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(54) **Title:** THE *DMI1* GENE ENCODES A PROTEIN THAT IS REQUIRED FOR THE EARLY STEPS OF BACTERIAL AND FUNGAL SYMBIOSIS

(57) **Abstract:** Mycorrhizal and rhizobial associations represent the two most important symbiotic relationships between higher plants and microorganisms, providing access to otherwise limiting supplies of phosphate and nitrogen, respectively. Although many higher plants are able to establish a symbiotic relationship with arbuscular mycorrhizal fungi, legumes are unusual among plants because they also form associations with nitrogen fixing soil bacteria called rhizobia. This symbiosis requires the production of bacterial signals, "Nod factors" that trigger several key developmental responses in the host plant (Dénarié et al., 1996). The *DMI1* gene of the model legume *M. truncatula* plays a major role both in the early steps of Nod factor signaling and in the establishment of mycorrhizal symbiosis. *Dmi1* mutants do not exhibit many of the early responses to Nod factors and are incapable of forming nitrogen fixing root nodules. Here we describe the cloning and preliminary characterization of *DMI1*. The*DMI1* gene encodes a novel protein with low global similarity to ligand-gated cation channels of archaea. The protein is highly conserved in angiosperms and ancestral to land plants. Interestingly a putative *A. thaliana DMI1* orthologous gene is expressed in roots. As *A. thaliana* is unable to establish a mycorrhizal symbiosis, this finding suggests that *DMI1* may also exhibit a function that is independent of symbiotic interactions.

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THE *DMI1* GENE ENCODES A PROTEIN THAT IS REQUIRED FOR THE EARLY STEPS OF BACTERIAL AND FUNGAL SYMBIOSIS

RELATED APPLICATION

This application claims priority to U.S. Patent Application No. 60/510,098 filed October 8, 2003 and U.S. Patent Application Serial No. 10/739,736, which are hereby incorporated by reference herein in their entirety.

GOVERNMENT RIGHTS

This invention was made with Government support under Grant (or Contract) Nos. DE-FG03-98ER20296 and DE-FG03-01ER15200, awarded by the Department of Energy. The Government has certain rights in this invention.

FIELD OF THE INVENTION

This invention is in the field of plant nitrogen fixation and phosphate uptake.

BACKGROUND OF THE INVENTION

Plants require nitrogen, phosphorus and certain other essential elements to survive and grow. Most fertilizers include both nitrogen, in the form of ammonium or nitrates, and phosphorus. However, not all of a fertilizer used in farming is taken up by the crops. A fair amount of the fertilizer is washed away and contaminates the ground water in the surrounding community. In addition, supplementing soil nutrients with fertilizer increases the cost to produce a crop. Nitrogen in the atmosphere represents a good source for nitrogen given its abundance. But only prokaryotes are able to "fix" atmospheric nitrogen into a form usable by plants. Certain plants, legumes in particular, have evolved a symbiotic relationship with nitrogen fixing bacteria. Such plants are often planted to replenish the soils for other crops. In addition, some of the same proteins that are involved in establishing this symbiosis also participate in establishing symbiosis with mycorrhizal fungi that assist in utilization of phosphorus. Thus there is a need for methods of enhancing a plant's symbiotic machinery to reduce the need for supplementing soil with added nutrients. In addition, there is a need for methods and compositions that enhance

the nutrients in the soil directly such as addition of bacteria that fix nitrogen in the absence of symbiosis with plants to lower costs associated with fertilizing during farming.

SUMMARY OF THE INVENTION

The present invention meets the needs for enhanced symbiosis in plants and methods and compositions for direct supplementation of soils with nitrogen and/or phosphorus.

Mutations in a genetic locus of the legume plant *Medicago truncatula* resulted in the inability to form root nodules that are produced in wild type *M. truncatula* from interaction with *Sinorhizobium meliloti* (rhizobia). The locus was mapped in the *M. truncatula* genome, and using positional cloning strategies the molecular identity of the gene was identified. In addition to conditioning the ability to allow formation of symbiotic root nodules identified, the gene controls establishment of symbiosis with mycorrhizal fungi.

Together, the rhizobial and mycorrhizal symbioses with legumes constitute key mechanisms by which plants acquire nitrogen and phosphorous. Identifying the genes that control these plant-microbe associations offers the potential for genetic manipulation of these important plant pathways. This invention allows one to control the development of symbiotic organs and tissues in interaction with beneficial bacteria and mycorrhizae. Furthermore, this invention permits genetic engineering of enhanced nitrogen and phosphorous acquisition in plants.

The present invention is directed to the finding that the *dmi1* gene controls the ability to establish rhizobial and mycorrhizal symbioses in *Medicago truncatula*.

The present invention is thus directed to an isolated nucleic acid encoding a DMI1 polypeptide. The recombinant nucleic acid may be operably linked to a promoter sequence. The nucleic acid of the invention may be present in a vector. The vector may be in a prokaryotic or eukaryotic host cell.

The "*dmi1* nucleic acid sequence" as defined herein refers to any sequence that hybridizes to the nucleic acid molecule of SEQ ID NO:11 or the complement thereof under at least low stringency, preferably moderate, high or very high stringency conditions, or is about 85%, 90%, 95%, or 97% identical in the nucleic

acid sequence of SEQ ID NO:11, or encodes a polypeptide having at least about 85%, 90%, 95%, or 97% sequence identity to the amino acid sequence of SEQ ID NO:12. This invention is directed to the *dmi1* nucleic acid sequence as described above. The invention is further directed to oligonucleotide primers that bind the nucleic acid sequence of SEQ ID NO:11.

The invention is further directed to recombinant constructs containing such isolated nucleic acids. The recombinant constructs may further comprise a promoter. The promoter may be a homologus or a heterologous promoter. The recombinant constructs may further be in a vector. By way of example but not in limitation, the vector may be a cloning, expression, transformation, or transfection vector. The recombinant construct may be introduced into a prokaryotic or eukaryotic host cell. The recombinant construct may be introduced into a plant so that the expression of the nucleic acid may be controlled or regulated. The introduction of the construct into the plant may be transient or stable. The control or regulation may include root-specific promoters designed to express the isolated nucleic acids in roots. Such regulation may be directed to constitutive expression. The regulation may be altered in response to various biotic, abiotic and artificial stimuli, relative to the native *dmi1* promoter.

The invention is further directed to isolated nucleic acids encoding the protein depicted in SEQ ID NO:12 and paralogs, homologs and orthologs of the protein. Yet another aspect of the present invention includes DMI1-related proteins and nucleic acids encoding such proteins. The DMI1-related proteins are proteins with structural homology to DMI1 proteins that have at least one DMI1 activity including the ability to regulate mycorrhizal and/or rhizobial symbiosis, the ability to increase available nitrogen and/or phosphorus in a cell or organism, the ability to increase nitrogen and/or phosphorus uptake into a cell or organism and/or the ability to regulate cation levels in or around a cell. Nucleic acids encoding DMI1-related proteins may be in a vector or transgenically expressed in plants. Such nucleic acids are preferably operably linked to a promoter that may be an inducible promoter, a regulated promoter, or a constitutive promoter. The DMI1-related protein coding sequences of the invention include those sequences that hybridize under at least low stringency and preferably moderate, high, or very high stringency conditions to the

nucleic acid of SEQ ID NO:11 or its complement. In another embodiment of the presenting invention, the DMI1-related protein coding sequences also include those sequences with at least 85% sequence identity and preferably at least 90%, or at least 95% sequence identity with a nucleotide sequence of SEQ ID NO:11. The present invention also includes isolated proteins having the protein sequence of SEQ ID NO:12 as well as protein sequences with at least 85% sequence identity and preferably at least 90%, or at least 95% sequence identity with the protein sequence of SEQ ID NO:12. The present invention further includes nucleic acid sequences encoding the above protein sequences.

The invention is also directed to antibodies and ligands that bind a polypeptide having at least about 85%, 90%, 95%, or 97% sequence identity to the amino acid sequence of SEQ ID NO:12. The invention is further directed to non-naturally occurring cation channels comprising the nucleic acid that encodes a polypeptide having at least about 85%, 90%, 95%, or 97% sequence identity to the amino acid sequence of SEQ ID NO:P2.

The invention is further directed to transgenic plants containing the isolated nucleic acids of the invention. The invention is further directed to seed produced from the transgenic plants of the invention. The present invention is further directed to methods of enhancing nitrogen and/or phosphorous acquisition in transgenic plants by transforming plants with the nucleic acids of the invention wherein the nucleic acids are operatively linked to a promoter. The invention is directed to a method of increasing root nodulation in a plant. The invention is further directed to use of these transgenic plants to increase available nitrogen in the soil. The invention is also directed to a fertilizer comprising cells or organisms comprising an increased accumulation of nitrogen and/or phosphorus wherein said increase is due to the presence of an exogenous *dmi1* nucleic acid sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the genetic and physical map of the *DMI1* locus.

(A) A comparative genetic map of *M. truncatula* and *M. sativa* was established by reciprocal transfer (arrows) of genetic markers between the two related genomes.

(B) Panel B presents a minimum tiling path of BAC clones covering the *DMI1* region. All five BAC clones were sequenced and annotated for candidate genes. The gap between mth2-80I8 and the telomere was filled by PCR and sequenced (Riely *et al.*, manuscript in preparation).

(C) The *Arabidopsis* and *M. truncatula* homologs, but not their rice counterpart (data not shown), reside in a region of conserved genome microsynteny, presumably indicative of the ancestral chromosomal context.

FIGURE 2 shows the complementation of the *dmi1* phenotype using *A. rhizogenes* based transformation.

Roots of *DMI1* and wild type plants were transformed with either the *DMI1* cDNA under control of the native promoter or with vector alone. Transformed roots were inoculated with *Sinorhizobium meliloti* and nodule development was scored visually after 15 days. A constitutively expressed *uidA* gene was used as a reporter to identify transformed tissues based on GUS staining. Among more than 80 independent *DMI1* transformants, only those transformed by the *DMI1* transgene were nodulated by *S. meliloti*, while transformation of wild type plants with either *DMI1* or control constructs had no effect on nodulation

FIGURE 3 shows the expression profiles of *DMI1* in *M. truncatula* and *A. thaliana*. RNAs were extracted using RNeasy Plant Minikit (Qiagen, Germany) and quantified with Ribogreen RNA quantitation kit (Molecular Probes, USA).

(A) *M. truncatula DMI1* and *A. thaliana At5g49960* cDNA analysis by semi-quantitative RT-PCR in different tissues. *DMI1* is expressed strongly in roots, but not in aerial tissues of *Medicago truncatula*. The *DMI1* ortholog in *Arabidopsis* is expressed in roots but not leaves.

(B) *M. truncatula DMI1* expression is not altered upon inoculation by wild type *S. meliloti*. RT-PCR experiments were performed using the PowerScript™ RT-PCR kit (Clontech laboratories, USA).

FIGURE 4 shows DMI1 proteins and homologs.

(A) Partial alignment of DMI1 homologous proteins sequences by means of ClustalW with default parameters. *A. thaliana* At5g49960 (SEQ ID NO:4) and rice BAB64102 DMI1 (SEQ ID NO:6) orthologs share about 80% similarity with the *M. truncatula* protein (SEQ ID NO:5). *A. thaliana* At5g02940 (SEQ ID NO:1), At5g43735

(SEQ ID NO:2) and rice AAN06856 (SEQ ID NO:3) proteins are 73% similar and share ~37% similarity with the orthologous grouping of DMI1-related proteins in *M. truncatula*, *Arabidopsis* and rice. *Mesorhizobium loti* NP_102608 (SEQ ID NO:10) and the *Streptomyces* (*S. avermitilis* NP821931 - SEQ ID NO:7; *S griseus* JL0032 – SEQ ID NO:8; *S. coelicolor* NP631245 - SEQ ID NO:9) proteins represent a third clade of proteins, with 60% similarity between bacterial homologs and ~37% similarity to the DMI1 orthologs. Transmembrane domains (dark gray) were predicted using THMM, TMpred and TopPred 2 software programs (us.expasy.org/tools/). The presence of signal peptides (underlined) was assessed using SignalP V2.0 software (at the cbs.dtu.dk/services/SignalP-2.0/ web site). The region homologous to cation channels was determined according to Anantharaman *et al.* (12) and annotated according to Jiang *et al.* (19).

(B) Unrooted phylogenetic tree and schematic diagrams of DMI1 homologs. Full-length protein sequences were aligned using ClustalW with default parameters. The branch-and-bound parsimony method was used to infer phylogenetic relationships, identifying two paralogous groups of plant genes and a single group of bacterial orthologs. Percentage bootstrap support is given to the side of each branch.

FIGURE 5 shows the nucleic acid sequence of DMI1 of *M. truncatula.*

FIGURE 6 shows the amino acid sequence of the DMI1 gene product.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

The following sequences are grouped according to the nature of the sequence. The list does not include sequences used as PCR primers or sequences used in sequence comparisons.

SEQ ID NO:11 is the protein encoding nucleotide sequence of DMI1 from the legume *Medicago truncatula*.

SEQ ID NO:13 is the portion of the protein encoding nucleotide sequence of DMI1 from the legume *Medicago truncatula* that is predicted to have cation channel activity as depicted in Figure 5.

SEQ ID NO:12 is the amino acid sequence of DMI1 from the legume *Medicago truncatula* as depicted in Figure 6.

SEQ ID NO:14 is the portion of the amino acid sequence of DMI1 from the legume *Medicago truncatula* that is predicted to have cation channel activity as depicted in Figure 6.

DETAILED DESCRIPTION OF THE INVENTION

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Throughout this disclosure, various publications, patents and published patent specifications are referenced. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of plant breeding, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook, Fritsch and Maniatis, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd edition (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, *et al.* eds., (1987); Plant Breeding: Principles and Prospects (Plant Breeding, Vol 1) M.D. Hayward, N.O. Bosemark, I. Romagosa; Chapman & Hall, (1993.); Coligan, Dunn, Ploegh, Speicher and Wingfeld, eds. (1995) CURRENT PROTOCOLS IN PROTEIN SCIENCE (John Wiley & Sons, Inc.); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995), Harlow and Lane, eds. (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE R.I. Freshney, ed. (1987).

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Lewin,

Genes V, published by Oxford University Press, 1994 (SBN 0-19-854287-9); Kendrew *et al.* (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (SBN 0-632-02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology, a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Ausubel *et al.* (1987) Current Protocols in Molecular Biology, Green Publishing; Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York. Definitions of common terms in plant biology may be found in Esau, Plant Anatomy, published by John Wiley & Sons (1977) (ISBN 0-471-24520-8); and Solomon *et al.*, Biology, published by Saunders College Publishing (1993).

Definitions

In order to facilitate review of the various embodiments of the invention, the following definitions are provided:

DMI1 protein or polypeptide, or *dmi1* gene: A DMI1 protein or DMI1 polypeptide is a protein encoded by the gene *dmi1*. In *Medicago truncatula*, mutations in DMI1 result in loss of the ability to form mycorrhizal or rhizobial symbiotic relationships.

The present invention may be practiced using nucleic acid sequences that encode full length DMI1 proteins as well as DMI1-derived proteins that retain DMI1 activity. The preferred DMI1 proteins are legume derived. DMI1-derived proteins which retain DMI1 biological activity include fragments of DMI1, generated either by chemical (e.g. enzymatic) digestion or genetic engineering means; chemically functionalized protein molecules obtained starting with the exemplified protein or nucleic acid sequences, and protein sequence variants, for example allelic variants and mutational variants, such as those produced by *in vitro* mutagenesis techniques, such as gene shuffling (Stemmer *et al.*, 1994a, 1994b). Thus, the term " DMI1 protein" encompasses full-length DMI1 proteins, as well as such DMI1 derived proteins that retain DMI1 activity. Depending upon the intended use of the DMI1 protein, the DMI1 biological activity may encompass the full activity of the DMI1 protein in mediating the rhizobial and fungus mycorrhizal symbiosis in plants. In certain situations, the DMI biological activity may encompass mediation of only one

of the two forms of symbiosis. For example, where the intended utility of the DMI1 protein is related to the enhancement of nitrogen fixation, then the relevant DMI1 biological activity will be the mediation of the rhizobial symbiosis. In still other situations, the DMI1 biological activity will be the cation channel activity.

Representative but non-limiting DMI1 sequences useful in the invention include SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:14.

