ccttcgagac atcttgaaca  | 840  |
| gtggaccaat aagttccacc aagataattc aaggagagca gcggaacggc atccaaagcc  | 900  |
| caagttaccg gtgcgcggca gcaagtatga ccaactggca agcggacgct gagagatgca  | 960  |
| tagagtggga agcccacaac cgtcaggcca ccgggatgac gcttgctttt ctactgcag   | 1020 |
| cggctaacga actcggatgc ccccttcctt gctgacacag ccatatttga agctgacatc  | 1080 |
| ggcgacactt gacagttagc gcgagcagtt gctgcatggt cagcgagcag gatggctaata | 1140 |
| cccttgctca aggatgactt cccagtctgc cccattatg tgatttataa ctgatgtata   | 1200 |
| ttagttgacc cagtcatacg gagcttgctc ccaactgtgtg atttaacttt taatctgaca | 1260 |
| ttagatgttc aagcatattg aactgcttgt gctgtaactt gtatttctgt agccgaaaga  | 1320 |
| tgtacactat ggtaaatgaa gacatatcat ttttcgtcat ggaaaaaaaaa aaaaaaaaaa | 1380 |
| aaaaaaaaa                                                          | 1389 |

<210> 8

M80696400.ST25

<211> 2652  
 <212> DNA  
 <213> *Lolium perenne*

<220>  
 <221> misc\_feature  
 <222> (3)..(3)  
 <223> n is a, c, g, or t

<220>  
 <221> misc\_feature  
 <222> (93)..(93)  
 <223> n is a, c, g, or t

<220>  
 <221> misc\_feature  
 <222> (2583)..(2583)  
 <223> n is a, c, g, or t

<220>  
 <221> misc\_feature  
 <222> (2649)..(2649)  
 <223> n is a, c, g, or t

<400> 8  
 aanttgtaca cgatttcact atagtggcga attggggccct ctatatgcat gctcgagcgg 60  
 ccgccagtgt gatggatatt tgcagaattc ttnaacatgt gctaaagtga tatcaccgct 120  
 taaccgggga gatatacgta cgagctcgcc ggcggtcaca ctacctatag acgtcttaag 180  
 ggccctttym tmyyyctcct ctcccttcgc atccccaacc gcaccactgt gcctctccga 240  
 ccccgggccgg ccgacgcgcg ctacgcctct ccgggaaark akrrrgagga gaggaaagcg 300  
 taggggttg cggtgtgaca cggagaggct ggggccggcc ggctgcgcgc gatgcggaga 360  
 cggcgtgdgg actggggrrg rrggaggaga kmgatcgatc rgyygggyys ggaggcggcg 420  
 atggcgcggg agaggcgcg gatcaagcgg gccgcachcc tgaccccycc yycctcctct 480  
 mkctagctag ycarccrrs cctccgccgc taccgcgccc tctccgcgct ctagttcgcc 540  
 atagagagcg cggcggcgcg ccaggtcacc ttctccaagc gccgcagggg cctcttcaag 600  
 aaggccgagg agctctccgt cctatgcgac tatctctcgc gccgccgcgc ggtccagtgg 660  
 aagaggttcg cggcgtcccc ggagaagttc ttccggctcc tcgagaggca ggatacgctg 720  
 gccgacgtcg cgctcatcgt cttctcgtcc acagggaagc tctcccagtt cgcaagctcc 780  
 agtatgaatg agatcatcga caagtacagc cggctgcagc gcgagtagca gaagagcagg 840  
 tgtcccttcg agagggtcaa gcgttcgagg tcatacttac tctagtagct gttcatgtcg 900  
 acccattcta agaacctggg gaaagcagac cagccttctc ttgacttgaa tttagaacat 960  
 agtaagtatg caaatctgaa cgatcagctt tgggtaagat tcttggaccc ctttcgtctg 1020  
 gtcggaagag aactgaactt aaatcttgta tcattcatal gtttagactt gctagtcgaa 1080  
 gcggaagcta gtctycgact tagacagatg agaggcgagg ggcttgaggg gttgavtggt 1140  
 gatgaactcc agcagttgga gaagaacctt cgccttcgat cagargctga atctgtctac 1200  
 tctccgctcc ccgaactccc caactbacaa ctacttgagg tcgtcaacct cttcttgga 1260

M80696400.ST25

|                                                                    |      |
|--------------------------------------------------------------------|------|
| gaaactggtc tgcacagggg gcttcagacg aaagatcaac aattcttggg gcagatcaat  | 1320 |
| gaattgcaac gaaagagctc acagctggca ctttgaccag acgtgtccca cgaagtctgc  | 1380 |
| tttctagttg ttaagaacct cgtctagtta cttaacgttg ctttctcgag tgtcgaccgt  | 1440 |
| gaggagaaca tgcaactgag gaaccaagta tcccagatac caatagctgg caagccagta  | 1500 |
| gttgctgata ccgaaaatgt tattgctgag ctctcttgt acgttgactc cttgggtcat   | 1560 |
| agggcttatg gttatcgacc gttcgggtcat caacgactat ggcttttaca ataacgactc | 1620 |
| gatgggcagt cctctgaatc tgtcatgacg gcgttgcaact cggaagctc acaggataac  | 1680 |
| gatgatgggt cagatgtatc cctgaaattg ctacccgtca ggagacttag acagtactgc  | 1740 |
| cgcaacgtga gcccttcgag tgtcctattg ctactaccaa gtctacatag ggactttaac  | 1800 |
| ggattaccct gcagtgcgtg gaagtaacta tataaaaycg tcrsttcaga tctttatgga  | 1860 |
| actgcccacr tcagtggaga agctcttgtg cctaattggga cgtcacgcac cttcattgat | 1920 |
| atatttttrgc agysaagtct agaaatacct tgacgggtgy agtcacctct tcgagaacac | 1980 |
| taatcsacaa acgtaccyga gctgcaataa tcttgcrct gaagcgagat cagttaacct   | 2040 |
| gatttatcat ccttggtggt gcatgacgtg attagstggt tgcattggrct cgacgttatt | 2100 |
| agaacgygca cttcgctcta gtcaattgga ctaaatagta ggaacaccga cgtactgcac  | 2160 |
| atgtttcccgt tyttacygtt tactaggatg ttaactaaac ttttagatcg atctgatgtc | 2220 |
| catcttatcc ccgttggcac tatttggtca tacaaggga araatgrcaa atgacctac    | 2280 |
| aattgatattg aaaatctagc tagactacag gtagaatagg ggcaaccgtg ataaacaagt | 2340 |
| tggtatccat gtaccttaac tgtcagtata tcttaamt atggtctata wwtkcwyw      | 2400 |
| crabrytarb yrrmvdwyg rtctrvtrrm accataggta catggaattg acagtcatat   | 2460 |
| agaatttkaa taccagatat wwamgwrrwc gytvyratyv ryykbhawrc yagaybayyk  | 2520 |
| dvmrmrmwma rwavmawrma wrdaragggg ccaattcgcc ctatagtga tcgtggtaca   | 2580 |
| aanttchbky kykwktywtb ktwyktyht ytccccggtt aagcgggata tcacttagca   | 2640 |
| ccatgtttna ag                                                      | 2652 |

<210> 9  
 <211> 228  
 <212> PRT  
 <213> Lolium perenne

<220>  
 <221> misc\_feature  
 <222> (119)..(119)  
 <223> Xaa can be any naturally occurring amino acid

<400> 9

Met Ala Arg Glu Arg Arg Glu Ile Lys Arg Ile Glu Ser Ala Ala Ala  
 1 5 10 15

Arg Gln Val Thr Phe Ser Lys Arg Arg Arg Gly Leu Phe Lys Lys Ala  
 20 25 30

M80696400.ST25

Glu Glu Leu Ser Val Leu Cys Asp Ala Asp Val Ala Leu Ile Val Phe  
35 40 45

Ser Ser Thr Gly Lys Leu Ser Gln Phe Ala Ser Ser Ser Met Asn Glu  
50 55 60

Ile Ile Asp Lys Tyr Ser Thr His Ser Lys Asn Leu Gly Lys Ala Asp  
65 70 75 80

Gln Pro Ser Leu Asp Leu Asn Leu Glu His Ser Lys Tyr Ala Asn Leu  
85 90 95

Asn Asp Gln Leu Ala Glu Ala Ser Leu Arg Leu Arg Gln Met Arg Gly  
100 105 110

Glu Gly Leu Glu Gly Leu Xaa Val Asp Glu Leu Gln Gln Leu Glu Lys  
115 120 125

Asn Leu Glu Thr Gly Leu His Arg Val Leu Gln Thr Lys Asp Gln Gln  
130 135 140

Phe Leu Glu Gln Ile Asn Glu Leu Gln Arg Lys Ser Ser Gln Leu Ala  
145 150 155 160

