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(54) Title: GENETIC SEQUENCES CONFERRING NEMATODE RESISTANCE IN PLANTS AND USES THEREFOR

(57) Abstract

The present invention relates generally to a nucleic acid molecule encoding, or complementary to a nucleic acid molecule encoding, a polypeptide which confers, enhances, or otherwise facilitates resistance to a nematode in a plant cell. The nucleic acid molecule of the present invention is useful in the isolation of related nematode resistance, or nematode resistance-like genetic sequences, from other plants. Furthermore, the present invention provides for the generation of plants carrying non-endogenous nematode resistance, or nematode resistance-like genetic sequences, said plants exhibiting enhanced tolerance to parasitic nematodes and related pathogens.

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GENETIC SEQUENCES CONFERRING NEMATODE RESISTANCE IN PLANTS AND USES THEREFOR

FIELD OF THE INVENTION

The present invention relates generally to genetic sequences, and more particularly to genetic sequences which confer, or otherwise facilitate or enhance, resistance in plants to plant parasitic nematodes, such as cyst nematodes and root knot nematodes. The present invention further provides for plants into which the subject genetic sequences have been introduced, generating enhanced resistance qualities to plant parasitic nematodes. The present invention is particularly useful in the development of plants resistant to plant parasitic nematodes such as food, fibre and ornamental plants.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence identity numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined after the bibliography.

Throughout this specification and the claims that follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

BACKGROUND TO THE INVENTION

Improvements in recombinant DNA technology have produced dramatic changes to the agricultural industry, in particular the approaches taken to improve crop productivity. A major concern is the effect of plant pests, such as plant parasitic nematodes, on productivity. Generically, plant parasitic nematodes invade a wide range of food, fibre and ornamental plants, causing damage to different plant tissues with varying severity on productivity. Parasitic

nematodes cost the agriculture and horticulture industries approximately US$78 billion per annum.

Plant parasitic nematodes are broadly classified as either migratory ectoparasites, sedentary ectoparasites, migratory ectoendoparasites, migratory endoparasites, or sedentary endoparasites, on the basis of their feeding patterns. Most crop damage is caused by sedentary endoparasites, for example the cyst nematodes *Heterodera sp.* and *Globodera sp.* and the root knot nematodes *Meloidogyne sp.*, through their devastating effect on root structures. Juvenile nematodes invade the plant root and migrate to the vascular tissue where they induce a multinucleate feeding structure or syncitium from which the nematode feeds.

The most cost-effective and sustainable method for control of plant pests is the development of resistant plants. However, the development of this method of control in relation to parasitic nematodes has faced many difficulties. For example, bioassays for nematodes, such as the cereal cyst nematode, are long and labour intensive. Although natural resistance to plant parasitic nematodes occurs in certain plant genotypes, the molecular basis of resistance was hitherto unknown. In particular, the molecular characterisation of genetic sequences encoding a polypeptide which confers nematode resistance on a plant, has not been a straightforward procedure. Furthermore, until the present invention, the chromosomal localisation of nematode resistance genes and genetic markers for nematode resistance in *Triticum tauschii*, were unknown.

SUMMARY OF THE INVENTION

In accordance with the present invention, genetic sequences conferring resistance to a plant pathogen, preferably a plant parasitic nematode, have been cloned from *Triticum tauschii*. The cloning of these sequences permits the generation of transgenic plants with *de novo*, improved or otherwise enhanced nematode resistance. The present invention also permits the screening through genetic or immunological means, similar nematode resistance genes in other plants for use in developing or enhancing nematode resistance in commercially and economically

important species.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a nucleic acid molecule which encodes a protein or derivative thereof, which confers, enhances, or otherwise facilitates resistance to a nematode in a plant.

In another embodiment, the present invention provides an isolated DNA molecule comprising a sequence of nucleotides which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) has at least about 40% nucleotide sequence similarity to any one or more of the sequences set forth in SEQ ID NOS: 1, 3, 5 or 7 or a part thereof.

In yet another embodiment, the present invention provides an isolated nucleic acid molecule which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NOS: 1, 3, 5 or 7 or to a complementary strand thereof.

In yet another embodiment, the invention provides an isolated nucleic acid molecule which is substantially the same as any one or more of the sequences set forth in SEQ ID NOS: 1, 3, 5 or 7 or is at least 40% identical thereto.

Another aspect of the invention provides a genetic construct comprising a sequence of nucleotides which encodes or is complementary to a nucleic acid molecule which encodes a protein or derivative thereof, which confers, enhances, or otherwise facilitates resistance to a nematode in a plant. According to one embodiment, the nucleic acid molecule is operably linked to a promoter sequence, thereby regulating expression of said nucleic acid molecule in

a eukaryotic cell, for example a plant cell, or a prokaryotic cell.

In yet another aspect, the present invention provides a genetic construct comprising an isolated promoter sequence from a gene which when expressed encodes a polypeptide that confers, enhances, or otherwise facilitates nematode resistance in a cell, or a functional part, derivative, fragment, homologue or analogue thereof, wherein said promoter is operably linked to the coding region isolated from a second genetic sequence.

The invention extends to the recombinant polypeptide product of said genetic construct.

The present invention also provides an oligonucleotide molecule of at least 10 nucleotides in length capable of hybridising under low stringency conditions to part of the nucleotide sequence, or to a complement of any one or more of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5 or 7.

The nucleic acid molecule and/or oligonucleotide of the present invention are useful in the isolation of nematode resistance or nematode resistance-like genetic sequences from other plants, using hybridisation and/or PCR-based approaches.

Accordingly, there is provided a method of identifying a nematode resistance genetic sequence or nematode resistance-like genetic sequence which method comprises contacting genomic DNA, or mRNA, or cDNA, or parts, or fragments thereof, or a source thereof, with a hybridisation effective amount of a genetic sequence encoding, or complementary to a genetic sequence encoding a polypeptide which confers, enhances or otherwise facilitates nematode resistance, or a part thereof, and then detecting said hybridisation.

There is also provided a method of identifying a nematode resistance genetic sequence or a nematode resistance-like genetic sequence in a plant cell, which method comprises contacting genomic DNA, mRNA, or cDNA with one or more oligonucleotide molecules to a genetic sequence from said plant for a period of time and under conditions sufficient to form a double-

stranded nucleic acid molecule and amplifying copies of the said genetic sequence in a polymerase chain reaction.

In another aspect, this invention also provides an isolated polypeptide which comprises an amino acid sequence which confers, enhances, or otherwise facilitates resistance to a nematode in a plant cell, or a functional mutant, derivative part, fragment, or analogue of said polypeptide.

The present invention extends to a synthetic peptide comprising at least 10 contiguous amino acids of the sequence set forth in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, or having at least 40% similarity to all or a part thereof.

The polypeptide and synthetic peptides of the present invention may be used to generate specific immuno-interactive molecules. Accordingly, the present invention also provides an antibody that binds to a polypeptide which confers, enhances or otherwise facilitates resistance to a nematode in a plant or a part or fragment thereof, wherein said polypeptide, part or fragment threof further comprises an amino acid sequence which is substantially the same as the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, or is at least 40% similar to all or a part thereof.

In yet another aspect of the present invention, there is provided a method of identifying a nematode resistance gene product or nematode resistance-like gene product in a plant cell, which method comprises contacting the antibody with an antigen from said plant for a period of time and under conditions sufficient to form an antibody-antigen complex and measuring the amount of said antibody-antigen complex formed.

The present invention is useful for the generation of plants with enhanced nematode resistance or nematode resistance-like characteristics and there is also provided a plant carrying a non-endogenous nucleic acid molecule encoding or complementary to a nucleic acid molecule encoding a polypeptide which confers, enhances, or otherwise facilitates nematode resistance in said plant. The present invention extends to the progeny derived from said plant.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical representation of an RFLP linkage and physical map of chromosome 2D produced from *Triticum tauschii* F2 progeny of the genetic crosses (a) CPI 110813 X CPI 110795; (b) AUS 18913 x CPI 110856. The map location of the nematode resistance genes *Ccn-D1* and *Ccn-D2* are indicated.

Figure 2 is a photographic representation of an agarose gel showing PCR amplification products obtained from a survey of hydroxylapatite-fractionated DNA enriched for low copy sequences. DNA samples were from bulked segregants of the *Triticum tauschii* cross CPI 110810 (resistant) x CPI 110825 (susceptible). Odd-numbered lanes contain DNA from resistant bulked segregants. Even-numbered lanes contain DNA from susceptible bulked segregants. Random primers used for each pair were OPF12 (lanes 1,2), OPF13 (lanes 3,4), OPG2 (lanes 5,6), OPG3 (lanes 7,8), OPG6 (lanes 9,10), OPG6 (lanes 11,12) and OPG13 (lanes 13,14).The arrow indicates the presence of a polymorphic PCR fragment present in lane 13 but absent from lane 14. Lane 15 is a size marker.

Figure 3 is a photographic representation of an agarose gel showing PCR amplification products obtained from total genomic DNA and hydroxylapatite-fractionated DNA enriched for low copy sequences. DNA samples were from parental and bulked segregants of the *Triticum tauschii* cross AUS 188913 (resistant) x CPI 110856 (susceptible).Amplification products were obtained using the random primers OPE20 (lanes 1-6) and OPF12 (lanes 7,8). Templates were from low copy bulked resistant segregant (lanes 1,7), low copy bulked susceptible segregant (lanes 2,8), total genomic bulked resistant segregant (lane 3), total genomic bulked susceptible segregant (lane 4), total genomic from AUS 188913 (lane 5), and total genomic from CPI 110856 (lane 6). The arrow indicates the presence of a polymorphic PCR fragment present in resistant bulked segregant (lane 1) but not in the susceptible bulked segregant (lane 2). The converse type of polymorphism is shown with primer OPF 12 in lanes 7 and 8.

Figure 4 is a photographic representation showing an autoradiograph of one euploid (lane 1)

and several nullitetrasomic lines (lanes 2-6) of *Triticum aestivum* cv Chinese Spring, showing RFLP patterns assayed with the cloned PCR fragment csE20-2. Cytogenetic stocks missing chromosome 2D are present in lanes 8 (nulli 2D tetra 2A) and 9 (nulli 2D tetra 2B).

Figure 5 is a photographic representation showing an autoradiograph of *Triticum tauschii* F2 individuals from the cross AUS 188913 (resistant) x CPI 110856 (susceptible) assayed with the cloned PCR fragment csE20-2. *Ccn-D1* resistance (R) and susceptibility (S) are indicated.

Figure 6 is a photographic representation showing linkage between the XcsE20 RFLP marker and *Cre3* (*Ccn-D1*) in *Triticum aestivum* resistant (R) and susceptible (S) backcross individuals.

Figure 7 is a schematic representation of a lambda clone containing the *Cre3* gene. The positions of the 6.5kb *Eco*RV RFLP fragment and PCR fragment csE20-2 are indicated. The position of the *Cre3* gene within the lambda clone is also indicated. Restriction enzyme sites *Bam*Hi(B) and *Eco*RV(E) are indicated.

Single letter abbreviations used for amino acid residues in the specification are defined in Table 1.

TABLE 1

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid	Xaa	X

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention comprises an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes a protein or derivative thereof, which confers, enhances or otherwise facilitates resistance to a nematode in a plant.

Hereinafter the term nematode "resistance gene" or "resistance-like gene", or similar term shall be used to define a nucleic acid molecule which upon expression confers, enhances, or otherwise facilitates resistance of a cell and/or organism to one or more plant parasitic pathogens. The term "nematode resistance gene" further defines a nucleic acid molecule which upon expression confers, enhances, or otherwise facilitates resistance to one or more plant parasitic nematode pathogens. Reference herein to a "gene" is to be taken in its broadest context and includes:

(i) a classical genomic gene consisting of a coding region optionally together with transcriptional and/or translational regulatory sequences and a coding region with or without non-translated sequences (i.e. introns, 5′- and 3′- untranslated sequences); or

(ii) mRNA or cDNA corresponding to the coding regions (i.e. exons) and optionally 5′- and 3′- untranslated sequences of the gene.

The term "gene" is also used to describe a synthetic or fusion molecule, or derivative which encodes, or is complementary to a molecule which encodes, all or part of a functional product. A functional product is one which confers, enhances or otherwise facilitates resistance of a cell to a parasitic nematode. Preferred nematode resistance-like genes are derived from a naturally occurring nematode resistance gene by standard recombinant techniques. Generally, a nematode resistance gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the nematode resistance gene of the present invention include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in

the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity.

The present invention extends to the isolated nucleic acid when integrated into a plant genome and to propagated plants containing same nucleic acid molecule.

Another aspect of the present invention is directed to a nucleic acid molecule which comprises a sequence of nucleotides corresponding or complementary to the nucleotide sequence set forth in any one or more of SEQ ID NOS: 1, 3, 5 or 7, or having at least 40% similarity to all or a part thereof and wherein said nucleic acid molecule encodes a protein that confers, enhances, or otherwise facilitates resistance to a nematode in a plant.

Preferably, the percentage similarity to a sequence set forth in SEQ ID NOS:1, 3, 5 or 7 is at least 50%. Even more preferably, the percentage similarity is at least 60-65%. Still more preferably, the percentage similarity is at least 70-75%. Yet still more preferably, the percentage similarity as at least 80-90%, including at least 91% or 93% or 95%.

For the purposes of nomenclature, the sequences shown in SEQ ID NOS: 1, 3, 5 and 7 relate to the *CRE 3* resistance gene of *Triticum tauschii* which controls resistance to cyst nematodes *Heterodera sp.* More preferably, SEQ ID NOS: 1 and 3 are nucleotide sequences of a genomic clone isolated from *Triticum tauschii*, containing the *Cre* 3 gene. The nucleotide sequence set forth in SEQ ID NO: 5 is a cDNA clone encoding *CRE* 3, which was isolated from *T. tauschii* AUS 18913 seedlings using genomic clone sequences. SEQ ID NO: 7 shows the nucleotide sequence of the promoter and complete open reading frame of the *Cre*3 gene, without introns.

The amino acid sequence of the complete CRE3 polypeptide is presented in SEQ ID NO: 8.

Preferably, the cyst nematode is the cereal cyst nematode (CCN) *Heterodera avenae*. More preferably, the cyst nematode is the Australian pathotype of *H. avenae*. The designation "*CRE 3*" is also synonymous with the designation "*Ccn-D1*" referred to by Eastwood *et al.* (1993), among others.

A further aspect of the present invention contemplates a nucleic acid molecule which encodes a protein that confers or otherwise facilitates nematode resistance in a plant and which is capable of hybridising under at least low stringency conditions to the nucleic acid molecule set forth in any one or more of SEQ ID NOS: 1, 3 , 5 or 7 or to a derivative homologue or analogue thereof.

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of clarification of parameters affecting hybridisation between nucleic acid molecules, reference can conveniently be made to pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

Genetic analysis indicates that specific interactions may occur between resistance genes and gene products of the parasitic nematode. Although not intending to limit the present invention to any one theory or mode of action, it is proposed that the genetic sequences of the present invention control host range *via* specific recognition of the gene products of the nematode pest, in a "gene-for-gene" interaction that is understood by one normally skilled in the art. Accordingly, the genetic sequences are useful in increasing the range of resistance of a plant to nematode pests, by providing *de novo* the required nematode resistance gene, or being introduced together with the corresponding nematode gene or genes, on, for example, a single

genetic cassette. Accordingly, these aspects of the invention are covered by the expression "conferring, improving, or otherwise enhancing nematode resistance" or other similar expression.

The present invention is particularly directed to resistance that is conferred, enhanced, or facilitated against a nematode, preferably a cereal cyst nematode, more preferably *Heterodera avenae*, even more preferably the Australian pathotype of *H. avenae* by a polypeptide encoded by genetic sequences from *Triticum tauschii*. Examples of genetic sequences in *Triticum tauschii* which confer resistance to a nematode include, but are not limited to the *Ccn* genes, *Ccn-D1* and *Ccn-D2* and the *Cre* 1. The subject invention clearly contemplates other sources of nematode resistance genes, such as but not limited to, other monocotyledonous plants, other *Triticum sp.*, barley, maize, rye, oats, and rice, amongst others.

The genetic sequences which encode a protein which confers, enhances, or otherwise facilitates nematode resistance may correspond to the naturally occurring sequence or may differ by one or more nucleotide substitutions, deletions and/or additions. Accordingly, the present invention extends to nematode resistance genes and any functional genes, mutants, derivatives, parts, fragments, homologues or analogues thereof or non-functional molecules but which are at least useful as, for example, genetic probes, or primer sequences in the enzymatic or chemical synthesis of said gene, or in the generation of immunologically interactive recombinant molecules.

In a particularly preferred embodiment, the nematode resistance genetic sequences or like genetic sequences are employed to identify and isolate similar genes, or nematode resistance-like genes from other plants. The present invention extends to the use of said genetic sequence, or a part thereof to detect polymorphisms of a nematode resistance genetic sequence or nematode resistance-like genetic sequence.

In this aspect of the invention, there is provided an oligonucleotide molecule of at least 10 nucleotides in length capable of hybridising under low stringency conditions to part of the

nucleotide sequence, or to a complement of any one or more of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5 or 7.

Accordingly there is contemplated a method for identifying a related nematode resistance genetic sequence or nematode resistance-like genetic sequence, said method comprising contacting genomic DNA, or mRNA, or cDNA, or parts, or fragments thereof, or a source thereof, with a hybridisation effective amount of a genetic sequence encoding or complementary to a genetic sequence encoding a polypeptide which confers, enhances or otherwise facilitates nematode resistance, or a part thereof, and then detecting said hybridisation.

The related nematode resistance genetic sequence or like sequence may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the related genetic sequence originates from *Triticum aestivum* or similar plant such as maize, barley, rye, oats, or rice and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same. In addition, the related genetic sequence may be bound to a support matrix, for example nylon, nitrocellulose, polyacrylamide, agarose, amongst others.

Preferably, the genetic sequence which encode a polypeptide which confers, enhances, or otherwise facilitates nematode resistance (i.e latter genetic sequence) is from *Triticum sp.*, or similar plant such as maize, barley, rye, oats, or rice. In a most preferred embodiment, the latter comprises a sequence of nucleotides set forth in any one or more of SEQ ID NOS: 1, 3, 5 or 7 or a homologue, derivative or analogue thereof.

Preferably, the latter genetic sequence is labelled with a reporter molecule capable of giving an identifiable signal (e.g. a radioisotope such as ^{32}P or ^{35}S or a biotintylated molecule).

An alternative method contemplated in the present invention involves hybridising a nucleic acid primer molecule of at least 10 nucleotides in length to a nucleic acid "template molecule", said template molecule herein defined as a nematode resistance genetic sequence, or resistance-like genetic sequence, or a functional part thereof, or its complementary sequence. Specific nucleic

acid molecule copies of the template molecule are amplified enzymatically in a polymerase chain reaction, a technique that is well known to one skilled in the art.