Promoter: A regulatory nucleic acid sequence, typically located upstream (5') of a gene or protein coding sequence that, in conjunction with various cellular proteins, is responsible for regulating the expression of the gene or protein coding sequence. The promoters suitable for use in the heterologous nucleic acids of this invention are functional in plants and in other host organisms used for expressing the inventive polynucleotides. Many plant promoters are publicly known. These include constitutive promoters, regulated promoters, inducible promoters, root-, tissue- and cell-specific promoters, and developmentally-regulated promoters. Exemplary promoters and fusion promoters are described, e.g., in WO 02/00894, U.S. 6,342,657 and 6,376,750.

The promoters may be those normally associated with a transgene of interest, or heterologous promoters which are derived from genes of other plants, viruses, and plant pathogenic bacteria and fungi. Those skilled in the art will be able without undue experimentation to select promoters that are suitable for use in practicing the subject invention.

Regulated promoter: As used herein, this term refers to any promoter functional in a plant that provides differential expression levels in response to stimuli internal to the plant such as developmental signals. This includes both promoters that increase expression and promoters that decrease expression in response to stimuli or changed external conditions. Many promoters that are regulated promoters are also inducible promoters. For example, promoters that are responsive to auxin are both because they will change levels of expression in response to developmental changes in auxin levels and in response to externally supplied auxin.

Examples of regulated promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds, or flowers. Exemplary promoters include the anther

specific promoter 5126 (U.S. Pat. Nos. 5,689,049 and 5,689,051), glob-1 promoter, and gamma-zein promoter. An exemplary promoter for leaf- and stalk-preferred expression is MS8-15 (see U.S. Patent No. 5,986,174, herein incorporated by reference). Examples of seed-preferred promoters included, but are not limited to, 27 kD gamma zein promoter and waxy promoter (Boronat et al. (1986); Reina et al. (1990); and Kloesgen et al. (1986)). Promoters that express in the embryo, pericarp, and endosperm are disclosed in U.S. Patents 6,342,657 and 6,376,750 both of which are hereby incorporated by reference in their entirety. The operation of a promoter may also vary depending on its location in the genome. Thus, a developmentally regulated promoter may become fully or partially constitutive in certain locations. A developmentally regulated promoter can also be modified, if necessary, for weak expression.

Sequence Identity: Sequences that show similarity to those described in this application can be identified by computer-based methods, using public domain sequence alignment algorithms and sequence similarity search tools to search sequence databases (public domain databases include Genbank, EMBL, Swiss-Prot, PIR and others).

Similarity searches retrieve and align sequences for comparison with a target sequence to be analyzed (i.e., a query sequence). The optimal alignment between local regions of the compared sequences is known as a local alignment. Sequence comparison algorithms use scoring matrices to assign an overall score to each of the alignments.

Polynucleotide and polypeptide sequences may be aligned, and percentage of identical residues in a specified region may be determined against other polynucleotide and polypeptide sequences, using computer algorithms that are publicly available. The percentage identity score is dependent on the length of the overlap region of the sequences being compared.

The similarity between two nucleic acid sequences, or two amino acid sequences may be expressed in terms of sequence identity (or, for proteins, also in terms of sequence similarity). Sequence identity is frequently measured in terms of percentage identity; the higher the percentage, the more similar the two sequences are. Homologs are nucleic acid or amino acid sequences that share a common

ancestry as assessed by phylogenetic methods. The term "homolog" does not imply a particular level of similarity, although homologs of recent ancestry are typically more similar than homologs of distant ancestry. Therefore, percent identity or percent similarity may be used as approximate measures of how related a given pair of genes are. We have identified DMI1 homologs throughout the plant kingdom. As described herein, homologs and variants of the DMI1 nucleic acid molecules may be used in the present invention. Closely related homologs and variants of these nucleic acid molecules will possess a relatively high degree of sequence identity when aligned using standard methods. Such homologs and variants will hybridize under high stringency conditions to one another.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman (1981); Needleman and Wunsch (1970); Pearson and Lipman (1988); Higgins and Sharp (1988); Higgins and Sharp (1989); Corpet *et al.* (1988); Huang *et al.* (1992); and Pearson *et al.* (1994). Altschul *et al.* (1994) presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. It can be accessed at the NCBI Website. A description of how to determine sequence identity using this program is available at the NCBI website.

Homologs of the disclosed protein sequences are typically characterized by possession of at least 40% sequence identity counted over the full length alignment with the amino acid sequence of the disclosed sequence using the NCBI Blast 2.0, gapped blastp set to default parameters. The adjustable parameters are preferably set with the following values: overlap span 1, overlap fraction = 0.125, word threshold (T) = 11. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity. Proteins with even greater similarity to the reference sequences will

show increasing percentage identities when assessed by this method, such as at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% or at least about 95% sequence identity.

Homologs of the disclosed nucleic acid sequences are typically characterized by possession of at least 40% sequence identity counted over the full length alignment with the amino acid sequence of the disclosed sequence using the NCBI Blast 2.0, gapped blastn set to default parameters. In addition, such sequences hybridize to homologous sequences under high stringency conditions. A preferred method utilizes the BLASTN module of WU-BLAST-2 (Altschul *et al.*, 1996); set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively. Nucleic acid sequences with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% or at least about 95% sequence identity.

The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the protein depicted in SEQ ID NO:12, it is understood that in one embodiment, the percentage of sequence identity will be determined based on the number of identical amino acids in relation to the total number of amino acids. Thus, for example, sequence identity of sequences shorter than that shown in the figures as discussed below, will be determined using the number of amino acids in the longer sequence, in one embodiment. In percent identity calculations relative weight is not assigned to various manifestations of sequence variation, such as, insertions, deletions, substitutions, *etc.*

In one embodiment, only identities are scored positively (+1) and all forms of sequence variation including gaps are assigned a value of "0", which obviates the need for a weighted scale or parameters as described herein for sequence similarity calculations. Percent sequence identity can be calculated, for example, by dividing the number of matching identical residues by the total number of residues of the

"shorter" sequence in the aligned region and multiplying by 100. The "longer" sequence is the one having the most actual residues in the aligned region.

Proteins can be classified according to their sequence relatedness to other proteins in the same genome (paralogs) or a different genome (orthologs). Ortholog genes are genes that evolved by speciation from a common ancestral gene. These genes normally retain the same function as they evolve. Paralog genes are genes that are duplicated within a genome. These genes may acquire new specificities or modified functions which may be related to the original one. Phylogenetic analysis methods are well-known to those with ordinary skill in bioinformatics.

As will be appreciated by those skilled in the art, the sequences of the present invention may contain sequencing errors. That is, there may be incorrect amino acid sequences, nucleotides, frameshifts, unknown nucleotides, or other types of sequencing errors in any of the sequences; however, the correct sequences will fall within the homology and stringency definitions herein for nucleic acids, and the protein homology described for proteins or polypeptides.

Stringency: Stringency refers to hybridization conditions chosen to optimize binding of polynucleotide sequences with different degrees of complementarity. Stringency is affected by factors such as temperature, salt conditions, the presence of organic solvents in the hybridization mixtures, and the lengths and base compositions of the sequences to be hybridized and the extent of base mismatching, and the combination of parameters is more important than the absolute measure of any one factor.

Very High Stringency: Very high stringency conditions refers to hybridization to filter-bound DNA in 5 X SSC, 2% sodium dodecyl sulfate (SDS), 100 µg/ml single stranded DNA at 55-65 ° C for 8 hours, and washing in 0.1 X SSC and 0.1% SDS at 60-65 ° C for thirty minutes.

High Stringency: High stringency conditions refers to hybridization to filter-bound DNA in 5 X SSC, 2% sodium dodecyl sulfate (SDS), 100 µg/ml single stranded DNA at 55-65 ° C for 8 hours, and washing in 0.2 X SSC and 0.2% SDS at 60-65 ° C for thirty minutes.

Moderate Stringency: Moderate stringency conditions refers to hybridization to filter-bound DNA in 5 X SSC, 2% sodium dodecyl sulfate (SDS), 100

µg/ml single stranded DNA at 55-65 ° C for 8 hours, and washing in 0.2 X SSC and 0.2% SDS at 50-55 ° C for thirty minutes.

Low Stringency: Low stringency conditions refers to hybridization to filter-bound DNA in 5 X SSC, 2% sodium dodecyl sulfate (SDS), 100 µg/ml single stranded DNA at 55-65 ° C for 8 hours, and washing in 2.0 X SSC and 0.2% SDS at 50-55 ° C for thirty minutes.

Construct: Unless otherwise stated, the term "construct" refers to a recombinant genetic molecule comprising one or more isolated polynucleotide sequences of the invention.

Genetic constructs used for transgene expression in a host organism comprise (in the 5'-3' direction): a gene promoter sequence; an open reading frame coding for at least a functional portion of a polypeptide of the present invention; and a gene termination sequence. The open reading frame may be orientated in either a sense or anti-sense direction. The construct may also comprise selectable marker gene(s) and other regulatory elements for gene expression.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter controls the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary, join two protein-coding regions in the same reading frame. With respect to polypeptides, two polypeptide sequences may be operably linked by covalent linkage, such as through peptide bonds or disulfide bonds.

Vector: The term "vector" refers to a nucleic acid molecule which is used to introduce a polynucleotide sequence into a host cell, thereby producing a transformed host cell. A "vector" may comprise genetic material in addition to the above-described genetic construct, e.g., one or more nucleic acid sequences that permit it to replicate in one or more host cells, such as origin(s) of replication, selectable marker genes and other genetic elements known in the art (e.g., sequences for integrating the genetic material into the genome of the host cell, and so on).

Transformed: A transformed cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, plant or animal cell, including transfection with viral vectors, transformation by *Agrobacterium*, with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration and includes transient as well as stable transformants.

Isolated: An "isolated" biological component (such as a nucleic acid or protein or organelle) has been substantially separated or purified away from other biological components in the cell or the organism in which the component naturally occurs, i.e., other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term embraces nucleic acids including chemically synthesized nucleic acids and also embraces proteins prepared by recombinant expression *in vitro* or in a host cell and recombinant nucleic acids as defined below. As an example, a gene in a large genomic DNA fragment such as a contig is not sufficiently purified away from other biological components to be considered isolated due to the relatively large amount of extra DNA found in the average contig. As outlined below "recombinant nucleic acids" and "recombinant proteins" also are "isolated" as described above

Recombinant: By "recombinant nucleic acid" herein is meant a nucleic acid that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of nucleic acids, e.g., by genetic engineering techniques, such as by the manipulation of at least one nucleic acid by a restriction enzyme, ligase, recombinase, and/or a polymerase. Once introduced into a host cell, a recombinant nucleic acid is replicated by the host cell; however, the recombinant nucleic acid once replicated in the cell remains a recombinant nucleic acid for purposes of this invention. By "recombinant protein" herein is meant a protein produced by a method employing a recombinant nucleic acid. As outlined above "recombinant nucleic acids" and "recombinant proteins" also are "isolated" as

described above. A gene in a large fragment such as a contig would not be a "recombinant nucleic acid" given that artificial combination does not relate to the gene. However, if sequences around or within a gene in a contig have been manipulated for purposes relating to that gene (i.e., not merely because the gene is near the end of the contig), then such a gene in a contig would constitute a "recombinant nucleic acid" due to the relative proximity of the recombinant portion of the nucleic acid to the gene in question.

Complementary DNA (cDNA): A piece of DNA that is synthesized in the laboratory by reverse transcription of an RNA, preferably an RNA extracted from cells. cDNA produced from mRNA may include 5′ and/or 3′ noncoding sequences (i.e., 5′ UTR, 3′ UTR) but typically lacks internal, non-coding segments (introns) and regulatory sequences, such as promoters, that determine transcription.

Open reading frame (ORF): A continuous coding sequence of a gene flanked by a start and stop codon. An ORF lacks internal termination codons and can usually be translated into an amino acid sequence.

Non-naturally Occurring Plant: A non-naturally occurring plant is a plant that does not occur in nature without human intervention. Non-naturally occurring plants include transgenic plants and plants produced by non-transgenic means such as plant breeding.

Transgenic plant: As used herein, this term refers to a plant or tree that contains recombinant genetic material not normally found in plants or trees of this type and which has been introduced into the plant in question (or into progenitors of the plant) by human manipulation. Thus, a plant into which recombinant DNA is introduced by transformation is a transgenic plant, as are all offspring of that plant that contain the introduced transgene (whether produced sexually or asexually). It is understood that the term transgenic plant encompasses the entire plant or tree and parts of the plant or tree, for instance grains, seeds, flowers, leaves, roots, fruit, pollen, stems *etc.*

Standard molecular biology methods and plant transformation techniques can be used to produce transgenic plants that produce plants having a recombinant *dmi1* gene.

Ortholog: Two nucleotide or amino acid sequences are orthologs of each other if they share a common ancestral sequence and diverged when a species carrying that ancestral sequence split into two species, sub-species, or cultivars. Orthologous sequences are also homologous sequences. Orthologous sequences hybridize to one another under high-stringency conditions. The term "polynucleotide", "oligonucleotide", or "nucleic acid" refers to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. The terms "polynucleotide" and "nucleotide" as used herein are used interchangeably. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. A "fragment" or "segment" of a nucleic acid is a small piece of that nucleic acid.

Gene: A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular protein after being transcribed and translated. In the present invention, the gene for DMI1 is described above.

Primer: The terms "primer" and "nucleic acid primer" are used interchangeably herein. A "primer" refers to a short polynucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product. The exact length of the primer will

depend upon many factors, including temperature, source of primer and use of the method.

Polymerase chain reaction: A "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using a "primer pair" or a "set of primers" consisting of an "forward" and a "reverse" primer, and a catalyst of polymerization, such as a DNA polymerase, and particularly a thermally stable polymerase enzyme. Methods for PCR are taught in U.S. Patent Nos. 4,683,195 (Mullis) and 4,683,202 (Mullis *et al.*). All processes of producing replicate copies of the same polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "amplification" or "replication".

Mycorrhizal symbiosis: Mycorrhizal symbiosis is the association of a plant root with specific fungi. The fungus invades the root but the root does not develop a pathologic response. The plant host is believed to be secreting sugars and other organic materials to the fungus, while the fungus appears to convert minerals in the soil (such as phosphorus) and decaying organic material into forms accessible to the host.

Rhizobial symbiosis: Rhizobial symbiosis is the association of the root with specific nitrogen-fixing bacteria. Symbiosis with the bacterial species *Rhizobium* typically results in the formation of nodules on the roots of the plants; the *Rhizobium* live in these nodules. The bacteria receive energy in the form of carbon compounds from the plant, while the plants receive nitrogen in a usable form.

Nitrogen fixation: Nitrogen fixation is the conversion of gaseous nitrogen (N_2) to ammonia (NH_3) or nitrate (NO_3^-).

The present invention meets the needs for enhanced symbiosis in plants and methods and compositions for direct supplementation of soils with nitrogen and/or phosphorus. The initiation of the symbiosis between the leguminous plants and rhizobial bacteria is controlled by a molecular dialog between the two partners. Legume roots secrete specific exudates, essentially flavonoids, which induce the secretion by the bacteria of lipo-chitooligosaccharidic signals called Nod factors (Dénarié *et al.*, 1996). Nod factors in turn, elicit plant responses that culminate in infection by rhizobia and the development of the root nodule. Extremely low concentrations (10^{-9} to 10^{-12} M) of purified Nod factors trigger many of the same

responses in the roots of legume hosts that are induced by symbiotic rhizobia. These responses include changes in ion fluxes, gene expression, and cell division (Long, 1996; Schultze and Kondorosi, 1998). Genetic screens in the model legume *Medicago truncatula* have identified mutants that are incapable of forming root nodules (Nod^- phenotype). (Sagan *et al.*, 1995; Cook *et al*, 1999; Penmetsa and Cook, 2000). Among these Nod^- mutants, those impaired pleiotropically for early Nod factor responses were selected and separated into six complementation groups corresponding to the *NFP*, *DMI1*, *DMI2*, *DMI3*, *NSP1* and *NSP2* loci (Catoira *et al.*, 2000; Ben Amor *et al.*, 2003, Oldroyd and Long, 2003).

Phenotypic analyses have placed these genes into a hierarchy. *NFP* resides at the top of the cascade, as *nfp* mutants do not exhibit any of the known responses to Nod factor. *DMI1* and *DMI2* genes are necessary for the induction of calcium spiking in root hairs indicating that these genes must play a role upstream of *DMI3*, *NSP1* and *NSP2* (Wais *et al.*, 2000), which are dispensable for this response.

Symbiosis with the bacterial species *Rhizobium* typically results in the formation of nodules on the roots of the plants. The *Rhizobia* live in these nodules and fix nitrogen. The bacteria receive energy in the form of organic compounds such as dicarboxyolic acids from the plant, while the plants receive nitrogen in a usable form, "fixed nitrogen." While not wishing to be bound by theory, it is thought that the nodule environment protects the nitrogenase enzyme from oxygen, which would impair the nitrogenase activity.