Glu Glu Asn Met Gln Leu Arg Asn Gln Val Ser Gln Ile Pro Ile Ala  
165 170 175

Gly Lys Pro Val Val Ala Asp Thr Glu Asn Val Ile Ala Glu Asp Gly  
180 185 190

Gln Ser Ser Glu Ser Val Met Thr Ala Leu His Ser Gly Ser Ser Gln  
195 200 205

Asp Asn Asp Asp Gly Ser Asp Val Ser Leu Lys Leu Gly Leu Pro Cys  
210 215 220

Ser Ala Trp Lys  
225

<210> 10  
<211> 683  
<212> DNA  
<213> Lolium perenne

<400> 10  
tggcgmggga gaggcgcgag atcaagcgga tagagagcgc ggcggcgcgc caggtcacct 60  
tctccaagcg ccgcaggggc ctcttcaaga aggccgagga gctctccgtm ctatgcgacg 120  
ccgacgtcgc gctcatcgtc ttctcgtcca cagggaagct ctcccagttc gcaagctcca 180  
gtatgaatga gatcatcgac aagtacagca cmcattctaa gaacctgggg aaagcagacc 240

M80696400.ST25

```

agccttctct tgacttgaay ttagaacata gtaagtatgc aaatctgaac gatcagcttg 300
cggaagctag tctycgactt agacagatga gaggcgaggg gcttgagggg ttgavtgttg 360
atgaactcca gcagttggag aagaaccttg aaactgggtct gcacaggggtg cttcagacga 420
aagatcaaca attcttggag cagatcaatg aattgcaacg aaagagctca cagctggcag 480
aggagaacat gcaactgagg aaccaagtat cccagatacc aatagctggc aagccagtag 540
ttgctgatac cgaaaatggt attgctgagg atgggcagtc ctctgaatct gtcattgacgg 600
cgttgcactc gggaagcksa caggataacg atgatgggtc agaygtatcc ctgaaattgg 660
gattaccctg cagtgcgtgg aag 683

```

<210> 11  
 <211> 447  
 <212> DNA  
 <213> *Lolium perenne*

```

<400> 11
gataccaata gctggcaagc cagtagttgc tgataccgaa aatggtattg ctgaggatgg 60
gcagtcctct gaatctgtca tgacggcggt gcactcggga agcksacagg ataacgatga 120
tggttcagay gtatccctga aattgggatt accctgcagt gcgtggaagt aactatataa 180
aaycgtcrst tcagatcttt atggaactgc ccacrtcagt ggagaagctc ttgtgtaatc 240
sacaaacgta ccygagctgc aataatcttg crgctgaagc gagatcagtt aacctgattt 300
atcatccttg tggctgcatg acgtgatgtt mccgttytta cygtttacta ggatgttaac 360
taaactttta gatcgatctg atgtccatct yatccccgtt grmactattw gttcatggta 420
wccatgtacc ttaactgtca gtatatc 447

```

<210> 12  
 <211> 228  
 <212> PRT  
 <213> *Lolium perenne*

<220>  
 <221> misc\_feature  
 <222> (119)..(119)  
 <223> Xaa can be any naturally occurring amino acid

<220>  
 <221> misc\_feature  
 <222> (207)..(207)  
 <223> Xaa can be any naturally occurring amino acid

<400> 12

Met Ala Arg Glu Arg Arg Glu Ile Lys Arg Ile Glu Ser Ala Ala Ala  
 1 5 10 15

Arg Gln Val Thr Phe Ser Lys Arg Arg Arg Gly Leu Phe Lys Lys Ala  
 20 25 30

Glu Glu Leu Ser Val Leu Cys Asp Ala Asp Val Ala Leu Ile Val Phe  
 35 40 45

M80696400.ST25

Ser Ser Thr Gly Lys Leu Ser Gln Phe Ala Ser Ser Ser Met Asn Glu  
50 55 60

Ile Ile Asp Lys Tyr Ser Thr His Ser Lys Asn Leu Gly Lys Ala Asp  
65 70 75 80

Gln Pro Ser Leu Asp Leu Asn Leu Glu His Ser Lys Tyr Ala Asn Leu  
85 90 95

Asn Asp Gln Leu Ala Glu Ala Ser Leu Arg Leu Arg Gln Met Arg Gly  
100 105 110

Glu Gly Leu Glu Gly Leu Xaa Val Asp Glu Leu Gln Gln Leu Glu Lys  
115 120 125

Asn Leu Glu Thr Gly Leu His Arg Val Leu Gln Thr Lys Asp Gln Gln  
130 135 140

Phe Leu Glu Gln Ile Asn Glu Leu Gln Arg Lys Ser Ser Gln Leu Ala  
145 150 155 160

Glu Glu Asn Met Gln Leu Arg Asn Gln Val Ser Gln Ile Pro Ile Ala  
165 170 175

Gly Lys Pro Val Val Ala Asp Thr Glu Asn Val Ile Ala Glu Asp Gly  
180 185 190

Gln Ser Ser Glu Ser Val Met Thr Ala Leu His Ser Gly Ser Xaa Gln  
195 200 205

Asp Asn Asp Asp Gly Ser Asp Val Ser Leu Lys Leu Gly Leu Pro Cys  
210 215 220

Ser Ala Trp Lys  
225

<210> 13  
<211> 162  
<212> PRT  
<213> Arabidopsis thaliana  
<400> 13

Asp Pro Leu Val Val Gly Arg Val Ile Gly Asp Val Leu Asp Met Phe  
1 5 10 15

Ile Pro Thr Ala Asn Met Ser Val Tyr Phe Gly Pro Lys His Ile Thr  
20 25 30

Asn Gly Cys Glu Ile Lys Pro Ser Thr Ala Val Asn Pro Pro Lys Val  
35 40 45

M80696400.ST25

Asn Ile Ser Gly His Ser Asp Glu Leu Tyr Thr Leu Val Met Thr Asp  
 50 55 60

Pro Asp Ala Pro Ser Pro Ser Glu Pro Asn Met Arg Glu Trp Val His  
 65 70 75 80

Trp Ile Val Val Asp Ile Pro Gly Gly Thr Asn Pro Ser Arg Gly Lys  
 85 90 95

Glu Ile Leu Pro Tyr Met Glu Pro Arg Pro Pro Val Gly Ile His Arg  
 100 105 110

Tyr Ile Leu Val Leu Phe Arg Gln Asn Ser Pro Val Gly Leu Met Val  
 115 120 125

Gln Gln Pro Pro Ser Arg Ala Asn Phe Ser Thr Arg Met Phe Ala Gly  
 130 135 140

His Phe Asp Leu Gly Leu Pro Val Ala Thr Val Tyr Phe Asn Ala Gln  
 145 150 155 160

Lys Glu

<210> 14  
 <211> 174  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 14

Met Ser Ile Asn Ile Arg Asp Pro Leu Ile Val Ser Arg Val Val Gly  
 1 5 10 15

Asp Val Leu Asp Pro Phe Asn Arg Ser Ile Thr Leu Lys Val Thr Tyr  
 20 25 30

Gly Gln Arg Glu Val Thr Asn Gly Leu Asp Leu Arg Pro Ser Gln Val  
 35 40 45

Gln Asn Lys Pro Arg Val Glu Ile Gly Gly Glu Asp Leu Arg Asn Phe  
 50 55 60

Tyr Thr Leu Val Met Val Asp Pro Asp Val Pro Ser Pro Ser Asn Pro  
 65 70 75 80

His Leu Arg Glu Tyr Leu His Trp Leu Val Thr Asp Ile Pro Ala Thr  
 85 90 95

Thr Gly Thr Thr Phe Gly Asn Glu Ile Val Cys Tyr Glu Asn Pro Ser  
 100 105 110

Pro Thr Ala Gly Ile His Arg Val Val Phe Ile Leu Phe Arg Gln Leu  
 115 120 125

Gly Arg Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Asn Phe Asn Thr  
 130 135 140

Arg Glu Phe Ala Glu Ile Tyr Asn Leu Gly Leu Pro Val Ala Ala Val  
 145 150 155 160