Preferably, the nucleic acid primer molecule or molecule effective in hybridisation is contained in an aqueous mixture of other nucleic acid primer molecules. More preferably, the nucleic acid primer molecule is in a substantially pure form. In a preferred embodiment, the nucleic acid primer molecule is from *Triticum sp.*, or similar plant such as maize, barley, rye, oats, or rice. In a most preferred embodiment, the nucleic acid primer molecule is any nucleotide sequence of at least 10 nucleotides in length derived from, or contained within any one or more of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5 or 7.

The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the related genetic sequence originates from *Triticum aestivum* or similar plant such as maize, barley, rye, oats, or rice and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

A further aspect of the present invention is directed to a genetic construct comprising an isolated nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes a protein, or derivative thereof, that confers, enhances, or otherwise facilitates resistance against a nematode in a plant cell. Preferably, the gene sequence is related to or a functional derivative, part fragment, homologue, or analogue of the nucleotide sequence defined by any one or more of SEQ ID NOS: 1, 3, 5 or 7. More preferably, the genetic construct comprises the entire open reading frame of the *Cre* 3 gene sequence.

The present invention extends to genetic constructs designed to assist expression of a nucleic acid molecule that confers, enhances or facilitates nematode resistance in a cell. Generally, the genetic construct comprises in addition to the subject nucleic acid molecule, a promoter and optional other regulatory sequences that modulate expression of the nucleic acid molecule. The promoter may be the *CRE 3* gene promoter, or a promoter from another genetic source.

Preferably, however, the promoter is capable of expression in a plant cell, in particular a root cell.

The subject nucleic acid molecule may be genomic DNA or cDNA and may correspond in sequence exactly with the nucleotide sequence as set forth in any one or more of SEQ ID NOS: 1, 3, 5 or 7 or it may contain one or more nucleotide substitutions, additions and/or deletions, either dispersed throughout, or clustered.

In an alternative embodiment, an isolated promoter sequence from a gene which, when expressed encodes a polypeptide that confers, enhances or otherwise facilitates nematode resistance in a cell, or a functional part, derivative, fragment, homologue or analogue thereof, is operably linked to the coding region of a second genetic sequence, for example the β-glucuronidase gene, or the chloramphenicol acetyltransferase gene, or the firefly luciferase gene, amongst others. Preferably, the promoter sequence is contained within nucleotides 1 to 1138 of the sequences set forth in SEQ ID NO: 1 or SEQ ID NO: 7.

Yet another aspect of the present invention provides for the expression of the subject genetic sequence in a suitable host (e.g. a prokaryote or eukaryote) to produce full length or non-full length recombinant nematode resistance gene products. Preferably, the nematode resistance gene product has a sequence that is identical to, or contained within an amino acid sequence set forth in any one or more of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8. More preferably, the nematode resistance gene product has a sequence that is identical to or contained within the amino acid sequence set forth in SEQ ID NO: 6 or SEQ ID NO: 8. The present invention extends also to a synthetic peptide fragment of a nematode resistance gene product, preferably the resistance gene product set forth in any one or more of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

The present invention provides an isolated polypeptide which comprises an amino acid sequence which confers, enhances, or otherwise facilitates resistance to a nematode in a plant cell, or a functional mutant, derivative part, fragment, or analogue of said polypeptide.

According to this aspect, the present invention also extends to the protein or polypeptide product of the *Triticum tauschii* nematode resistance gene *Cre3* and the weaker resistance gene *Ccn-D2*. This is done, however, with the understanding that the subject invention extends to a range of resistance genes for nematode and other pathogens. In fact, the present invention extends to a nematode resistance gene characterised by said gene encoding a product having at least one imperfect leucine rich repeat region. Preferably, the leucine rich repeat region is located at the C-terminal end of the protein molecule and has at least 60% similarly to amino acid residues 185 to 412 of the amino acid sequence set forth in SEQ ID NO: 4, or residues 308 to 768 of SEQ ID NO: 6 and even more preferably is at least 80% similar thereto. Still more preferably, the leucine rich region corresponds to amino acid residues 185 to 412 of the amino acid sequence set forth in SEQ ID NO: 4 or residues 308 to 768 of SEQ ID NO: 6.

Alternatively or in addition to, the nematode resistance gene product further contains a p-Loop, or kinase-1a motif, having the sequence:

GV(G/S)GSGKST

and more particularly

GIHGV(G/S)GSGKST,

or having one or more amino acid substitutions, insertions and/or deletions thereto provided that such derivatives still function as a p-Loop in conferring nematode resistance in a cell. A p-Loop is involved in ATP/GTP binding.

Alternatively, or in addition to, the nematode resistance gene product further contains a kinase-2 motif, having the sequence:

XXXD,

where X is any hydrophobic amino acid residue, and more particularly:

KLDGKRFLL(I/V)LDDVWC,

or having one or more amino acid substitutions, deletions, and/or insertions thereto provided that such derivatives still function as a kinase-2 motif. A kinase-2 motif functions in nucleotide binding, preferably in binding of ATP/GTP.

The present invention extends to a recombinant gene product that contains the p-Loop, and/or kinase-2, and/or imperfect leucine-rich repeat sequence in any relative combination, or frequency, provided that said recombinant gene product confers, enhances, or facilitates nematode resistance in a cell.

The present invention also extends to a synthetic peptide comprising any part of the amino acid sequence set forth in any one or more of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, or having at least 40% similarity to all or a part thereof.

The recombinant nematode resistance gene product, nematode resistance-like gene product, or functional derivative thereof, may be used to produce immunologically interactive molecules, such as antibodies, or functional derivatives thereof, the only requirement being that the recombinant products are immunologically interactive with antibodies to all or part of said gene product.

According to this aspect, there is provided an antibody that binds to a polypeptide comprising an amino acid sequence which:

(i) confers, enhances, or otherwise facilitates resistance to a nematode in a plant; or

(ii) is substantially the same as the amino acid sequence set forth in any one or more of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, or having at least 40% similarity to all or a part thereof.

Antibodies to a recombinant nematode resistance gene product are particularly useful in the screening of plants for the presence of said gene product. Another aspect of the present invention is, therefore, directed to antibodies to a recombinant nematode resistance gene product or part or fragment thereof. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to a nematode resistance gene product or may be specifically raised to a recombinant nematode resistance gene product. In the case of the latter, the nematode resistance gene product may first need to be associated with a carrier molecule.

Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies and/or the recombinant nematode resistance gene products of the present invention are particularly useful for the immunological screening of nematode resistance gene products in various plants, in monitoring expression of nematode resistance genetic sequences in transgenic plants and as a proprietary tagging system.

In one embodiment, specific antibodies are used to screen for nematode resistance gene products or nematode resistance-like gene products in plants. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of a recombinant nematode resistance gene product.

Both polyclonal and monoclonal antibodies are obtainable by immunisation with a recombinant nematode resistance gene product and either type is utilisable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of recombinant nematode resistance gene product, or antigenic or immunointeractive parts thereof, collecting serum from the animal and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilisable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitised against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art (see, for example, Douillard and Hoffman, 1981; Kohler and Milstein, 1975; Kohler and Milstein, 1976).

The presence of a nematode resistance gene product or nematode resistance-like gene product in a plant or more commonly plant extract may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilised on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time and under conditions sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule.

In this case, the first antibody is raised to a recombinant nematode resistance gene product and the antigen is a nematode resistance gene product in a plant.

The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain nematode resistance gene product and include crude or purified plant extract such as extracts of leaves, roots and stems.

In the typical forward sandwich assay, a first antibody raised against a recombinant nematode resistance gene product is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking, covalent binding or physically adsorption, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any antigen present in the sample to the antibody. Following the incubation period, the reaction locus is washed and dried and incubated with a second antibody specific for a portion of the first antibody. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilising the target molecules in the biological sample and then exposing the immobilised target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detected by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The

complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. The term "reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in enzyme immunoassays

(EIA), the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescene and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

It will be readily apparent to the skilled technician how to vary the above assays and all such variations are encompassed by the present invention.

The present invention further extends to a plant such as a crop plant carrying a non-endogenous nucleic acid molecule encoding or complementary to a nucleic acid molecule encoding a polypeptide which confers, enhances, or otherwise facilitates nematode resistance in said plant. Preferably, the plant is a monocot plant. More preferably the transgenic plant is one or more of the following: *Triticum aestivum, Triticum tauschii*, maize, barley, rye, oats, rice, sorghum, amongst others. Other species are not excluded.

The non-endogenous genetic sequence or transgene may originate from any plant species. Preferably, said genetic sequence is identical to any one or more of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5 or 7, or a functional derivative, fragment, part, complement, homologue, or analogue thereof.

Further, where said genetic sequence or transgene is a cDNA molecule such as set forth in SEQ ID NO: 5 or other nucleic acid molecule which lacks a functional promoter, it may be placed operably under control of the *Cre3* promoter sequence, or under the control of a heterologous promoter sequence. The expression of the transgene may be constitutive or inducible by an external stimulus such as physiological stress, or by addition of a chemical compound, or the expression may be developmentally-regulated, or expressed in a tissue- or cell-specific pattern. Furthermore the transgene may be inserted into or fused to a particular endogenous genetic

sequence. Methods for placing a structural gene operably under the control of a promoter sequence are well-known to those skilled in the art.

A non-endogenous nucleic acid molecule encoding, or complementary to a nucleic acid molecule encoding a polypeptide which confers, enhances or otherwise facilitates nematode resistance in a recipient plant may be introduced into said plant by any one, or a combination of procedures, including *Agrobacterium*-mediated transformation, microparticle bombardment, PEG fusion, electroporation, introgression *via* conventional breeding program, amongst others. It will be readily apparent to one skilled in the art how to produce plants carrying a non-endogenous genetic sequence and perform variations to said procedures.

The present invention extends to the progeny and clonal derivatives of said plant.

The present invention is further described in the following Examples. The embodiments exemplified hereinafter are in no way to be taken as limiting the subject invention.

EXAMPLE 1

PLANT MATERIAL

Experiments were conducted in four resistant and three susceptible accessions of *Triticum tauschii*, as indicated in Table 2.

TABLE 2. Parental *Triticum tauschii* lines used to create crosses for bulked segregant analysis

Accession	Taxon	Reaction to *Heterodera avenae*
AUS 18912	ssp. *eusquarrosa* var. *meyeri*	resistant (*Ccn-D1*)
AUS 18913	ssp. *eusquarrosa* var. *meyeri*	resistant (*Ccn-D1*)
CPI 110810	ssp. *eusquarrosa* var. *typica*, intermediate	resistant (*Ccn-D1*)
CPI 110813	ssp. *eusquarrosa* var. *typica*, intermediate	resistant (*Ccn-D2*)
CPI 110856	ssp. *eusquarrosa* var. *typica*	susceptible
CPI 110825	Intermediate	susceptible
CPI 110795	Intermediate	susceptible

The four segregating progeny analysed were from the following crosses:

1. CPI 110813 x CPI 110795
2. CPI 110810 x CPI 110825
3. AUS 18913 x CPI 110856
4. AUS 18912 x CPI 110856

Segregation for resistance to the nematode *Heterodera avenae* was determined for the F2 progeny of each cross and for 10-12 individuals within each F3 family. A total of 2472 individuals were assessed for reactions to *Heterodera avenae*. Fifty eight to sixty two F2 plants from each cross were chosen on the basis of availability of F3 data and recovery of sufficient DNA to determine marker segregation. Chromosomal locations of polymorphic DNA markers were determined by nullitetrasomic and ditelocentric lines of *Triticum aestivum* cv. Chinese

Spring and wheat-barley addition lines (Islam *et al.*, 1981; Sears, 1966).

EXAMPLE 2

PLANT RESISTANCE

Resistance to the nematode *Heterodera avenae* was assessed using the method of Eastwood *et al.* (1991), except that the white female nematodes were washed from the roots onto a 300 micron sieve, decanted from the sand and counted under a magnifying lamp.

The F3 families in crosses 2,3,4 (see Table 2) segregated for reaction to *Heterodera avenae* at the *Ccn-D1* locus in the ratio 1:2:1 (homozygous resistant: heterozygous resistant: homozygous susceptible), consistent with the ratio expected for Mendelian inheritance of a single dominant autosomal gene.

An average of 0.14 +/- 0.1 white females (cysts) were produced per homozygous resistant *Ccn-D1* plant, compared to 1.38 +/- 0.46 (range 0-8) per resistant line homozygous for the weaker *Ccn-D2* gene. The susceptible lines carried a significantly greater number of cysts, in the range of 10-90 cysts.

EXAMPLE 3

RFLP SEGREGATION ANALYSIS

The prior art teachings of Andersen and Andersen (1973), Slootmaker *et al.* (1974), Rivoal *et al.* (1986), and Aseidu *et al.* (1990) indicated the presence of resistance loci to *Heterodera avenae* on group 2 and group 6 homeologous chromosomes of bread wheat *Triticum aestivum*. One-half of the 60 RFLP markers used were selected because they map to these chromosome locations, thus maximising the probability of selecting an RFLP linked to the *Ccn* loci.

A total of 35 RFLP loci were analysed for linkage to *Ccn-D2* using the segregants from cross 1, and 34 loci for linkage to *Ccn-D1*, using the segregants from cross 2. A total of 17 polymorphic loci were identified on groups 2 and 6 chromosomes, of which 11 segregated with the *Ccn-D1* locus and 6 segregated with the *Ccn-D2* locus.

Multipoint analysis of joint F2/F3 segregation of RFLP loci and *Ccn* resistance revealed a loose linkage between *Ccn-D2* and chromosome 2 markers (Figure 1). No methods used were able to identify polymorphisms to map further, the *Ccn-D2* locus.

Two-point RFLP linkage data set for cross 1 showed 5cM map units between *Ccn-D2* resistance and the RFLP markers *ksuH9* (Gill *et al.*, 1991) and *cslH52* (Lagudah *et al.*,1993), where 1cM is herein defined as 1% recombination between two genetic loci or markers, in a randomly segregating population. *Ccn-D1* was linked to *ksuH9* only (Figure 1).

EXAMPLE 4

PCR AMPLIFICATION OF DNA FROM BULKED F2 SEGREGANTS TO IDENTIFY DNA PRODUCTS LINKED TO *Ccn-D1*

Parental lines and individuals F_2 plants were processed for the isolation of leaf DNA as described in Lagudah *et al.* (1991). Bulked DNA pools for resistance and susceptibility to *Heterodera avenae* were generated from F_2 populations from cross 2 and cross 3 (Table 2). Pooled samples from cross 2 were created by bulking together DNA from 12 homozygous resistant and 13 homozygous susceptible F_2 lines, and in cross 3 from 10 resistant and 13 susceptible homozygotes. Two hundred micrograms of genomic DNA from each sample was sonicated for 6 seconds to give a size range of 0.5-6 kb (Clarke *et al.* 1992). A second set of unsonicated bulked DNA segregants from population 3 was included in the study. Each sample was ethanol precipitated and resuspended in 400 µL of 0.12 M phosphate buffer (pH 6.8).

Molecular markers were generated from bulked homozygous resistant and susceptible F2 DNA pools of cross 2 (Table 2), by PCR amplification of genomic DNA using 260 random 10-mer oligonucleotides (Operon Technologies, Alameda, California) (OPA-01 to OPM 20). Other oligonucleotides included were 4 semirandom primers of 15-18 nucleotides in length, based on the consensus nucleotide sequences of intron-exon splice junctions for plant genes (Weining and Langridge, 1991), designated ISJR1, ISJR2, ISJE3 and ISJE4.

PCRs were performed in 10 µL of a reaction mix containing about 30 ng of template DNA, 0.5 units of Taq polymerase (Boehringer Mannheim GMBH, Germany), 15 ng of primer, 1.5 mM $MgCI_2$ and 1 x reaction buffer (0.2 mM dNTPs, 67 mM Tris-HCl (pH 8.8), 16 nM $(NH_4)_2$ SO_4 0.01% (w/v) gelatin, and 0.45% (v/v) Triton X-100). Samples were loaded into capillary tips and run on a thermocycler (FTS-1 Thermal Sequencer, Corbett Research, Sydney, Australia) under the following conditions: 1. Five cycles of denaturation at 93°C for 30 seconds, annealing at 35°C for 120 seconds, and extension at 72°C for 90 seconds;and

2. Thirty five cycles of denaturation at 92°C for 5 seconds, annealing at 40°C for 20 seconds,

and extension at 72°C for 90 seconds; and

3. One cycle of denaturation at 92°C for 10 seconds, annealing at 40°C for 20 seconds, and extension at 72°C for 5 minutes. When longer and specific oligonucleotide (24 bases) primer pairs were used the annealing temperature was 55°C.

The reaction products were visualised in 1.5% (w/v) agarose gels containing ethidium bromide, using UV light.

EXAMPLE 5

FRACTIONATION OF DNA TO ENRICH FOR LOW-COPY SEQUENCES

Wheat genomic DNA has a large proportion of highly-repeated DNA sequences, which may reduce the probability of detecting low-copy sequences in the genome, using PCR. To improve the intensity of the PCR band generated using ISJE3, DNA was fractionated on hydroxylapatite to remove highly repetitive DNA sequences.

Enrichment for low copy sequences from total genomic DNA was achieved by reannealing the heat denatured DNA (100°C for 10 minutes) at 61°C for at least 20 hours to a $C_o t$ (=moles nucleotide/litre x incubation time [seconds]) value of greater than 100 (Smith and Flavell, 1975). Resistant and susceptible bulks in segregants of cross 2 (Table 2) were annealed to a $C_o t$ value of 145, while those in bulked segregants of cross 3 were annealed for a $C_o t$ value of 120. The samples were then loaded into a 10 mm diameter hydroxylapatite (Biorad DNA grade, Bio-gel HTP) column maintained at 60°C that had been prewashed with several volumes of 0.01M phosphate buffer (pH 6.8). The column was rinsed with 3 mL of 0.01M phosphate buffer and the single-stranded DNA was eluted with one column volume of 0.15M phosphate buffer (60°C) and collected in 15 x 0.5 mL aliquots. The DNA concentration in each aliquot was determined with UV (260 nm) spectrophotometry and three to four of the 0.5 mL aliquotes that contained most of the DNA were further concentrated with butan-2-ol extractions (Sambrook

et al. 1989) and each sample of three to four tubes finally reduced to a single sample of 100-120 μL. Sodium phosphates were removed from the DNA using a Sephadex 50 column equilibrated with TEN buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 8.0). DNA was recovered by ethanol precipitation and diluted to a concentration of 300 ng/μL in TE ready for use in PCR amplification reactions.

The average proportion of DNA recovered after hydroxylapatite fractionation in bulked segregants from cross 2 and cross 3 was 17% and 25%, respectively.