The *dmi1*, *dmi2* and *dmi3* mutants are also affected for the establishment of the mycorrhizal symbiosis (Nod^- Myc^- mutants) indicating that the signaling pathways of nodulation and mycorrhization share common components (Catoira *et al.*, 2000; Geurts and Bisseling, 2002). In mycorrhizal symbiosis, specific fungi associate with the roots of a plant. The fungi invade the root but the root does not develop a pathologic response. The plant host is believed to be secreting sugars and other organic materials to the fungus, while the fungus appears to convert minerals (such as phosphorus) and other soil nutrients into forms accessible to the host. The molecular details of mycorrhizal symbiosis are not as well understood as those of rhizobial symbiosis.

Uses of the Invention

By way of example and not of limitation, the following uses of the invention are described and are therefore included in the scope of the invention:

The nucleic acid of the invention will be useful in identifying *dmi1* and *dmi1*-related genes in other species. In addition, the *dmi1* nucleic acid will be useful in designing probes that may be used to detect *dmi1* and specific variants of *dmi1*. Such probes may be useful in breeding plants with particular *dmi1* genes. Antisense nucleic acids and RNA inteference (RNAi) nucleic acids of the invention may be used to decrease the level of DMI1 transcripts in the cell, thereby decreasing the level of DMI1 protein in the cell, presumably altering the cell or organism's response to stimuli.

Transformation or transfection of prokaryotic or eukaryotic host cells with the nucleic acid of the *dmi1* gene will be useful in amplifying, modifying, and characterizing the *dmi1* gene and its encoded DMI1 protein. The primers and vectors of the invention will be useful for the same purposes. Modification of the *dmi1* nucleic acid and the DMI1 amino acid sequence may entail mutagenesis, deletions, additions, fusions, or other alterations of various parts of the gene or protein in order to change its activity, thereby altering the effect of DMI1 on plant symbioses with mycorrhizae and rhizobia. Such mutations, deletions, substitutions, additions, and fusions of the *dmi1* gene and protein are within the scope of the invention. *dmi1* fusions may include the use of heterologous promoters to alter the regulation of the *dmi1* gene.

It has been discovered that two nitrogen-fixing symbiants, *Mesorhizobium loti* and *Streptomyces* spp, contain relatively close homologs of *dmi1*. The nucleic acid of the *dmi1* gene will be useful in determining what function if any *dmi1* plays in these organisms.

The antibodies of the invention will be useful in identifying species with polypeptides having similar structural characteristics to the DMI1 polypeptide. Additionally the antibodies of the invention may be used to impair the activity of DMI1 in vitro or in vivo, thereby altering the cell or organism's response to stimuli. The DMI1 protein of the invention will be helpful in isolating other members of the

pathways controlling plant mycorrhizal and rhizobial symbioses. For instance, the DMI1 protein may be used to identify ligands in the pathways.

The nucleic acid of the invention is useful in generating transgenic plants. The transgenic plants of the invention are useful in that such plants may exhibit improved levels of plant nutrition, as the plant will have increased levels of available phosphorus and/or nitrogen to utilize. Such transgenic plants may also improve the surrounding soil's nutrient quality, particularly with regards to levels of available nitrogen and phosphorus. Such transgenic plants may also be useful in decreasing the levels of nitrogen and phosphorus, where such levels are higher in a local environment than is desirable. Plants comprising mutations, deletions, substitutions, additions, and fusions of the *dmi1* gene and protein may exhibit altered effects on plant symbioses with mycorrhizae and rhizobia. Such effect may result in altered plant nutrition, as the plant will have different levels of available phosphorus and/or nitrogen to utilize. Of particular interest is generation of important crop plants such as corn, wheat, barley, sorghum, oat, and rye that are capable of rhizobial symbiosis. Generating transgenic forms of such important crop plants expressing DMI1 as well as other genes in the symbiosis pathway may enable such important crop plants to form nodules with nitrogen fixing bacteria.

It is also of particular interest to increase the nitrogen-fixing capability of plants which already do so. Such plants include, but are not limited to soybean, common bean, pea, peanut, lentil, chickpea, cowpea, pigeon peas, alfalfa, and clover. In addition to the human and animal food produced by these plants, nitrogen-fixing legumes are also used as cover crops in which they are overseeded onto other crops such as wheat or oats, or grown in between crops to increase nitrogen content in the soil. Such cover or rotation crops include but are not limited to, crimson clover, hairy vetch, field peas, subterranean clover, red clover, white clover and sweetclover. There are also woody legumes such as Acacia and Albizia of which the leaves are consumed in a number of countries. Increasing the nitrogen fixation capability of these plants would also increase the usefulness of these nitrogen-fixing legumes as rotation or cover crops.

This invention will be better understood by reference to the following non-limiting embodiments.

Constructs

The present invention includes various aspects of nucleic acid sequences encoding DMI1 proteins. The simplest form of nucleic acid of the present invention is an isolated nucleic acid encoding a DMI1 protein or fragment thereof having some relevant DMI1 biological activity. Examples of such nucleic acids include nucleic acids that hybridize to the DMI1 nucleic acid disclosed herein under low, moderate, high or very high stringency, nucleic acids with 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identity to the DMI1 nucleic acids disclosed herein, and nucleic acids encoding a DMI protein with 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identity to the DMI1 proteins disclosed herein. In addition, the nucleic acids may include nucleic acids that encode proteins that share conserved regions with other DMI1 proteins when aligned with DMI1 protein families such as the *A. thaliana, O. sativa*, and *M. truncatula* proteins. Such conserved regions may share 70%, 75%, 80%, 85%, 90%, 95%, or 97% identity. Examples of conserved regions with 95% or greater identity in SEQ ID NO:12 include residues 345-376, 379-402, and 413-468.

In addition, the present invention includes the above nucleic acid sequences operably linked to a promoter. The preferred promoter is a heterologous promoter. The choice of promoter will be dictated by the target cell in which the DMI1 protein is to be expressed. Selection of an appropriate promoter functional in a desired target cell is routine in the art. One of skill in the art can use, for example, a constitutive promoter, an inducible promoter or a regulated promoter depending upon the desired pattern of expression. In addition to natural promoters, mutant promoters and artificial promoters created by splicing distinct regulatory elements may be used.

Another aspect of the present invention is vectors including the nucleic acids and promoter linked constructs described above. There are a wide range of vectors available to one of skill in the art. Such vectors can include, without limitation, expression vectors, cloning vectors, shuttle vectors, etc. which can include, but are not limited to, the following vectors or their derivatives: human, animal, or plant viruses such as vaccinia virus, adenovirus, cauliflower mosaic virus (CaMV), geminivirus, brome mosaic virus, and tobacco mosaic virus; insect viruses such as

baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid (e.g. the Ti plasmid of Agrobacterium tumefaciens) and cosmid DNA vectors, to name but a few. Selection of the appropriate vector will be dictated by the target cells, desired expression mode (e.g., transient expression versus permanent integration into the genome versus independently replicating vectors will cause one of skill in the art to select different vectors), and ease of recombinant manipulation. In some circumstances, one of skill in the art would use a shuttle vector that is functional in at least two organisms so that the nucleic acid may be manipulated in one organism and then transferred into the other.

Cells

The present invention encompasses cells containing the above constructs. The cells may be generated by standard molecular biology techniques discussed below. Preferred embodiments of the present invention include transgenic plants, plant cells, plant parts, and plant seeds. Preferred plants include, without limitation, monocots and dicots such as maize, sunflower, Brassica, cotton, sorghum, wheat, barley, millet, rice, cassava, potato, Arabidopsis, tomato, pepper, apple, spinach, or lettuce, legumes such as soybean, alfalfa, common bean, pea, peanut, lentil, chickpea, cowpea, pigeon peas, and clover. Other preferred embodiments include transgenic bacteria capable of fixing nitrogen.

Methods

The present invention also includes methods of making the constructs, vectors, transgenic cells and plants discussed above. The constructs and vectors may be generated using standard molecular biology techniques

Introduction of the selected construct into plants is typically achieved using standard transformation techniques. The basic approach is to: (a) clone the construct into a transformation vector, which (b) is then introduced into plant cells by one of a number of techniques (e.g., electroporation, microparticle bombardment, *Agrobacterium* infection); (c) identify the transformed plant cells and regenerate whole plants from the identified plant cells, and (d) select progeny plants containing the introduced construct.

Preferably all or part of the transformation vector will stably integrate into the genome of the plant cell. That part of the transformation vector which integrates into the plant cell and which contains the introduced recombinant sequence may be referred to as the recombinant expression cassette.

Selection of progeny plants containing the introduced transgene may be made based upon the detection of the recombinant *dmi1* gene in transgenic plants, upon the detection of the recombinant DMI1 or DMI1-related protein coding sequence or upon enhanced resistance to a chemical agent (such as an antibiotic) as a result of the inclusion of a selectable marker gene incorporated into the transformation vector.

Successful examples of the modification of plant characteristics by transformation with cloned nucleic acid sequences are replete in the technical and scientific literature. Selected examples, which serve to illustrate the knowledge in this field of technology include: U.S. Patent No.5,571,706 ("Plant Virus Resistance Gene and Methods"); U.S. Patent No.5,677,175 ("Plant Pathogen Induced Proteins"); U.S. Patent No. 5,510,471 ("Chimeric Gene for the Transformation of Plants"); U.S. Patent No.5,750,386 ("Pathogen-Resistant Transgenic Plants"); U.S. Patent No.5,597,945 ("Plants Genetically Enhanced for Disease Resistance"); U.S. Patent No.5,589,615 ("Process for the Production of Transgenic Plants with Increased Nutritional Value Via the Expression of Modified 2S Storage Albumins"); U.S. Patent No.5,750,871 ("Transformation and Foreign Gene Expression in Brassica Species"); U.S. Patent No. 5,268,526 ("Overexpression of Phytochrome in Transgenic Plants"); U.S. Patent No. 5,780,708 ("Fertile Transgenic Corn Plants"); U.S. Patent No. 5,538,880 ("Method for Preparing Fertile Transgenic Corn Plants"); U.S. Patent No. 5,773,269 ("Fertile Transgenic Oat Plants"); U.S. Patent No. 5,736,369 ("Method for Producing Transgenic Cereal Plants"); U.S. Patent No. 5,610,049 ("Methods for Stable Transformation of Wheat"); U.S. Patent No. 6,235,529 ("Compositions and Methods for Plant Transformation and Regeneration") all of which are hereby incorporated by reference in their entirety. These examples include descriptions of transformation vector selection, transformation techniques and the construction of constructs designed to express an introduced transgene.

The transgene-expressing constructs of the present invention may be usefully expressed in a wide range of higher plants where an altered mycorrhizal and/or rhizobial symbiosis response is useful.

Methods for the transformation and regeneration of monocotyledonous and dicotyledonous plant cells are known, and the appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods may include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG-mediated transformation); transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium*-mediated transformation. Typical procedures for transforming and regenerating plants are described in the patent documents listed above.

Following transformation, transformants are preferably selected using a dominant selectable marker. Typically, such a marker will confer antibiotic or herbicide resistance on the seedlings of transformed plants, and selection of transformants can be accomplished by exposing the seedlings to appropriate concentrations of the antibiotic or herbicide. Suitable markers include, without limitation, those genes coding for resistance to the antibiotic spectinomycin or streptomycin (e.g., the aada gene), the streptomycin phosphotransferase (SPT) gene coding for streptomycin resistance, the neomycin phosphotransferase (NPTII) gene encoding kanamycin or geneticin resistance, the hygromycin phosphotransferase (HPT) gene coding for hygromycin resistance. After transformed plants are selected and grown the plant can be assayed for expression of recombinant proteins.

Proteins

The present invention further includes isolated DMI1 proteins and fragments thereof with DMI1 biological activity. The proteins may be isolated by routine techniques available to one of ordinary skill in the art. Such techniques include overexpression in desired target cells and purification therefrom. Standard methods

of protein purification include chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Such standard techniques may be found in Robert K. Scopes, Protein Purification: Principles and Practice, Springer Verlag, 3rd Ed. 1996. In addition, affinity tags may be affixed to the protein via molecular biology to ease purification. Examples include his-tagging and flag-tagging the protein. The functional properties may be evaluated using any suitable assay

Ligands

The present invention includes ligands that interact with the above described proteins. Such ligands include small molecules, antibodies and other proteins. Antibodies may be generated by standard molecular biology techniques. Small molecule ligands may be identified by standard techniques available to one of ordinary skill in the art. With the automated screening techniques available today, large libraries may be screened with ease once pure protein is available. Such ligands may merely bind to the proteins while others may down regulate or completely inhibit the DMI1 biological activity or upregulate or activate the DMI1 biological activity.

Antibodies can be raised to a protein of the present invention, including individual, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms and in recombinant forms. Additionally, antibodies are raised to these proteins in either their native configurations or in non-native configurations. Anti-idiotypic antibodies can also be generated. Many methods of making antibodies are known to persons of skill.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies are found in, e.g., Basic and Clinical Immunology, 4th ed., Stites et al., Eds., Lange Medical Publications, Los Altos, Calif., and references cited therein; Harlow and Lane, Supra; Goding, Monoclonal Antibodies: Principles and Practice, 2nd ed., Academic Press, New York, N.Y. (1986); and Kohler and Milstein, Nature 256: 495-497 (1975).

Other suitable techniques involve selection of libraries of recombinant antibodies in phage or similar vectors (see, e.g., Huse et al., Science 246:1275-1281 (1989); and Ward, et al., Nature 341:544-546 (1989); and Vaughan et al., Nature Biotechnology, 14:309-314 (1996)). Alternatively, high avidity human monoclonal antibodies can be obtained from transgenic mice comprising fragments of the unrearranged human heavy and light chain Ig loci (i.e., minilocus transgenic mice). Fishwild et al., Nature Biotech., 14:845-851 (1996). Also, recombinant immunoglobulins may be produced. See, Cabilly, U.S. Pat. No. 4,816,567; and Queen et al., Proc. Nat'l Acad. Sci. 86:10029-10033 (1989).

The antibodies of this invention can be used for affinity chromatography in isolating proteins of the present invention, for screening expression libraries for particular expression products such as normal or abnormal protein, for raising anti-idiotypic antibodies which are useful for detecting or diagnosing various pathological conditions related to the presence of the respective antigens, for detecting expression of the DMI1 protein or allelic variants when breeding plants, and for down regulating or up regulating the activity of the DMI1 protein.

Kits

The present invention also includes kits useful for detecting the presence of the dmi1 nucleic acids and proteins of the present invention. Such kits may include molecules for the detection of the DMI1 genes and nucleic acids of the present invention such as nucleic acid probes for hybridization or primers for amplification and detection of DMI1. Alternatively, such kits may include molecules for the detection of the DMI1 proteins of the present invention such as the antibodies and ligands described above.

The present invention further includes kits useful in generating transgenic plants expressing the DMI1 protein. Such kits will include the constructs or vectors described above. In addition, the kits may contain additional materials useful for plant transformation as described above under methods.

Example 1: Cloning DMI1 *from* M. truncatula

By means of genetic and cytogenetic analyses *DMI1* was previously localized to one extremity of *M. truncatula* chromosome 2 (Ané *et al.* 2002). Genetic marker 1N1R, which defined the telomere end of linkage group 2 and was invariantly linked to the *DMI1* locus in an F2 population of 499 Nod$^-$ individuals, was used to seed a chromosome walk towards *DMI1*. In parallel to physical mapping, knowledge of chromosomal synteny between *M. truncatula* and *M. sativa* (Choi *et al.* 2003) was used to systematically position new genetic markers between 1N1R and the telomere. Genetic mapping of 1N1R in *M. sativa* revealed a single genetic marker, U212D, that was telomeric of 1N1R in both species (Figure 1A). Using U212D as probe, additional BAC clones (e.g., mth2-50E3) of *M. truncatula* were identified and a 1.2 Mb contig extending from genetic marker 88P13S to the adjacent telomere of chromosome 2 was rapidly completed (Figure 1).