Phe Tyr Asn Cys Gln Arg Glu Ser Gly Cys Gly Gly Arg Arg  
 165 170

<210> 15  
 <211> 174  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 15

Met Ser Leu Ser Arg Arg Asp Pro Leu Val Val Gly Ser Val Val Gly  
 1 5 10 15

Asp Val Leu Asp Pro Phe Thr Arg Leu Val Ser Leu Lys Val Thr Tyr  
 20 25 30

Gly His Arg Glu Val Thr Asn Gly Leu Asp Leu Arg Pro Ser Gln Val  
 35 40 45

Leu Asn Lys Pro Ile Val Glu Ile Gly Gly Asp Asp Phe Arg Asn Phe  
 50 55 60

Tyr Thr Leu Val Met Val Asp Pro Asp Val Pro Ser Pro Ser Asn Pro  
 65 70 75 80

His Gln Arg Glu Tyr Leu His Trp Leu Val Thr Asp Ile Pro Ala Thr  
 85 90 95

Thr Gly Asn Ala Phe Gly Asn Glu Val Val Cys Tyr Glu Ser Pro Arg  
 100 105 110

Pro Pro Ser Gly Ile His Arg Ile Val Leu Val Leu Phe Arg Gln Leu  
 115 120 125

Gly Arg Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Gln Phe Asn Thr  
 130 135 140

Arg Glu Phe Ala Glu Ile Tyr Asn Leu Gly Leu Pro Val Ala Ala Ser  
 145 150 155 160

Tyr Phe Asn Cys Gln Arg Glu Asn Gly Cys Gly Gly Arg Arg  
 165 170

<210> 16

M80696400.ST25

&lt;211&gt; 171

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 16

Met Gly Thr Arg Val Ile Glu Pro Leu Ile Met Gly Arg Val Val Gly  
 1 5 10 15

Asp Val Leu Asp Phe Phe Thr Pro Thr Thr Lys Met Asn Val Ser Tyr  
 20 25 30

Asn Lys Lys Gln Val Ser Asn Gly His Glu Leu Phe Pro Ser Ser Val  
 35 40 45

Ser Ser Lys Pro Arg Val Glu Ile His Gly Gly Asp Leu Arg Ser Phe  
 50 55 60

Phe Thr Leu Val Met Ile Asp Pro Asp Val Pro Gly Pro Ser Asp Pro  
 65 70 75 80

Phe Leu Lys Glu His Leu His Trp Ile Val Thr Asn Ile Pro Gly Thr  
 85 90 95

Thr Asp Ala Thr Phe Gly Lys Glu Val Val Ser Tyr Glu Leu Pro Arg  
 100 105 110

Pro Ser Ile Gly Ile His Arg Phe Val Phe Val Leu Phe Arg Gln Lys  
 115 120 125

Gln Arg Arg Val Ile Phe Pro Asn Ile Pro Ser Arg Asp His Phe Asn  
 130 135 140

Thr Arg Lys Phe Ala Val Glu Tyr Asp Leu Gly Leu Pro Val Ala Ala  
 145 150 155 160

Val Phe Phe Asn Ala Gln Arg Glu Thr Ala Ala  
 165 170

&lt;210&gt; 17

&lt;211&gt; 166

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 17

Asp Pro Leu Met Val Gly Arg Val Ile Gly Asp Val Val Asp Asn Cys  
 1 5 10 15

Leu Gln Ala Val Lys Met Thr Val Thr Tyr Asn Ser Asp Lys Gln Val  
 20 25 30

Tyr Asn Gly His Glu Leu Phe Pro Ser Val Val Thr Tyr Lys Pro Lys  
 35 40 45

M80696400.ST25

Val Glu Val His Gly Gly Asp Met Arg Ser Phe Phe Thr Leu Val Met  
50 55 60

Thr Asp Pro Asp Val Pro Gly Pro Ser Asp Pro Tyr Leu Arg Glu His  
65 70 75 80

Leu His Trp Ile Val Thr Asp Ile Pro Gly Thr Thr Asp Val Ser Phe  
85 90 95

Gly Lys Glu Ile Ile Gly Tyr Glu Met Pro Arg Pro Asn Ile Gly Ile  
100 105 110

His Arg Phe Val Tyr Leu Leu Phe Lys Gln Thr Arg Arg Gly Ser Val  
115 120 125

Val Ser Val Pro Ser Tyr Arg Asp Gln Phe Asn Thr Arg Glu Phe Ala  
130 135 140

His Glu Asn Asp Leu Gly Leu Pro Val Ala Ala Val Phe Phe Asn Cys  
145 150 155 160

Gln Arg Glu Thr Ala Ala  
165

<210> 18  
<211> 165  
<212> PRT  
<213> Lolium perenne

<400> 18

Glu Pro Leu Ile Val Gly Arg Val Ile Gly Glu Val Leu Asp Pro Phe  
1 5 10 15

Asn Pro Cys Val Lys Met Val Ala Thr Tyr Asn Ser Asn Lys Leu Val  
20 25 30

Phe Asn Gly His Glu Leu Tyr Pro Ser Ala Val Val Ser Lys Pro Arg  
35 40 45

Val Glu Val Gln Gly Gly Asp Leu Arg Ser Leu Phe Thr Leu Val Met  
50 55 60

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

Leu His Trp Ile Val Ser Asn Ile Pro Gly Thr Thr Asp Ala Ser Phe  
85 90 95

Gly Gly Glu Val Met Ser Tyr Glu Ser Pro Lys Pro Asn Ile Gly Ile  
100 105 110

M80696400.ST25

His Arg Phe Ile Phe Val Leu Phe Lys Gln Lys Arg Arg Gln Thr Val  
 115 120 125

Ser Val Pro Ser Phe Arg Asp His Phe Asn Thr Arg Gln Phe Ala Val  
 130 135 140

Asp Asn Asp Leu Gly Leu Pro Val Ala Ala Val Tyr Phe Asn Cys Gln  
 145 150 155 160

Arg Glu Thr Ala Ala  
 165

<210> 19  
 <211> 164  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 19

Glu Pro Leu Ile Val Gly Arg Val Ile Gly Asp Val Leu Glu Met Phe  
 1 5 10 15

Asn Pro Ser Val Thr Met Arg Val Thr Phe Asn Ser Asn Thr Ile Val  
 20 25 30

Ser Asn Gly His Glu Leu Ala Pro Ser Leu Leu Leu Ser Lys Pro Arg  
 35 40 45

Val Glu Ile Gly Gly Gln Asp Leu Arg Ser Phe Phe Thr Leu Ile Met  
 50 55 60

Met Asp Pro Asp Ala Pro Ser Pro Ser Asn Pro Tyr Met Arg Glu Tyr  
 65 70 75 80

Leu His Trp Met Val Thr Asp Ile Pro Gly Thr Thr Asp Ala Ser Phe  
 85 90 95

Gly Arg Glu Ile Val Arg Tyr Glu Thr Pro Lys Pro Val Ala Gly Ile  
 100 105 110

His Arg Tyr Val Phe Ala Leu Phe Lys Gln Arg Gly Arg Gln Ala Val  
 115 120 125

Lys Ala Ala Pro Glu Thr Arg Glu Cys Phe Asn Thr Asn Ala Phe Ser  
 130 135 140

Ser Tyr Phe Gly Leu Ser Gln Pro Val Ala Ala Val Tyr Phe Asn Ala  
 145 150 155 160

Gln Arg Glu Thr

<210> 20

M80696400.ST25

<211> 72  
 <212> PRT  
 <213> Lolium perenne

<400> 20

Val Leu Tyr Leu Arg Pro Gln Pro Pro Val Gly Ile His Arg Tyr Val  
 1 5 10 15

Leu Val Leu Phe Gln Gln Lys Ser Arg Ile Val Asp Gly Tyr Ala Ala  
 20 25 30

Pro Pro Ala Asp Arg Pro Tyr Phe Asn Thr Arg Ala Phe Ala Phe Asn  
 35 40 45

His Glu Leu Gly Leu Pro Val Ala Val Val Tyr Phe Asn Ser Gln Arg  
 50 55 60

Glu Pro Ser Gly Asn Arg Arg Arg  
 65 70

<210> 21  
 <211> 77  
 <212> PRT  
 <213> Lolium perenne

<400> 21

Val Ser Phe Gly Thr Glu Val Val Cys Tyr Glu Ser Pro Arg Pro Val  
 1 5 10 15

Leu Gly Ile His Arg Leu Val Phe Leu Leu Phe Gln Gln Leu Gly Arg  
 20 25 30

Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Asn Phe Ser Thr Arg Asp  
 35 40 45