EXAMPLE 6

PCR AMPLIFICATION OF DNA ENRICHED FOR LOW-COPY SEQUENCES FROM BULKED F2 SEGREGANTS

Polymorphic amplification products were obtained using four random 10-mer primers, including ISJE3, in the bulked segregants of cross 2 (see Table 2), an increase in the detectable level of polymorphism from 0.45% to 2%. In each case, the polymorphic PCR product obtained was associated with the presence of a DNA band in the resistant bulk, that was absent from the susceptible bulk (Figure 2).

Polymorphic amplification products were also obtained using eight random 10-mer primers, in the bulked segregants of cross 3 (Table 2). In seven of the polymorphisms, the polymorphic PCR product was associated with the susceptible bulk and only one (OPE-20) was associated with the resistant bulk (Figure 3). Primer OPE-20 produced a consistent polymorphisms in DNA enriched for low-copy nucleotide sequences, but not for total wheat genomic DNA of either the parental genotypes, or the bulked segregating progeny (Figure 3).

EXAMPLE 7

CHROMOSOME LOCATION AND GENETIC LINKAGE OF POLYMORPHIC PCR PRODUCTS

The polymorphic PCR products present in the resistant bulk plus the DNA amplified in the susceptible bulk using primer OPF12 (Figure 2, Figure 3) were excised from low-melting agarose gels, radiolabelled and hybridised to membrane filters containing DNA of parental lines from all populations that had been digested with restriction enzymes.

Polymorphism was observed using a 1kb amplified DNA fragment, designated E-20. All *Ccn-D1* resistant parents showed one to two major hybridising DNA fragments, while the susceptible lines were characterised by a single minor hybridising fragment. The E-20 fragment was subsequently cloned into a "T-overhang" pUC118 plasmid vector, to produce the recombinant plasmid csE20-2.

Genomic and cDNA clones used in the construction of *Triticum tauschii*, wheat, and barley genetic maps (Sharp *et al.*, 1989; Gill *et al.*, 1991; Lagudah *et al.*,1991a; Heun *et al.*, 1991), as well as the csE20-2 clone were used as RFLP markers to analyse joint segregation with *CCN* resistance/susceptibility in the F_2 progenies. Procedures for RFLP analysis were as described by Lagudah *et al.* (1991a). As an aid in selecting potential markers to target the *CCN* resistance region, the genetic map of *Triticum tauschii* produced from the main mapping population (cross F) reported by Lagudah *et al.* (1991a, 1993) was aligned with common reference RFLP loci mapped in the *CCN* population (Figure 1). Linkage analysis of segregating RFLP loci and *CCN* resistance derived from all F2 progenies were carried out using the MAPMAKER program (Lander *et al.*, 1987).

Digested DNA from nullitetrasomic and ditelocentric lines of *Triticum aestivum* cv Chinese Spring were hybridised with the csE20-2 fragment, to determine its chromosome location. The csE20-2 hybridising band was present in all lines except those deleted for chromosome 2D

(Figure 4), including the ditelo 2DS line, indicating that the csE20-2 clone maps to the long arm of chromosome 2D.

The csE20-2 RFLP patterns of parental genotypes of *Triticum tauschii* and resistance or susceptibility to *Heterodera avenae* infestation reveal a complete linkage between *Ccn-D1* and the csE20-2 RFLP marker, for segregating progeny of crosses 2,3, and 4 (Table 2, see Figure 5). Pooled crosses were based on the RFLP analysis of 178 F2 lines and the *Heterodera avenae* reactions observed for 2020 individual F3 plants.

Further RFLP markers were mapped on chromosome 2D, in cross 3. As shown in Figure 1b, both *Ccn-D1* and csE20-2 are approximately 13.8cM from the RFLP marker WG645 (Kleinhofs *et al.*, 1993), 26.3cM from csIH57-1 (Lagudah *et al.*, 1991b), and 46.1cM from ksu H9 (Gill *et al.*, 1991), which have previously been localised on the long arm of chromosome 2. The *Ccn-D1* parent CPI 110813 was mapped in cross 1, and the RFLP variant of csE20-2 shown also to be linked distally, 25cM from csIH57-1, but independent of the *Ccn-D2* locus (Figure 1), suggesting that *Ccn-D1* and *Ccn-D2* are non-allelic nematode resistance genes.

EXAMPLE 8

INTROGRESSION OF THE *Cre3* GENE INTO BREAD WHEAT, *Triticum tauschii*

The *Cre3* gene from *Triticum tauschii* was introgressed into bread wheat *Triticum aestivum* by repeated backcrossing to produce backcross one F4 lines. The csE20-2 RFLP marker was used as a probe to check for linkage among 30 progeny lines, between csE-20 and reaction to *Heterodera avenae* (Figure 6). The 6.5kb RFLP fragment detectable by hybridisation with csE-20 in resistant parental lines of *Triticum tauschii*, was observed in all resistant homozygous and heterozygous progeny of *Triticum aestivum* (Figure 6, lanes 4,7,9,14,15). In contrast, bread wheat lines that were susceptible to infestation with *Heterodera avenae* all lacked the 6.5kb RFLP fragment and exhibited identical RFLP patterns to the parental susceptible line (Figure

6, lanes 3,5,6, 8,10,11,12,13,16,17).

Thus, the introgressed *Cre3* gene was able to confer nematode resistance in bread wheat, *Triticum aestivum*.

EXAMPLE 9

POSITIONAL CLONING OF THE *Cre3*GENE

Data from bulked segregant analysis provide indications of the genetic distance between loci, but no indication of the physical distance, in kb, which may be in the order of several megabases in a highly recombinogenic region such as the 2DL chromosome of *Triticum tauschii*. To determine the physical size (kb) per unit of genetic recombination (cM) in the *Cre3* gene region, a series of chromosome deletion lines for the long arm of chromosome 2D (Endo, 1990) were employed. Genetic data from one deletion line, with 24% of the 2DL chromosome deleted, indicated that there were approximately 300kb of DNA per cM in the *Cre3* region of 2DL. Southern analysis on 178 F2 families, using the csE20-2 RFLP marker as a probe, showed complete cosegregation between *Cre3* and the 6.5kb *Eco*RV RFLP band that hybridises to csE20-2 (0.0% recombination, p=0.05). Thus, the *Cre3* gene was estimated to be within 15kb of csE20-2.

DNA from *Triticum tauschii* **line** AUS 18913 carrying the *Cre3* gene, was size-fractionated to isolate fragments in the range 15-20kb in length, and used to construct a lambda genomic library. The insert from csE20-2 was radioactively labelled and used to isolate three genomic clones, which were sub-cloned for further analysis. One sub-clone, designated CCN4, was shown to overlap 4kb at the 5' end of the 6.5kb *Eco*RV RFLP band (Figure 7). This subclone was also shown to cosegregate with *Ccn* resistance.

The sequenced region of CCN4 clone contains the nucleotide sequences of the 889bp PCR

amplification product E-20, between nucleotide positions 194 and 1082 of the nucleotide sequence set forth in SEQ ID NO: 1. The sequenced region of CCN4 also contains two overlapping reading frames (exons) from nucleotides 1138 to 1614 of the nucleotide sequence set forth in SEQ ID NO: 1 and from nucleotides 1 to 1238 of the nucleotide sequence set forth in SEQ ID NO: 3, with no stop codon at the end of the second exon, suggesting that the clone contains a partial *Cre3* gene sequence.

The first exon encodes a p-Loop (or kinase-1a) motif between nucleotides 1414 and 1437 of the nucleotide sequence set forth in SEQ ID NO: 1, with the amino acid sequence GVGGSGKS. The second exon encodes another nucleotide binding site, kinase-2, between nucleotide positions 73 and 87 and an imperfect leucine-rich repeat sequence from nucleotides 682 to 1238 of the nucleotide sequence set forth in SEQ ID NO: 3.

EXAMPLE 10

ISOLATION OF A ROOT-EXPRESSED NEMATODE RESISTANCE cDNA CLONE

Total RNA was extracted from pooled root samples taken from seedlings of *T. tauschii*, AUS 18913, (resistant source of *Cre*3 gene) grown for 10, 15 and 25 days. Polyadenylated mRNA was prepared using an oligodT sequence coupled to a magnetic bead (Dynal®). First and second cDNA strand synthesis was performed using manufacturers instructions (Stratagene®) and cloned into a lambda ZAP vector. The *T. tauschii* AUS18913 cDNA library was screened using the genomic clone containing the nucleotide sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 3. Eight size classes of cDNA clones ranging from 1.7 to 2.6 kb with strong hybridising intensity to SEQ ID NO: 1 and SEQ ID NO: 3 sequences were identified. The complete nucleotide sequence of the 2.6kb cDNA was determined and compared with sequences from the remaining seven clones. The sequence comparison revealed that all seven clones were identical and represented shorter fragments of the 2.6kb cDNA clone.

Nucleotide sequence comparison between the 2.6kb cDNA and the SEQ ID NO: 1 and SEQ ID NO: 3 sequences revealed 87% identity. The regions of sequence identity to the 2.6kb cDNA was localised to the reading frames present in SEQ ID NO: 1 and SEQ ID NO: 3. Thus the csE20 fragment which lacks an open reading frame was incapable of detecting candidate *Cre*3 nematode resistance genes expressed in roots.

The 2.6kb cDNA sequence set forth as SEQ ID NO: 5 represents a partial gene and encodes a reading frame of 768 amino acids in addition to 324 baes of the 3′ untranslated region. The 768 amino acids contains a P loop (base positions 191 to 217) or ATP/GTP binding site, kinase 2 domain (base positions 443 to 457) and a leucine rich region (base positions 1202 to 2260). When the 2.6kb root cDNA clone was used as a hybridisation probe to *Dra* I restricted genomic DNA of the bread wheat variety, Chinese Spring, and its nulliitetrasomic chromosome stocks, an identical hybridisation pattern to SEQ ID NO: 1 and SEQ ID NO: 3 occurred. This analysis confirmed the chromosome 2D location of SEQ ID NO: 5 sequence as identical to SEQ ID NO: 1 and SEQ ID NO: 3. The SEQ ID NO: 1 and SEQ ID NO: 3 sequences cosegregates with cereal cyst nematode resistance at the *Cre*3 locus in 178 F2 families of *T. tauschii*. Thus the root expressed gene sequence, SEQ ID NO: 5, is clustered with the genomic sequence, SEQ ID NO: 1 and SEQ ID NO: 3 at the *Cre*3 locus.

EXAMPLE 11

COMPLEMENTATION ANALYSIS

Wheat are transformed with a genetic construct comprising the complete open reading frame of the *Cre*3 gene, essentially according to the established transformation and regeneration procedures of Weeks *et al*, 1993, Nehra *et al*, 1994 and Becker *et al*, 1994. The complete genomic sequence of the *Cre*3 gene including at least lkb of 5′ untranslated sequence comprising nucleotide sequence information required for efficient transcription and the 3′ untranslated region, are cloned into a plasmid vector.

Scutellar tissue of immature wheat embryos derived from the cereal cyst nematode susceptible cultiva Gabo are co-transformed with the *Cre*3 gene construct and the plasmid pEmuKON (Chamberlain *et al*, 1994) which comprises an efficient promoter for gene expression in cereal cells operably linked to the *npt*II gene (conferring resistance to aminoglycoside antibiotics) and a termination signal.

Stable tansformants are selected on paromomycin-containing media and the presence of the *Cre*3 gene construct verified subsequently by standard procedures (polymerase chain reaction Northern blotting and Southern blotting using *Cre*3-derived nucleotide sequences; Ausubel *et al*, 1987). Transformed tissue containing the introduced *Cre*3 gene sequences are placed on regeneration medium and regenerated into whole plants. Transformed plants are retained for further analysis. The roots of transformed plants are assayed for expression of the introduced *Cre*3 gene, using northern blot hybridisation, reverse-transcription PCR or other procedure suitable for the detection of *Cre*3 gene transcripts in root tissue. Such methods are well-known to those skilled in the art.

Roots expressing the *Cre*3 gene are inoculated with juvenile cereal cyst nematodes of the Australian pathotype, essentially as described by Eastwood *et al.* (1991). Non-transformed isogenic wheat are similarly infected in a parallel experiment. Juveniles of cyst nematodes normally invade plant roots and migrate to the vascular tissue where they induce syncytia formation in a compatible host plant interaction. Alternatively, in resistance plants, host plant mechanisms lead to breakdown of syncytia with the production of large, vacuolated syncytia which possesses degenerated membranes in the roots of infected plants, at about 15 days post-invasion of the roots by the juvenile nematode.

Significantly fewer cysts (0-8) are observed on the roots of wheat plants expressing the introduced *Cre*3 gene sequence, compared to high cyst counts (>40) on untransformed wheat lines, confirming the ability of the introdued *Cre*3 gene to confer resistance to the cereal cyst nematode in a compatible interaction.

EXAMPLE 12

PRODUCTION OF POLYCLONAL ANTIBODIES AGAINST CRE3

Antibodies are raised against an *E. coli* fusion protein composed of the carboxy-terminal part of glutathione S-transferase and the 768 amino acids of the CRE3 protein set forth in SEQ ID NO: 6 or alternatively, the 797 amino acids of the full-length CRE 3 protein set forth in SEQ ID NO: 8. The vectors encoding this construct are generated by cloning the 2654 bp fragment of the cDNA shown in SEQ ID NO: 5 or the entire *Cre*3 open reading frame of SEQ ID NO: 7, into a pGEX plasmid (Pharmacia, Uppsala) producing an in-frame fusion with a partial cDNA encoding about 250 amino acids (27.5 kD) of glutathione S-transferase, placed operably under the control of the *lac* promoter. The construct is transformed into *E. coli* cells. After induction with IPTG (1mM final concentration) the expressed fusion protein is purified on Glutathione-Sepharose 4B (Pharmacia, Uppsala) according to the manufacturer's instruction. The apparent molecular weight of the fusion polypeptide in SDS-PAGE is approximately 110-115kD.

The purified fusion protein (100 µg) is subcutaneously injected into a female rabbit using Freund's adjuvant as described by Harlow and Lane (1988). After the second boost, antiserum is collected and used in Western blotting and ELISA tests.

Antisera are screened by ELISA (Enzyme-Linked Immunosorbent Assay) using purified CRE-GST fusion polypeptide. The ELISA is performed as follows: partially purified CRE-GST fusion polypeptide in coating buffer (Na_2CO_3, 15 mM; $NaHCO_3$, 35 mM, $CaCl_2$, 0.1 mM; final pH 9.2) is incubated overnight at 4°C in Nunc-Immuno Plate Maxisorb (Nunc-Kamstrup, Denmark). After 4 rinses in washing butter (Tris/HCl, 20 mM; NaCl, 120 mM; Tween 20, 0.05%; final pH 7.4), the primary antibody (diluted serially between 1:250 and 1:5000) is added for 1 hour at 37°C, wells are washed 4 times with washing buffer, and then incubated with peroxidase-conjugated goat anti-rabbit IgG (Sigma, St. Louis, MO) at a 1:1000 dilution for an additional 1 hour at 37°C. Wells are washed 4 times using washing buffer substrate solution

(0-phenylenediamine dihydrochloride, 0.05 ml, 0.4 mg/ml, Sigma) dissolved in 0.1 M sodium citrate (pH 4.5) containing H202 (0.0006%) is added, and color is allowed to develop. The reaction is stopped by adding H_2SO_4 (0.025 ml; 8N), and absorbance at 490 mm is then measured.

For Western blotting, total lysate of *E. coli* clones expressing either the pGEX encoded GST protein alone or the CRE3-GST fusion protein before and after induction with IPTG are separated on SDS-PAGE. The bacteria are harvested by centrifugation and resuspended in Laemmli sample buffer (4% SDS, 125 mM Tris pH 6.8, 10% β-mercapto ethanol, 10% glyceol, 0.02% bromphenol blue) to a concentration of $2x10^7$ cells/µl of sample buffer. After boiling for 5 min, 10 µl of this SDS lysate are applied to SDS-PAGE (10% polyacrylamide), transferred to nitrocellulose using standard techniques (Sambrook *et al.*, (1989), 200 mA, 40 min), blocked with PBS containing 2% nonfat dry milk, 0.02% Tween 20, washed (PBS 0.02% Tween 30, 0.2% gelatin) and probed using polyclonal antiserum raised against the CRE3-GST fusion protein preimmune serum (both sera diluted at least 1:1000 in PBS containing 0.05% Tween 20 and 0.2% gelatine). A second antibody (horseradish peroxidase coupled goat anti-rabbit IgG (BioRad, Munich)) is diluted 1:20000 fold in PBS 0.02% Tween 20, 0.2% gelatin. Peroxidase reaction is performed using the ECL Kit (Amersham International) to detect bound antibody. The antiserum strongly recognizes a band at ~11O-115 kD corresponding to the molecular weight of the CRE3-GST fusion protein, which is not detected by the preimmune serum. There is no cross reactivity of the anti-CRE3-GST antiserum with the recombinant GST protein.

EXAMPLE 13

WESTERN BLOT ANALYSIS OF THE CRE3 PROTEIN

Root protein extracts are obtained from infected plants of the wheat cultivar Gabo, transformed with the *Cre3* gene as described in Example 11 and which has improved resistance to the

Australian pathotype of the cereal cyst nematode compared to untransformed *Triticum aestivum* cv Gabo. Root protein extracts are also obtained from infected untransformed Gabo plants. Plants are infected with juvenile nematodes, according to Eastwood *et al* (1991). Soluble protein is fractionated on SDS-PAGE, transferred to nitrocellulose and probed with antisera to CRE3, as described in Example 12, to identify the CRE3 polypeptide. A cross-reactive band of approximately 80kDa molecular weight, corresponding to the CRE3 polypeptide, is only observed in protein extracts obtained from transformed plants which express the introduced *Cre*3 gene.