Genotyping of >1,500 individuals identified a single recombination event that delimited *DMI1* to a 550 kbp interval between genetic marker 57N18S and the telomere. The candidate gene approach was used to identify the gene. A minimum tiling path composed of 5 BAC clones (~550kb in length) was sequenced using a BAC sublibrary shotgun strategy. Fgenesh (at the softberry.com/berry.phtml web site) predicted 85 genes in the sequenced region, roughly half of which were supported by unigene assignments in the *M. truncatula* EST data. Oligonucleotide primers were designed for candidate genes and the polymerase chain reaction was used to amplify both genomic DNA and cDNA isolated from independent *DMI1* alleles, including 3 ethylmethane sulfonate (EMS) and 2 fast neutron bombardment (FNB) mutants. A single unknown protein gene on BAC mth2-54A24 was altered in each of five independent alleles (Table 1) with strong affects on the transcript and/or predicted protein structure. To confirm the identity of this gene as *DMI1*, we verified the ability of a wild type *DMI1* transgene to complement the Nod$^-$ phenotype of *DMI1* alleles C71 and Y6 (Figure 2).

A full-length *DMI1* cDNA was cloned from *M. truncatula* root mRNA and determined to encode a 2,649 bp open reading frame with a deduced protein of 883 amino acids. Predicted features of the protein include 4 trans-membrane domains, the first two of which encompass a putative leucine zipper (LZ) motif, and a proline

rich domain that could facilitate protein–protein interactions (Bornberg-Bauer *et al.* 1998). Other than these general features, the DMI1 protein lacks even moderate similarity to functionally characterized proteins or protein sub-domains in plants, although as described below the protein is well conserved throughout most lineages of land plants that have been sampled by EST and genome sequencing projects.

Example 2: Transgenic complementation of DMI1 *in plant root*

To confirm that the identified gene was *DMI1*, we analyzed the ability of wild type *DMI1* gene to complement the Nod$^-$ Myc$^-$ phenotype of the mutants. In a previous study, we have shown that the nodulation phenotype of the *dmi* mutants is determined by the genotype of the root (Ané *et al.*, 2002). As such it was possible to use the *Agrobacterium rhizogenes* mediated transformation to complement the *dmi1* mutation (Boisson-Dernier *et al.*, 2001).

The full length wild type *DMI1* cDNA was amplified in a pCR2.1 vector (Invitrogen). 1.6 kb of the promoter sequence was also amplified from mth2-54A24 and cloned upstream of the cDNA. The whole construct was introduced into the pCAMBIA-1303 binary vector (clone pCAMBIA-DMI1). *A. rhizogenes* strain Arqua1 containing the pCH32 vector carrying *virE* and *virG* virulence genes was used for transformation. pCAMBIA-DMI1 and pCAMBIA-1303 (as a negative control) were introduced into Arqua1 (pCH32) strain. Roots of *DMI1* and wild type plants were transformed with either the *DMI1* cDNA under control of the native promoter or with vector alone. Transformed roots were inoculated with *Sinorhizobium meliloti* and nodule development was scored visually after 15 days. A constitutively expressed *uidA* gene was used as a reporter to identify transformed tissues based on GUS staining. Among more than 80 independent *DMI1* transformants, only those transformed by the *DMI1* transgene were nodulated by *S. meliloti*, while transformation of wild type plants with either *DMI1* or control constructs had no effect on nodulation. The restoration of the Nod$^+$ phenotype clearly confirmed that we had cloned *DMI1* (Figure 2).

Example 3: Expression analysis of DMI1

The expression of *DMI1* was analyzed by means of semi-quantitative RT-PCR (Figure 3A). RNAs were extracted using RNeasy Plant Minikit (Qiagen, Germany) and

quantified with Ribogreen RNA quantitation kit (Molecular Probes, USA). RT-PCR experiments were performed using the PowerScript™ RT-PCR kit (Clontech laboratories, USA). The results indicated that *DMI1* is constitutively expressed in roots, with lower levels of transcript detected in pods, flowers, leaves and stems. Treatments known to activate expression of plant "nodulin genes", including inoculation with the compatible symbiotic bacterium *Sinorhizobium meliloti* (Figure 3B) or 10^{-9} M cognate Nod factors (data not shown), had no effect on *DMI1* transcript levels.

Example 4: Analysis of the gene structure and homologs

A growing body of evidence indicates that the signalling pathway leading to nodulation is highly conserved across legume species and that diverse legume species are likely to contain orthologous signalling components (Endre *et al.* 2002). Southern blot experiments indicate that *DMI1* is a single copy gene *in M. truncatula*, with homologs present in many other leguminous plants, including *Medicago sativa*, *Melilotus alba*, *Vicia hirsuta*, *Lotus japonicus*, *Sesbania*, *Cassia*, *Trifolium*, *Desmodium*, *Vigna*, *Macroptilium* and *Vigna radiata* (data not shown). BLASTN analyses identified highly similar ESTs in soybean and *L. japonicus* indicating that the sequence homologs in these species represent expressed genes.

BLASTX and TBLASTN searches of the NCBI non-redundant and EST databases revealed with highly similar proteins predicted in over 28 monocot and dicot species, and a more distant homolog in the non-vascular plant *Physcomitrella patens*. Related sequences were not identified in the fully sequenced genomes of other eukaryotes, including animals, fungi, or green algae. Instead, relatively close homologs were identified in two eubacterial genera, namely the nitrogen fixing symbiont *Mesorhizobium loti* and *Streptomyces* spp. Surprisingly, homologous genes were not evident in any other sequenced bacterial genome, including close relatives of *M. loti* such as *Sinorhizobium meliloti* and *Agrobacterium tumefaciens*. Phylogenetic reconstruction based on parsimony analysis was used to infer the evolutionary history of this gene family. Analysis of several full-length deduced proteins from *M. truncatula*, *Arabidopsis*, rice and bacterial species yielded three well supported clades, corresponding to two paralogous groups of plant genes and a single group of bacterial genes (Figure 4B).

The complete sequence of *DMI1* putative orthologous genes was available for the *A. thaliana* At5g49960 and rice BAB64102 genes, corresponding to single copy genes on chromosomes 5 and 1 respectively. The *Arabidopsis* and *M. truncatula* loci, but not their rice counterpart, reside in a region of conserved genome microsynteny, presumably indicative of the ancestral chromosomal context (Figure 1C). The *A. thaliana* and rice predicted proteins share 80% identity with *DMI1*, with the highest similarity found in the C-terminal region. Although ESTs corresponding to the *A. thaliana* gene were absent from the public databases semi-quantitative RT-PCR analysis indicates that the Arabidopsis gene is expressed in roots but not in leaves (Figure 3A).

Comparison of the deduced partial protein *Physcomitrella patens* with either group of angiosperm paralogs indicates that all three groups are roughly equally diverged from one another (i.e., 26-28% identity and 53-56% similarity), consistent with an origin in the non-vascular plants. Taken together with the absence of homologs in the fungal, animal and algal lineages, these results suggest that the DMI1 protein represents a plant-specific innovation that potentially arose near the base of the land plant lineage. Interestingly, the fact that the *M. loti* and *Streptomyces* sequences also branch from the base of the DMI1 lineage, near the inferred plant duplication, is suggestive of horizontal transfer from an ancient plant genome(s) to a limited number of bacterial species.

DMI1 is predicted to act in genetic proximity to two additional *M. truncatula* genes, *DMI2* and *DMI3*, which together comprise the set of known genes common to both mycorrhizal and rhizobial symbioses (Catoira *et al.* 2000). All three *DMI* genes are implicated in transduction of the Nod factor signal; DMI1 and DMI2 are required for Nod factor-induced calcium spiking, which by analogy to animal systems is predicted to have a causal role in the Nod factor signal transduction cascade. Despite the fact that DMI1 homologs have been identified in the bulk sequencing projects of many plant species, DMI1 represents the first member of this gene family with an assigned phenotype and verified gene structure. As with the previously identified NORK receptor kinase (*DMI2*) (Endre *et al.* 2002), *DMI1* is predicted to be a membrane spanning protein and may participate in the formation of a receptor-complex for symbiotic signals. The fact that proline-rich and leucine zipper domains

often participate in protein-protein interactions supports the idea of such a protein complex, but these motifs are present in the N-terminus of DMI1, a region of the protein that is poorly conserved among the various homologs. Together, the homologs of *Arabidopsis*, rice, *Mesorhizobium* and *Streptomyces* proteins comprise the DUF1012 protein family in the Pfam database (at the sanger.ac.uk/Software/Pfam/ web site), without functional assignment. Pfam identifies the so-called DUF1012 domain as a defining feature of this protein family, which in the case of DMI1 overlaps substantially with a domain of low, but broad similarity to the NAD-binding TrkA domain of certain bacterial potassium channels (Anantharaman *et al.* 2001). The central portion of DMI1, commencing at the third predicted transmembrane domain through the entire TrkA homology region, is predicted to share distant homology with the well-characterised potassium channels of certain archaea (Figure 4A). More generally, proteins of this family are implicated as cation channels in both prokaryotes and eukaryotes. The crystal structure of the *Methanobacterium thermoautotrophicum* protein MthK reveals a multimeric K^+ channel that is ligand-gated by Ca^{2+} (Jiang *et al.* 2002). DMI1 is conserved throughout both the pore and ring domains that are thought to constitute the functional cation channel (Jiang *et al.* 2002), but degenerate in the filter region, located between transmembrane domains 3 and 4, where structural modifications have been correlated with altered cation specificity (Maser *et al.* 2002). Whether DMI1 and its close phylogenetic relatives in plants and bacteria might also function as ligand-gated cation channels remains uncertain, but the possibility is particularly intriguing given the genetic requirement for DMI1 function in Nod factor induced calcium oscillations within *Medicago* root hair cells.

If DMI1 is membrane-localised, it will be important to resolve the cellular localisation (intracellular or extracellular) of the DMI1 N- and C-termini. An extracellular ligand-binding domain could facilitate perception of symbiotic signals, while an intracellular location might suggest a role in downstream signal transduction events. Similarly, identifying DMI1-interacting proteins should also expand our understanding of the role of the *DMI1* protein in symbiotic interactions. Candidate proteins to interact with *DMI1* include LYK3 and 4/NFR1 and 5 (Limpens *et al.* 2003, Radutoiu *et al.* 2003, Madsen *et al.* 2003, Parniske *et al.* 2003), NFP

(Amor *et al.* 2003), DMI2 (Endre *et al.* 2002, Stracke *et al.* 2002), and DMI3 (Catoira *et al.* 2000), in addition to other proteins not yet identified in molecular or genetic screens.

Approximately 80% of terrestrial plants establish mycorrhizal symbioses. These beneficial plant-fungal associations act to expand the effective root-soil interface; in addition to their importance in natural and agricultural ecosystems, they may have facilitated the colonization of land by plants by increasing access to vital soil nutrients in the primitive root-like structures of non-vascular plants (Heckman *et al.* 2001). The high degree of conservation for DMI1 orthologs among angiosperms (>80% similarity among rice, Arabidopsis and *Medicago* proteins) and the fact that only a single gene with high similarity to DMI1 is present in the fully sequenced genomes of rice and *Arabidopsis*, suggests that the biological and molecular function of this group of proteins is also likely to be conserved. By comparison, other genes that have been recently identified for Nod factor signalling (i.e., LYK homologs, DMI2) are less well conserved among angiosperms, and in the case of LYK homologs and DMI2 appear to be members of large, fast evolving gene families. It is widely anticipated that nodulation in legumes arose from an ancient, conserved pathway for mycorrhizal associations, and we suggest that DMI1 is a pivotal component of this pathway.

References and Notes

The following references and notes are hereby incorporated by reference in their entirety:

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Table 1: Summary of *M. truncatula dmi1* alleles

Mutagenesis	*DMI1* mutant	Nature of mutations
EMS	B129	G1068A point mutation that results in a premature stop codon truncating the protein at amino acid position 356
EMS	C71 (domi)	G1264A point mutation that occurs at the 5' splice site of the third intron and that causes a mis-splicing event
EMS	Y6	C913T point mutation that creates a premature stop codon truncating the protein at amino acid position 305
FNB	GY15-3F-4	Large deletion of all the 5′ of the gene and the promoter
FNB	GY15-1B-5.1	Large deletion of all the 5′ of the gene and the promoter

We claim:

1. An isolated nucleic acid comprising a sequence of nucleotides having at least about 95% sequence identity to the sequence of SEQ ID NO:11 or its complement.

2. An isolated nucleic acid that hybridizes to the nucleic acid sequence of SEQ ID NO:11 or its complement under hybridization conditions that include at least one wash in 0.1 X SSC and 0.1% SDS at 60-65 ° C for thirty minutes.

3. An isolated nucleic acid encoding a polypeptide comprising a sequence of amino acids having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:12.

4. A recombinant nucleic acid comprising the nucleic acid of claim 3, wherein said recombinant nucleic acid is operably linked to a promoter sequence.

5. A prokaryotic or eukaryotic host cell comprising the nucleic acid of claim 3.

6. A vector comprising a nucleic acid of claim 3.

7. A prokaryotic or eukaryotic host cell comprising the vector of claim 6.

8. A transgenic plant comprising a nucleic acid selected chosen from an exogenous nucleic acid sequence comprising a sequence of nucleotides having at least about 95% sequence identity to the sequence of SEQ ID NO:11 or its complement, an exogenous nucleic acid sequence encoding a protein comprising a sequence of amino acids having at least about 95% sequence identity to the sequence of SEQ ID NO:12, and an exogenous nucleic acid that hybridizes to the nucleic acid sequence of SEQ ID NO:11 or its complement under hybridization conditions that include at least one wash in 0.1 X SSC and 0.1% SDS at 60-65 ° C for thirty minutes.

9. A seed produced from the transgenic plant of claim 8.

10. A method of increasing root nodulation in a plant comprising expressing the nucleic acid chosen from an exogenous nucleic acid sequence comprising a sequence of nucleotides having at least about 95% sequence identity to the sequence of SEQ ID NO:11 or its complement, an exogenous nucleic acid sequence encoding a protein comprising a sequence of amino acids having at least about 95% sequence identity to the sequence of SEQ ID NO:12 and an exogenous nucleic acid hybridizing to the nucleic acid sequence of SEQ ID NO:11 or its complement under hybridization conditions that include at least one wash in 0.1 X SSC and 0.1% SDS at 60-65 ° C for thirty minutes.

11. A cell or organism with an increased accumulation of nitrogen or phosphorus, wherein said increased accumulation is relative to a similar cell or organism, wherein the cell or organism with said increased accumulation of nitrogen or phosphorus differs in comprising the nucleic acid chosen from: an exogenous nucleic acid sequence comprising a sequence of nucleotides having at least about 95% sequence identity to the sequence of SEQ ID NO:11 or its complement, an exogenous nucleic acid sequence encoding a protein comprising a sequence of amino acids having at least about 95% sequence identity to the sequence of SEQ ID NO:12, and an exogenous nucleic acid hybridizing to the nucleic acid sequence of SEQ ID NO:11 or its complement under hybridization conditions that include at least one wash in 0.1 X SSC and 0.1% SDS at 60-65 ° C for thirty minutes.

12. A fertilizer comprising the cells or organisms of claim 11.

13. A nucleic acid that binds to the nucleic acid of claim 3, wherein said nucleic acid is an oligonucleotide primer.

14. An antibody that binds a polypeptide comprising at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:12.

15. A ligand that binds a polypeptide comprising at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:12.

16. An isolated nucleic acid encoding a non-naturally occurring cation channel comprising a nucleic acid sequence chosen from a nucleic acid comprising a sequence of nucleotides having at least about 95% sequence identity to the sequence of SEQ ID NO:13 or its complement, a nucleic acid encoding a protein comprising a sequence of amino acids having at least about 95% sequence identity to the sequence of SEQ ID NO:14, and an exogenous nucleic acid hybridizing to the nucleic acid sequence of SEQ ID NO:13 or its complement under hybridization conditions that include at least one wash in 0.1 X SSC and 0.1% SDS at 60-65 ° C for thirty minutes.

17. The nucleic acid of claim 16, wherein the cation is calcium or potassium.

18.. A transgenic plant wherein at least a part of said plant comprises a recombinant nucleic acid encoding a DMI1 polypeptide.

19.. The transgenic plant of claim 18 wherein said part is a seed.