Phe Ala Glu Leu Tyr Asn Leu Gly Ser Pro Val Ala Ala Val Tyr Phe  
 50 55 60

Asn Cys Gln Arg Glu Ser Gly Thr Gly Gly Arg Arg Met  
 65 70 75

<210> 22  
 <211> 77  
 <212> PRT  
 <213> Lolium perenne

<400> 22

Val Ser Phe Gly Thr Glu Val Val Cys Tyr Glu Ser Pro Arg Pro Val  
 1 5 10 15

Leu Gly Ile His Arg Leu Val Phe Leu Leu Phe Gln Gln Leu Gly Arg  
 20 25 30

M80696400.ST25

Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Asn Phe Ser Thr Arg Asp  
           35                                  40                                  45

Phe Ala Glu Leu Tyr Asn Leu Gly Ser Pro Val Ala Ala Val Tyr Phe  
       50                                  55                                  60

Asn Cys Gln Arg Glu Ser Gly Thr Gly Gly Arg Arg Met  
   65                                  70                                  75

<210> 23  
 <211> 170  
 <212> PRT  
 <213> Lolium perenne

<400> 23

Met Ala Arg Val Val Glu Pro Leu Ile Val Gly Lys Val Ile Gly Glu  
   1                                  5                                  10                                  15

Val Ile Asp Asn Phe Thr Pro Thr Glu Lys Met Thr Val Thr Tyr Ser  
           20                                  25                                  30

Ser Asn Lys Gln Val Phe Asn Gly His Glu Phe Phe Pro Ser Ala Val  
           35                                  40                                  45

Val Ser Lys Pro Arg Ile Glu Val Gln Gly Gly Asp Met Arg Ser Phe  
       50                                  55                                  60

Phe Thr Leu Val Met Thr Asp Pro Asp Val Pro Gly Pro Ser Asp Pro  
   65                                  70                                  75                                  80

Tyr Leu Arg Glu His Leu His Trp Ile Val Asn Asn Ile Pro Gly Thr  
           85                                  90                                  95

Thr Asp Ala Ser Phe Gly Lys Glu Val Val Ser Tyr Glu Ser Pro Lys  
          100                                 105                                 110

Pro Asn Ile Gly Ile His Arg Phe Thr Phe Val Leu Phe Lys Gln Lys  
          115                                 120                                 125

Gln Arg Gln Thr Met Ser Pro Pro Ser Ser Arg Asp Tyr Phe Asn Thr  
      130                                 135                                 140

Arg Arg Phe Ala Ala Ala Asn Asp Leu Gly Leu Pro Val Ala Ala Val  
  145                                 150                                 155                                 160

Tyr Phe Asn Ala Gln Arg Glu Thr Ala Ala  
          165                                 170

<210> 24  
 <211> 171  
 <212> PRT  
 <213> Lolium perenne

M80696400.ST25

&lt;400&gt; 24

Met Ser Arg Ala Leu Glu Pro Leu Val Val Gly Lys Val Ile Gly Glu  
 1 5 10 15

Val Leu Asp Ser Phe Asn Pro Thr Val Lys Met Thr Ala Thr Tyr Ser  
 20 25 30

Ser Asn Lys Gln Val Phe Asn Gly His Glu Phe Phe Pro Ser Ala Ile  
 35 40 45

Ala Ala Lys Pro Arg Val Glu Val Gln Gly Gly Asp Leu Arg Ser Phe  
 50 55 60

Phe Thr Leu Val Met Thr Asp Pro Asp Val Pro Gly Pro Ser Asp Pro  
 65 70 75 80

Tyr Leu Arg Glu His Leu His Trp Ile Val Thr Asp Ile Pro Gly Thr  
 85 90 95

Thr Asp Ala Ser Phe Gly Lys Glu Val Val His Tyr Glu Ser Pro Lys  
 100 105 110

Pro Asn Ile Gly Ile His Arg Phe Ile Leu Val Leu Phe Gln Gln Thr  
 115 120 125

His Arg Gly Ser Val Lys Asn Thr Pro Ser Ser Arg Asp Arg Phe Arg  
 130 135 140

Thr Arg Glu Phe Ala Lys Asp Asn Glu Leu Gly Leu Pro Val Ala Ala  
 145 150 155 160

Val Tyr Phe Asn Ala Gln Arg Glu Thr Ala Ala  
 165 170

&lt;210&gt; 25

&lt;211&gt; 120

&lt;212&gt; PRT

&lt;213&gt; Lolium perenne

&lt;400&gt; 25

Met Thr Asp Pro Ala Ala Pro Ser Arg Lys Glu Pro Lys Phe Arg Glu  
 1 5 10 15

Trp His His Trp Leu Val Gly Asn Val Pro Glu Cys Asp Val Ala Lys  
 20 25 30

Gly Glu Thr Leu Ser Glu Tyr Ile Gly Ser Gly Pro Pro Pro Asp Thr  
 35 40 45

Gly Leu His Arg Tyr Val Phe Leu Val Tyr Leu Gln Pro Gly Lys Ile  
 50 55 60

M80696400.ST25

Asp Phe Lys Asp Val Pro Arg Leu Thr Asn Arg Ser Gly Asp Asn Arg  
65 70 75 80

Ala Asn Phe Lys Ile Gln Ala Phe Ala Asp Lys Tyr Asn Leu Gly Asp  
85 90 95

Pro Val Ala Ser Ala Tyr Tyr Arg Ala Glu Tyr Asp Asp Tyr Val Pro  
100 105 110

Ile Leu Tyr Lys Gln Leu Gly Ala  
115 120

<210> 26  
<211> 176  
<212> PRT  
<213> Oryza sativa

<400> 26

Met Ser Gly Arg Gly Arg Gly Asp Pro Leu Val Leu Gly Arg Val Val  
1 5 10 15

Gly Asp Val Val Asp Pro Phe Val Arg Arg Val Ala Leu Arg Val Ala  
20 25 30

Tyr Gly Ala Arg Glu Val Ala Asn Gly Cys Glu Leu Arg Pro Ser Ala  
35 40 45

Val Ala Asp Gln Pro Arg Val Ala Val Gly Gly Pro Asp Met Arg Thr  
50 55 60

Phe Tyr Thr Leu Val Met Val Asp Pro Asp Ala Pro Ser Pro Ser Asp  
65 70 75 80

Pro Asn Leu Arg Glu Tyr Leu His Trp Leu Val Thr Asp Ile Pro Ala  
85 90 95

Thr Thr Gly Val Ser Phe Gly Thr Glu Val Val Cys Tyr Glu Ser Pro  
100 105 110

Arg Pro Val Leu Gly Ile His Arg Leu Val Phe Leu Leu Phe Glu Gln  
115 120 125

Leu Gly Arg Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Asn Phe Ser  
130 135 140

Thr Arg Asp Phe Ala Glu Leu Tyr Asn Leu Gly Leu Pro Val Ala Ala  
145 150 155 160

Val Tyr Phe Asn Cys Gln Arg Glu Ser Gly Thr Gly Gly Arg Arg Met  
165 170 175

M80696400.ST25

<210> 27  
 <211> 178  
 <212> PRT  
 <213> Oryza sativa

<400> 27

Met Ser Gly Val Pro Thr Val Glu Pro Leu Val Leu Ala His Val Ile  
 1 5 10 15

His Asp Val Leu Asp Pro Phe Arg Pro Thr Met Pro Leu Arg Ile Thr  
 20 25 30

Tyr Asn Asp Arg Leu Leu Leu Ala Gly Ala Glu Leu Lys Pro Ser Ala  
 35 40 45

Thr Val His Lys Pro Arg Val Asp Ile Gly Gly Thr Asp Leu Arg Val  
 50 55 60

Phe Tyr Thr Leu Val Leu Val Asp Pro Asp Ala Pro Ser Pro Ser Asn  
 65 70 75 80

Pro Ser Leu Gly Glu Tyr Leu His Met Val Ile Asp Ile Pro Gly Thr  
 85 90 95

Thr Gly Val Asn Cys Gln Asp Leu Met Leu Tyr Glu Arg Pro Glu Leu  
 100 105 110

Arg Tyr Gly Ile His Arg Met Val Phe Val Leu Phe Arg Gln Leu Gly  
 115 120 125

Arg Gly Thr Val Phe Ala Pro Glu Met Arg His Asn Phe His Cys Arg  
 130 135 140

Ser Phe Ala Gln Gln Tyr His Leu Asp Ile Val Ala Ala Thr Tyr Phe  
 145 150 155 160