Those skilled in the art will appreciate that the invention described herein 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 compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

(ii) TITLE OF INVENTION: Genetic Sequences conferring nematode resistance in plants and uses therefor

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT International

(B) FILING DATE: 29-MAR-1996

(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1614 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1138..1614

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATCCGGAGTC GTGGTTGTGG CCGCTGTCTT CCACTTGTGT ACGGTTTTCC CGTTGTCGAG 60

TGGCCTTCCT TCTTTCGGCC TTGCGGTTAA CAGGTTAGGG GCGCCGGTAG TGTTGTGGTG 120

TGTCATCTTT GGTGAGCTTA TTAATGTCGG CTTGTGGTGT CACATGGTGG TGGGTTCTCA 180

TTTAACGTCG AGGCGGTGAC CTCAGGTGGC GGTCCGTCAA GTCGGCTTCT CAACAAGCGT 240

CTTGGCGGCG GCCGTGGTGG CATTGTTTGG TCGTGTGGAC GGCGGAGGAT GCTAAGTTGG 300

GTGATCCTAG TGTCGGTGGT GCTTTGGCAC TGGCGGTGCC CAGATCGTGT TCTAGTGGTC 360

TGGCTTGGTA GTGACATGTT CACCTCGGTG TGGGCTGGTG CTCGGGAGGC CTAGTGTGGC 420

GTGGAAGAGT GCAACAAGGT CCGGCGATTT TCCTTGGAGC GAACTTTCAT CTTTGTTGGT 480

AGTTTAGGTA GCTTTGTGTT AGGGTGTGGT TCCTCCTATT TTCTTGTTTT TCTTTGATCT 540

GCTTTGTAAG AGGGTCTCCT CATCACCTTG TATCTCTTTG GTCGTGGTTC TTTATATATA 600

AAGCGGGGCC GAAGTAATTT TTGGTAGGAT TCACCAACAT CATGAGAACA AAGCACGAAA 660

ATATAGTAGT ACGGTAGTAG AGAATGTTAA TTCCTCTTGT ATCCAATGTT ATCTCTTGTA 720

TACCGTGATT CTTGCCCATC AGTATTCTCT TAGGCTTCTG TTAGCGAAAC AAAATTCCTT 780

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CTTCCAAATT ACCAAACTTC TAGCTCATGA GTATGTTCAT ATAGTGCGCG GAGGATGTGC      840

GTGCCACATG CGTGCGCATG ATGGTGTTGA TAGACTAACA TGTGTGTGTG GTTTCTGTGT      900

GACTGCCTTG TGTTCTCTGC AAAACTAGGC TTTTGGCAAG TCAGTCTAGA TCCCTCGGCG      960

TATTTTTTAG AAGTATACCG GAGAGTAGAC GAATTCCCTA TATTACATTA GTCTTTTTTC     1020

TTTATTTAGT GTCATGATAG TTTATGTGAA GATAAAATCT CTCTTCTGTA ATGGTCACCT     1080

ATAATTTATT TTTTAAAGAT TTCTCTCTTG TTATTTGGGG TCTCGCAGGA GAGTGGC        1137

ATG TCA AAG AAA AAG TTG ATA GAC AGC CTG AAG AAG ATA GAA GAC AAT        1185
Met Ser Lys Lys Lys Leu Ile Asp Ser Leu Lys Lys Ile Glu Asp Asn
 1               5                  10                  15

ATA AAT GAA GCA CAC CAA ATT CTG GAT AAG CTT AAC TTG TCA AGC ATA       1233
Ile Asn Glu Ala His Gln Ile Leu Asp Lys Leu Asn Leu Ser Ser Ile
            20                  25                  30

AGT GAT GGA AAT AGA AGA CAT GTA ATG GAT GCT AAT CGT CCT ACT ACT       1281
Ser Asp Gly Asn Arg Arg His Val Met Asp Ala Asn Arg Pro Thr Thr
        35                  40                  45

GCA GTT TCT CCG CAT AAA GTA CTT GGT CGA GAT AAT GAG CGC GAC AAG       1329
Ala Val Ser Pro His Lys Val Leu Gly Arg Asp Asn Glu Arg Asp Lys
    50                  55                  60

ATC ATA AAA ATG CTT CAC AAA AAT GAA GGT GGT GTT CAA CCA AGC ACC       1377
Ile Ile Lys Met Leu His Lys Asn Glu Gly Gly Val Gln Pro Ser Thr
65                  70                  75                  80

AGC AAC AGT CTA TGC TTT TCT GTA ATT GGC ATA CAT GGA GTT GGT GGG       1425
Ser Asn Ser Leu Cys Phe Ser Val Ile Gly Ile His Gly Val Gly Gly
                85                  90                  95

TCA GGG AAA TCT ACC CTT GCA CAA TTG GTT TAT GCC CAT GAG GAA AAA       1473
Ser Gly Lys Ser Thr Leu Ala Gln Leu Val Tyr Ala His Glu Glu Lys
            100                 105                 110
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GAC AAG AAA GAC AAC AAG GAA GGT CAC TTC GAC CTG GTT ATG TGG GTC 1521
Asp Lys Lys Asp Asn Lys Glu Gly His Phe Asp Leu Val Met Trp Val
115 120 125

CAT GTC TCT CAG AAT TTT AGT GTG GGC GAC ATC TTC AAG GAG TTG TAT 1569
His Val Ser Gln Asn Phe Ser Val Gly Asp Ile Phe Lys Glu Leu Tyr
130 135 140

GAG GCA GCT TCA GAG CCT AAG GTT CCA TGC CAT TCA ATA ACA TG 1614
Glu Ala Ala Ser Glu Pro Lys Val Pro Cys His Ser Ile Thr
145 150 155

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 158 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Lys Lys Lys Leu Ile Asp Ser Leu Lys Lys Ile Glu Asp Asn
1 5 10 15

Ile Asn Glu Ala His Gln Ile Leu Asp Lys Leu Asn Leu Ser Ser Ile
20 25 30

Ser Asp Gly Asn Arg Arg His Val Met Asp Ala Asn Arg Pro Thr Thr
35 40 45

Ala Val Ser Pro His Lys Val Leu Gly Arg Asp Asn Glu Arg Asp Lys
50 55 60

Ile Ile Lys Met Leu His Lys Asn Glu Gly Gly Val Gln Pro Ser Thr
65 70 75 80

Ser Asn Ser Leu Cys Phe Ser Val Ile Gly Ile His Gly Val Gly Gly
85 90 95

Ser Gly Lys Ser Thr Leu Ala Gln Leu Val Tyr Ala His Glu Glu Lys
100 105 110

Asp Lys Lys Asp Asn Lys Glu Gly His Phe Asp Leu Val Met Trp Val
115 120 125

His Val Ser Gln Asn Phe Ser Val Gly Asp Ile Phe Lys Glu Leu Tyr
130 135 140

Glu Ala Ala Ser Glu Pro Lys Val Pro Cys His Ser Ile Thr
145 150 155

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1238 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1238

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGT TCC ATG CCA TTC AAT AAC ATG AAT TCC TTG GGA AAA GAA TTG GAA 48
Gly Ser Met Pro Phe Asn Asn Met Asn Ser Leu Gly Lys Glu Leu Glu
1 5 10 15

AGG AAA CTA GAT GGA AAG CGA TTC CTT CTG ATA CTG GAT GAT GTC TGG 96
Arg Lys Leu Asp Gly Lys Arg Phe Leu Leu Ile Leu Asp Asp Val Trp
20 25 30

TGC AAT AAG GAT GTC AGC GAT CAG AAT CTA CCA GAG TTA CTT TCT CCA 144
Cys Asn Lys Asp Val Ser Asp Gln Asn Leu Pro Glu Leu Leu Ser Pro
35 40 45

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TTG AAG GTT GGA AAG AGA GGA AGC AAG ATC CTA GTG ACG ACT CGA AGT        192
Leu Lys Val Gly Lys Arg Gly Ser Lys Ile Leu Val Thr Thr Arg Ser
     50                  55                  60

AAA TAT GCA TTA CCG GTT CTA GGT CCT GGT GTG AGA TGT ACT GCC ATA        240
Lys Tyr Ala Leu Pro Val Leu Gly Pro Gly Val Arg Cys Thr Ala Ile
 65                  70                  75                  80

CCA GTA CCT GAG TTT GAT GAT ACC GCC TTC TTC GAG CTA TTC ATG CAC        288
Pro Val Pro Glu Phe Asp Asp Thr Ala Phe Phe Glu Leu Phe Met His
                 85                  90                  95

TAT GCC CTG GAA GAA GGC CAA GAT CAG AGC CTG TTC TGT ATA ATT GGT        336
Tyr Ala Leu Glu Glu Gly Gln Asp Gln Ser Leu Phe Cys Ile Ile Gly
            100                 105                 110

GAG GAG ATA GCG AAA AAG CTG AAG GGG TCA CCT CTA GCT GCC AGA ACA        384
Glu Glu Ile Ala Lys Lys Leu Lys Gly Ser Pro Leu Ala Ala Arg Thr
        115                 120                 125

GTG GGA GGA AAT TTA CGT CGA CAA CCA GAT GTC GAC CAT TGG AGA AGA        432
Val Gly Gly Asn Leu Arg Arg Gln Pro Asp Val Asp His Trp Arg Arg
    130                 135                 140

GTC AGA GAT CAA GAC CTT TTC AAG GTA TGG GGA GGG CCT CTG TGG TGG        480
Val Arg Asp Gln Asp Leu Phe Lys Val Trp Gly Gly Pro Leu Trp Trp
145                 150                 155                 160

AGC TAC TAT CAG CTT GGT GAG CAG GCT AGG CGT TGC TTT GCT TAT TGC        528
Ser Tyr Tyr Gln Leu Gly Glu Gln Ala Arg Arg Cys Phe Ala Tyr Cys
                165                 170                 175

AGT ATT TTT CCT AGG AGA CAT CGC CTG TAC CGT GAT GAC CTA GTT AGA        576
Ser Ile Phe Pro Arg Arg His Arg Leu Tyr Arg Asp Asp Leu Val Arg
            180                 185                 190

CTT TGG GTT GCA GAA GGG TTC ATA AGA AGC ACA GAT GAA GGG GCG GAT        624
Leu Trp Val Ala Glu Gly Phe Ile Arg Ser Thr Asp Glu Gly Ala Asp
        195                 200                 205
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ATT GAA GAT GTT GGT CAG GAA ATA TTT AAT GAA CTA TTG TCG ATC TCG      672
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    210                 215                 220

TTT CTT CAA CCA GGA GGC ACG AAC AAC TCT TAT CTC GCC GGC ATT TAT      720
Phe Leu Gln Pro Gly Gly Thr Asn Asn Ser Tyr Leu Ala Gly Ile Tyr
225                 230                 235                 240

TAT GGC AAG GAA TAC TAT TTA GTT CAT GAT CTG CTG CAC GAT TTA GCA      768
Tyr Gly Lys Glu Tyr Tyr Leu Val His Asp Leu Leu His Asp Leu Ala
                245                 250                 255

GAG GCA GTA GCT GGC AGT GAC TGC TTC AGA ATT GAC AAT AAC GCG AGC      816
Glu Ala Val Ala Gly Ser Asp Cys Phe Arg Ile Asp Asn Asn Ala Ser
            260                 265                 270

CAG AAA GGA GGA GGA TGG ACA AGA GAT GTT CCC CGA GAC GTT CGG CAT      864
Gln Lys Gly Gly Gly Trp Thr Arg Asp Val Pro Arg Asp Val Arg His
        275                 280                 285

CTT TTT GTT CAG AGT TAT GAT GCA ACA TTG ATT ACT GAA AAG ATT CTT      912
Leu Phe Val Gln Ser Tyr Asp Ala Thr Leu Ile Thr Glu Lys Ile Leu
    290                 295                 300

GAA TTG AGA AAG TTA CAC ACT CTT ATC ATT TAT AGT GTT GGA GGG GAT      960
Glu Leu Arg Lys Leu His Thr Leu Ile Ile Tyr Ser Val Gly Gly Asp
305                 310                 315                 320

ACA CCA GTT GAG GAA ATA GTC ATC AAG AAC ATA CTC AAG AGT CTG CCA     1008
Thr Pro Val Glu Glu Ile Val Ile Lys Asn Ile Leu Lys Ser Leu Pro
                325                 330                 335

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Lys Leu Arg Val Leu Ala Ile Ala Ser Ser Leu Glu Asp Ser Ala Phe
            340                 345                 350

ATT TGG AAA CCA GAT ACA TTC TCT GTC CCA GAA TCT GTT GGT CAA TTG     1104
Ile Trp Lys Pro Asp Thr Phe Ser Val Pro Glu Ser Val Gly Gln Leu
        355                 360                 365
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AAA CAT CTG CGC TAT CTT GCT TTC CGG ACA GAT AGA GGA TGC CGA GTA      1152
Lys His Leu Arg Tyr Leu Ala Phe Arg Thr Asp Arg Gly Cys Arg Val
    370                 375                 380

ATT TTA CCA AGC AGT CTA AAC CAG CTT TAC CAG ATG CAA CTG CTA GAT      1200
Ile Leu Pro Ser Ser Leu Asn Gln Leu Tyr Gln Met Gln Leu Leu Asp
385                 390                 395                 400

TTT GGT CAA TGC CAT GAT TTG GTA TTT TGC TGT GAT GA                   1238
Phe Gly Gln Cys His Asp Leu Val Phe Cys Cys Asp
                405                 410
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 412 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Gly Ser Met Pro Phe Asn Asn Met Asn Ser Leu Gly Lys Glu Leu Glu
  1               5                  10                  15

Arg Lys Leu Asp Gly Lys Arg Phe Leu Leu Ile Leu Asp Asp Val Trp
             20                  25                  30

Cys Asn Lys Asp Val Ser Asp Gln Asn Leu Pro Glu Leu Leu Ser Pro
         35                  40                  45

Leu Lys Val Gly Lys Arg Gly Ser Lys Ile Leu Val Thr Thr Arg Ser
     50                  55                  60

Lys Tyr Ala Leu Pro Val Leu Gly Pro Gly Val Arg Cys Thr Ala Ile
 65                  70                  75                  80