20. The transgenic plant of claim 18 wherein said DMI1 polypeptide is from *Medicago truncatula,*

21. The transgenic plant of claim 18 wherein said recombinant nucleic acid is operatively linked to a promoter.

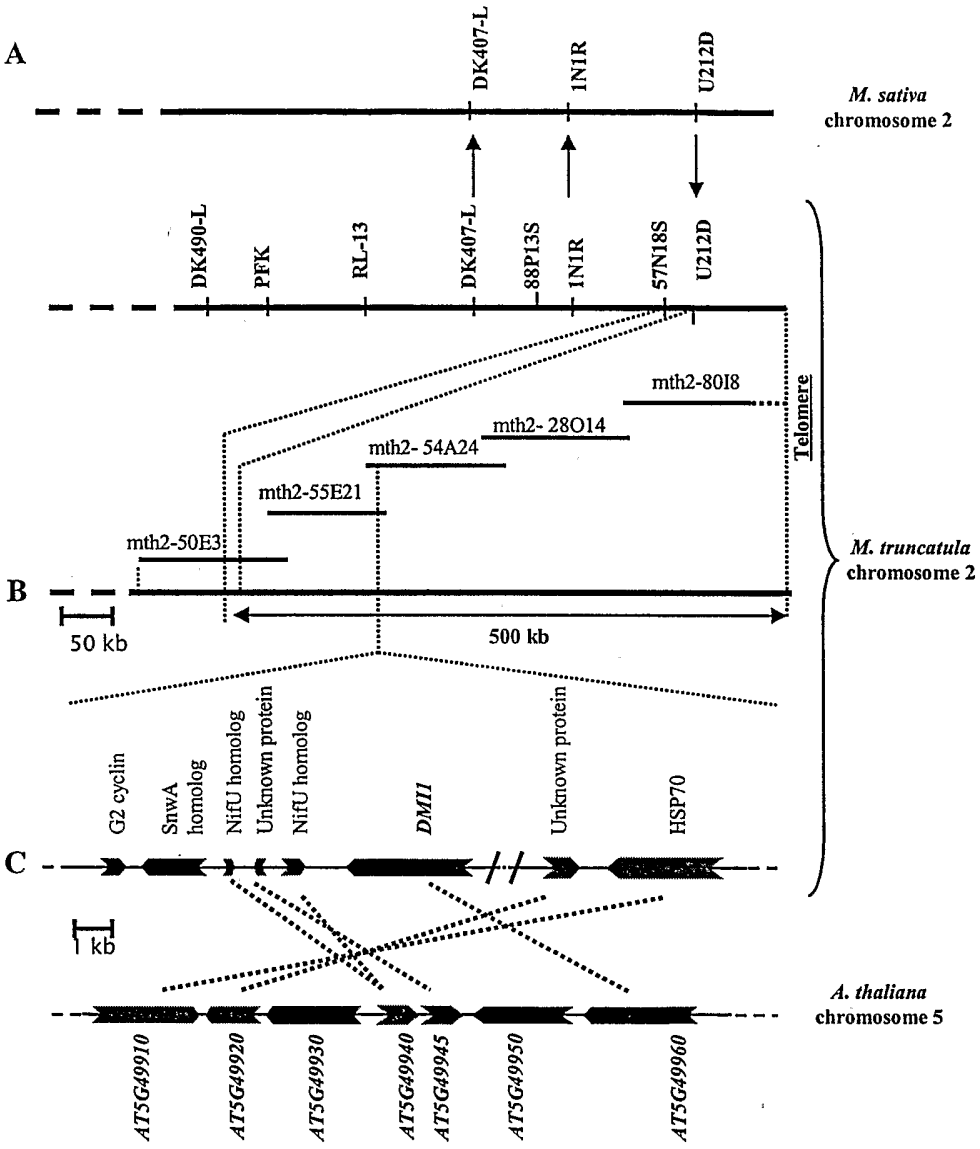
22. An isolated nucleic acid encoding a DMI1 polypeptide.

23. A recombinant nucleic acid comprising the nucleic acid of claim22, wherein said recombinant nucleic acid is operably linked to a promoter sequence.

24. A prokaryotic or eukaryotic host cell comprising the nucleic acid of claim 22.

25. A vector comprising the nucleic acid of claim 3.

26. A prokaryotic or eukaryotic host cell comprising the vector of claim 25.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DK407-L
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U212D
M. sativa
chromosome 2
DK490-L
PFK
RL-13
DK407-L
88P13S
1N1R
57N18S
U212D
mth2-80I8
mth2- 28O14
mth2- 54A24
mth2-55E21
mth2-50E3
Telomere
M. truncatula
chromosome 2
B
50 kb
500 kb
G2 cyclin
SnwA homolog
NifU homolog
Unknown protein
NifU homolog
DMI1
Unknown protein
HSP70
C
1 kb
A. thaliana
chromosome 5
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AT5G49930
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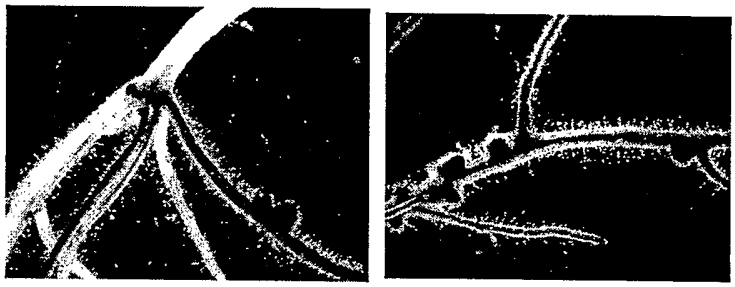
FIGURE 1

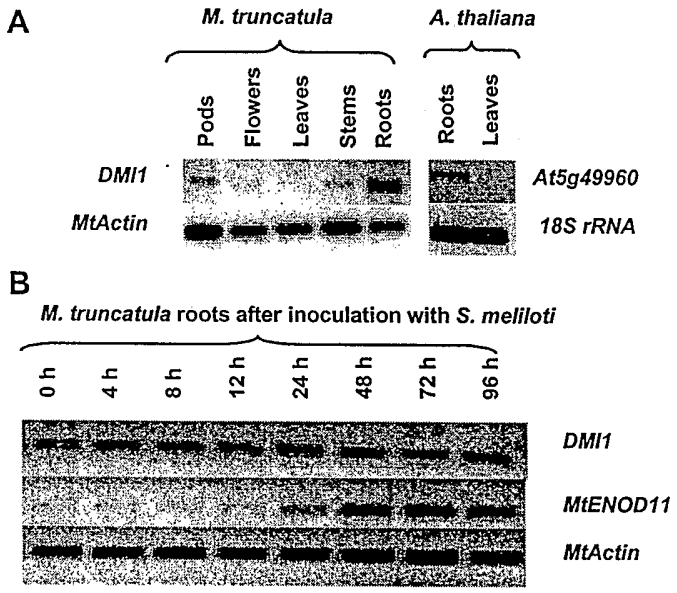
A



B

FIGURE 2

Figure 3


A
M. truncatula
A. thaliana
Pods
Flowers
Leaves
Stems
Roots
Roots
Leaves
DMI1
MtActin
At5g49960
18S rRNA
B
M. truncatula roots after inoculation with S. meliloti
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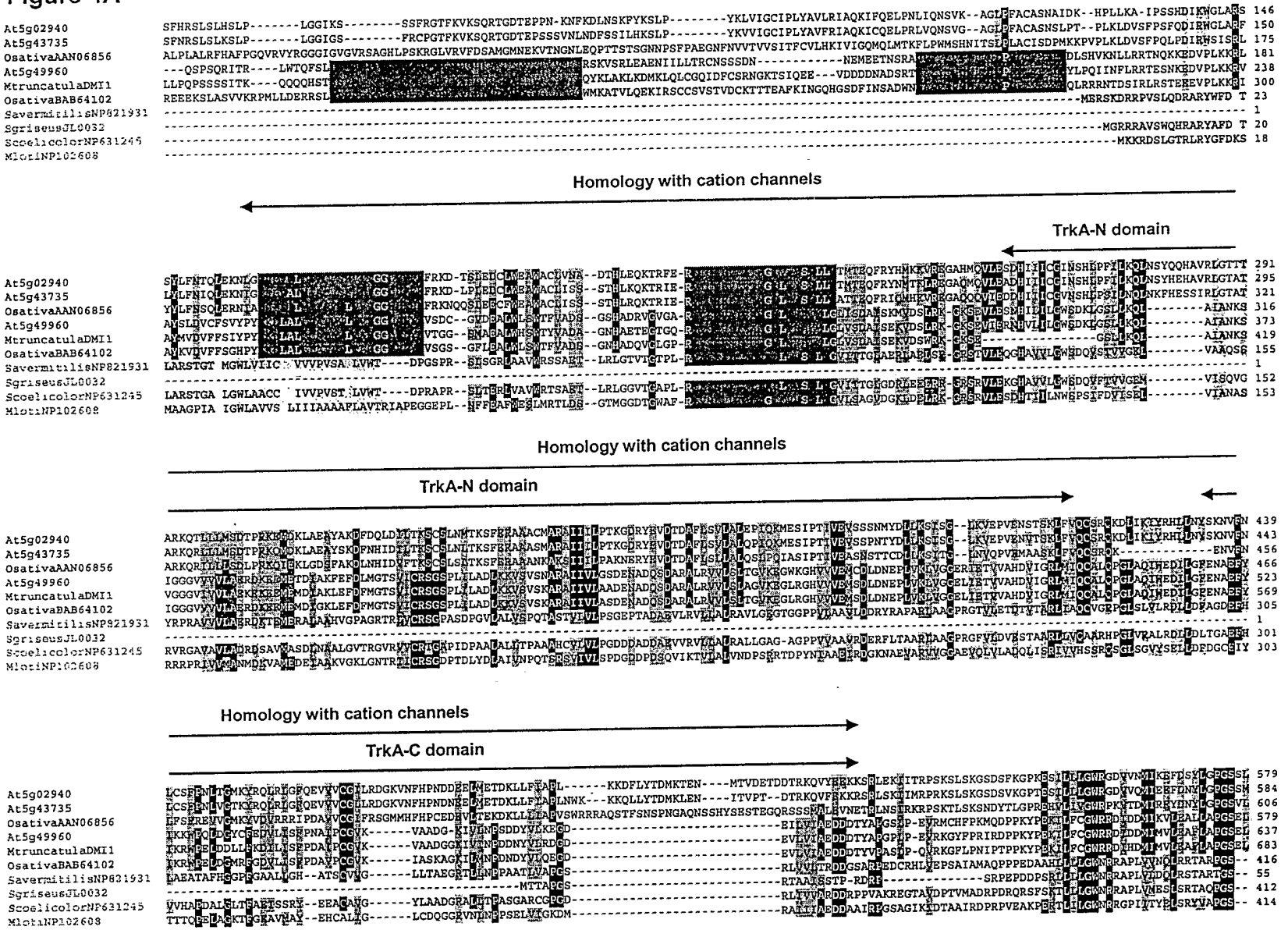
8 h
12 h
24 h
48 h
72 h
96 h
DMI1
MtENOD11
MtActin

Figure 4A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Homology with cation channels
TrkA-N domain
Homology with cation channels
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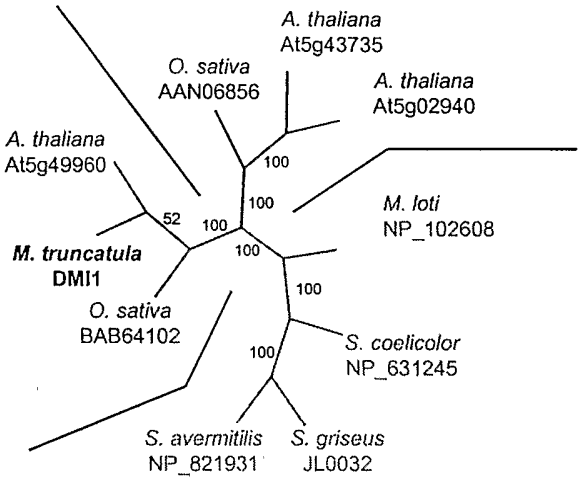
Homology with cation channels
TrkA-C domain

Figure 4B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O. sativa
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NP_631245
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JL0032
100
100
52
100
100
100
100

Figure 5

Nucleic Acid sequence of DMI1 of *M. truncatula*:

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Figure 6

amino acid sequence of DMI1 gene product:

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121 QQQQHSTSSPIFYLLVICCIILVPYSAYLQYKLAKLKDMKLQLCGQIDFCSRNGKTSIQE
181 EVDDDDNADSRTIALYIVLFTLILPFVLYKYLDYLPQIINFLRRTESNKEDVPLKKRVAY
241MVDVFFSIYPYAKLLALLCATLFLIAFGGLALYAVTGGSMAEALWHSWTYVADAGNHAET
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661ERERKLAAGELDVFGLENIKLVHREGNAVIRRHLESLPLETFDSILILADESVEDSVAHS
721DSRSLATLLLRDIQFLYPGSVIMYYPYELVSMALAMVAEDKQINRVLEELFAEEGNEMC
781IKPAEFYLFDQEELCFYDIMIRGRTRKEIVIGYRLANQERAIINPSEKSVPRKWSLDDVF
841VVLASGE (The full sequence is SEQ ID NO: 12)(SEQ ID NO: 14 is underlined).

SEQUENCE LISTING

<110> The Regents of the University of California
Cook, Douglas R.
Penmetsa, Ramachandra V.
Kiss, Gyorgy B.
Ane, Jean-Michel
Denarie, Jean

<120> THE DMI1 GENE ENCODES A PROTEIN THAT IS REQUIRED FOR THE EARLY STEPS OF BACTERIAL AND FUNGAL SYMBIOSES

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Ser	Phe	His	Arg	Ser	Leu	Ser	Leu	His	Ser	Leu	Pro	Leu	Gly	Gly	Ile
1				5					10					15	
Lys	Ser	Ser	Ser	Phe	Arg	Gly	Thr	Phe	Lys	Val	Lys	Ser	Gln	Arg	Thr
			20					25					30		
Gly	Asp	Thr	Glu	Pro	Pro	Asn	Lys	Asn	Phe	Lys	Asp	Leu	Asn	Ser	Lys
		35					40					45			
Phe	Tyr	Lys	Ser	Leu	Pro	Tyr	Lys	Leu	Val	Ile	Gly	Cys	Ile	Pro	Leu
	50					55					60				
Tyr	Ala	Val	Leu	Arg	Ile	Ala	Gln	Lys	Ile	Phe	Gln	Glu	Leu	Pro	Asn
65					70					75					80
Leu	Ile	Gln	Asn	Ser	Val	Lys	Ala	Gly	Leu	Pro	Phe	Ala	Cys	Ala	Ser
				85					90					95	
Asn	Ala	Ile	Asp	Lys	His	Pro	Leu	Leu	Lys	Ala	Ile	Pro	Ser	Ser	His
			100					105					110		
Asp	Ile	Lys	Trp	Gly	Leu	Ala	Arg	Ser	Ser	Tyr	Leu	Phe	Asn	Thr	Gln
		115					120					125			
Leu	Glu	Lys	Asn	Leu	Gly	Thr	Val	Phe	Val	Val	Leu	Leu	Ile	Thr	Cys
	130					135					140				
Phe	Ser	Phe	Val	Ile	Ile	Gly	Gly	Leu	Phe	Phe	Phe	Lys	Phe	Arg	Lys
145					150					155					160
Asp	Thr	Ser	Leu	Glu	Asp	Cys	Leu	Trp	Glu	Ala	Trp	Ala	Cys	Leu	Val
				165					170					175	
Asn	Ala	Asp	Thr	His	Leu	Glu	Gln	Lys	Thr	Arg	Phe	Glu	Arg	Leu	Ile
			180					185					190		
Gly	Phe	Val	Leu	Ala	Ile	Trp	Gly	Ile	Val	Phe	Tyr	Ser	Arg	Leu	Leu
		195					200					205			
Ser	Thr	Met	Thr	Glu	Gln	Phe	Arg	Tyr	His	Met	Lys	Lys	Val	Arg	Glu
	210					215					220				
Gly	Ala	His	Met	Gln	Val	Leu	Glu	Ser	Asp	His	Ile	Ile	Ile	Cys	Gly
225					230					235					240
Ile	Asn	Ser	His	Leu	Pro	Phe	Ile	Leu	Lys	Gln	Leu	Asn	Ser	Tyr	Gln

```
                245                 250                 255
Gln His Ala Val Arg Leu Gly Thr Thr Thr Ala Arg Lys Gln Thr Leu
            260                 265                 270
Leu Leu Met Ser Asp Thr Pro Arg Lys Glu Met Asp Lys Leu Ala Glu
        275                 280                 285
Ala Tyr Ala Lys Asp Phe Asp Gln Leu Asp Ile Leu Thr Lys Ser Cys
    290                 295                 300
Ser Leu Asn Met Thr Lys Ser Phe Glu Arg Ala Ala Ala Cys Met Ala
305                 310                 315                 320
Arg Ala Ile Ile Ile Leu Pro Thr Lys Gly Asp Arg Tyr Glu Val Asp
                325                 330                 335
Thr Asp Ala Phe Leu Ser Val Leu Ala Leu Glu Pro Ile Gln Lys Met
            340                 345                 350
Glu Ser Ile Pro Thr Ile Val Glu Val Ser Ser Ser Asn Met Tyr Asp
        355                 360                 365
Leu Leu Lys Ser Ile Ser Gly Leu Lys Val Glu Pro Val Glu Asn Ser
    370                 375                 380
Thr Ser Lys Leu Phe Val Gln Cys Ser Arg Gln Lys Asp Leu Ile Lys
385                 390                 395                 400
Ile Tyr Arg His Leu Leu Asn Tyr Ser Lys Asn Val Phe Asn Leu Cys
                405                 410                 415
Ser Phe Pro Asn Leu Thr Gly Met Lys Tyr Arg Gln Leu Arg Leu Gly
            420                 425                 430
Phe Gln Glu Val Val Val Cys Gly Ile Leu Arg Asp Gly Lys Val Asn
        435                 440                 445
Phe His Pro Asn Asp Asp Glu Glu Leu Met Glu Thr Asp Lys Leu Leu
    450                 455                 460
Phe Ile Ala Pro Leu Lys Lys Asp Phe Leu Tyr Thr Asp Met Lys Thr
465                 470                 475                 480
Glu Asn Met Thr Val Asp Glu Thr Asp Asp Thr Arg Lys Gln Val Tyr
                485                 490                 495
Glu Glu Lys Lys Ser Arg Leu Glu Lys Ile Ile Thr Arg Pro Ser Lys
            500                 505                 510
Ser Leu Ser Lys Gly Ser Asp Ser Phe Lys Gly Pro Lys Glu Ser Ile
        515                 520                 525
Leu Leu Leu Gly Trp Arg Gly Asp Val Val Asn Met Ile Lys Glu Phe
    530                 535                 540
Asp Ser Tyr Leu Gly Pro Gly Ser Ser Leu
545                 550
```

