



US007604972B2

(12) **United States Patent**
Mori et al.

(10) **Patent No.:** **US 7,604,972 B2**

(45) **Date of Patent:** **Oct. 20, 2009**

(54) **NICOTIANAMINE SYNTHASE**

A01H 9/00 (2006.01)

A01H 5/00 (2006.01)

(75) Inventors: **Satoshi Mori**, Chiba-ken (JP); **Kyoko Higuchi**, Gunma (JP); **Kazuya Suzuki**, Tokyo (JP); **Naoko Nishizawa**, Tokyo (JP); **Hiroshi Nakanishi**, Tokyo (JP)

(52) **U.S. Cl.** **435/193**; 435/183; 530/350; 800/295; 800/320; 800/320.2

(58) **Field of Classification Search** None
See application file for complete search history.

(73) Assignee: **Japan Science and Technology Corporation**, Saitama (JP)

(56) **References Cited**

OTHER PUBLICATIONS

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

Chica et al. *Curr Opin Biotechnol.* Aug. 2005;16(4):378-84.*
Accession Q10MI9. Aug. 22, 2006.*
Sen et al. *Appl Biochem Biotechnol.* Dec. 2007;143(3):212-23.*
* cited by examiner

(21) Appl. No.: **11/702,690**

Primary Examiner—Christian L Fronda

(22) Filed: **Feb. 5, 2007**

(74) *Attorney, Agent, or Firm*—Edwards Angell Palmer & Dodge LLP; Peter F. Corless; Jonathan M. Sparks

(65) **Prior Publication Data**

US 2008/0233627 A1 Sep. 25, 2008

(57) **ABSTRACT**

Related U.S. Application Data

(62) Division of application No. 09/674,337, filed as application No. PCT/JP99/02305 on Apr. 30, 1999, now Pat. No. 7,192,755.

A nicotianamine synthase is isolated and purified. Then the gene of this enzyme is cloned and the base sequence and amino acid sequence thereof are determined. This gene is employed in constructing plants, in particular, grass plants highly tolerant to iron-deficiency. A nicotianamine synthase involved in the mugineic acid biosynthesis pathway; the amino acid sequence thereof; a gene encoding the same; a vector containing this gene; cells transformed by the vector; a process for producing nicotianamine by using the same; plants transformed by the gene encoding the nicotianamine synthase; and an antibody against the nicotianamine synthase.

(30) **Foreign Application Priority Data**

Apr. 30, 1998 (JP) 10-137685

4 Claims, 14 Drawing Sheets

(51) **Int. Cl.**

C12N 9/00 (2006.01)
C12N 9/10 (2006.01)
C07K 14/00 (2006.01)