Pro Val Pro Glu Phe Asp Asp Thr Ala Phe Phe Glu Leu Phe Met His
                 85                  90                  95
```

Tyr Ala Leu Glu Glu Gly Gln Asp Gln Ser Leu Phe Cys Ile Ile Gly
100 105 110

Glu Glu Ile Ala Lys Lys Leu Lys Gly Ser Pro Leu Ala Ala Arg Thr
115 120 125

Val Gly Gly Asn Leu Arg Arg Gln Pro Asp Val Asp His Trp Arg Arg
130 135 140

Val Arg Asp Gln Asp Leu Phe Lys Val Trp Gly Gly Pro Leu Trp Trp
145 150 155 160

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

Ser Ile Phe Pro Arg Arg His Arg Leu Tyr Arg Asp Asp Leu Val Arg
180 185 190

Leu Trp Val Ala Glu Gly Phe Ile Arg Ser Thr Asp Glu Gly Ala Asp
195 200 205

Ile Glu Asp Val Gly Gln Glu Ile Phe Asn Glu Leu Leu Ser Ile Ser
210 215 220

Phe Leu Gln Pro Gly Gly Thr Asn Asn Ser Tyr Leu Ala Gly Ile Tyr
225 230 235 240

Tyr Gly Lys Glu Tyr Tyr Leu Val His Asp Leu Leu His Asp Leu Ala
245 250 255

Glu Ala Val Ala Gly Ser Asp Cys Phe Arg Ile Asp Asn Asn Ala Ser
260 265 270

Gln Lys Gly Gly Gly Trp Thr Arg Asp Val Pro Arg Asp Val Arg His
275 280 285

Leu Phe Val Gln Ser Tyr Asp Ala Thr Leu Ile Thr Glu Lys Ile Leu
290 295 300

Glu Leu Arg Lys Leu His Thr Leu Ile Ile Tyr Ser Val Gly Gly Asp
305 310 315 320

Thr Pro Val Glu Glu Ile Val Ile Lys Asn Ile Leu Lys Ser Leu Pro
325 330 335

Lys Leu Arg Val Leu Ala Ile Ala Ser Ser Leu Glu Asp Ser Ala Phe
340 345 350

Ile Trp Lys Pro Asp Thr Phe Ser Val Pro Glu Ser Val Gly Gln Leu
355 360 365

Lys His Leu Arg Tyr Leu Ala Phe Arg Thr Asp Arg Gly Cys Arg Val
370 375 380

Ile Leu Pro Ser Ser Leu Asn Gln Leu Tyr Gln Met Gln Leu Leu Asp
385 390 395 400

Phe Gly Gln Cys His Asp Leu Val Phe Cys Cys Asp
405 410

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2627 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum tauschii
(B) STRAIN: AUS 18913
(F) TISSUE TYPE: Root

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..2305

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 2306..2627

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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G TCA AGC ATA AGT GAT GGC AAT ATA CGA CAC ACA ATG GTT GTC AAT          46
  Ser Ser Ile Ser Asp Gly Asn Ile Arg His Thr Met Val Val Asn
    1               5                  10                  15

CCT ACG ACT ACC GCA GTT TCC CCG CAA AAA GTT TTT GGT CGA GAT AAT        94
Pro Thr Thr Thr Ala Val Ser Pro Gln Lys Val Phe Gly Arg Asp Asn
                20                  25                  30

GAT CGC GAC AAG ATC ATA GCA ATG CTT CAT GAA AAG GAA GGT GGT CTT        142
Asp Arg Asp Lys Ile Ile Ala Met Leu His Glu Lys Glu Gly Gly Leu
            35                  40                  45

GAT CCA AGC ACT AGC AAA GGT CTA TGT TTT TCT GTA ATT GGC ATA CAT        190
Asp Pro Ser Thr Ser Lys Gly Leu Cys Phe Ser Val Ile Gly Ile His
        50                  55                  60

GGA GTC AGC GGG TCT GGG AAA TCT ACC CTT GCA CAG CTT GTT TAT GCC        238
Gly Val Ser Gly Ser Gly Lys Ser Thr Leu Ala Gln Leu Val Tyr Ala
    65                  70                  75

CAC GAG AAA AAT GAC AAG CAA GAC AAC AAG GAA GAC CAT TTC GAC CTT        286
His Glu Lys Asn Asp Lys Gln Asp Asn Lys Glu Asp His Phe Asp Leu
80                  85                  90                  95

GTT ATG TGG GTT CAT GTC TCT CAG GAT TTT AGT GTG TGG GGC ATC TTC        334
Val Met Trp Val His Val Ser Gln Asp Phe Ser Val Trp Gly Ile Phe
                100                 105                 110

ANG GAG TTG TAT GAG GCA GCT TCA GAT CCT AAG GTT CCA TGC CCT CAA        382
Xaa Glu Leu Tyr Glu Ala Ala Ser Asp Pro Lys Val Pro Cys Pro Gln
            115                 120                 125

TTT AAT AAC TTG ANT GCC TTG GAA GAA GAA CTG GAG AGG AAA CTA GAT        430
Phe Asn Asn Leu Xaa Ala Leu Glu Glu Glu Leu Glu Arg Lys Leu Asp
        130                 135                 140
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GGA AAG CGA TTC CTT CTG GTA CTG GAT GAT GTC TGG TGC AAT GCG GAT      478
Gly Lys Arg Phe Leu Leu Val Leu Asp Asp Val Trp Cys Asn Ala Asp
    145                 150                 155

GTT GGT AAC CAG GAG CTA CCA AAG TTA CTT TCT CCA CTG AAG AAA GGA      526
Val Gly Asn Gln Glu Leu Pro Lys Leu Leu Ser Pro Leu Lys Lys Gly
160                 165                 170                 175

AAG AAA GGA AGC AAG ATC CTA GTG ACA ACT CGA AGT AAA TAT GCA CTA      574
Lys Lys Gly Ser Lys Ile Leu Val Thr Thr Arg Ser Lys Tyr Ala Leu
                180                 185                 190

CCG GAT CTA TGT CCT GGT GTG AGA TAT ACT GCC ATG CCG ATA ACT GAG      622
Pro Asp Leu Cys Pro Gly Val Arg Tyr Thr Ala Met Pro Ile Thr Glu
            195                 200                 205

GTT GAT GAT ACC GCC TTC TTT GAG TTG TTC ATG CAT TAT GCC CTC GAA      670
Val Asp Asp Thr Ala Phe Phe Glu Leu Phe Met His Tyr Ala Leu Glu
        210                 215                 220

GAT GGC CAA GAT CAA AGC ATG TTC CAG AAC ATT GGG GTT GAG ATT GCA      718
Asp Gly Gln Asp Gln Ser Met Phe Gln Asn Ile Gly Val Glu Ile Ala
    225                 230                 235

AAA AAG CTG AAG GGG TCA CCT TTA GCA GCT AGA ACA GTG GGT GGA AAT      766
Lys Lys Leu Lys Gly Ser Pro Leu Ala Ala Arg Thr Val Gly Gly Asn
240                 245                 250                 255

TTA CGT CGA CAG CAA GAT GTT GAC CAT TGG AGA AGA GTC GGA GAT CAA      814
Leu Arg Arg Gln Gln Asp Val Asp His Trp Arg Arg Val Gly Asp Gln
                260                 265                 270

GAC CTT TTC AAG GTA TGG ACG GGA CCT CTG TGG TGG AGC TAC TAT CAG      862
Asp Leu Phe Lys Val Trp Thr Gly Pro Leu Trp Trp Ser Tyr Tyr Gln
            275                 280                 285

CTT GGT GAG CAG GCT AGG CGT TGC TTT GCT TAC TGC AGT ATT TTT CCT      910
Leu Gly Glu Gln Ala Arg Arg Cys Phe Ala Tyr Cys Ser Ile Phe Pro
        290                 295                 300
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AGG AGA CAT CGC TTG TAC CGY GAT GAA TTA GTT AGA CTC TGG ATG GCA 958
Arg Arg His Arg Leu Tyr Arg Asp Glu Leu Val Arg Leu Trp Met Ala
305 310 315

GAA GGG TTC ATA AGA AAC ACA GAT GAA GGG GCG GAT GCT GAA GAC GTT 1006
Glu Gly Phe Ile Arg Asn Thr Asp Glu Gly Ala Asp Ala Glu Asp Val
320 325 330 335

GGT CTG GGA ATA TTT AAT GAA CTA TTG TCG ATA TCA TTT CTT CAA CCA 1054
Gly Leu Gly Ile Phe Asn Glu Leu Leu Ser Ile Ser Phe Leu Gln Pro
340 345 350

GGA GGC CAG GAC TGG TAC AAT CAT GGC AAG GAA TAC TAT TTA GTT CAT 1102
Gly Gly Gln Asp Trp Tyr Asn His Gly Lys Glu Tyr Tyr Leu Val His
355 360 365

GAT TTG CTG TAT GAT TTA GCA GGG GCA GYA GCT GGA ACT GAC TGC TTC 1150
Asp Leu Leu Tyr Asp Leu Ala Gly Ala Xaa Ala Gly Thr Asp Cys Phe
370 375 380

AGA ATT GAC AAT AAC ATG ATC CAG ACA GGA GAA AGC TGG GCA AAA GAT 1198
Arg Ile Asp Asn Asn Met Ile Gln Thr Gly Glu Ser Trp Ala Lys Asp
385 390 395

GTT CCC AGA GAC GTT CGC CAT CTT TTT GTT CAG AGT TAT GAT GCA NCN 1246
Val Pro Arg Asp Val Arg His Leu Phe Val Gln Ser Tyr Asp Ala Xaa
400 405 410 415

TTG AGT ACA GGG AGA TTG CTT GTA TTG GAG GAN TTA CAC ACA CTC GTC 1294
Leu Ser Thr Gly Arg Leu Leu Val Leu Glu Xaa Leu His Thr Leu Val
420 425 430

ATT TAT AGT GTT GGA GGG GAT ACA ACA GTT GAG GAA ATA GTC ATC AAG 1342
Ile Tyr Ser Val Gly Gly Asp Thr Thr Val Glu Glu Ile Val Ile Lys
435 440 445

AAC ATA CTC AAG AGT CTG CCT AAA CTG CGG GTA CTA GCA ATA GCT TTA 1390
Asn Ile Leu Lys Ser Leu Pro Lys Leu Arg Val Leu Ala Ile Ala Leu
450 455 460

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TGT CTG GAA AAG GAT GGA TTT ATN TGT AGA CCA AAT ATA TTG TCT GTT      1438
Cys Leu Glu Lys Asp Gly Phe Xaa Cys Arg Pro Asn Ile Leu Ser Val
 465                 470                 475

CCA GAA TCT ATT AGT CAA TTA AAA CAT CTA CGA TAT CTT GCT TTC CGG      1486
Pro Glu Ser Ile Ser Gln Leu Lys His Leu Arg Tyr Leu Ala Phe Arg
480                 485                 490                 495

ACA GAT ATT GAA TGC AGA GTA ATT TTA CCA AGC AGT CTA AAC CAG CTT      1534
Thr Asp Ile Glu Cys Arg Val Ile Leu Pro Ser Ser Leu Asn Gln Leu
            500                 505                 510

TAC CAG ATG CAA CTG CTA GAT TTT GGT GTC TGC ATG AAT TTG GTA TTT      1582
Tyr Gln Met Gln Leu Leu Asp Phe Gly Val Cys Met Asn Leu Val Phe
        515                 520                 525

TCC TGT GGT GAT CTT ATC AAC TTG CGG CAT GTA TGC AGC GGT CCT GGA      1630
Ser Cys Gly Asp Leu Ile Asn Leu Arg His Val Cys Ser Gly Pro Gly
    530                 535                 540

TTG CAA TTT TCA AAC ATC GGT AGG CTT GTC TCA CTC CAA ACA ATC CCA      1678
Leu Gln Phe Ser Asn Ile Gly Arg Leu Val Ser Leu Gln Thr Ile Pro
545                 550                 555

GCA TTC AAA GTA AGT CAT GAA CAA GGA CAT GAG GCA AAG CAG TTG AGG      1726
Ala Phe Lys Val Ser His Glu Gln Gly His Glu Ala Lys Gln Leu Arg
560                 565                 570                 575

TAC CTA AAC AGG CTC AGC GGC GAA CTG AGT ATA TAT GGT CTC CAA AGT      1774
Tyr Leu Asn Arg Leu Ser Gly Glu Leu Ser Ile Tyr Gly Leu Gln Ser
            580                 585                 590

GTT GAA AGC AGA GAG GAA GCT CTT GCA TTC GAT CTA GCT GCC AAG AAA      1822
Val Glu Ser Arg Glu Glu Ala Leu Ala Phe Asp Leu Ala Ala Lys Lys
        595                 600                 605

CGG CTC GCA GAA CTA ACA CTA TCA TTC GGT GGA AGT TCA GAA GTT GCA      1870
Arg Leu Ala Glu Leu Thr Leu Ser Phe Gly Gly Ser Ser Glu Val Ala
    610                 615                 620
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GCA GAG GTA CTT GAG GGC CTT TGT CCT CCC GTG GGG CTT GTA ACA CTC      1918
Ala Glu Val Leu Glu Gly Leu Cys Pro Pro Val Gly Leu Val Thr Leu
    625                 630                 635

GAC ATC CGT GAC TAC GAT GGT TTG GTA TAC CCA AAG TGG ATG GTG GGC      1966
Asp Ile Arg Asp Tyr Asp Gly Leu Val Tyr Pro Lys Trp Met Val Gly
640                 645                 650                 655

AGG CAA AAT GGC GCA CCA GAG AAG CTG CAA CAA CTT GGT CTC TCA GGA      2014
Arg Gln Asn Gly Ala Pro Glu Lys Leu Gln Gln Leu Gly Leu Ser Gly
                660                 665                 670

TGG AGC CAG CCA GGA CCT GCT CCT GCA CTG AAG GCT TTC AAT CAT CTT      2062
Trp Ser Gln Pro Gly Pro Ala Pro Ala Leu Lys Ala Phe Asn His Leu
            675                 680                 685

CGT TGC CTC AAT CTG ATG CAC TGC AGC TGG AAC GCC TTG CCA TGC AAT      2110
Arg Cys Leu Asn Leu Met His Cys Ser Trp Asn Ala Leu Pro Cys Asn
        690                 695                 700

ATG GAG CAC CTC AGC TCG CTC GAA ACA GTA ATC ATT ATT AAA TGT TTG      2158
Met Glu His Leu Ser Ser Leu Glu Thr Val Ile Ile Ile Lys Cys Leu
    705                 710                 715

AAT ATC CGG TCG CTT CCA ACG CTG CCA CAG TCT CTT ACG TAT TTT TGG      2206
Asn Ile Arg Ser Leu Pro Thr Leu Pro Gln Ser Leu Thr Tyr Phe Trp
720                 725                 730                 735

CTC CTG AAG TGC GAC GAT GGG TTC ATG GAG TCT TGT CAA ACA GTT GGA      2254
Leu Leu Lys Cys Asp Asp Gly Phe Met Glu Ser Cys Gln Thr Val Gly
                740                 745                 750

CAT CCA AAC TGG AAA AAG ATT CAA CAC ATC TGC AGG AAA TAT TTT AGT      2302
His Pro Asn Trp Lys Lys Ile Gln His Ile Cys Arg Lys Tyr Phe Ser
            755                 760                 765

GAA TGACGCGGGC TTGGAATCGG AGTCAGAGTA CTTACTTATG GCCCCTAACT           2355
Glu

TGAGACCTGC ATGCCGCTGC AGCTATTTTA TTCCAATTGG AGTCAAGACA AGAGTATTTA    2415
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CTCGAGATTC ATAATCTATT CCTGGGTGGA TCTTCTCTTG TGAGTCTGAA AACCTACCAG 2475

TGCCAGTCTG CAATATTGTA AGGAAAGGAG TACATCTATA GTGTCAGTGC ATATACAGTG 2535

TCTGAATCAT GCACTTCCGT TTCTGTATTT CACCGTATTA TTGATTAAAC AGTGCATGTG 2595

CACGTGCACA ATATATATTT CCCGAATCTT CT 2627

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 768 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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

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

Arg Asp Lys Ile Ile Ala Met Leu His Glu Lys Glu Gly Gly Leu Asp
35 40 45

Pro Ser Thr Ser Lys Gly Leu Cys Phe Ser Val Ile Gly Ile His Gly
50 55 60

Val Ser Gly Ser Gly Lys Ser Thr Leu Ala Gln Leu Val Tyr Ala His
65 70 75 80

Glu Lys Asn Asp Lys Gln Asp Asn Lys Glu Asp His Phe Asp Leu Val
85 90 95

Met Trp Val His Val Ser Gln Asp Phe Ser Val Trp Gly Ile Phe Xaa
100 105 110

Glu Leu Tyr Glu Ala Ala Ser Asp Pro Lys Val Pro Cys Pro Gln Phe
115 120 125

Asn Asn Leu Xaa Ala Leu Glu Glu Glu Leu Glu Arg Lys Leu Asp Gly
130 135 140

Lys Arg Phe Leu Leu Val Leu Asp Asp Val Trp Cys Asn Ala Asp Val
145 150 155 160

Gly Asn Gln Glu Leu Pro Lys Leu Leu Ser Pro Leu Lys Lys Gly Lys
165 170 175

Lys Gly Ser Lys Ile Leu Val Thr Thr Arg Ser Lys Tyr Ala Leu Pro
180 185 190

Asp Leu Cys Pro Gly Val Arg Tyr Thr Ala Met Pro Ile Thr Glu Val
195 200 205

Asp Asp Thr Ala Phe Phe Glu Leu Phe Met His Tyr Ala Leu Glu Asp
210 215 220

Gly Gln Asp Gln Ser Met Phe Gln Asn Ile Gly Val Glu Ile Ala Lys
225 230 235 240

Lys Leu Lys Gly Ser Pro Leu Ala Ala Arg Thr Val Gly Gly Asn Leu
245 250 255

Arg Arg Gln Gln Asp Val Asp His Trp Arg Arg Val Gly Asp Gln Asp
260 265 270

Leu Phe Lys Val Trp Thr Gly Pro Leu Trp Trp Ser Tyr Tyr Gln Leu
275 280 285

Gly Glu Gln Ala Arg Arg Cys Phe Ala Tyr Cys Ser Ile Phe Pro Arg
290 295 300

Arg His Arg Leu Tyr Arg Asp Glu Leu Val Arg Leu Trp Met Ala Glu
305 310 315 320

Gly Phe Ile Arg Asn Thr Asp Glu Gly Ala Asp Ala Glu Asp Val Gly
325 330 335

Leu Gly Ile Phe Asn Glu Leu Leu Ser Ile Ser Phe Leu Gln Pro Gly
340 345 350

Gly Gln Asp Trp Tyr Asn His Gly Lys Glu Tyr Tyr Leu Val His Asp
355 360 365

Leu Leu Tyr Asp Leu Ala Gly Ala Xaa Ala Gly Thr Asp Cys Phe Arg
370 375 380

Ile Asp Asn Asn Met Ile Gln Thr Gly Glu Ser Trp Ala Lys Asp Val
385 390 395 400

Pro Arg Asp Val Arg His Leu Phe Val Gln Ser Tyr Asp Ala Xaa Leu
405 410 415

Ser Thr Gly Arg Leu Leu Val Leu Glu Xaa Leu His Thr Leu Val Ile
420 425 430

Tyr Ser Val Gly Gly Asp Thr Thr Val Glu Glu Ile Val Ile Lys Asn
435 440 445

Ile Leu Lys Ser Leu Pro Lys Leu Arg Val Leu Ala Ile Ala Leu Cys
450 455 460

Leu Glu Lys Asp Gly Phe Xaa Cys Arg Pro Asn Ile Leu Ser Val Pro
465 470 475 480

Glu Ser Ile Ser Gln Leu Lys His Leu Arg Tyr Leu Ala Phe Arg Thr
485 490 495

Asp Ile Glu Cys Arg Val Ile Leu Pro Ser Ser Leu Asn Gln Leu Tyr
500 505 510

Gln Met Gln Leu Leu Asp Phe Gly Val Cys Met Asn Leu Val Phe Ser
515 520 525

Cys Gly Asp Leu Ile Asn Leu Arg His Val Cys Ser Gly Pro Gly Leu
530 535 540

Gln Phe Ser Asn Ile Gly Arg Leu Val Ser Leu Gln Thr Ile Pro Ala
545 550 555 560

Phe Lys Val Ser His Glu Gln Gly His Glu Ala Lys Gln Leu Arg Tyr
565 570 575

Leu Asn Arg Leu Ser Gly Glu Leu Ser Ile Tyr Gly Leu Gln Ser Val
580 585 590

Glu Ser Arg Glu Glu Ala Leu Ala Phe Asp Leu Ala Ala Lys Lys Arg
595 600 605

Leu Ala Glu Leu Thr Leu Ser Phe Gly Gly Ser Ser Glu Val Ala Ala
610 615 620

Glu Val Leu Glu Gly Leu Cys Pro Pro Val Gly Leu Val Thr Leu Asp
625 630 635 640

Ile Arg Asp Tyr Asp Gly Leu Val Tyr Pro Lys Trp Met Val Gly Arg
645 650 655

Gln Asn Gly Ala Pro Glu Lys Leu Gln Gln Leu Gly Leu Ser Gly Trp
660 665 670

Ser Gln Pro Gly Pro Ala Pro Ala Leu Lys Ala Phe Asn His Leu Arg
675 680 685

Cys Leu Asn Leu Met His Cys Ser Trp Asn Ala Leu Pro Cys Asn Met
690 695 700

Glu His Leu Ser Ser Leu Glu Thr Val Ile Ile Ile Lys Cys Leu Asn
705 710 715 720

Ile Arg Ser Leu Pro Thr Leu Pro Gln Ser Leu Thr Tyr Phe Trp Leu
725 730 735

Leu Lys Cys Asp Asp Gly Phe Met Glu Ser Cys Gln Thr Val Gly His
740 745 750

Pro Asn Trp Lys Lys Ile Gln His Ile Cys Arg Lys Tyr Phe Ser Glu
755 760 765

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3850 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum tauschii
(B) STRAIN: AUS 18913
(F) TISSUE TYPE: Root

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1138..3528

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATCCGGAGTC GTGGTTGTGG CCGCTGTCTT CCACTTGTGT ACGGTTTTCC CGTTGTCGAG 60

TGGCCTTCCT TCTTTCGGCC TTGCGGTTAA CAGGTTAGGG GCGCCGGTAG TGTTGTGGTG 120

TGTCATCTTT GGTGAGCTTA TTAATGTCGG CTTGTGGTGT CACATGGTGG TGGGTTCTCA 180

TTTAACGTCG AGGCGGTGAC CTCAGGTGGC GGTCCGTCAA GTCGGCTTCT CAACAAGCGT 240

CTTGGCGGCG GCCGTGGTGG CATTGTTTGG TCGTGTGGAC GGCGGAGGAT GCTAAGTTGG 300

GTGATCCTAG TGTCGGTGGT GCTTTGGCAC TGGCGGTGCC CAGATCGTGT TCTAGTGGTC 360

TGGCTTGGTA GTGACATGTT CACCTCGGTG TGGGCTGGTG CTCGGGAGGC CTAGTGTGGC 420

GTGGAAGAGT GCAACAAGGT CCGGCGATTT TCCTTGGAGC GAACTTTCAT CTTTGTTGGT 480

AGTTTAGGTA GCTTTGTGTT AGGGTGTGGT TCCTCCTATT TTCTTGTTTT TCTTTGATCT 540

GCTTTGTAAG AGGGTCTCCT CATCACCTTG TATCTCTTTG GTCGTGGTTC TTTATATATA 600

AAGCGGGGCC GAAGTAATTT TTGGTAGGAT TCACCAACAT CATGAGAACA AAGCACGAAA 660

ATATAGTAGT ACGGTAGTAG AGAATGTTAA TTCCTCTTGT ATCCAATGTT ATCTCTTGTA 720

TACCGTGATT CTTGCCCATC AGTATTCTCT TAGGCTTCTG TTAGCGAAAC AAAATTCCTT 780

CTTCCAAATT ACCAAACTTC TAGCTCATGA GTATGTTCAT ATAGTGCGCG GAGGATGTGC 840

GTGCCACATG CGTGCGCATG ATGGTGTTGA TAGACTAACA TGTGTGTGTG GTTTCTGTGT 900

GACTGCCTTG TGTTCTCTGC AAAACTAGGC TTTTGGCAAG TCAGTCTAGA TCCCTCGGCG 960

TATTTTTTAG AAGTATACCG GAGAGTAGAC GAATTCCCTA TATTACATTA GTCTTTTTTC 1020

TTTATTTAGT GTCATGATAG TTTATGTGAA GATAAAATCT CTCTTCTGTA ATGGTCACCT 1080

ATAATTTATT TTTTAAAGAT TTCTCTCTTG TTATTTGGGG TCTCGCAGGA GAGTGGC 1137

ATG TCA AAG AAA AAG TTG ATA GAC AGC CTG AAG AAG ATA GAA GAC AAT 1185
Met Ser Lys Lys Lys Leu Ile Asp Ser Leu Lys Lys Ile Glu Asp Asn
1 5 10 15

ATA AAT GAA GCA CAC CAA ATT CTG GAT AAG CTT AAC TTG TCA AGC ATA 1233
Ile Asn Glu Ala His Gln Ile Leu Asp Lys Leu Asn Leu Ser Ser Ile
20 25 30

AGT GAT GGC AAT ATA CGA CAC ACA ATG GTT GTC AAT CCT ACG ACT ACC 1281
Ser Asp Gly Asn Ile Arg His Thr Met Val Val Asn Pro Thr Thr Thr
35 40 45

GCA GTT TCC CCG CAA AAA GTT TTT GGT CGA GAT AAT GAT CGC GAC AAG 1329
Ala Val Ser Pro Gln Lys Val Phe Gly Arg Asp Asn Asp Arg Asp Lys
50 55 60

ATC ATA GCA ATG CTT CAT GAA AAG GAA GGT GGT CTT GAT CCA AGC ACT 1377
Ile Ile Ala Met Leu His Glu Lys Glu Gly Gly Leu Asp Pro Ser Thr
65 70 75 80

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AGC AAA GGT CTA TGT TTT TCT GTA ATT GGC ATA CAT GGA GTC AGC GGG      1425
Ser Lys Gly Leu Cys Phe Ser Val Ile Gly Ile His Gly Val Ser Gly
                 85                  90                  95

TCT GGG AAA TCT ACC CTT GCA CAG CTT GTT TAT GCC CAC GAG AAA AAT      1473
Ser Gly Lys Ser Thr Leu Ala Gln Leu Val Tyr Ala His Glu Lys Asn
            100                 105                 110

GAC AAG CAA GAC AAC AAG GAA GAC CAT TTC GAC CTT GTT ATG TGG GTT      1521
Asp Lys Gln Asp Asn Lys Glu Asp His Phe Asp Leu Val Met Trp Val
        115                 120                 125

CAT GTC TCT CAG GAT TTT AGT GTG TGG GGC ATC TTC ANG GAG TTG TAT      1569
His Val Ser Gln Asp Phe Ser Val Trp Gly Ile Phe Xaa Glu Leu Tyr
    130                 135                 140

GAG GCA GCT TCA GAT CCT AAG GTT CCA TGC CCT CAA TTT AAT AAC TTG      1617
Glu Ala Ala Ser Asp Pro Lys Val Pro Cys Pro Gln Phe Asn Asn Leu
145                 150                 155                 160

ANT GCC TTG GAA GAA GAA CTG GAG AGG AAA CTA GAT GGA AAG CGA TTC      1665
Xaa Ala Leu Glu Glu Glu Leu Glu Arg Lys Leu Asp Gly Lys Arg Phe
                165                 170                 175

CTT CTG GTA CTG GAT GAT GTC TGG TGC AAT GCG GAT GTT GGT AAC CAG      1713
Leu Leu Val Leu Asp Asp Val Trp Cys Asn Ala Asp Val Gly Asn Gln
            180                 185                 190

GAG CTA CCA AAG TTA CTT TCT CCA CTG AAG AAA GGA AAG AAA GGA AGC      1761
Glu Leu Pro Lys Leu Leu Ser Pro Leu Lys Lys Gly Lys Lys Gly Ser
        195                 200                 205

AAG ATC CTA GTG ACA ACT CGA AGT AAA TAT GCA CTA CCG GAT CTA TGT      1809
Lys Ile Leu Val Thr Thr Arg Ser Lys Tyr Ala Leu Pro Asp Leu Cys
    210                 215                 220

CCT GGT GTG AGA TAT ACT GCC ATG CCG ATA ACT GAG GTT GAT GAT ACC      1857
Pro Gly Val Arg Tyr Thr Ala Met Pro Ile Thr Glu Val Asp Asp Thr
225                 230                 235                 240
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GCC TTC TTT GAG TTG TTC ATG CAT TAT GCC CTC GAA GAT GGC CAA GAT      1905
Ala Phe Phe Glu Leu Phe Met His Tyr Ala Leu Glu Asp Gly Gln Asp
                245                 250                 255

CAA AGC ATG TTC CAG AAC ATT GGG GTT GAG ATT GCA AAA AAG CTG AAG      1953
Gln Ser Met Phe Gln Asn Ile Gly Val Glu Ile Ala Lys Lys Leu Lys
            260                 265                 270

GGG TCA CCT TTA GCA GCT AGA ACA GTG GGT GGA AAT TTA CGT CGA CAG      2001
Gly Ser Pro Leu Ala Ala Arg Thr Val Gly Gly Asn Leu Arg Arg Gln
        275                 280                 285

CAA GAT GTT GAC CAT TGG AGA AGA GTC GGA GAT CAA GAC CTT TTC AAG      2049
Gln Asp Val Asp His Trp Arg Arg Val Gly Asp Gln Asp Leu Phe Lys
    290                 295                 300

GTA TGG ACG GGA CCT CTG TGG TGG AGC TAC TAT CAG CTT GGT GAG CAG      2097
Val Trp Thr Gly Pro Leu Trp Trp Ser Tyr Tyr Gln Leu Gly Glu Gln
305                 310                 315                 320

GCT AGG CGT TGC TTT GCT TAC TGC AGT ATT TTT CCT AGG AGA CAT CGC      2145
Ala Arg Arg Cys Phe Ala Tyr Cys Ser Ile Phe Pro Arg Arg His Arg
                325                 330                 335

TTG TAC CGY GAT GAA TTA GTT AGA CTC TGG ATG GCA GAA GGG TTC ATA      2193
Leu Tyr Arg Asp Glu Leu Val Arg Leu Trp Met Ala Glu Gly Phe Ile
            340                 345                 350

AGA AAC ACA GAT GAA GGG GCG GAT GCT GAA GAC GTT GGT CTG GGA ATA      2241
Arg Asn Thr Asp Glu Gly Ala Asp Ala Glu Asp Val Gly Leu Gly Ile
        355                 360                 365

TTT AAT GAA CTA TTG TCG ATA TCA TTT CTT CAA CCA GGA GGC CAG GAC      2289
Phe Asn Glu Leu Leu Ser Ile Ser Phe Leu Gln Pro Gly Gly Gln Asp
    370                 375                 380

TGG TAC AAT CAT GGC AAG GAA TAC TAT TTA GTT CAT GAT TTG CTG TAT      2337
Trp Tyr Asn His Gly Lys Glu Tyr Tyr Leu Val His Asp Leu Leu Tyr
385                 390                 395                 400
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GAT TTA GCA GGG GCA GYA GCT GGA ACT GAC TGC TTC AGA ATT GAC AAT      2385
Asp Leu Ala Gly Ala Xaa Ala Gly Thr Asp Cys Phe Arg Ile Asp Asn
                405                 410                 415

AAC ATG ATC CAG ACA GGA GAA AGC TGG GCA AAA GAT GTT CCC AGA GAC      2433
Asn Met Ile Gln Thr Gly Glu Ser Trp Ala Lys Asp Val Pro Arg Asp
            420                 425                 430

GTT CGC CAT CTT TTT GTT CAG AGT TAT GAT GCA NCN TTG AGT ACA GGG      2481
Val Arg His Leu Phe Val Gln Ser Tyr Asp Ala Xaa Leu Ser Thr Gly
        435                 440                 445

AGA TTG CTT GTA TTG GAG GAN TTA CAC ACA CTC GTC ATT TAT AGT GTT      2529
Arg Leu Leu Val Leu Glu Xaa Leu His Thr Leu Val Ile Tyr Ser Val
    450                 455                 460

GGA GGG GAT ACA ACA GTT GAG GAA ATA GTC ATC AAG AAC ATA CTC AAG      2577
Gly Gly Asp Thr Thr Val Glu Glu Ile Val Ile Lys Asn Ile Leu Lys
465                 470                 475                 480

AGT CTG CCT AAA CTG CGG GTA CTA GCA ATA GCT TTA TGT CTG GAA AAG      2625
Ser Leu Pro Lys Leu Arg Val Leu Ala Ile Ala Leu Cys Leu Glu Lys
                485                 490                 495

GAT GGA TTT ATN TGT AGA CCA AAT ATA TTG TCT GTT CCA GAA TCT ATT      2673
Asp Gly Phe Xaa Cys Arg Pro Asn Ile Leu Ser Val Pro Glu Ser Ile
            500                 505                 510

AGT CAA TTA AAA CAT CTA CGA TAT CTT GCT TTC CGG ACA GAT ATT GAA      2721
Ser Gln Leu Lys His Leu Arg Tyr Leu Ala Phe Arg Thr Asp Ile Glu
        515                 520                 525

TGC AGA GTA ATT TTA CCA AGC AGT CTA AAC CAG CTT TAC CAG ATG CAA      2769
Cys Arg Val Ile Leu Pro Ser Ser Leu Asn Gln Leu Tyr Gln Met Gln
    530                 535                 540

CTG CTA GAT TTT GGT GTC TGC ATG AAT TTG GTA TTT TCC TGT GGT GAT      2817
Leu Leu Asp Phe Gly Val Cys Met Asn Leu Val Phe Ser Cys Gly Asp
545                 550                 555                 560
```