Asn Cys Gln Arg Glu Ala Gly Ser Gly Gly Arg Arg Phe Arg Ser Glu  
 165 170 175

Ser Ser

<210> 28  
 <211> 170  
 <212> PRT  
 <213> Lolium perenne

<400> 28

Met Ser Arg Ser Val Glu Pro Leu Ile Val Gly Arg Val Ile Gly Glu  
 1 5 10 15

Val Leu Asp Pro Phe Asn Pro Cys Val Lys Met Val Ala Thr Tyr Asn  
 20 25 30

M80696400.ST25

Ser Asn Lys Leu Val Phe Asn Gly His Glu Leu Tyr Pro Ser Ala Val  
           35                          40                          45  
 Val Ser Lys Pro Arg Val Glu Val Gln Gly Gly Asp Leu Arg Ser Leu  
           50                          55                          60  
 Phe Thr Leu Val Met Thr Asp Pro Asp Val Pro Gly Pro Ser Asp Pro  
           65                          70                          75                          80  
 Tyr Leu Arg Glu His Leu His Trp Ile Val Ser Asn Ile Pro Gly Thr  
                           85                          90                          95  
 Thr Asp Ala Ser Phe Gly Gly Glu Val Met Ser Tyr Glu Ser Pro Lys  
                           100                          105                          110  
 Pro Asn Ile Gly Ile His Arg Phe Ile Phe Val Leu Phe Lys Gln Lys  
                           115                          120                          125  
 Arg Arg Gln Thr Val Ser Val Pro Ser Phe Arg Asp His Phe Asn Thr  
           130                          135                          140  
 Arg Gln Phe Ala Val Asp Asn Asp Leu Gly Leu Pro Val Ala Ala Val  
           145                          150                          155                          160  
 Tyr Phe Asn Cys Gln Arg Glu Thr Ala Ala  
                           165                          170

<210> 29  
 <211> 170  
 <212> PRT  
 <213> Oryza sativa

<400> 29

Met Ser Arg Ser Val Glu Pro Leu Val Val Gly Arg Val Ile Gly Glu  
   1                          5                          10                          15  
 Val Ile Asp Ser Phe Asn Pro Cys Thr Lys Met Ile Val Thr Tyr Asn  
           20                          25                          30  
 Ser Asn Lys Leu Val Phe Asn Gly His Glu Phe Tyr Pro Ser Ala Val  
           35                          40                          45  
 Val Ser Lys Pro Arg Val Glu Val Gln Gly Gly Asp Met Arg Ser Phe  
           50                          55                          60  
 Phe Thr Leu Val Met Thr Asp Pro Asp Val Pro Gly Pro Ser Asp Pro  
           65                          70                          75                          80  
 Tyr Leu Arg Glu His Leu His Trp Ile Val Thr Asp Ile Pro Gly Thr  
                           85                          90                          95

M80696400.ST25

Thr Asp Ala Ser Phe Gly Arg Glu Ile Ile Ser Tyr Glu Ser Pro Lys  
 100 105 110

Pro Ser Ile Gly Ile His Arg Phe Val Phe Val Leu Phe Lys Gln Lys  
 115 120 125

Arg Arg Gln Ala Val Val Val Pro Ser Ser Arg Asp His Phe Asn Thr  
 130 135 140

Arg Gln Phe Ala Glu Glu Asn Glu Leu Gly Leu Pro Val Ala Ala Val  
 145 150 155 160

Tyr Phe Asn Ala Gln Arg Glu Thr Ala Ala  
 165 170

<210> 30  
 <211> 178  
 <212> PRT  
 <213> Oryza sativa

<400> 30

Met Ala Gly Ser Gly Arg Asp Asp Pro Leu Val Val Gly Arg Ile Val  
 1 5 10 15

Gly Asp Val Leu Asp Pro Phe Val Arg Ile Thr Asn Leu Ser Val Ser  
 20 25 30

Tyr Gly Ala Arg Ile Val Ser Asn Gly Cys Glu Leu Lys Pro Ser Met  
 35 40 45

Val Thr Gln Gln Pro Arg Val Val Val Gly Gly Asn Asp Met Arg Thr  
 50 55 60

Phe Tyr Thr Leu Val Met Val Asp Pro Asp Ala Pro Ser Pro Ser Asn  
 65 70 75 80

Pro Asn Leu Arg Glu Tyr Leu His Trp Leu Val Thr Asp Ile Pro Gly  
 85 90 95

Thr Thr Gly Ala Thr Phe Gly Gln Glu Val Met Cys Tyr Glu Ser Pro  
 100 105 110

Arg Pro Thr Met Gly Ile His Arg Leu Val Phe Val Leu Phe Gln Gln  
 115 120 125

Leu Gly Arg Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Asn Phe Ser  
 130 135 140

Thr Arg Asn Phe Ala Glu Leu Tyr Asn Leu Gly Ser Pro Val Ala Thr  
 145 150 155 160

M80696400.ST25

```
<210> 31
<211> 180
<212> PRT
<213> Oryza sativa
```

<400> 31

Met Ser Ser Ala Asn Ser Leu Val Leu Gly Arg Val Ile Gly Asp Val  
1 5 10 15Val Asp Leu Phe Ser Pro Glu Val Thr Leu Arg Val Met Tyr Asn Gly  
20 25 30Val Arg Val Val Asn Gly Glu Asp Leu Arg Pro Ser Ala Val Ser Ala  
35 40 45

Arg Pro Ser Val Glu Val Gly Gly Asp Leu His Gln Phe Tyr Thr Ile  
50 55 60

Val Met Val Asp Pro Asp Ala Pro Asn Pro Ser Asn Pro Thr Leu Arg  
65 70 75 80

Glu Tyr Leu His Trp Leu Val Thr Asp Ile Pro Gly Thr Thr Asp Ala  
85 90 95

Asn Tyr Gly Arg Glu Val Val Cys Tyr Glu Ser Pro Arg Pro Ala Ala

Gly Ile His Arg Val Ala Val Val Leu Phe Arg Gln Met Ala Arg Gly  
115 120 125

Gly Val Asp Gln Pro Pro Leu Leu Arg His Asn Phe Ser Thr Arg Gly  
130 135 140

Phe 145 Ala Asp Asp His 150 Ala Leu Gly Ala Pro 155 Val Ala Ala Ala Phe 160

Thr Cys Lys Pro **Glu** Gly Gly Thr Gly **Gly** Arg Arg Phe Arg Pro Pro  
165 170 175

Ser Arg His Ser  
180

|       |              |
|-------|--------------|
| <210> | 32           |
| <211> | 173          |
| <212> | PRT          |
| <213> | Oryza sativa |

M80696400.ST25

&lt;400&gt; 32

Met Ser Arg Ser Val Glu Pro Leu Val Val Gly Arg Val Ile Gly Glu  
 1 5 10 15  
 Val Leu Asp Thr Phe Asn Pro Cys Met Lys Met Ile Val Thr Tyr Asn  
 20 25 30  
 Ser Asn Lys Leu Val Phe Asn Gly His Glu Leu Tyr Pro Ser Ala Val  
 35 40 45  
 Val Ser Lys Pro Arg Val Glu Val Gln Gly Gly Asp Leu Arg Ser Phe  
 50 55 60  
 Phe Thr Leu Val Met Thr Asp Pro Asp Val Pro Gly Pro Ser Asp Pro  
 65 70 75 80  
 Tyr Leu Arg Glu His Leu His Trp Ile Val Thr Asp Ile Pro Gly Thr  
 85 90 95  
 Thr Asp Ala Ser Phe Gly Arg Glu Val Ile Ser Tyr Glu Ser Pro Lys  
 100 105 110  
 Pro Asn Ile Gly Ile His Arg Phe Ile Phe Val Leu Phe Lys Gln Lys  
 115 120 125  
 Arg Arg Gln Thr Val Ile Val Pro Ser Phe Arg Asp His Phe Asn Thr  
 130 135 140  
 Arg Arg Phe Ala Glu Glu Asn Asp Leu Gly Leu Pro Val Ala Ala Val  
 145 150 155 160  
 Tyr Phe Asn Ala Gln Arg Glu Thr Ala Ala Arg Arg Arg  
 165 170

&lt;210&gt; 33

&lt;211&gt; 174

&lt;212&gt; PRT

&lt;213&gt; Oryza sativa

&lt;400&gt; 33

Met Ala Arg Phe Val Asp Pro Leu Val Val Gly Arg Val Ile Gly Glu  
 1 5 10 15  
 Val Val Asp Leu Phe Val Pro Ser Ile Ser Met Thr Ala Ala Tyr Gly  
 20 25 30  
 Asp Arg Asp Ile Ser Asn Gly Cys Leu Val Arg Pro Ser Ala Ala Asp  
 35 40 45  
 Tyr Pro Pro Leu Val Arg Ile Ser Gly Arg Arg Asn Asp Leu Tyr Thr  
 50 55 60