<210> 2
<211> 557
<212> PRT
<213> Arabidopsis thaliana

<400> 2

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

```
Ile Gln Leu Glu Lys Asn Ile Gly Thr Phe Leu Val Ala Leu Met Ile
    130                 135                 140
Ala Cys Val Ser Phe Val Ile Ile Gly Gly Leu Leu Phe Phe Lys Phe
145                 150                 155                 160
Arg Lys Asp Leu Pro Leu Glu Asp Cys Leu Trp Glu Ala Trp Ala Cys
                165                 170                 175
Leu Ile Ser Ser Ser Thr His Leu Lys Gln Lys Thr Arg Ile Glu Arg
            180                 185                 190
Val Ile Gly Phe Val Leu Ala Ile Trp Gly Ile Leu Phe Tyr Ser Arg
        195                 200                 205
Leu Leu Ser Thr Met Thr Glu Gln Phe Arg Tyr Asn Met Thr Lys Leu
    210                 215                 220
Arg Glu Gly Ala Gln Met Gln Val Leu Glu Ala Asp His Ile Ile Ile
225                 230                 235                 240
Cys Gly Ile Asn Ser His Leu Pro Phe Ile Leu Lys Gln Leu Asn Ser
                245                 250                 255
Tyr His Glu His Ala Val Arg Leu Gly Thr Ala Thr Ala Arg Lys Gln
            260                 265                 270
Arg Leu Leu Leu Met Ser Asp Thr Pro Arg Lys Gln Met Asp Lys Leu
        275                 280                 285
Ala Glu Ala Tyr Ser Lys Asp Phe Asn His Ile Asp Ile Leu Thr Lys
    290                 295                 300
Ser Cys Ser Leu Asn Leu Thr Lys Ser Phe Glu Arg Ala Ala Ala Ser
305                 310                 315                 320
Met Ala Arg Ala Ile Ile Ile Leu Pro Thr Lys Gly Asp Arg Tyr Glu
                325                 330                 335
Val Asp Thr Asp Ala Phe Leu Ser Val Leu Ala Leu Gln Pro Ile Gln
            340                 345                 350
Lys Met Glu Ser Ile Pro Thr Ile Val Glu Val Ser Ser Pro Asn Thr
        355                 360                 365
Tyr Asp Leu Leu Lys Ser Ile Ser Gly Leu Lys Val Glu Pro Val Glu
    370                 375                 380
Asn Val Thr Ser Lys Leu Phe Val Gln Cys Ser Arg Gln Lys Asp Leu
385                 390                 395                 400
Ile Lys Ile Tyr Arg His Leu Leu Asn Tyr Ser Lys Asn Val Phe Asn
                405                 410                 415
Leu Cys Ser Phe Pro Asn Leu Val Gly Thr Lys Tyr Arg Gln Leu Arg
            420                 425                 430
Leu Gly Phe Gln Glu Val Val Val Cys Gly Leu Leu Arg Asp Gly Lys
        435                 440                 445
Val Asn Phe His Pro Asn Asp Asn Glu Glu Leu Met Glu Thr Asp Lys
    450                 455                 460
Leu Leu Phe Ile Ala Pro Leu Asn Trp Lys Lys Lys Gln Leu Leu Tyr
465                 470                 475                 480
Thr Asp Met Lys Leu Glu Asn Ile Thr Val Pro Thr Asp Thr Arg Lys
                485                 490                 495
Gln Val Phe Glu Lys Lys Arg Ser Arg Leu Ser Lys Ile Ile Met Arg
            500                 505                 510
Pro Arg Lys Ser Leu Ser Lys Gly Ser Asp Ser Val Lys Gly Pro Thr
        515                 520                 525
Glu Ser Ile Leu Leu Leu Gly Trp Arg Gly Asp Val Val Gln Met Ile
    530                 535                 540
Glu Glu Phe Asp Asn Tyr Leu Gly Pro Gly Ser Ser Met
545                 550                 555
```

<210> 3
<211> 581
<212> PRT
<213> Oryza sativa

<400> 3
Ala Leu Pro Leu Ala Leu Arg Phe His Ala Phe Pro Gly Gln Val Arg

```
 1               5                  10                  15
Val Tyr Arg Gly Gly Gly Ile Gly Val Gly Val Arg Ser Ala Gly His
            20                  25                  30
Leu Pro Ser Lys Arg Gly Leu Val Arg Val Phe Asp Ser Ala Met Gly
        35                  40                  45
Met Asn Glu Lys Val Thr Asn Gly Asn Leu Glu Gln Pro Thr Thr Ser
    50                  55                  60
Thr Ser Gly Asn Asn Pro Ser Phe Pro Ala Glu Gly Asn Phe Asn Val
65                  70                  75                  80
Val Thr Val Val Ser Ile Thr Phe Cys Val Leu His Lys Ile Val Ile
                85                  90                  95
Gly Gln Met Gln Leu Met Thr Lys Phe Leu Pro Trp Met Ser His Asn
            100                 105                 110
Ile Thr Ser Leu Pro Leu Ala Cys Ile Ser Asp Pro Met Lys Lys Pro
        115                 120                 125
Val Pro Leu Lys Leu Asp Val Ser Phe Pro Gln Leu Pro Asp Ile Arg
    130                 135                 140
Trp Ser Ile Ser Arg Leu Tyr Tyr Leu Phe Asn Ser Gln Leu Glu Arg
145                 150                 155                 160
Asn Ile Ala Leu Ser Ile Ile Thr Leu Met Ile Thr Cys Phe Ser Leu
                165                 170                 175
Val Val Val Gly Gly Phe Leu Phe His Lys Phe Arg Lys Asn Gln Gln
            180                 185                 190
Ser Leu Glu Glu Cys Phe Trp Glu Ala Trp Ala Cys Leu Ile Ser Ser
        195                 200                 205
Ser Thr His Leu Arg Gln Lys Thr Arg Ile Glu Arg Val Leu Gly Phe
    210                 215                 220
Phe Leu Ala Ile Trp Gly Ile Leu Phe Tyr Ser Arg Leu Leu Ser Ala
225                 230                 235                 240
Thr Thr Glu Gln Phe Arg Ile Gln Met His Lys Val Arg Glu Gly Ala
                245                 250                 255
Gln Gln Gln Val Ile Glu Asp Asp His Ile Ile Ile Cys Gly Val Asn
            260                 265                 270
Ser His Leu Pro Ser Ile Leu Asn Gln Leu Asn Lys Phe His Glu Ser
        275                 280                 285
Ser Ile Arg Leu Gly Thr Ala Thr Ala Arg Lys Gln Arg Ile Leu Leu
    290                 295                 300
Leu Ser Asp Leu Pro Arg Lys Gln Ile Glu Lys Leu Gly Asp Ser Phe
305                 310                 315                 320
Ala Lys Asp Leu Asn His Ile Asp Val Phe Thr Lys Ser Cys Ser Leu
                325                 330                 335
Ser Leu Thr Lys Ser Phe Glu Arg Ala Ala Ala Asn Lys Ala Lys Ser
            340                 345                 350
Ile Ile Ile Leu Pro Ala Lys Asn Glu Arg Tyr Glu Val Asp Thr Asp
        355                 360                 365
Ala Phe Leu Ser Leu Leu Ala Leu Gln Ser Leu Pro Gln Ile Ala Ser
    370                 375                 380
Ile Pro Thr Ile Val Glu Ala Ser Asn Ser Thr Thr Cys Asp Leu Leu
385                 390                 395                 400
Lys Ser Ile Thr Gly Leu Asn Val Gln Pro Val Glu Met Ala Ala Ser
                405                 410                 415
Lys Leu Phe Val Gln Cys Ser Arg Gln Lys Glu Asn Val Phe Asn Leu
            420                 425                 430
Phe Ser Phe Arg Glu Val Val Gly Met Lys Tyr Val Asp Val Arg Arg
        435                 440                 445
Arg Ile Pro Asp Ala Val Val Cys Gly Ile Phe Arg Ser Gly Met Met
    450                 455                 460
His Phe His Pro Cys Glu Asp Glu Val Leu Thr Glu Lys Asp Lys Leu
465                 470                 475                 480
Leu Leu Ile Ala Pro Val Ser Trp Arg Arg Arg Ala Gln Ser Thr Phe
                485                 490                 495
Ser Asn Ser Pro Asn Gly Ala Gln Asn Ser Ser His Tyr Ser Glu Ser
            500                 505                 510
```

```
Thr Glu Gly Gln Arg Ser Ser Ser Met Ala Leu Glu Val Asn Glu Thr
        515                 520                 525
Arg Leu Asn Ser Ile Arg Lys Arg Pro Ser Lys Thr Leu Ser Lys Ser
    530                 535                 540
Asn Asp Tyr Thr Leu Gly Pro Arg Glu His Val Leu Ile Val Gly Trp
545                 550                 555                 560
Arg Pro Lys Val Thr Asp Met Ile Arg Glu Tyr Asp Asn Tyr Leu Gly
                565                 570                 575
Pro Gly Ser Val Leu
            580
```

<210> 4
<211> 512
<212> PRT
<213> Arabidopsis thaliana

<400> 4

```
Gln Ser Pro Ser Gln Arg Ile Thr Arg Leu Trp Thr Gln Phe Ser Leu
 1               5                  10                  15
Thr His Cys Leu Lys Phe Ile Cys Ser Cys Ser Phe Thr Tyr Val Met
            20                  25                  30
Phe Leu Arg Ser Lys Val Ser Arg Leu Glu Ala Glu Asn Ile Ile Leu
        35                  40                  45
Leu Thr Arg Cys Asn Ser Ser Ser Asp Asn Asn Glu Met Glu Glu Thr
    50                  55                  60
Asn Ser Arg Ala Val Val Phe Phe Ser Val Ile Ile Thr Phe Val Leu
65                  70                  75                  80
Pro Phe Leu Leu Tyr Met Tyr Leu Asp Asp Leu Ser His Val Lys Asn
                85                  90                  95
Leu Leu Arg Arg Thr Asn Gln Lys Lys Glu Asp Val Pro Leu Lys Lys
            100                 105                 110
Arg Leu Ala Tyr Ser Leu Asp Val Cys Phe Ser Val Tyr Pro Tyr Ala
        115                 120                 125
Lys Leu Leu Ala Leu Leu Leu Ala Thr Val Val Leu Ile Val Tyr Gly
    130                 135                 140
Gly Leu Ala Leu Tyr Ala Val Ser Asp Cys Gly Val Asp Glu Ala Leu
145                 150                 155                 160
Trp Leu Ser Trp Thr Phe Val Ala Asp Ser Gly Ser His Ala Asp Arg
                165                 170                 175
Val Gly Val Gly Ala Arg Ile Val Ser Val Ala Ile Ser Ala Gly Gly
            180                 185                 190
Met Leu Ile Phe Ala Thr Met Leu Gly Leu Ile Ser Asp Ala Ile Ser
        195                 200                 205
Lys Met Val Asp Ser Leu Arg Lys Gly Lys Ser Glu Val Leu Glu Ser
    210                 215                 220
Asn His Ile Leu Ile Leu Gly Trp Ser Asp Lys Leu Gly Ser Leu Leu
225                 230                 235                 240
Lys Gln Leu Ala Ile Ala Asn Lys Ser Ile Gly Gly Gly Val Val Val
                245                 250                 255
Val Leu Ala Glu Arg Asp Lys Glu Glu Met Glu Thr Asp Ile Ala Lys
            260                 265                 270
Phe Glu Phe Asp Leu Met Gly Thr Ser Val Ile Cys Arg Ser Gly Ser
        275                 280                 285
Pro Leu Ile Leu Ala Asp Leu Lys Lys Val Ser Val Ser Asn Ala Arg
    290                 295                 300
Ala Ile Ile Val Leu Gly Ser Asp Glu Asn Ala Asp Gln Ser Asp Ala
305                 310                 315                 320
Arg Ala Leu Arg Val Val Leu Ser Leu Thr Gly Val Lys Glu Gly Trp
                325                 330                 335
Lys Gly His Val Val Val Glu Met Cys Asp Leu Asp Asn Glu Pro Leu
            340                 345                 350
Val Lys Leu Val Gly Gly Glu Arg Ile Glu Thr Val Val Ala His Asp
```

```
        355                 360                 365
Val Ile Gly Arg Leu Met Ile Gln Cys Ala Leu Gln Pro Gly Leu Ala
    370                 375                 380
Gln Ile Trp Glu Asp Ile Leu Gly Phe Glu Asn Ala Glu Phe Tyr Ile
385                 390                 395                 400
Lys Lys Trp Pro Gln Leu Asp Gly Tyr Cys Phe Glu Asp Val Leu Ile
                405                 410                 415
Ser Phe Pro Asn Ala Ile Pro Cys Gly Val Lys Val Ala Ala Asp Gly
            420                 425                 430
Lys Ile Val Leu Asn Pro Ser Asp Asp Tyr Val Leu Lys Glu Gly Asp
        435                 440                 445
Glu Ile Leu Val Ile Ala Glu Asp Asp Asp Thr Tyr Ala Pro Gly Ser
    450                 455                 460
Leu Pro Glu Val Arg Met Cys His Phe Pro Lys Met Gln Asp Pro Pro
465                 470                 475                 480
Lys Tyr Pro Glu Lys Ile Leu Phe Cys Gly Trp Arg Arg Asp Ile Asp
                485                 490                 495
Asp Met Ile Lys Val Leu Glu Ala Leu Leu Ala Pro Gly Ser Glu Leu
            500                 505                 510
```