CTT ATC AAC TTG CGG CAT GTA TGC AGC GGT CCT GGA TTG CAA TTT TCA 2865
Leu Ile Asn Leu Arg His Val Cys Ser Gly Pro Gly Leu Gln Phe Ser
565 570 575

AAC ATC GGT AGG CTT GTC TCA CTC CAA ACA ATC CCA GCA TTC AAA GTA 2913
Asn Ile Gly Arg Leu Val Ser Leu Gln Thr Ile Pro Ala Phe Lys Val
580 585 590

AGT CAT GAA CAA GGA CAT GAG GCA AAG CAG TTG AGG TAC CTA AAC AGG 2961
Ser His Glu Gln Gly His Glu Ala Lys Gln Leu Arg Tyr Leu Asn Arg
595 600 605

CTC AGC GGC GAA CTG AGT ATA TAT GGT CTC CAA AGT GTT GAA AGC AGA 3009
Leu Ser Gly Glu Leu Ser Ile Tyr Gly Leu Gln Ser Val Glu Ser Arg
610 615 620

GAG GAA GCT CTT GCA TTC GAT CTA GCT GCC AAG AAA CGG CTC GCA GAA 3057
Glu Glu Ala Leu Ala Phe Asp Leu Ala Ala Lys Lys Arg Leu Ala Glu
625 630 635 640

CTA ACA CTA TCA TTC GGT GGA AGT TCA GAA GTT GCA GCA GAG GTA CTT 3105
Leu Thr Leu Ser Phe Gly Gly Ser Ser Glu Val Ala Ala Glu Val Leu
645 650 655

GAG GGC CTT TGT CCT CCC GTG GGG CTT GTA ACA CTC GAC ATC CGT GAC 3153
Glu Gly Leu Cys Pro Pro Val Gly Leu Val Thr Leu Asp Ile Arg Asp
660 665 670

TAC GAT GGT TTG GTA TAC CCA AAG TGG ATG GTG GGC AGG CAA AAT GGC 3201
Tyr Asp Gly Leu Val Tyr Pro Lys Trp Met Val Gly Arg Gln Asn Gly
675 680 685

GCA CCA GAG AAG CTG CAA CAA CTT GGT CTC TCA GGA TGG AGC CAG CCA 3249
Ala Pro Glu Lys Leu Gln Gln Leu Gly Leu Ser Gly Trp Ser Gln Pro
690 695 700

GGA CCT GCT CCT GCA CTG AAG GCT TTC AAT CAT CTT CGT TGC CTC AAT 3297
Gly Pro Ala Pro Ala Leu Lys Ala Phe Asn His Leu Arg Cys Leu Asn
705 710 715 720

CTG ATG CAC TGC AGC TGG AAC GCC TTG CCA TGC AAT ATG GAG CAC CTC 3345
Leu Met His Cys Ser Trp Asn Ala Leu Pro Cys Asn Met Glu His Leu
725 730 735

AGC TCG CTC GAA ACA GTA ATC ATT ATT AAA TGT TTG AAT ATC CGG TCG 3393
Ser Ser Leu Glu Thr Val Ile Ile Ile Lys Cys Leu Asn Ile Arg Ser
740 745 750

CTT CCA ACG CTG CCA CAG TCT CTT ACG TAT TTT TGG CTC CTG AAG TGC 3441
Leu Pro Thr Leu Pro Gln Ser Leu Thr Tyr Phe Trp Leu Leu Lys Cys
755 760 765

GAC GAT GGG TTC ATG GAG TCT TGT CAA ACA GTT GGA CAT CCA AAC TGG 3489
Asp Asp Gly Phe Met Glu Ser Cys Gln Thr Val Gly His Pro Asn Trp
770 775 780

AAA AAG ATT CAA CAC ATC TGC AGG AAA TAT TTT AGT GAA TGACGCGGGC 3538
Lys Lys Ile Gln His Ile Cys Arg Lys Tyr Phe Ser Glu
785 790 795

TTGGAATCGG AGTCAGAGTA CTTACTTATG GCCCCTAACT TGAGACCTGC ATGCCGCTGC 3598

AGCTATTTTA TTCCAATTGG AGTCAAGACA AGAGTATTTA CTCGAGATTC ATAATCTATT 3658

CCTGGGTGGA TCTTCTCTTG TGAGTCTGAA AACCTACCAG TGCCAGTCTG CAATATTGTA 3718

AGGAAAGGAG TACATCTATA GTGTCAGTGC ATATACAGTG TCTGAATCAT GCACTTCCGT 3778

TTCTGTATTT CACCGTATTA TTGATTAAAC AGTGCATGTG CACGTGCACA ATATATATTT 3838

CCCGAATCTT CT 3850

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 797 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Lys Lys Lys Leu Ile Asp Ser Leu Lys Lys Ile Glu Asp Asn
1 5 10 15

Ile Asn Glu Ala His Gln Ile Leu Asp Lys Leu Asn Leu Ser Ser Ile
20 25 30

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

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

Ile Ile Ala Met Leu His Glu Lys Glu Gly Gly Leu Asp Pro Ser Thr
65 70 75 80

Ser Lys Gly Leu Cys Phe Ser Val Ile Gly Ile His Gly Val Ser Gly
85 90 95

Ser Gly Lys Ser Thr Leu Ala Gln Leu Val Tyr Ala His Glu Lys Asn
100 105 110

Asp Lys Gln Asp Asn Lys Glu Asp His Phe Asp Leu Val Met Trp Val
115 120 125

His Val Ser Gln Asp Phe Ser Val Trp Gly Ile Phe Xaa Glu Leu Tyr
130 135 140

Glu Ala Ala Ser Asp Pro Lys Val Pro Cys Pro Gln Phe Asn Asn Leu
145 150 155 160

Xaa Ala Leu Glu Glu Glu Leu Glu Arg Lys Leu Asp Gly Lys Arg Phe
165 170 175

Leu Leu Val Leu Asp Asp Val Trp Cys Asn Ala Asp Val Gly Asn Gln
180 185 190

Glu Leu Pro Lys Leu Leu Ser Pro Leu Lys Lys Gly Lys Lys Gly Ser
195 200 205

Lys Ile Leu Val Thr Thr Arg Ser Lys Tyr Ala Leu Pro Asp Leu Cys
210 215 220

Pro Gly Val Arg Tyr Thr Ala Met Pro Ile Thr Glu Val Asp Asp Thr
225 230 235 240

Ala Phe Phe Glu Leu Phe Met His Tyr Ala Leu Glu Asp Gly Gln Asp
245 250 255

Gln Ser Met Phe Gln Asn Ile Gly Val Glu Ile Ala Lys Lys Leu Lys
260 265 270

Gly Ser Pro Leu Ala Ala Arg Thr Val Gly Gly Asn Leu Arg Arg Gln
275 280 285

Gln Asp Val Asp His Trp Arg Arg Val Gly Asp Gln Asp Leu Phe Lys
290 295 300

Val Trp Thr Gly Pro Leu Trp Trp Ser Tyr Tyr Gln Leu Gly Glu Gln
305 310 315 320

Ala Arg Arg Cys Phe Ala Tyr Cys Ser Ile Phe Pro Arg Arg His Arg
325 330 335

Leu Tyr Arg Asp Glu Leu Val Arg Leu Trp Met Ala Glu Gly Phe Ile
340 345 350

Arg Asn Thr Asp Glu Gly Ala Asp Ala Glu Asp Val Gly Leu Gly Ile
355 360 365

Phe Asn Glu Leu Leu Ser Ile Ser Phe Leu Gln Pro Gly Gly Gln Asp
370 375 380

Trp Tyr Asn His Gly Lys Glu Tyr Tyr Leu Val His Asp Leu Leu Tyr
385 390 395 400

Asp Leu Ala Gly Ala Xaa Ala Gly Thr Asp Cys Phe Arg Ile Asp Asn
405 410 415

Asn Met Ile Gln Thr Gly Glu Ser Trp Ala Lys Asp Val Pro Arg Asp
420 425 430

Val Arg His Leu Phe Val Gln Ser Tyr Asp Ala Xaa Leu Ser Thr Gly
435 440 445

Arg Leu Leu Val Leu Glu Xaa Leu His Thr Leu Val Ile Tyr Ser Val
450 455 460

Gly Gly Asp Thr Thr Val Glu Glu Ile Val Ile Lys Asn Ile Leu Lys
465 470 475 480

Ser Leu Pro Lys Leu Arg Val Leu Ala Ile Ala Leu Cys Leu Glu Lys
485 490 495

Asp Gly Phe Xaa Cys Arg Pro Asn Ile Leu Ser Val Pro Glu Ser Ile
500 505 510

Ser Gln Leu Lys His Leu Arg Tyr Leu Ala Phe Arg Thr Asp Ile Glu
515 520 525

Cys Arg Val Ile Leu Pro Ser Ser Leu Asn Gln Leu Tyr Gln Met Gln
530 535 540

Leu Leu Asp Phe Gly Val Cys Met Asn Leu Val Phe Ser Cys Gly Asp
545 550 555 560

Leu Ile Asn Leu Arg His Val Cys Ser Gly Pro Gly Leu Gln Phe Ser
565 570 575

Asn Ile Gly Arg Leu Val Ser Leu Gln Thr Ile Pro Ala Phe Lys Val
580 585 590

Ser His Glu Gln Gly His Glu Ala Lys Gln Leu Arg Tyr Leu Asn Arg
595 600 605

Leu Ser Gly Glu Leu Ser Ile Tyr Gly Leu Gln Ser Val Glu Ser Arg
610 615 620

Glu Glu Ala Leu Ala Phe Asp Leu Ala Ala Lys Lys Arg Leu Ala Glu
625 630 635 640

Leu Thr Leu Ser Phe Gly Gly Ser Ser Glu Val Ala Ala Glu Val Leu
645 650 655

Glu Gly Leu Cys Pro Pro Val Gly Leu Val Thr Leu Asp Ile Arg Asp
660 665 670

Tyr Asp Gly Leu Val Tyr Pro Lys Trp Met Val Gly Arg Gln Asn Gly
675 680 685

Ala Pro Glu Lys Leu Gln Gln Leu Gly Leu Ser Gly Trp Ser Gln Pro
690 695 700

Gly Pro Ala Pro Ala Leu Lys Ala Phe Asn His Leu Arg Cys Leu Asn
705 710 715 720

Leu Met His Cys Ser Trp Asn Ala Leu Pro Cys Asn Met Glu His Leu
725 730 735

Ser Ser Leu Glu Thr Val Ile Ile Ile Lys Cys Leu Asn Ile Arg Ser
740 745 750

Leu Pro Thr Leu Pro Gln Ser Leu Thr Tyr Phe Trp Leu Leu Lys Cys
755 760 765

Asp Asp Gly Phe Met Glu Ser Cys Gln Thr Val Gly His Pro Asn Trp
770 775 780

Lys Lys Ile Gln His Ile Cys Arg Lys Tyr Phe Ser Glu
785 790 795

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding, or complementary to a sequence encoding a polypeptide which confers, enhances, or otherwise facilitates resistance to a nematode in a plant or a part or fragment of said polypeptide.