M80696400.ST25

Leu Ile Met Thr Asp Pro Asp Ala Pro Ser Pro Ser Asp Pro Ser Met  
65 70 75 80

Arg Glu Phe Leu His Trp Ile Val Val Asn Ile Pro Gly Gly Thr Asp  
85 90 95

Ala Ser Lys Gly Glu Glu Met Val Glu Tyr Met Gly Pro Arg Pro Thr  
100 105 110

Val Gly Ile His Arg Tyr Val Leu Val Leu Tyr Glu Gln Lys Ala Arg  
115 120 125

Phe Val Asp Gly Ala Leu Met Pro Pro Ala Asp Arg Pro Asn Phe Asn  
130 135 140

Thr Arg Ala Phe Ala Ala Tyr His Gln Leu Gly Leu Pro Thr Ala Val  
145 150 155 160

Val His Phe Asn Ser Gln Arg Glu Pro Ala Asn Arg Arg Arg  
165 170

<210> 34  
<211> 173  
<212> PRT  
<213> Oryza sativa

<400> 34

Met Ser Arg Val Leu Glu Pro Leu Val Val Gly Lys Val Ile Gly Glu  
1 5 10 15

Val Ile Asp Asn Phe Asn Pro Thr Val Lys Met Thr Ala Thr Tyr Ser  
20 25 30

Ser Asn Lys Gln Val Phe Asn Gly His Glu Leu Phe Pro Ser Ala Val  
35 40 45

Val Ser Lys Pro Arg Val Glu Val Gln Gly Gly Asp Leu Arg Ser Phe  
50 55 60

Phe Thr Leu Val Met Thr Asp Pro Asp Val Pro Gly Pro Ser Asp Pro  
65 70 75 80

Tyr Leu Arg Glu His Leu His Trp Ile Val Thr Asp Ile Pro Gly Thr  
85 90 95

Thr Asp Ala Ser Phe Gly Arg Glu Val Val Ser Tyr Glu Ser Pro Lys  
100 105 110

Pro Asn Ile Gly Ile His Arg Phe Val Leu Val Leu Phe Lys Gln Lys  
115 120 125

M80696400.ST25

Arg Arg Gln Ala Val Thr Pro Pro Ser Ser Arg Asp Tyr Phe Ser Thr  
 130 135 140

Arg Arg Phe Ala Ala Asp Asn Asp Leu Gly Leu Pro Val Ala Ala Val  
 145 150 155 160

Tyr Phe Asn Ala Gln Arg Glu Thr Ala Ala Arg Arg Arg  
 165 170

<210> 35  
 <211> 185  
 <212> PRT  
 <213> Oryza sativa

<400> 35

Met Ala Asn Asp Ser Leu Ala Thr Gly Arg Val Ile Gly Asp Val Leu  
 1 5 10 15

Asp Pro Phe Ile Ser Thr Val Asp Leu Thr Val Met Tyr Gly Asp Asp  
 20 25 30

Gly Met Pro Val Ile Ser Gly Val Glu Leu Arg Ala Pro Ala Val Ala  
 35 40 45

Glu Lys Pro Val Val Glu Val Gly Gly Asp Asp Leu Arg Val Ala Tyr  
 50 55 60

Thr Leu Val Met Val Asp Pro Asp Ala Pro Asn Pro Ser Asn Pro Thr  
 65 70 75 80

Leu Arg Glu Tyr Leu His Trp Met Val Thr Asp Ile Pro Ala Ser Thr  
 85 90 95

Asp Ala Thr Tyr Gly Arg Glu Val Val Cys Tyr Glu Ser Pro Asn Pro  
 100 105 110

Thr Thr Gly Ile His Arg Met Val Leu Val Leu Phe Arg Gln Leu Gly  
 115 120 125

Arg Glu Thr Val Tyr Ala Pro Ala Val Arg His Asn Phe Thr Thr Arg  
 130 135 140

Ala Phe Ala Arg Arg Tyr Asn Leu Gly Ala Pro Val Ala Ala Val Tyr  
 145 150 155 160

Phe Asn Cys Gln Arg Gln Ala Gly Ser Gly Gly Arg Arg Phe Thr Gly  
 165 170 175

Pro Tyr Thr Ser Arg Arg Arg Gln Ala  
 180 185

<210> 36

M80696400.ST25

<211> 179  
 <212> PRT  
 <213> Oryza sativa

<400> 36

Met Ala Gly Ser Gly Arg Asp Arg Asp Pro Leu Val Val Gly Arg Val  
 1 5 10 15

Val Gly Asp Val Leu Asp Ala Phe Val Arg Ser Thr Asn Leu Lys Val  
 20 25 30

Thr Tyr Gly Ser Lys Thr Val Ser Asn Gly Cys Glu Leu Lys Pro Ser  
 35 40 45

Met Val Thr His Gln Pro Arg Val Glu Val Gly Gly Asn Asp Met Arg  
 50 55 60

Thr Phe Tyr Thr Leu Val Met Val Asp Pro Asp Ala Pro Ser Pro Ser  
 65 70 75 80

Asp Pro Asn Leu Arg Glu Tyr Leu His Trp Leu Val Thr Asp Ile Pro  
 85 90 95

Gly Thr Thr Ala Ala Ser Phe Gly Gln Glu Val Met Cys Tyr Glu Ser  
 100 105 110

Pro Arg Pro Thr Met Gly Ile His Arg Leu Val Phe Val Leu Phe Gln  
 115 120 125

Gln Leu Gly Arg Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Asn Phe  
 130 135 140

Asn Thr Lys Asp Phe Ala Glu Leu Tyr Asn Leu Gly Ser Pro Val Ala  
 145 150 155 160

Ala Val Tyr Phe Asn Cys Gln Arg Glu Ala Gly Ser Gly Gly Arg Arg  
 165 170 175

Val Tyr Pro

<210> 37  
 <211> 177  
 <212> PRT  
 <213> Lolium perenne

<400> 37

Met Ala Gly Arg Asp Arg Asp Pro Leu Val Val Gly Arg Val Val Gly  
 1 5 10 15

Asp Val Leu Asp Pro Phe Ile Arg Thr Thr Asn Leu Arg Val Thr Phe  
 20 25 30

M80696400.ST25

Gly Asn Arg Ala Val Ser Asn Gly Cys Glu Leu Lys Pro Ser Met Val  
 35 40 45  
 Thr His Gln Pro Arg Val Glu Val Gly Gly Asn Asp Met Arg Thr Phe  
 50 55 60  
 Tyr Thr Leu Val Met Val Asp Pro Asp Ala Pro Ser Pro Ser Asp Pro  
 65 70 75 80  
 Asn Leu Arg Glu Tyr Leu His Trp Leu Val Thr Asp Ile Pro Gly Thr  
 85 90 95  
 Thr Gly Ala Ser Phe Gly Gln Glu Val Met Cys Tyr Glu Ser Pro Arg  
 100 105 110  
 Pro Asn Met Gly Ile His Arg Phe Val Leu Val Leu Phe Gln Gln Leu  
 115 120 125  
 Gly Arg Gln Thr Val Tyr Ala Pro Gly Trp Arg Gln Asn Phe Asn Thr  
 130 135 140  
 Arg Asp Phe Ala Glu Leu Tyr Asn Leu Gly Pro Pro Val Ala Ala Val  
 145 150 155 160  
 Tyr Phe Asn Cys Gln Arg Glu Ala Gly Ser Gly Asp Arg Arg Met Tyr  
 165 170 175

Asn

<210> 38  
 <211> 177  
 <212> PRT  
 <213> Lolium perenne

&lt;400&gt; 38

Met Ala Gly Arg Asp Arg Asp Pro Leu Val Val Gly Arg Val Val Gly  
 1 5 10 15  
 Asp Val Leu Asp Pro Phe Ile Arg Thr Thr Asn Leu Arg Val Thr Phe  
 20 25 30  
 Gly Asn Arg Ala Val Ser Asn Gly Cys Glu Leu Lys Pro Ser Met Val  
 35 40 45  
 Thr His Gln Pro Arg Val Glu Val Gly Gly Asn Asp Met Arg Thr Phe  
 50 55 60  
 Tyr Thr Leu Val Met Val Asp Pro Asp Ala Pro Ser Pro Ser Asp Pro  
 65 70 75 80