<210> 5
<211> 529
<212> PRT
<213> Medicago truncatula

<400> 5

```
Leu Leu Pro Gln Pro Ser Ser Ser Ser Ile Thr Lys Gln Gln Gln Gln
 1               5                  10                  15
His Ser Thr Ser Ser Pro Ile Phe Tyr Leu Leu Val Ile Cys Cys Ile
            20                  25                  30
Ile Leu Val Pro Tyr Ser Ala Tyr Leu Gln Tyr Lys Leu Ala Lys Leu
        35                  40                  45
Lys Asp Met Lys Leu Gln Leu Cys Gly Gln Ile Asp Phe Cys Ser Arg
    50                  55                  60
Asn Gly Lys Thr Ser Ile Gln Glu Glu Val Asp Asp Asp Asp Asn Ala
65                  70                  75                  80
Asp Ser Arg Thr Ile Ala Leu Tyr Ile Val Leu Phe Thr Leu Ile Leu
                85                  90                  95
Pro Phe Val Leu Tyr Lys Tyr Leu Asp Tyr Leu Pro Gln Ile Ile Asn
            100                 105                 110
Phe Leu Arg Arg Thr Glu Ser Asn Lys Glu Asp Val Pro Leu Lys Lys
        115                 120                 125
Arg Val Ala Tyr Met Val Asp Val Phe Phe Ser Ile Tyr Pro Tyr Ala
    130                 135                 140
Lys Leu Leu Ala Leu Leu Cys Ala Thr Leu Phe Leu Ile Ala Phe Gly
145                 150                 155                 160
Gly Leu Ala Leu Tyr Ala Val Thr Gly Gly Ser Met Ala Glu Ala Leu
                165                 170                 175
Trp His Ser Trp Thr Tyr Val Ala Asp Ala Gly Asn His Ala Glu Thr
            180                 185                 190
Glu Gly Thr Gly Gln Arg Ile Val Ser Val Ser Ile Ser Ala Gly Gly
        195                 200                 205
Met Leu Ile Phe Ala Met Met Leu Gly Leu Val Ser Asp Ala Ile Ser
    210                 215                 220
Glu Lys Val Asp Ser Leu Arg Lys Gly Lys Ser Glu Val Ile Glu Arg
225                 230                 235                 240
Asn His Val Leu Ile Leu Gly Trp Ser Asp Lys Leu Gly Ser Leu Leu
                245                 250                 255
Lys Gln Leu Ala Ile Ala Asn Lys Ser Val Gly Gly Gly Val Ile Val
            260                 265                 270
Val Leu Ala Glu Lys Glu Lys Glu Glu Met Glu Met Asp Ile Ala Lys
        275                 280                 285
```

```
Leu Glu Phe Asp Phe Met Gly Thr Ser Val Ile Cys Arg Ser Gly Ser
    290                 295                 300
Pro Leu Ile Leu Ala Asp Leu Lys Lys Val Ser Val Ser Lys Ala Arg
305                 310                 315                 320
Ala Ile Ile Val Leu Ala Ala Asp Glu Asn Ala Asp Gln Ser Asp Ala
                325                 330                 335
Arg Ala Leu Arg Val Val Leu Ser Leu Ala Gly Val Lys Glu Gly Leu
            340                 345                 350
Arg Gly His Val Val Val Glu Met Ser Asp Leu Asp Asn Glu Pro Leu
        355                 360                 365
Val Lys Leu Val Gly Gly Glu Leu Ile Glu Thr Val Val Ala His Asp
    370                 375                 380
Val Ile Gly Arg Leu Met Ile Gln Cys Ala Leu Gln Pro Gly Leu Ala
385                 390                 395                 400
Gln Ile Trp Glu Asp Ile Leu Gly Phe Glu Asn Ala Glu Phe Tyr Ile
                405                 410                 415
Lys Arg Trp Pro Glu Leu Asp Asp Leu Leu Phe Lys Asp Ile Leu Ile
            420                 425                 430
Ser Phe Pro Asp Ala Ile Pro Cys Gly Val Lys Val Ala Ala Asp Gly
        435                 440                 445
Gly Lys Ile Val Ile Asn Pro Asp Asp Asn Tyr Val Leu Arg Asp Gly
    450                 455                 460
Asp Glu Val Leu Val Ile Ala Glu Asp Asp Asp Thr Tyr Ala Pro Gly
465                 470                 475                 480
Pro Leu Pro Glu Val Arg Lys Gly Tyr Phe Pro Arg Ile Arg Asp Pro
                485                 490                 495
Pro Lys Tyr Pro Glu Lys Ile Leu Phe Cys Gly Trp Arg Arg Asp Ile
            500                 505                 510
Asp Asp Met Ile Met Val Leu Glu Ala Phe Leu Ala Pro Gly Ser Glu
        515                 520                 525
Leu
```

<210> 6
<211> 533
<212> PRT
<213> Oryza sativa

```
<400> 6
Arg Glu Glu Glu Lys Ser Leu Ala Ser Val Val Lys Arg Pro Met Leu
 1               5                  10                  15
Leu Asp Glu Arg Arg Ser Leu Ser Pro Pro Pro Pro Gln Gln Arg Ala
            20                  25                  30
Pro Arg Phe Asp Leu Ser Pro Tyr Leu Val Leu Met Leu Val Val Thr
        35                  40                  45
Val Ile Ser Phe Ser Leu Ala Ile Trp Gln Trp Met Lys Ala Thr Val
    50                  55                  60
Leu Gln Glu Lys Ile Arg Ser Cys Cys Ser Val Ser Thr Val Asp Cys
65                  70                  75                  80
Lys Thr Thr Thr Glu Ala Phe Lys Ile Asn Gly Gln His Gly Ser Asp
                85                  90                  95
Phe Ile Asn Ser Ala Asp Trp Asn Leu Ala Ser Cys Ser Arg Met Leu
            100                 105                 110
Val Phe Ala Ile Pro Val Phe Leu Val Lys Tyr Ile Asp Gln Leu Arg
        115                 120                 125
Arg Arg Asn Thr Asp Ser Ile Arg Leu Arg Ser Thr Glu Glu Glu Val
    130                 135                 140
Pro Leu Lys Lys Arg Ile Ala Tyr Lys Val Asp Val Phe Phe Ser Gly
145                 150                 155                 160
His Pro Tyr Ala Lys Leu Leu Ala Leu Leu Leu Ala Thr Ile Ile Leu
                165                 170                 175
Ile Ala Ser Gly Gly Ile Ala Leu Tyr Val Val Ser Gly Ser Gly Phe
```

```
            180                 185                 190
Leu Glu Ala Leu Trp Leu Ser Trp Thr Phe Val Ala Asp Ser Gly Asn
        195                 200                 205
His Ala Asp Gln Val Gly Leu Gly Pro Arg Ile Val Ser Val Ser Ile
    210                 215                 220
Ser Ser Gly Gly Met Leu Val Phe Ala Thr Met Leu Gly Leu Val Ser
225                 230                 235                 240
Asp Ala Ile Ser Glu Lys Val Asp Ser Trp Arg Lys Gly Lys Ser Glu
                245                 250                 255
Gly Ser Leu Leu Lys Gln Leu Ala Ile Ala Asn Lys Ser Ile Gly Gly
            260                 265                 270
Gly Val Val Val Val Leu Ala Glu Arg Asp Lys Glu Glu Met Glu Met
        275                 280                 285
Asp Ile Gly Lys Leu Glu Phe Asp Phe Met Gly Thr Ser Val Ile Cys
    290                 295                 300
Arg Ser Gly Ser Pro Leu Ile Leu Ala Asp Leu Lys Lys Val Ser Val
305                 310                 315                 320
Ser Lys Ala Arg Ala Ile Ile Val Leu Ala Ser Asp Glu Asn Ala Asp
                325                 330                 335
Gln Ser Asp Ala Arg Ala Leu Arg Val Val Leu Ser Leu Thr Gly Val
            340                 345                 350
Lys Glu Gly Leu Arg Gly His Val Val Val Glu Met Ser Asp Leu Asp
        355                 360                 365
Asn Glu Pro Leu Val Lys Leu Val Gly Gly Glu Leu Ile Glu Thr Val
    370                 375                 380
Val Ala His Asp Val Ile Gly Arg Leu Met Ile Gln Cys Ala Leu Gln
385                 390                 395                 400
Pro Gly Leu Ala Gln Ile Trp Glu Asp Ile Leu Gly Phe Glu Asn Ala
                405                 410                 415
Glu Phe Tyr Ile Lys Arg Trp Pro Glu Leu Asp Gly Met Arg Phe Gly
            420                 425                 430
Asp Val Leu Ile Ser Phe Pro Asp Ala Val Pro Cys Gly Val Lys Ile
        435                 440                 445
Ala Ser Lys Ala Gly Lys Ile Leu Met Asn Pro Asp Asn Asp Tyr Val
    450                 455                 460
Leu Gln Glu Gly Asp Glu Val Leu Val Ile Ala Glu Asp Asp Asp Thr
465                 470                 475                 480
Tyr Val Pro Ala Ser Leu Pro Gln Val Arg Lys Gly Phe Leu Pro Asn
                485                 490                 495
Ile Pro Thr Pro Pro Lys Tyr Pro Glu Lys Ile Leu Phe Cys Gly Trp
            500                 505                 510
Arg Arg Asp Ile His Asp Met Ile Met Val Leu Glu Ala Phe Leu Ala
        515                 520                 525
Pro Gly Ser Glu Leu
    530
```

<210> 7
<211> 416
<212> PRT
<213> Streptomyces avermitilis

```
<400> 7
Met Glu Arg Ser Lys Asp Arg Arg Pro Val Ser Leu Gln Asp Arg Ala
 1               5                  10                  15
Arg Tyr Trp Phe Asp Arg Thr Leu Ala Arg Ser Thr Gly Thr Leu Met
            20                  25                  30
Gly Trp Leu Val Ile Ile Cys Leu Ala Val Val Val Pro Val Ser Ala
        35                  40                  45
Leu Leu Val Trp Thr Asp Pro Gly Ser Pro Arg Ser Leu Ser Gly Arg
    50                  55                  60
Leu Ala Ala Val Trp Arg Ser Ser Ala Glu Thr Leu Arg Leu Gly Thr
65                  70                  75                  80
```

```
Val Thr Gly Thr Pro Leu Arg Met Leu Leu Ser Ala Leu Leu Gly Leu
                85                  90                  95
Val Ala Leu Leu Cys Val Ser Thr Leu Val Gly Val Ile Thr Thr Gly
            100                 105                 110
Leu Ala Glu Arg Leu Ala Glu Leu Ser Arg Gly Arg Ser Thr Val Leu
        115                 120                 125
Glu Gln Gly His Ala Val Val Leu Gly Trp Ser Asp Gln Val Ser Thr
    130                 135                 140
Val Val Gly Glu Leu Val Ala Ala Gln Ser Ser Tyr Arg Pro Arg Ala
145                 150                 155                 160
Val Val Val Leu Ala Glu Arg Asp Lys Thr Glu Met Glu Arg Ala Leu
                165                 170                 175
Ala Ala His Val Gly Pro Ala Gly Arg Thr Arg Leu Val Cys Arg Ser
            180                 185                 190
Gly Pro Ala Ser Asp Pro Gly Val Leu Ala Leu Val Ser Pro Gln Thr
        195                 200                 205
Ala Ser Thr Val Leu Val Leu Pro Ser Gly Glu Pro Thr Ala Asp Ala
    210                 215                 220
Glu Val Leu Arg Val Leu Leu Ala Leu Arg Ala Val Leu Gly Glu Gly
225                 230                 235                 240
Thr Gly Gly Pro Pro Val Leu Ala Ala Val Leu Asp Asp Arg Tyr Arg
                245                 250                 255
Ala Pro Ala Arg Leu Ala Ala Gly Pro Arg Gly Thr Val Leu Glu Thr
            260                 265                 270
Asp Thr Val Thr Ala Arg Leu Ile Ala Gln Cys Val Gly Arg Pro Gly
        275                 280                 285
Leu Ser Leu Val Leu Arg Asp Leu Leu Asp Phe Ala Gly Asp Glu Phe
    290                 295                 300
His Leu Ala Glu Ala Thr Ala Phe His Gly Gly Pro Phe Gly Ala Ala
305                 310                 315                 320
Leu Leu Gly His Ala Thr Ser Cys Val Val Gly Leu Leu Thr Ala Glu
                325                 330                 335
Gly Arg Thr Leu Leu Asn Pro Pro Ala Ala Thr Leu Val Ala Pro Gly
            340                 345                 350
Ser Arg Leu Val Val Leu Thr Arg Asp Asp Gly Ser Ala Arg Pro Glu
        355                 360                 365
Asp Cys Arg His Leu Val Glu Pro Ser Ala Ile Ala Met Ala Gln Pro
    370                 375                 380
Pro Pro Glu Asp Ala Ala His Leu Leu Leu Leu Gly Trp Asn Arg Arg
385                 390                 395                 400
Ala Pro Leu Val Val Asn Gln Leu Arg Arg Thr Ala Arg Pro Gly Ser
                405                 410                 415
```

<210> 8
<211> 55
<212> PRT
<213> Streptomyces griseus

<400> 8

```
Met Thr Thr Ala Pro Gly Ser Arg Thr Ala Ala Ile Ser Ser Thr Pro
 1               5                  10                  15
Arg Asp Arg Pro Ser Arg Pro Glu Pro Asp Asp Pro Ser Arg Leu Leu
            20                  25                  30
Leu Leu Gly Trp Asn Arg Arg Ala Pro Leu Val Leu Asp Gln Leu Arg
        35                  40                  45
Ser Thr Ala Arg Thr Gly Ser
    50                  55
```