2. An isolated nucleic acid molecule according to claim 1, wherein said polypeptide encoded thereof comprises at least one amino acid sequence motif selected from the group consisting of p-Loop, kinase-2, or leucine-rich repeat motifs as hereinbefore defined.

3. An isolated nucleic acid molecule according to claim 1 or 2 wherein said nucleic acid is DNA.

4. An isolated nucleic acid molecule according to claim 1 or 2 or 3 wherein the polypeptide product of said nucleic acid is of plant origin.

5. An isolated nucleic acid molecule according to claim 4, wherein the plant is a monocotyledonous plant selected from the group consisting of *Triticum tauschii*, wheat, maize, rice, oats, barley and rye and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

6. An isolated nucleic acid molecule according to claim 5 wherein said nucleic acid molecule comprises a nucleotide sequence or complementary nucleotide sequence which is substantially the same as the nucleotide sequence set forth in SEQ ID NO: 1 or is at least about 40% similar to all or a part thereof.

7. An isolated nucleic acid molecule according to claim 5 wherein said nucleic acid molecule comprises a nucleotide sequence or complementary nucleotide sequence which is substantially the same as the nucleotide sequence set forth in SEQ ID NO: 3 or is at least

about 40% similar to all or a part thereof.

8. An isolated nucleic acid molecule according to claim 5 wherein said nucleic acid molecule comprises a nucleotide sequence or complementary nucleotide sequence which is substantially the same as the nucleotide sequence set forth in SEQ ID NO: 5 or is at least about 40% similar to all or a part thereof.

9. An isolated nucleic acid molecule according to claim 5 wherein said nucleic acid molecule comprises a nucleotide sequence or complementary nucleotide sequence which is substantially the same as the nucleotide sequence set forth in SEQ ID NO: 7 or is at least about 40% similar to all or a part thereof.

10. An isolated DNA molecule comprising a sequence of nucleotides which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) has at least about 40% nucleotide sequence similarity to SEQ ID NO: 1 or a part thereof.

11. An isolated DNA molecule comprising a sequence of nucleotides which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) has at least about 40% nucleotide sequence similarity to SEQ ID NO: 3 or a part thereof.

12. An isolated DNA molecule comprising a sequence of nucleotides which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) has at least about 40% nucleotide sequence similarity to SEQ ID NO: 5 or a part thereof.

13. An isolated DNA molecule comprising a sequence of nucleotides which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) has at least about 40% nucleotide sequence similarity to SEQ ID NO: 7 or a part thereof.

14. An isolated DNA molecule according to claim 10 comprising a nucleotide sequence substantially as the same as any one or more of SEQ ID NO: 1.

15. An isolated DNA molecule according to claim 11 comprising a nucleotide sequence substantially as the same as any one or more of SEQ ID NO: 3.

16. An isolated DNA molecule according to claim 12 comprising a nucleotide sequence substantially as the same as any one or more of SEQ ID NO: 5.

17. An isolated DNA molecule according to claim 13 comprising a nucleotide sequence substantially as the same as any one or more of SEQ ID NO: 7.

18. An isolated nucleic acid molecule which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 1 or to a complementary strand thereof.

19. An isolated nucleic acid molecule which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 3 or to a complementary strand thereof.

20. An isolated nucleic acid molecule which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 5 or to a complementary strand thereof.

21. An isolated nucleic acid molecule which:

(i) encodes or is complementary to a sequence encoding a polypeptide of plant origin which confers, enhances, or otherwise facilitates nematode resistance in a plant; and

(ii) hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 7 or to a complementary strand thereof.

22. An isolated nucleic acid molecule having a nucleotide sequence substantially the same as the sequence set forth in SEQ ID NO: 1 or a complementary strand thereof or at least 40% identical thereto.

23. An isolated nucleic acid molecule having a nucleotide sequence substantially the same as the sequence set forth in SEQ ID NO: 3 or a complementary strand thereof or at least 40% identical thereto.

24. An isolated nucleic acid molecule having a nucleotide sequence substantially the same as the sequence set forth in SEQ ID NO: 5 or a complementary strand thereof or at least 40% identical thereto.

25. An isolated nucleic acid molecule having a nucleotide sequence substantially the same as the sequence set forth in SEQ ID NO: 7 or a complementary strand thereof or at least 40% identical thereto.

26. A genetic construct comprising the nucleic acid molecule according to claim 1.

27. A genetic construct according to claim 26 wherein the nucleic acid molecule is operably linked to a promoter sequence.

28. A genetic construct according to claim 27 wherein the promoter sequence comprises a sequence of nucleotides having at least 40% nucleotide sequence similarity to nucleotides 1 to 1138 set forth in SEQ ID NO: 1, or a functional part thereof.

29. A genetic construct according to claim 26 or 27 or 28 capable of being expressed in a plant cell.

30. A genetic construct according to claim 29 wherein the plant cell is a root cell.

31. A genetic construct according to claim 26 or 27 or 28 capable of being expressed in a prokaryotic cell.

32. A genetic construct according to claim 29 or 30 wherein the plant cell is a monocotyledonous plant cell selected from the group consisting of *Triticum tauschii*, what, maize, rice, oats, barley and rye and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

33. A genetic construct comprising an isolated promoter sequence which originates from a gene which when expressed encodes a polypeptide that confers, enhances, or otherwise facilitates nematode resistance in a cell, or a functional part, derivative, fragment, homologue or analogue thereof, wherein said promoter is operably linked to the coding region isolated from a second genetic sequence.

34. A genetic construct according to claim 33 wherein the promoter sequence comprises a sequence of nucleotides having at least 40% nucleotide sequence similarity to nucleotides 1 to 1138 set forth in SEQ ID NO: 1, or a functional part thereof.

35. A genetic construct according to claim 33 or 34 wherein the plant cell is a monocotyledonous plant cell selected from the group consisting of *Triticum tauschii,* wheat, maize, rice, oats, barley and rye and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

36. An oligonucleotide molecule of at least 10 nucleotides in length capable of hybridising under low stringency conditions to part of the nucleotide sequence, or to a complement of the nucleotide sequence set forth in SEQ ID NO: 1.

37. An oligonucleotide molecule of at least 10 nucleotides in length capable of hybridising under low stringency conditions to part of the nucleotide sequence, or to a complement of the nucleotide sequence set forth in SEQ ID NO: 3.

38. An oligonucleotide molecule of at least 10 nucleotides in length capable of hybridising under low stringency conditions to part of the nucleotide sequence, or to a complement of the nucleotide sequence set forth in SEQ ID NO: 5.

39. An oligonucleotide molecule of at least 10 nucleotides in length capable of hybridising under low stringency conditions to part of the nucleotide sequence, or to a complement of the nucleotide sequence set forth in SEQ ID NO: 7.

40. A recombinant polypeptide product of the genetic construct according to any one of claims 26 to 35.

41. A recombinant polypeptide according to claim 40 wherein said polypeptide comprises at least one amino acid sequence motif selected from the group consisting of p-Loop, kinase-2 or leucine-rich repeat motifs as hereinbefore defined.

42. An isolated polypeptide which comprises an amino acid sequence which confers, enhances, or otherwise facilitates resistance to a nematode in a plant cell, or a functional

mutant, derivative part, fragment, or analogue of said polypeptide.

43. An isolated polypeptide according to claim 42 being of plant origin.

44. An isolated polypeptide according to claim 43 wherein the plant is a monocotyledonous plant selected from the group consisting of *Triticum tauschii,* wheat maize, rice, oats, barley and rye and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

45. An isolated polypeptide according claim 42 or 43 or 44 having an amino acid sequence substantially the same as the amino acid sequence set forth in SEQ ID NO: 2 or a part thereof.

46. An isolated polypeptide according to claim 42 or 43 or 44 having an amino acid sequence substantially the same as the amino acid sequence set forth in SEQ ID NO: 4 or a part thereof.

47. An isolated polypeptide according to claim 42 or 43 or 44 having an amino acid sequence substantially the same as the amino acid sequence set forth in SEQ ID NO: 6 or a part thereof.

48. An isolated polypeptide according to claim 42 or 43 or 44 having an amino acid sequence substantially the same as the amino acid sequence set forth in SEQ ID NO: 8 or a part thereof.

49. An isolated polypeptide according to claim 42 or 43 or 44 having an amino acid sequence which is at least 40% similar to the amino acid sequence set forth in SEQ ID NO: 2 or a part thereof.

50. An isolated polypeptide according to claim 42 or 43 or 44 having an amino acid

sequence which is at least 40% similar to the amino acid sequence set forth in SEQ ID NO: 4 or a part thereof.

51. An isolated polypeptide according to claim 42 or 43 or 44 having an amino acid sequence which is at least 40% similar to the amino acid sequence set forth in SEQ ID NO: 6 or a part thereof.

52. An isolated polypeptide according to claim 42 or 43 or 44 having an amino acid sequence which is at least 40% similar to the amino acid sequence set forth in SEQ ID NO: 8 or a part thereof.

53. A synthetic peptide comprising at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 2 or having at least 40% similarity to all or a part thereof.

54. A synthetic peptide comprising at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 4 or having at least 40% similarity to all or a part thereof.

55. A synthetic peptide comprising at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 6 or having at least 40% similarity to all or a part thereof.

56. A synthetic peptide comprising at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 8 or having at least 40% similarity to all or a part thereof.

57. An antibody that binds to a polypeptide which confers, enhances, or otherwise facilitates resistance to a nematode in a plant or a part or fragment thereof, wherein said polypeptide further comprises a sequence of amino acids is substantially the same as the amino acid sequence set forth in SEQ ID NO: 2 or is at least 40% similar to all or a part thereof.

58. An antibody that binds to a polypeptide which confers, enhances, or otherwise

facilitates resistance to a nematode in a plant or a part or fragment thereof, wherein said polypeptide further comprises a sequence of amino acids is substantially the same as the amino acid sequence set forth in SEQ ID NO: 4 or is at least 40% similar to all or a part thereof.

59. An antibody that binds to a polypeptide which confers, enhances or otherwise facilitates resistance to a nematode in a plant or a part or fragment thereof, wherein said polypeptide, part or fragment further comprises a sequence of amino acids is substantially the same as the amino acid sequence set forth in SEQ ID NO: 6 or is at least 40% similar to all or a part thereof.

60. An antibody that binds to a polypeptide which confers, enhances, or otherwise facilitates resistance to a nematode in a plant or a part or fragment thereof, wherein said polypeptide further comprises a sequence of amino acids is substantially the same as the amino acid sequence set forth in SEQ ID NO: 8 or is at least 40% similar to all or a part thereof.

61. An antibody according to claim 57 or 58 or 59 or 60 whiich is a polyclonal antibody.

62. An antibody according to claim 57 or 58 or 59 or 60 which is a monoclonal antibody.

63. A method of identifying a nematode resistance gene product or nematode resistance-like gene product in a plant cell, which method comprises contacting the antibody of any one of claims 57 to 62 with an antigen from said plant for a period of time and under conditions sufficient to form an antibody-antigen complex and measuring the amount of said antibody-antigen complex formed.

64. A method according to claim 63 wherein the antigen is obtained from the roots of a monocotyledonous plant selected from the group consisting of *Triticum tauschii*, wheat,

maize, rice, oats, barley and rye and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

65. A method according to claim 63 or 64, wherein said step of measuring the amount of antibody-antigen complex formed is by an immunoassay.

66. A method according to claim 65, wherein said immunoassay is an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay (RIA).

67. A method of identifying a nematode resistance genetic sequence or nematode resistance-like genetic sequence which method comprises contacting genomic DNA, or mRNA, or cDNA, or parts, or fragments thereof, or a source thereof, with a hybridisation effective amount of a genetic sequence encoding, or complementary to a genetic sequence encoding a polypeptide which confers, enhances or otherwise facilitates nematode resistance, or a part thereof, and then detecting said hybridisation.

68. A method of identifying a nematode resistance genetic sequence or a nematode resistance-like genetic sequence in a plant cell, which method comprises contacting genomic DNA, mRNA, or cDNA with one or more oligonucleotide molecules of claim 36 or 37 or 38 or 39 to a genetic sequence from said plant for a period of time and under conditions sufficient to form a double-stranded nucleic acid molecule and amplifying copies of the said genetic sequence in a polymerase chain reaction.

69. A method according to claim 68 wherein the plant is a monocotyledonous plant selected from the group consisting of *Triticum tauschii,* wheat, maize, rice, oats, barley and rye, and/or wilde varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

70. A plant carrying a non-endogenous nucleic acid molecule encoding or complementary to a nucleic acid molecule encoding a polypeptide which confers, enhances,

or otherwise facilitates nematode resistance in said plant.

71. A plant according to claim 70 wherein said non-endogenous nucleic acid molecule comprises at least 40% nucleotide sequence similarity to all or a part of the nucleotide sequence set forth in SEQ ID NO: 1 or hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 1 or to a complementary strand thereof.

72. A plant according to claim 70 wherein said non-endogenous nucleic acid molecule comprises at least 40% nucleotide sequence similarity to all or a part of the nucleotide sequence set forth in SEQ ID NO: 3 or hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 3 or to a complementary strand thereof.

73. A plant according to claim 70 wherein said non-endogenous nucleic acid molecule comprises at least 40% nucleotide sequence similarity to all or a part of the nucleotide sequence set forth in SEQ ID NO: 5 or hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO:5 or to a complementary strand thereof.

74. A plant according to claim 70 wherein said non-endogenous nucleic acid molecule comprises at least 40% nucleotide sequence similarity to all or a part of the nucleotide sequence set forth in SEQ ID NO: 7 or hybridises under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 7 or to a complementary strand thereof.

75. A plant according to claim 70 or 71 or 72 or 73 or 74 wherein the non-endogenous nucleic acid molecule is introduced into the plant by any one or a combination of procedures selected from the group consisting of *Agrobacterium*-mediated transformation, microparticle bombardment, PEG fusion, electroporation or introgression.