Asn

26

14

21

14

Page 28

M80696400.ST25

<220>  
<223> synthetic primer

<400> 43  
catgaaatgt gcagaacagg 20

<210> 44  
<211> 21  
<212> DNA  
<213> Artificial

<220>  
<223> synthetic primer

<400> 44  
tggttgactc tccacatctt c 21

<210> 45  
<211> 23  
<212> DNA  
<213> Artificial

<220>  
<223> synthetic primer

<400> 45  
gctgttttcc ctagcattgt tgg 23

<210> 46  
<211> 23  
<212> DNA  
<213> Artificial

<220>  
<223> synthetic primer

<400> 46  
ataagagaat ccgtgagatc ccg 23

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/AU2004/000634**

## A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. 7: C12N 15/29, A01H 5/00

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
**SEE BELOW**

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
DGENE, SWISS PROT, EMBL, GENBANK, PIR: SEQ ID NO: 1-7, 10, 11 (BLASTN, BLASTX)  
WPIDS, AGRICOLA, MEDLINE, CA: Gigantea, Flowering Locus T, Terminal Flower, Short Vegetative Phase, MADS, Ryegrass, Lolium, Fescue, Festuca.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                            | Relevant to claim No.                         |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| X         | <p><b>WO 2002/044390 A</b> (E. I. DU PONT DE NEMOURS) 6 June 2002</p> <p>SEQ ID NO:7 of citation displays 67% identity with SEQ ID NO:1 and 73% identity with SEQ ID NO:2.</p> <p>SEQ ID NO:31 of citation displays 67% identity with SEQ ID NOs:4 and 5.</p> | <p>1, 3, 4, 6-9,<br/>11-18, 20-22,<br/>24</p> |

☒ Further documents are listed in the continuation of Box C ☒ See patent family annex

|                                                                                                                                                                         |                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                                |                                                                                                                                                                                                                                                  |
| "A" document defining the general state of the art which is not considered to be of particular relevance                                                                | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
| "E" earlier application or patent but published on or after the international filing date                                                                               | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                                                                     |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means                                                                                            | "&" document member of the same patent family                                                                                                                                                                                                    |
| "P" document published prior to the international filing date but later than the priority date claimed                                                                  |                                                                                                                                                                                                                                                  |

|                                                                                                                                                                                        |                                                                               |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Date of the actual completion of the international search<br>2 June 2004                                                                                                               | Date of mailing of the international search report<br>18 JUN 2004             |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized officer<br><br><b>TERRY MOORE</b><br>Telephone No : (02) 6283 2632 |

# INTERNATIONAL SEARCH REPORT

International application No.

**PCT/AU2004/000634**

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                             |                                 |
|-------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Category*                                             | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                          | Relevant to claim No.           |
| X                                                     | <p><b>WO 2003/000904 A</b> (SYNGENTA PARTICIPATIONS AG) 3 January 2003</p> <p>SEQ ID NO:19 of citation displays 89% identity with fragment of SEQ ID NO:4 (bp 96-620) and 81% identity with fragment of SEQ ID NO:5 (bp 134-655).</p> <p>SEQ ID NO:37 of citation displays 75% identity with fragment of SEQ ID NOs:1 and 2 (bp 1-233).</p> | 1, 3, 4, 6-9, 11-18, 20-22, 24  |
| E, X                                                  | <p><b>WO 2003/048319 A</b> (SYNGENTA PARTICIPATIONS AG) 12 June 2003</p> <p>SEQ ID NO:43 of citation displays 86% identity with fragment of SEQ ID NOs:1 and 2 (bp 1-236).</p>                                                                                                                                                              | 1, 3, 4, 6-9, 11-18, 20-22, 24  |
| E, X                                                  | <p><b>EP 1342785 A</b> (NATIONAL INSTITUTE OF AGROBIOLOGICAL SCIENCES) 10 September 2003</p> <p>SEQ ID NO:23 of citation displays 66% identity with SEQ ID NOs:1 and 2.</p>                                                                                                                                                                 | 1, 3, 4, 6-9, 11-18, 20-22, 24  |
| X                                                     | <p><b>WO 2002/033091 A</b> (AGRICULTURE VICTORIA SERVICES PTY LTD) 25 April 2002</p> <p>Figure 2 - 99% identity with SEQ ID NO:9.<br/>Figure 49 - 62% identity with SEQ ID NO:5.</p>                                                                                                                                                        | 1-24                            |
| X                                                     | <p><b>WO 1999/049064 A</b> (PLANT BIOSCIENCE LIMITED) 30 September 1999</p> <p>Figure 11 - 85% identity with fragment of SEQ ID 7 (bp 1-1059).</p>                                                                                                                                                                                          | 2, 3, 5-8, 10-17, 19-21, 23, 24 |
| X                                                     | <p><b>US 2001/051335 A</b> (LALGUDI, et al.) 13 December 2001</p> <p>SEQ ID NO:5753 of citation displays 85% identity with fragment of SEQ ID NO:7 (bp 165-475).</p>                                                                                                                                                                        | 2, 3, 5-8, 10-17, 19-21, 23, 24 |
| X                                                     | <p><b>US 6476212 B</b> (LALGUDI, et al.) 5 November 2002</p> <p>SEQ ID NO:999 of citation displays 77% identity with fragment of SEQ ID NO:7 (bp 34-315).</p>                                                                                                                                                                               | 2, 3, 5-8, 10-17, 19-21, 23, 24 |
| X                                                     | <p><b>WO 2001/026459</b> (MENDEL BIOTECHNOLOGY INC) 19 April 2001</p> <p>SEQ ID NO:25 of citation displays 56% identity (77% similarity) with SEQ ID NO:9.</p>                                                                                                                                                                              | 2, 3, 5-8, 10-17, 19-21, 23, 24 |

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/AU2004/000634**

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                 |
|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                                 | Relevant to claim No.           |
| X                                                    | <p><b>Genbank Accession Number AF290457</b>, Taylor, C. et al. (2000), <i>Lolium perenne</i> clone LpV532</p> <p>97% identity with SEQ ID NO:4 (543 bp fragment).</p>                                                                                                                                                                                                                                                                                                                                              | 1, 3, 4, 6-9, 11-18, 20-22, 24  |
| X                                                    | <p><b>Genbank Accession Number AF316419 and AAG31808</b></p> <p>Jensen, C.S. et al., 2001, A TERMINAL FLOWER1-like gene from perennial ryegrass involved in floral transition and axillary meristem identity, <i>Plant Physiology</i>, 125(3): 1517-28.</p> <p>SEQ ID NO:4, 5 display 58% and 64% identity with AF316419.</p> <p>BLASTX - putative protein encoded by SEQ ID NO: 4 (frame= +3) displays 79% identity with AAG31808; putative protein encoded by SEQ ID NO:5 (frame= +2) displays 82% identity.</p> | 1, 3, 4, 6-9, 11-18, 20-22, 24  |
| X                                                    | <p><b>Genbank Accession Number AAD42896</b>, Cong,B. et al. (1999), Cen-like protein FDR2 (<i>Oryza sativa</i>).</p> <p>BLASTX - protein encoded by SEQ ID NO:5 (frame= +2) displays 82% identity with AAD42896.</p>                                                                                                                                                                                                                                                                                               | 1, 3, 4, 6-9, 11-18, 20-22, 24  |
| X                                                    | <p><b>Genbank Accession Number AAM46142</b>, Boss,P.K. and Thomas,M.R. (2002), Terminal flower-like protein 1 (<i>Vitis vinifera</i>)</p> <p>BLASTX - putative protein encoded by SEQ ID NO:4 (frame= +3) displays 72% identity with AAM46142; putative protein encoded by SEQ ID NO:5 (frame= +2) displays 82% identity.</p>                                                                                                                                                                                      | 1, 3, 4, 6-9, 11-18, 20-22, 24  |
| X                                                    | <p><b>Genbank Accession Number AF411229 and AAL08497</b>, Dunford, R.P. and Laurie,D.A. (2002), <i>Hordeum vulgare</i> gigantea-like protein.</p> <p>AF411229 displays 88% identity with SEQ ID NO:6, 7.</p> <p>BLASTX - AAL08497 displays 85% identity with the putative protein encoded by SEQ ID NO: 6, 7 (frame = +3).</p>                                                                                                                                                                                     | 2, 3, 5-8, 10-17, 19-21, 23, 24 |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2004/000634