<210> 9
<211> 412
<212> PRT

<213> Streptomyces coelicolor

<400> 9

```
Met Gly Arg Arg Arg Ala Val Ser Trp Gln His Arg Ala Arg Tyr Ala
 1               5                  10                  15
Phe Asp Arg Thr Leu Ala Arg Ser Thr Gly Ala Leu Leu Gly Trp Leu
            20                  25                  30
Ala Ala Cys Cys Leu Ala Ile Val Val Pro Val Ser Thr Leu Leu Val
        35                  40                  45
Trp Thr Asp Pro Arg Ala Pro Arg Ser Leu Thr Glu Arg Leu Val Ala
    50                  55                  60
Val Trp Arg Thr Ser Ala Glu Thr Leu Arg Leu Gly Gly Val Thr Gly
65                  70                  75                  80
Ala Pro Leu Arg Met Leu Leu Ser Val Phe Leu Gly Leu Ile Ala Leu
                85                  90                  95
Leu Cys Val Ser Thr Leu Val Gly Val Ile Thr Thr Gly Leu Gly Asp
            100                 105                 110
Arg Leu Glu Glu Leu Arg Arg Gly Arg Ser Arg Val Leu Glu Lys Gly
        115                 120                 125
His Ala Val Val Leu Gly Trp Ser Asp Gln Val Phe Thr Val Val Gly
    130                 135                 140
Glu Met Val Ile Ser Gln Val Gly Arg Val Arg Gly Ala Val Ala Val
145                 150                 155                 160
Leu Ala Asp Arg Asp Ser Ala Val Met Ala Ser Asp Leu Asn Ala Ala
                165                 170                 175
Leu Gly Val Thr Arg Gly Val Arg Val Val Cys Arg Thr Gly Ala Pro
            180                 185                 190
Ile Asp Pro Ala Ala Leu Ala Leu Leu Thr Pro Ala Ala Ala His Cys
        195                 200                 205
Val Leu Val Leu Pro Gly Asp Asp Asp Ala Asp Asp Ala Glu Val Val
    210                 215                 220
Arg Val Leu Leu Ala Leu Arg Ala Leu Leu Gly Ala Gly Ala Gly Pro
225                 230                 235                 240
Pro Val Val Ala Ala Val Arg Asp Glu Arg Phe Leu Thr Ala Ala Arg
                245                 250                 255
Leu Ala Ala Gly Pro Arg Gly Phe Val Leu Asp Val Glu Ser Thr Ala
            260                 265                 270
Ala Arg Leu Leu Val Gln Ala Ala Arg His Pro Gly Leu Val Arg Ala
        275                 280                 285
Leu Arg Asp Leu Leu Asp Leu Thr Gly Ala Glu Phe His Val Val His
    290                 295                 300
Ala Pro Asp Ala Leu Gly Leu Thr Phe Ala Glu Ile Ser Ser Arg Tyr
305                 310                 315                 320
Glu Glu Ala Cys Ala Val Gly Tyr Leu Ala Ala Asp Gly Arg Ala Leu
                325                 330                 335
Leu Thr Pro Ala Ser Gly Ala Arg Cys Gly Pro Gly Asp Arg Leu Ile
            340                 345                 350
Val Val Ala Arg Asp Asp Arg Pro Pro Val Ala Lys Arg Glu Gly Thr
        355                 360                 365
Ala Val Asp Pro Thr Val Met Ala Asp Arg Pro Asp Arg Gln Arg Ser
    370                 375                 380
Phe Ser Lys Thr Leu Leu Leu Gly Trp Asn Arg Arg Ala Pro Leu Val
385                 390                 395                 400
Met Glu Ser Leu Ser Arg Thr Ala Gln Pro Gly Ser
                405                 410
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<210> 10
<211> 414
<212> PRT
<213> Mesorhizobium loti

<400> 10

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Lys Ser Met Ala Ala Gly Pro Ile Ala Leu Ile Gly Trp Leu Ala Val
            20                  25                  30
Val Ser Leu Leu Ile Ile Ile Ala Ala Ala Ala Phe Leu Ala Val Thr
        35                  40                  45
Arg Ile Ala Pro Glu Gly Gly Glu Pro Leu Asn Phe Phe Glu Ala Phe
    50                  55                  60
Trp Glu Ser Leu Met Arg Thr Leu Asp Ser Gly Thr Met Gly Gly Asp
65                  70                  75                  80
Thr Gly Trp Ala Phe Arg Leu Val Met Leu Val Val Thr Leu Ala Gly
                85                  90                  95
Ile Phe Val Val Ser Ala Leu Ile Gly Val Leu Ser Ala Gly Val Asp
            100                 105                 110
Gly Lys Leu Asp Glu Leu Arg Lys Gly Arg Ser Arg Val Leu Glu Ser
        115                 120                 125
Asp His Thr Ile Ile Leu Asn Trp Ser Pro Ser Ile Phe Asp Val Ile
    130                 135                 140
Ser Glu Leu Val Ile Ala Asn Ala Ser Arg Arg Arg Pro Arg Ile Val
145                 150                 155                 160
Val Met Ala Asn Met Asp Lys Val Ala Met Glu Asp Glu Ile Ala Ala
                165                 170                 175
Lys Val Gly Lys Leu Gly Asn Thr Arg Ile Ile Cys Arg Ser Gly Asp
            180                 185                 190
Pro Thr Asp Leu Tyr Asp Leu Ala Ile Val Asn Pro Gln Thr Ser Arg
        195                 200                 205
Ser Val Ile Val Leu Ser Pro Asp Gly Asp Asp Pro Asp Ser Gln Val
    210                 215                 220
Ile Lys Thr Val Leu Ala Leu Val Asn Asp Pro Ser Arg Arg Thr Asp
225                 230                 235                 240
Pro Tyr Asn Ile Ala Ala Glu Ile Arg Asp Gly Lys Asn Ala Glu Val
                245                 250                 255
Ala Arg Val Val Gly Gly Ala Glu Val Gln Leu Val Leu Ala Asp Gln
            260                 265                 270
Leu Ile Ser Arg Ile Val Val His Ser Ser Arg Gln Ser Gly Leu Ser
        275                 280                 285
Gly Val Tyr Ser Glu Leu Leu Asp Phe Asp Gly Cys Glu Ile Tyr Thr
    290                 295                 300
Thr Thr Gln Pro Glu Leu Ala Gly Lys Thr Phe Gly Glu Ala Val Met
305                 310                 315                 320
Ala Tyr Glu His Cys Ala Leu Ile Gly Leu Cys Asp Gln Gly Gly Arg
                325                 330                 335
Val Asn Leu Asn Pro Pro Ser Glu Leu Val Ile Gly Lys Asp Met Arg
            340                 345                 350
Ala Ile Ile Ile Ala Glu Asp Asp Ala Ala Ile Arg Pro Gly Ser Ala
        355                 360                 365
Gly Ile Lys Ile Asp Thr Ala Ala Ile Arg Asp Pro Arg Pro Val Glu
    370                 375                 380
Ala Lys Pro Glu Arg Thr Leu Ile Leu Gly Trp Asn Arg Arg Gly Pro
385                 390                 395                 400
Ile Ile Thr Tyr Glu Leu Ser Arg Tyr Val Ala Pro Gly Ser
                405                 410
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<210> 11
<211> 2646
<212> DNA
<213> Medicago truncatula

<400> 11
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gacaacaatg gaattggagg aacttcaact actaaaactg atttctcaga acaacaatgg 180

aactaccctt ctttccttgg cattggcagc acctccagaa aaagaagaca accacccccct 240
cctccttcca aacctcctgt aaacctcatt cctcctcatc cccgtcccct ctccgtcaac 300
gaccacaaca aaaccacctc ctcacttctt ccacaacctt cctcttcctc catcaccaaa 360
caacaacaac aacactctac ctcctctccc atcttctatc ttttagttat ctgttgtatt 420
attcttgtac cctattcagc ttatttacaa tacaaacttg ccaaactcaa ggatatgaaa 480
cttcaactct gtggtcaaat tgatttttgt tcccgtaacg gaaaaacatc catacaagaa 540
gaggttgatg acgatgataa tgcagatagt agaacaatag ctttatatat tgtgcttttc 600
acattgattt tgccttttgt attgtacaaa tatcttgatt atcttcctca aataattaat 660
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gaatga 2646

<210> 12
<211> 847
<212> PRT
<213> Medicago truncatula

<400> 12

Met	Ala	Lys	Ser	Asn	Glu	Glu	Ser	Ser	Asn	Leu	Asn	Val	Met	Asn	Lys
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Pro	Pro	Leu	Lys	Lys	Thr	Lys	Thr	Leu	Pro	Ser	Leu	Asn	Leu	Arg	Val
		20					25					30			
Ser	Val	Thr	Pro	Pro	Asn	Pro	Asn	Asp	Asn	Asn	Gly	Ile	Gly	Gly	Thr
	35					40					45				
Ser	Thr	Thr	Lys	Thr	Asp	Phe	Ser	Glu	Gln	Gln	Trp	Asn	Tyr	Pro	Ser
50					55					60					
Phe	Leu	Gly	Ile	Gly	Ser	Thr	Ser	Arg	Lys	Arg	Arg	Gln	Pro	Pro	Pro
65				70					75					80	
Pro	Pro	Ser	Lys	Pro	Pro	Val	Asn	Leu	Ile	Pro	Pro	His	Pro	Arg	Pro
			85					90					95		
Leu	Ser	Val	Asn	Asp	His	Asn	Lys	Thr	Thr	Ser	Ser	Leu	Leu	Pro	Gln
		100					105					110			

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Pro Ser Ser Ser Ser Ile Thr Lys Gln Gln Gln Gln His Ser Thr Ser
        115                 120                 125
Ser Pro Ile Phe Tyr Leu Leu Val Ile Cys Cys Ile Ile Leu Val Pro
    130                 135                 140
Tyr Ser Ala Tyr Leu Gln Tyr Lys Leu Ala Lys Leu Lys Asp Met Lys
145                 150                 155                 160
Leu Gln Leu Cys Gly Gln Ile Asp Phe Cys Ser Arg Asn Gly Lys Thr
                165                 170                 175
Ser Ile Gln Glu Glu Val Asp Asp Asp Asp Asn Ala Asp Ser Arg Thr
            180                 185                 190
Ile Ala Leu Tyr Ile Val Leu Phe Thr Leu Ile Leu Pro Phe Val Leu
        195                 200                 205
Tyr Lys Tyr Leu Asp Tyr Leu Pro Gln Ile Ile Asn Phe Leu Arg Arg
    210                 215                 220
Thr Glu Ser Asn Lys Glu Asp Val Pro Leu Lys Lys Arg Val Ala Tyr
225                 230                 235                 240
Met Val Asp Val Phe Phe Ser Ile Tyr Pro Tyr Ala Lys Leu Leu Ala
                245                 250                 255
Leu Leu Cys Ala Thr Leu Phe Leu Ile Ala Phe Gly Gly Leu Ala Leu
            260                 265                 270
Tyr Ala Val Thr Gly Gly Ser Met Ala Glu Ala Leu Trp His Ser Trp
        275                 280                 285
Thr Tyr Val Ala Asp Ala Gly Asn His Ala Glu Thr Glu Gly Thr Gly
    290                 295                 300
Gln Arg Ile Val Ser Val Ser Ile Ser Ala Gly Gly Met Leu Ile Phe
305                 310                 315                 320
Ala Met Met Leu Gly Leu Val Ser Asp Ala Ile Ser Glu Lys Val Asp
                325                 330                 335
Ser Leu Arg Lys Gly Lys Ser Glu Val Ile Glu Arg Asn His Val Leu
            340                 345                 350
Ile Leu Gly Trp Ser Asp Lys Leu Gly Ser Leu Leu Lys Gln Leu Ala
        355                 360                 365
Ile Ala Asn Lys Ser Val Gly Gly Gly Val Ile Val Val Leu Ala Glu
    370                 375                 380
Lys Glu Lys Glu Glu Met Glu Met Asp Ile Ala Lys Leu Glu Phe Asp
385                 390                 395                 400
Phe Met Gly Thr Ser Val Ile Cys Arg Ser Gly Ser Pro Leu Ile Leu
                405                 410                 415
Ala Asp Leu Lys Lys Val Ser Val Ser Lys Ala Arg Ala Ile Ile Val
            420                 425                 430
Leu Ala Ala Asp Glu Asn Ala Asp Gln Lys Leu Ile Leu Ser Gln Cys
        435                 440                 445
Phe Pro Arg Ile Cys Leu Gln Ser Asp Ala Arg Ala Leu Arg Val Val
    450                 455                 460
Leu Ser Leu Ala Gly Val Lys Glu Gly Leu Arg Gly His Val Val Val
465                 470                 475                 480
Glu Met Ser Asp Leu Asp Asn Glu Pro Leu Val Lys Leu Val Gly Gly
                485                 490                 495
Glu Leu Ile Glu Thr Val Val Ala His Asp Val Ile Gly Arg Leu Met
            500                 505                 510
Ile Gln Cys Ala Leu Gln Pro Gly Leu Ala Gln Ile Trp Glu Asp Ile
        515                 520                 525
Leu Gly Phe Glu Asn Ala Glu Phe Tyr Ile Lys Arg Trp Pro Glu Leu
    530                 535                 540
Asp Asp Leu Leu Phe Lys Asp Ile Leu Ile Ser Phe Pro Asp Ala Ile
545                 550                 555                 560
Pro Cys Gly Val Lys Val Ala Ala Asp Gly Gly Lys Ile Val Ile Asn
                565                 570                 575
Pro Asp Asp Asn Tyr Val Leu Arg Asp Gly Asp Glu Val Leu Val Ile
            580                 585                 590
Ala Glu Asp Asp Asp Thr Tyr Ala Pro Gly Pro Leu Pro Glu Val Arg
        595                 600                 605
Lys Gly Tyr Phe Pro Arg Ile Arg Asp Pro Pro Lys Tyr Pro Glu Lys
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    610                 615                 620
Ile Leu Phe Cys Gly Trp Arg Arg Asp Ile Asp Asp Met Ile Met Val
625                 630                 635                 640
Leu Glu Ala Phe Leu Ala Pro Gly Ser Glu Leu Trp Met Phe Asn Glu
                645                 650                 655
Val Pro Glu Lys Glu Arg Glu Arg Lys Leu Ala Ala Gly Glu Leu Asp
            660                 665                 670
Val Phe Gly Leu Glu Asn Ile Lys Leu Val His Arg Glu Gly Asn Ala
        675                 680                 685
Val Ile Arg Arg His Leu Glu Ser Leu Pro Leu Glu Thr Phe Asp Ser
    690                 695                 700
Ile Leu Ile Leu Ala Asp Glu Ser Val Glu Asp Ser Val Ala His Ser
705                 710                 715                 720
Asp Ser Arg Ser Leu Ala Thr Leu Leu Leu Ile Arg Asp Ile Gln Phe
                725                 730                 735
Leu Tyr Pro Gly Ser Val Ile Met Tyr Tyr Pro Tyr Glu Leu Val Ser
            740                 745                 750
Met Ala Leu Ala Met Val Ala Glu Asp Lys Gln Ile Asn Arg Val Leu
        755                 760                 765
Glu Glu Leu Phe Ala Glu Glu Gly Asn Glu Met Cys Ile Lys Pro Ala
    770                 775                 780
Glu Phe Tyr Leu Phe Asp Gln Glu Glu Leu Cys Phe Tyr Asp Ile Met
785                 790                 795                 800
Ile Arg Gly Arg Thr Arg Lys Glu Ile Val Ile Gly Tyr Arg Leu Ala
                805                 810                 815
Asn Gln Glu Arg Ala Ile Ile Asn Pro Ser Glu Lys Ser Val Pro Arg
            820                 825                 830
Lys Trp Ser Leu Asp Asp Val Phe Val Val Leu Ala Ser Gly Glu
        835                 840                 845
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<210> 13
<211> 1050
<212> DNA
<213> Medicago truncatula

<400> 13
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ggtttagcgt tgtatgcggt tactggtggt agcatggctg aagcactttg gcattcttgg 120
acttatgtag ctgacgcagg aaatcacgct gaaacagaag gaaccggcca gagaatcgtc 180
tctgtctcaa ttagtgcggg tggcatgctt atatttgcca tgatgcttgg gcttgtttcg 240
gatgctatat cagagaaggt tgattcactt agaaaaggaa agagcgaagt catcgaaaga 300
aaccatgtac tcatccttgg ctggagtgac aaattgggct cacttttgaa gcagctagca 360
atagccaata agagtgttgg tggtggtgtt attgtggtgc ttgcagaaaa ggaaaaggag 420
gaaatggaaa tggatattgc aaagctcgaa ttcgatttca tggggacatc agtaatatgt 480
agaagtggca gtccactaat acttgctgac ctaaagaagg tttcagtttc aaaggcacgt 540
gcaatcattg ttttagctgc ggacgaaaat gcagatcaga aacttattct tagtcagtgt 600
tttcctcgaa tttgtctcca gagtgatgca cgtgctttga gagttgttct tagcttagct 660
ggtgtaaagg agggcttaag ggggcatgtt gttgtagaga tgagcgacct agacaatgaa 720
cccctagtga aacttgttgg tggagaactc attgaaacag ttgttgcaca tgatgtgatt 780
ggacgtttga tgattcagtg tgctctacag cctggccttg cacagatatg ggaggacatt 840
ctaggatttg agaatgctga gttttacata aaaagatggc ctgaactgga tgatcttctt 900
ttcaaagaca tattaatttc atttcctgat gcaataccgt gtggagttaa ggttgctgca 960
gatggaggga agattgtcat aaatccagat gataattatg ttctgagaga tggtgatgaa 1020
gtccttgtta tagctgagga tgatgacact 1050

<210> 14
<211> 357
<212> PRT
<213> Medicago truncatula

<400> 14
Met Val Asp Val Phe Phe Ser Ile Tyr Pro Tyr Ala Lys Leu Leu Ala

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 1               5                  10                  15
Leu Leu Cys Ala Thr Leu Phe Leu Ile Ala Phe Gly Gly Leu Ala Leu
            20                  25                  30
Tyr Ala Val Thr Gly Gly Ser Met Ala Glu Ala Leu Trp His Ser Trp
        35                  40                  45
Thr Tyr Val Ala Asp Ala Gly Asn His Ala Glu Thr Glu Gly Thr Gly
    50                  55                  60
Gln Arg Ile Val Ser Val Ser Ile Ser Ala Gly Gly Met Leu Ile Phe
65                  70                  75                  80
Ala Met Met Leu Gly Leu Val Ser Asp Ala Ile Ser Glu Lys Val Asp
                85                  90                  95
Ser Leu Arg Lys Gly Lys Ser Glu Val Ile Glu Arg Asn His Val Leu
            100                 105                 110
Ile Leu Gly Trp Ser Asp Lys Leu Gly Ser Leu Leu Lys Gln Leu Ala
        115                 120                 125
Ile Ala Asn Lys Ser Val Gly Gly Gly Val Ile Val Val Leu Ala Glu
    130                 135                 140
Lys Glu Lys Glu Glu Met Glu Met Asp Ile Ala Lys Leu Glu Phe Asp
145                 150                 155                 160
Phe Met Gly Thr Ser Val Ile Cys Arg Ser Gly Ser Pro Leu Ile Leu
                165                 170                 175
Ala Asp Leu Lys Lys Val Ser Val Ser Lys Ala Arg Ala Ile Ile Val
            180                 185                 190
Leu Ala Ala Asp Glu Asn Ala Asp Gln Lys Leu Ile Leu Ser Gln Cys
        195                 200                 205
Phe Pro Arg Ile Cys Leu Gln Ser Asp Ala Arg Ala Leu Arg Val Val
    210                 215                 220
Leu Ser Leu Ala Gly Val Lys Glu Gly Leu Arg Gly His Val Val Val
225                 230                 235                 240
Glu Met Ser Asp Leu Asp Asn Glu Pro Leu Val Lys Leu Val Gly Gly
                245                 250                 255
Glu Leu Ile Glu Thr Val Val Ala His Asp Val Ile Gly Arg Leu Met
            260                 265                 270
Ile Gln Cys Ala Leu Gln Pro Gly Leu Ala Gln Ile Trp Glu Asp Ile
        275                 280                 285
Leu Gly Phe Glu Asn Ala Glu Phe Tyr Ile Lys Arg Trp Pro Glu Leu
    290                 295                 300
Asp Asp Leu Leu Phe Lys Asp Ile Leu Ile Ser Phe Pro Asp Ala Ile
305                 310                 315                 320
Pro Cys Gly Val Lys Val Ala Ala Asp Gly Gly Lys Ile Val Ile Asn
                325                 330                 335
Pro Asp Asp Asn Tyr Val Leu Arg Asp Gly Asp Glu Val Leu Val Ile
            340                 345                 350
Ala Glu Asp Asp Asp
        355
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