76. The progeny derived from the plant of any one of claims 70 to 75.

GENETIC LINKAGE OF csE20 TO CCNR

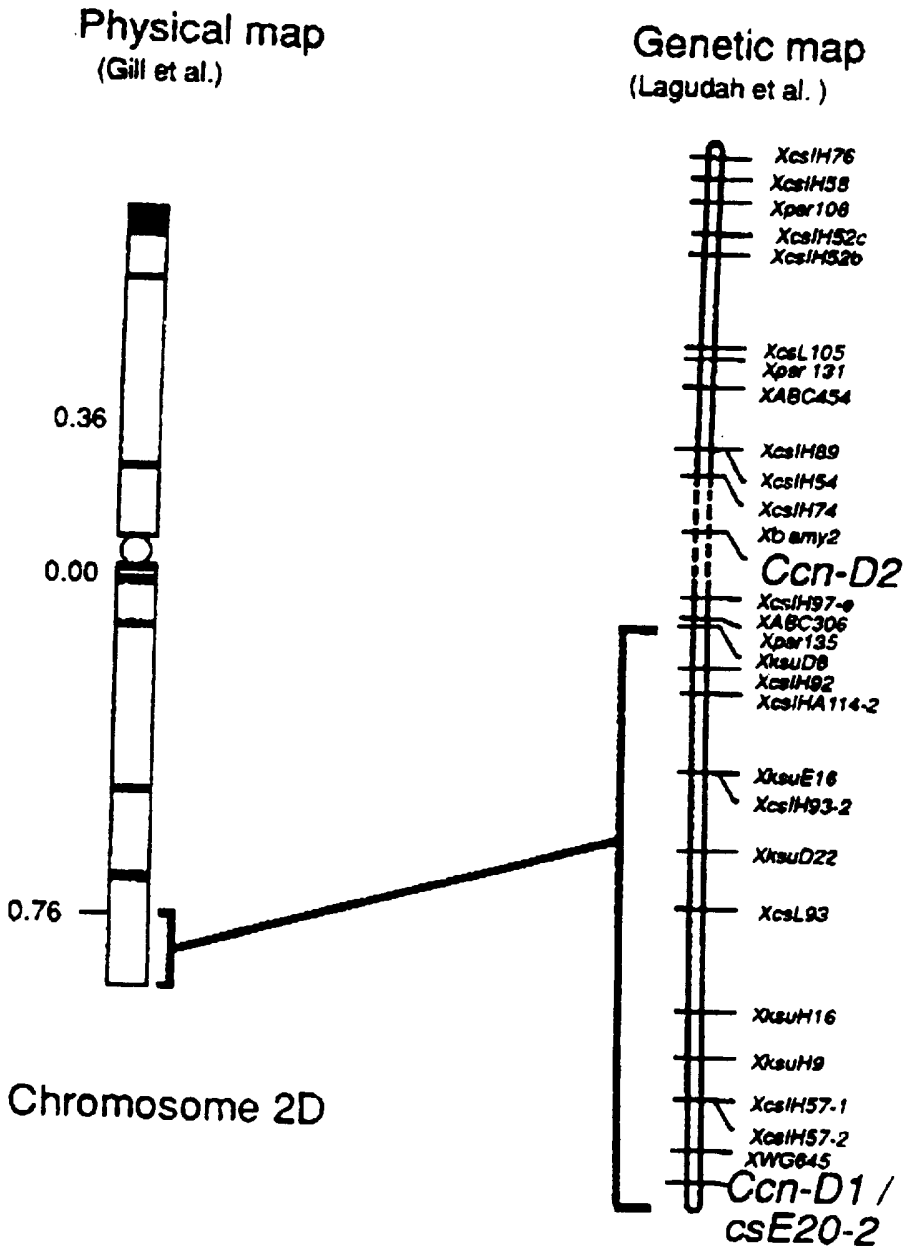


FIG. 1

RAPD ANALYSIS ON LOW COPY TEMPLATE FROM BULKED "R" AND "S" SAMPLES

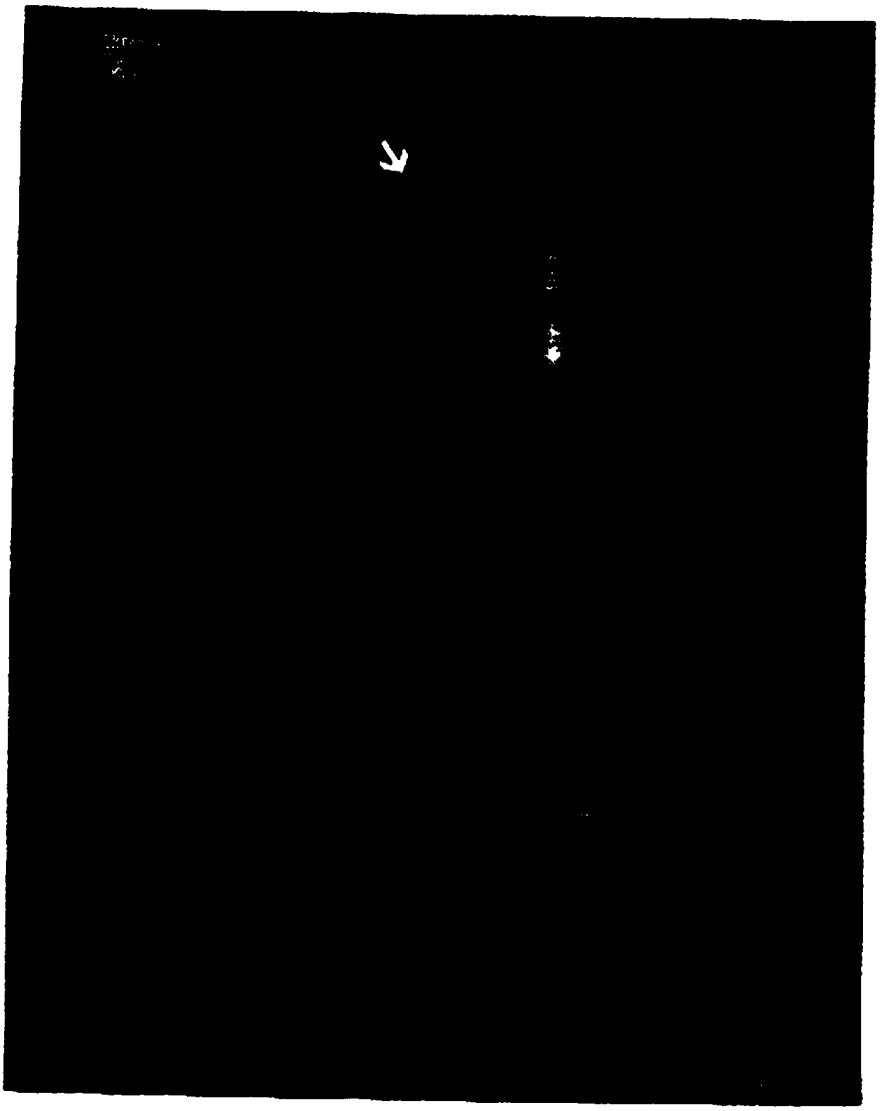


FIG. 2

Comparison of **total DNA** vs **Cot fraction** in a bulked F2 segregants-RAPD analysis

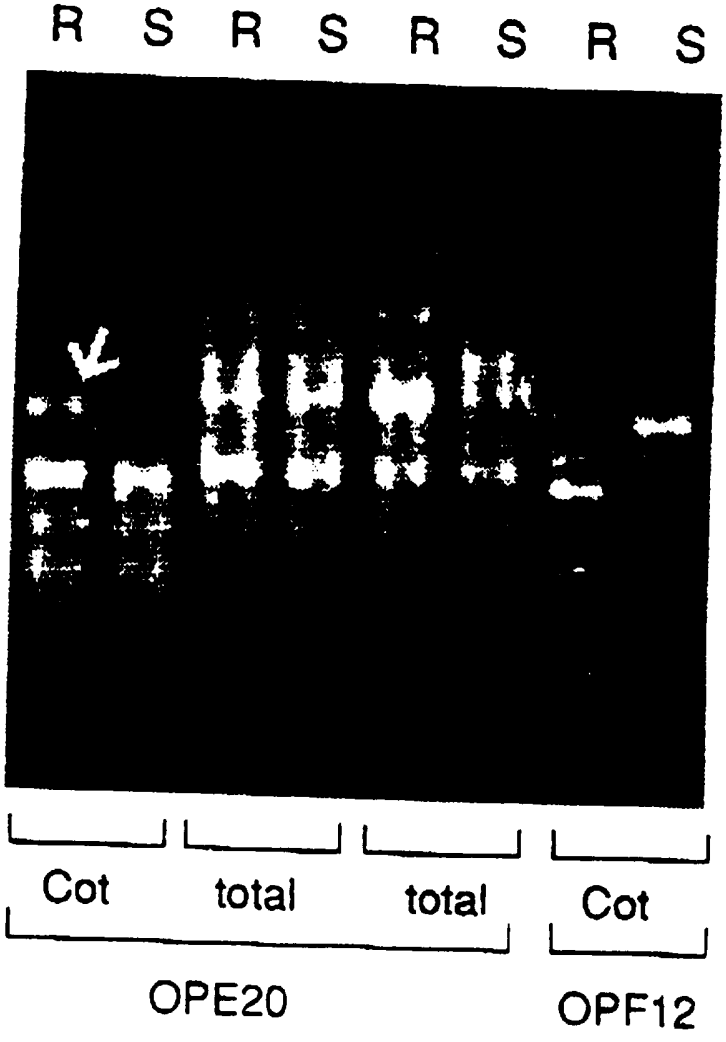


FIG. 3

● a nulli-tetra analysis using the Sears chromosomal stocks in cv Chinese Spring demonstrated a chromosome 2D location

missing chromosome 2D

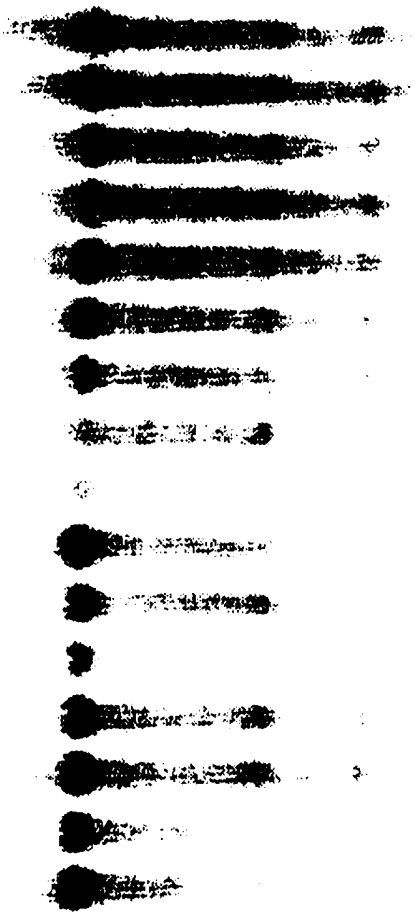
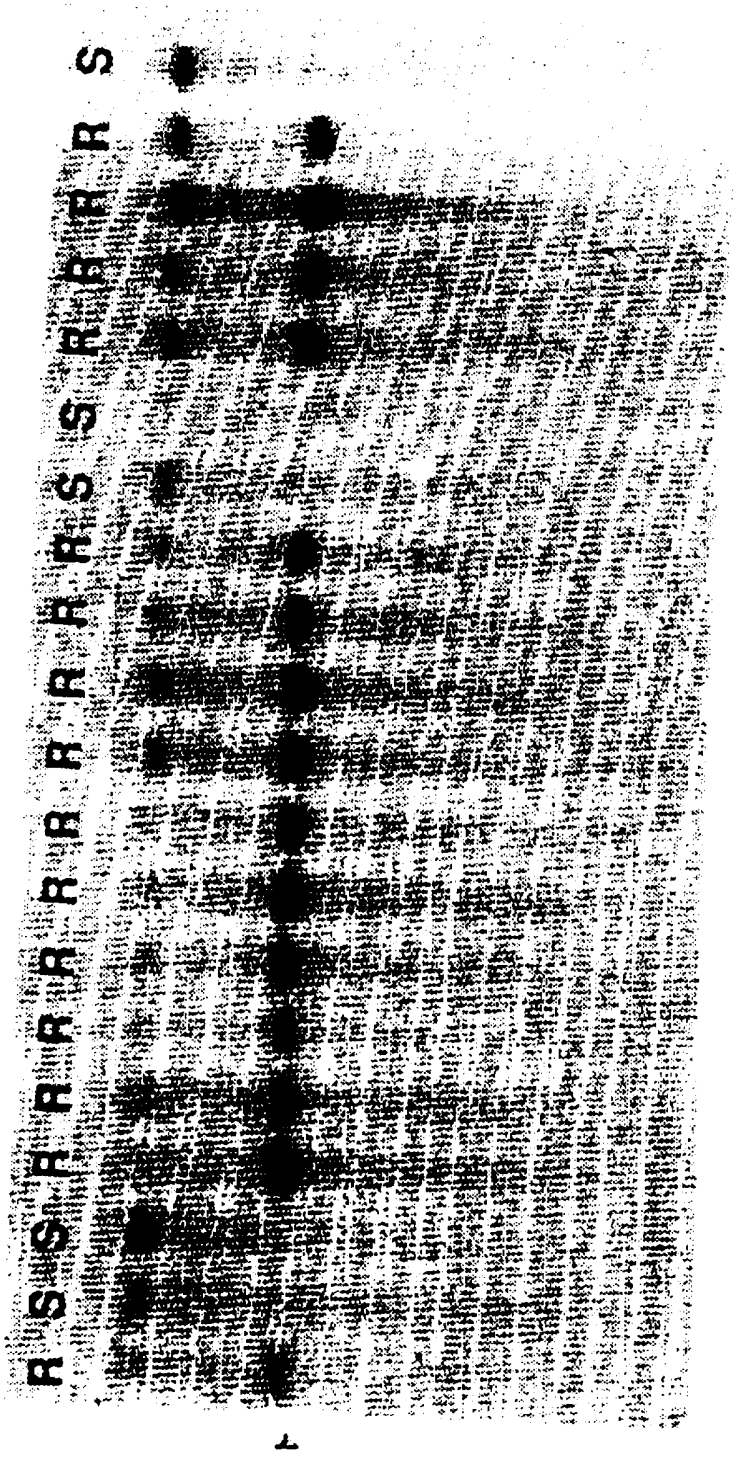


FIG. 4

CLONE *XCSRE20* IS TIGHTLY LINKED TO *CCN-D1*



DNA from a subset of *T. tauschii* individuals in an F2 population in which *Ccn-D1* is segregating

FIG. 5

The csE20 sequence is approximately 120 base pairs from the start of transcription of Cre3

R = Resistance S = Susceptible

- **linkage between XcsE20 RFLP marker and *CcnD1* is complete in bread wheat backcross derivatives produced in a program to introduce this gene into commercial cultivars**

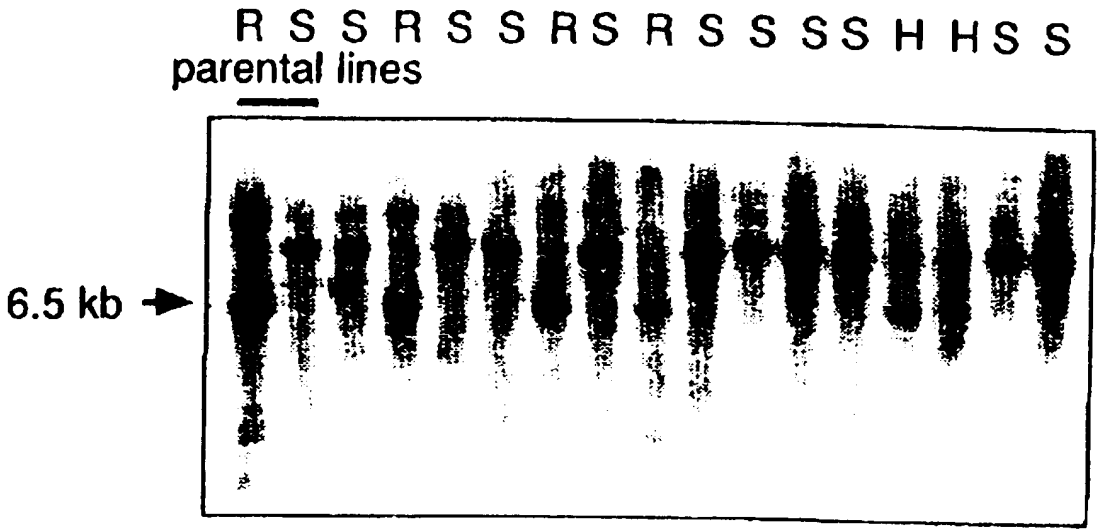


FIG. 6

λ CCNE20 clone

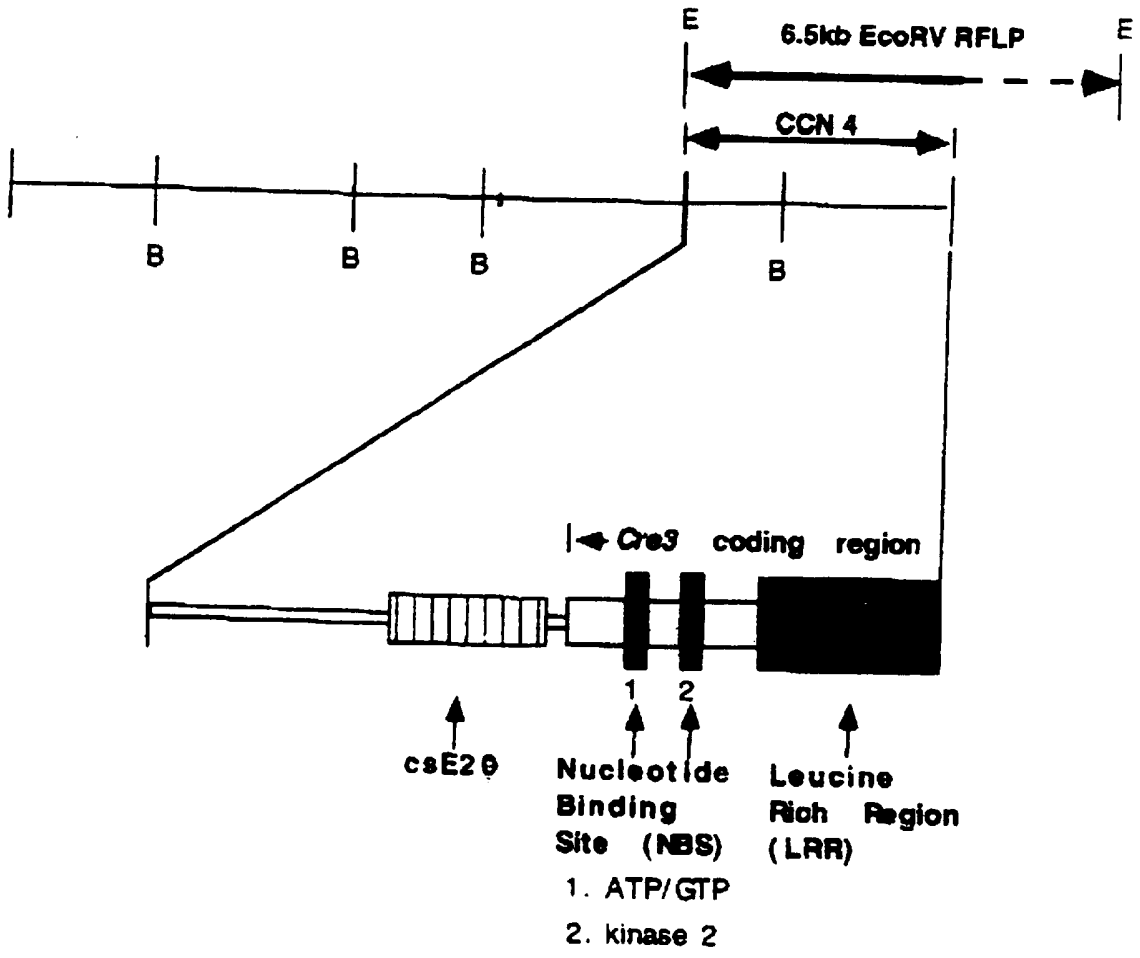


FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00181

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/29; A01H 5/00; C07K 16/16, 14/415; C07H 21/04; C12Q 1/68; G01N 33/53; A01N 37/18, 63/00

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)
IPC6: C12N 15/29

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: nematod:, heterodera, globodera, meloidogyne, resist:,C12N - 015/29/1C
CHEMICAL ABSTRACTS: STN sequence search and keywords as follows: nematod?, heterodera, globodera, meloidogyne, resist?/ IT, gene#/ IT, genetic/ IT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Euphytica, Volume 23, 1974 L.A.J. Slootmaker et al "Monosamic Analysis in Bread Wheat of Resistance to Cereal Root Eelworm" pages 497-503 Cited in the application Whole Document	6-25, 28, 34, 36-39, 45-66, 68, 69, 71-76
X	Genome, Volume 37, Number 1, February 1994 R.F. Eastwood et al "A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticum tauschii*" pages 311-319 Whole Document	6-25, 28, 34, 36-39, 45-66, 68, 69, 71-76

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

[ ] See patent family annex

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search
14 June 1996

Date of mailing of the international search report
29TH JUNE 1996.

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00181

Box 1 Observations where certain claims were found unsearchable (Continuation of item 1 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.: 1-5, 26, 27, 29-33, 35, 40-44, 67 and 70
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:

the scope of these claims is so broad, they are considered to be unsearchable.

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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00181

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Theor. Appl. Genet. Volume 89, 1994, K J Williams et al "Identification of RFLP markers linked to the cereal cyst nematode resistance gene (*Cre*) in wheat" pages 927-930 Whole Document	6-25, 28, 34, 36-39, 45-56, 68, 69, 71-76
Y	Aust. J. Agric Res. Volume 42, 1991, R. F. Eastwood et al. "*Triticum tauschii:* a Novel Source of Resistance to Cereal Cyst Nematode (*Heterodera avenae)*" pages 69-67 Cited in the application Whole Document	6-25, 28, 34, 36-39, 45-56, 68, 69, 71-76
A	Genome, Volume 34, 1991, E.S Lagudah et al "The molecular-genetic analysis of *Triticum tauschii*, the D-genome donor to hexaploid wheat" pages 375-386 Cited in the application Whole Document	