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                 |
|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                                           | Relevant to claim No.           |
| X                                                    | <p><b>Genbank Accession Number AJ133787 and CAB56058</b>, <i>Oryza sativa gigantea</i> homologue.</p> <p>Fowler, S. et al., 1999, GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains, <i>EMBO Journal</i>, 18:4679-4688</p> <p>AJ133787 displays 85% identity with SEQ ID NO:6, 7.</p> <p>BLASTX - CAB56058 displays 81% identity with the putative protein encoded by SEQ ID NO:6, 7 (frame = +3).</p> | 2, 3, 5-8, 10-17, 19-21, 23, 24 |
| X                                                    | <p><b>Genbank Accession Number AAF00023</b>, Park, D.H. et al. (1999), GIGANTEA (<i>Arabidopsis thaliana</i>).</p> <p>BLASTX - AAF00023 displays 63% identity with the putative protein encoded by SEQ ID NO:6, 7 (frame = +3).</p>                                                                                                                                                                                                                                                                                          | 2, 3, 5-8, 10-17, 19-21, 23, 24 |
| X                                                    | <p><b>Genbank Accession Number CAD23439 and CAD23409</b>, Putative MADS-domain transcription factor (<i>Zea mays</i>).</p> <p>Muenster, T. et al., 2002, Maize MADS-box genes galore, <i>Maydica</i>, 47: 287-301</p> <p>CAD23439 and CAD23409 display 91% identity with SEQ ID NO:9.</p>                                                                                                                                                                                                                                    | 2, 3, 5-8, 10-17, 19-21, 23, 24 |
| X                                                    | <p><b>Genbank Accession Number BAA81880</b>, Shinozuka, Y. et al., (2002), MADS box-like protein (<i>Oryza sativa</i> (japonica cultivar-group)).</p> <p>BAA81880 displays 90% identity with SEQ ID NO:9.</p>                                                                                                                                                                                                                                                                                                                | 2, 3, 5-8, 10-17, 19-21, 23, 24 |

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/AU2004/000634**

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
  - a. type of material
    - ☒ a sequence listing
    - ☐ table(s) related to the sequence listing
  - b. format of material
    - ☐ in written format
    - ☒ in computer readable form
  - c. time of filing/furnishing
    - ☐ contained in the international application as filed
    - ☒ filed together with the international application in computer readable form
    - ☐ furnished subsequently to this Authority for the purposes of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2004/000634

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

### See Supplemental Box I

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

### Remark on Protest

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

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2004/000634

## Supplemental Box I

(To be used when the space in any of Boxes I to VIII is not sufficient)

### Continuation of Box No III:

The problem addressed by the present application is the inhibition of flowering in a wide range of plant species, particularly forage plants. The solution provided by the applicants resides in the isolation of 10 different polynucleotides from ryegrass or fescue. It appears that the only common feature shared by all the nucleic acid molecules described is that the sequences have been isolated from the plant species *Lolium* or *Festuca*. However, the species of origin is not a unifying special technical feature.

There appears to be no other technical relationship between the nucleic acid molecules, therefore it is considered that the present application relates to four groups of inventions as described below.

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

Claims 1, 3, 4, 6-9, 11-18, 20-22, 24 (all partially) directed to an isolated nucleic acid fragment from ryegrass or fescue encoding a FLOWERING LOCUS T (FT) polypeptide, an FT-like polypeptide, or functionally active fragment or variant thereof, the presence of which inhibits flowering; a construct comprising the nucleic acid molecule mentioned above; a host cell comprising said vector; an isolated polypeptide encoded by the above mentioned nucleic acid molecule; and methods using the above mentioned nucleic acid molecules or polypeptides.

Claims 1, 3, 4, 6-9, 11-18, 20-22, 24 (all partially) relating to an isolated nucleic acid fragment from ryegrass or fescue encoding a Terminal Flower (TFL) polypeptide, and TFL-like polypeptide, or functionally active fragment or variant thereof; the presence of which inhibits flowering; a construct comprising the nucleic acid molecule mentioned above; a host cell comprising said vector; an isolated polypeptide encoded by the above mentioned nucleic acid molecule; and methods using the above mentioned nucleic acid molecules or polypeptides.

Claims 2, 3, 5-8, 10-17, 19-21, 23, 24 (all partially) relating to an isolated nucleic acid fragment from ryegrass or fescue encoding a Gigantea (GI) polypeptide, and GI-like polypeptide, or functionally active fragment or variant thereof; a construct comprising the nucleic acid molecule mentioned above; a host cell comprising said vector; an isolated polypeptide encoded by the above mentioned nucleic acid molecule; and methods using the above mentioned nucleic acid molecules or polypeptides.

Claims 2, 3, 5-8, 10-17, 19-21, 23, 24 (all partially) relating to an isolated nucleic acid fragment from ryegrass or fescue encoding a Short Vegetative Phase (SVP) polypeptide, and SVP-like polypeptide, or functionally active fragment or variant thereof; a construct comprising the nucleic acid molecule mentioned above; a host cell comprising said vector; an isolated polypeptide encoded by the above mentioned nucleic acid molecule; and methods using the above mentioned nucleic acid molecules or polypeptides.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2004/000634

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report |            | Patent Family Member |            |    |            |    |            |
|----------------------------------------|------------|----------------------|------------|----|------------|----|------------|
| WO                                     | 0244390    | AU                   | 41502/02   | CA | 2436778    | EP | 1364033    |
| WO                                     | 03000904   | EP                   | 1399561    | EP | 1402038    | EP | 1402042    |
|                                        |            | EP                   | 1409696    | US | 2003135888 | US | 2004010815 |
|                                        |            | US                   | 2004016025 | WO | 03000897   | WO | 03000905   |
|                                        |            | WO                   | 03000906   | WO | 03007699   | WO | 03008540   |
|                                        |            | WO                   | 03027249   |    |            |    |            |
| WO                                     | 03049319   | EP                   | 1317151    |    |            |    |            |
| EP                                     | 1342785    | AU                   | 24080/02   | CA | 2429679    | JP | 2002153283 |
|                                        |            | US                   | 2004088763 | WO | 0242475    |    |            |
| WO                                     | 0233091    | AU                   | 10243/02   |    |            |    |            |
| WO                                     | 9949064    | AU                   | 31765/99   | CA | 2324515    | EP | 1064385    |
| US                                     | 2001051335 |                      |            |    |            |    |            |
| US                                     | 6476212    |                      |            |    |            |    |            |
| WO                                     | 0126459    | AU                   | 15698/01   | AU | 16101/01   | AU | 17656/01   |
|                                        |            | AU                   | 17671/01   | AU | 17682/01   | AU | 17683/01   |
|                                        |            | AU                   | 19199/01   | AU | 25669/99   | AU | 41577/97   |
|                                        |            | AU                   | 44528/00   | AU | 86617/01   | BR | 0014750    |
|                                        |            | BR                   | 0015628    | BR | 0015631    | BR | 0015632    |
|                                        |            | BR                   | 0015633    | BR | 0015634    | BR | 0015635    |
|                                        |            | BR                   | 9908523    | CA | 2319714    | CA | 2367408    |
|                                        |            | CA                   | 2386170    | CA | 2390594    | CA | 2390597    |
|                                        |            | CA                   | 2390600    | CA | 2391443    | CA | 2391446    |
|                                        |            | CA                   | 2391569    | CA | 2456972    | CA | 2456979    |
|                                        |            | EP                   | 0955807    | EP | 1053328    | EP | 1192255    |
|                                        |            | EP                   | 1229780    | EP | 1229781    | EP | 1229782    |
|                                        |            | EP                   | 1230256    | EP | 1230344    | EP | 1230345    |
|                                        |            | EP                   | 1231835    | EP | 1406483    | EP | 1420630    |
|                                        |            | US                   | 5891859    | US | 5892009    | US | 5965705    |
|                                        |            | US                   | 6417428    | US | 6706866    | US | 2002157136 |
|                                        |            | US                   | 2003041356 | US | 2003046723 | US | 2003061637 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

**PCT/AU2004/000634**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in<br>Search Report                                                                                 |    |            |    | Patent Family Member |    |            |  |
|---------------------------------------------------------------------------------------------------------------------------|----|------------|----|----------------------|----|------------|--|
| WO 0126459<br><br>continued                                                                                               | US | 2003093837 | US | 2003101481           | US | 2003121070 |  |
|                                                                                                                           | US | 2003131386 | US | 2003217383           | US | 2003226173 |  |
|                                                                                                                           | US | 2003229915 | US | 2003233680           | US | 2004019925 |  |
|                                                                                                                           | US | 2004019927 | US | 2004045049           | WO | 0060089    |  |
|                                                                                                                           | WO | 0135725    | WO | 0135726              | WO | 0135727    |  |
|                                                                                                                           | WO | 0136444    | WO | 0136597              | WO | 0136598    |  |
|                                                                                                                           | WO | 0215675    | WO | 9809521              | WO | 9938977    |  |
|                                                                                                                           | WO | 02077185   | WO | 03013227             | WO | 03013228   |  |
|                                                                                                                           | WO | 03014327   | WO | 2004031349           |    |            |  |
| Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. |    |            |    |                      |    |            |  |
| END OF ANNEX                                                                                                              |    |            |    |                      |    |            |  |