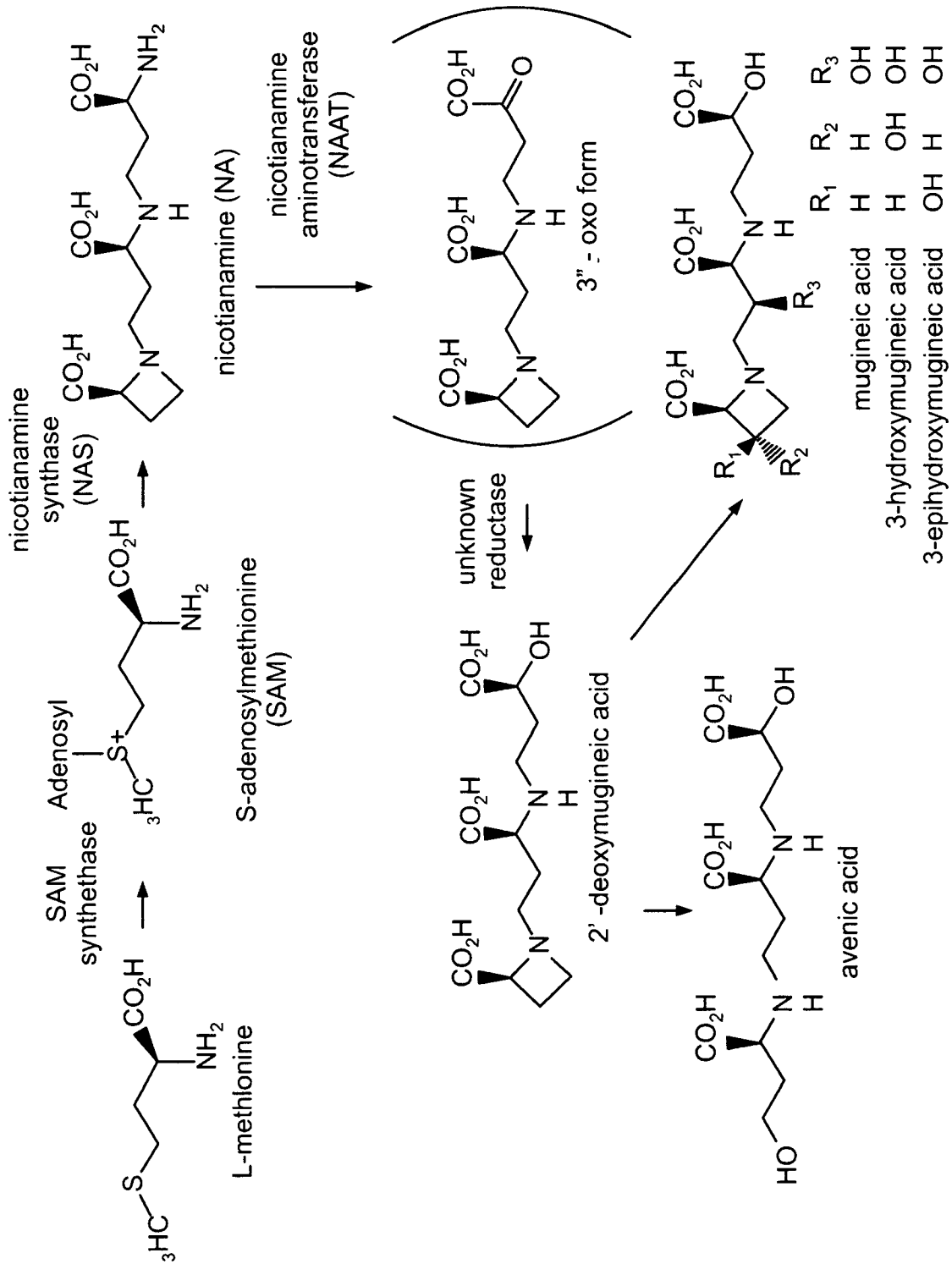


FIG. 1

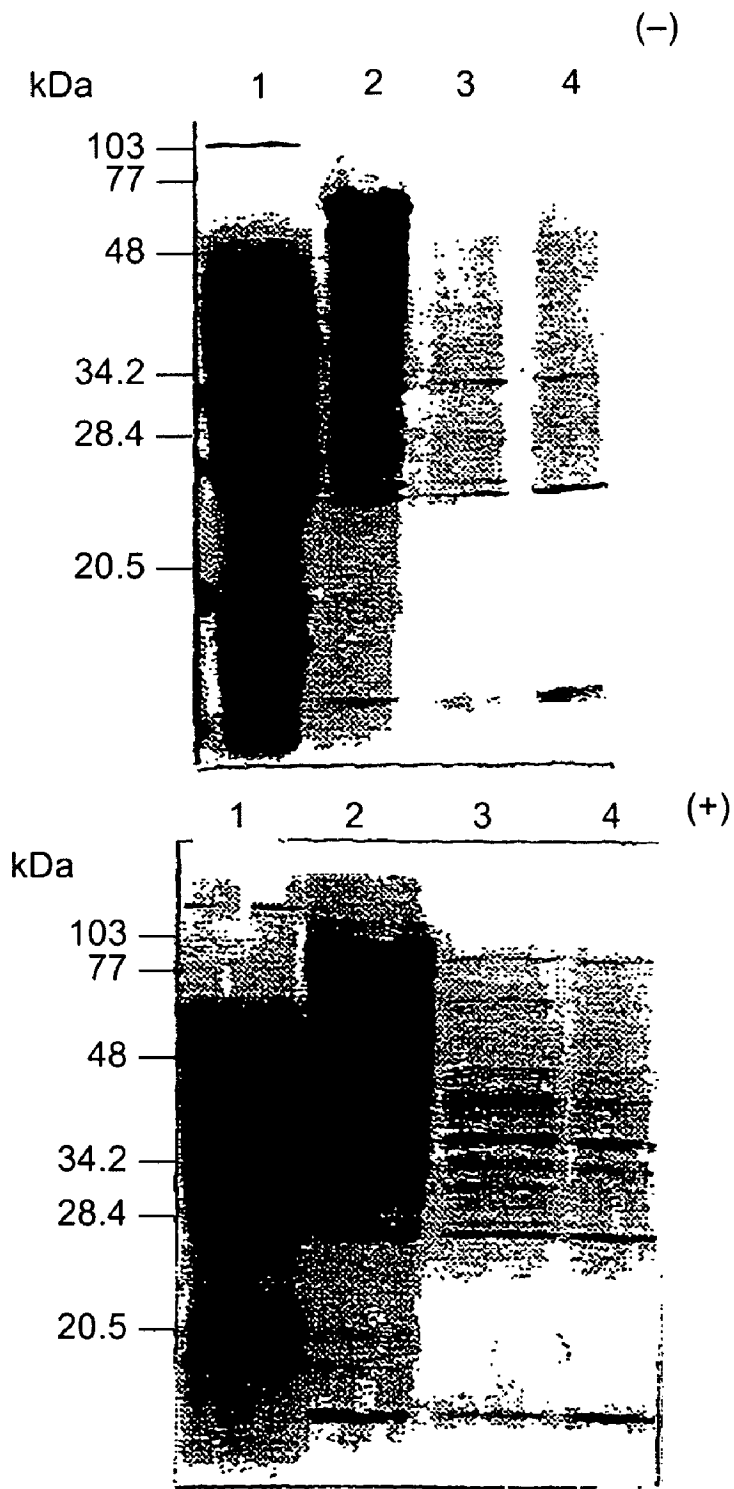


FIG. 2

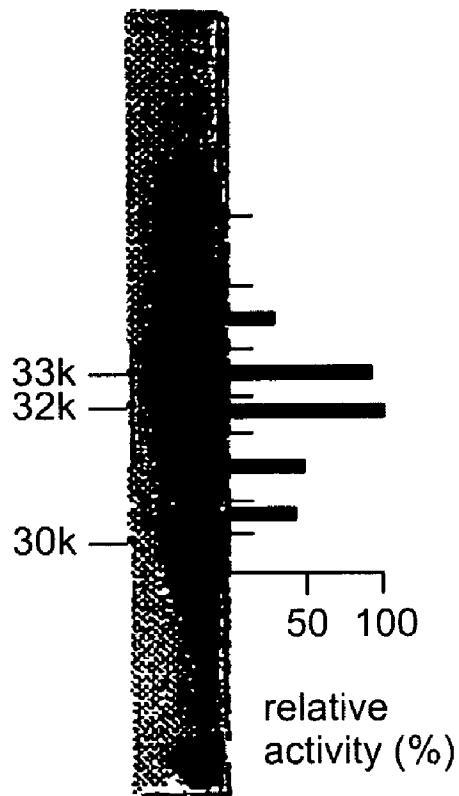


FIG. 3

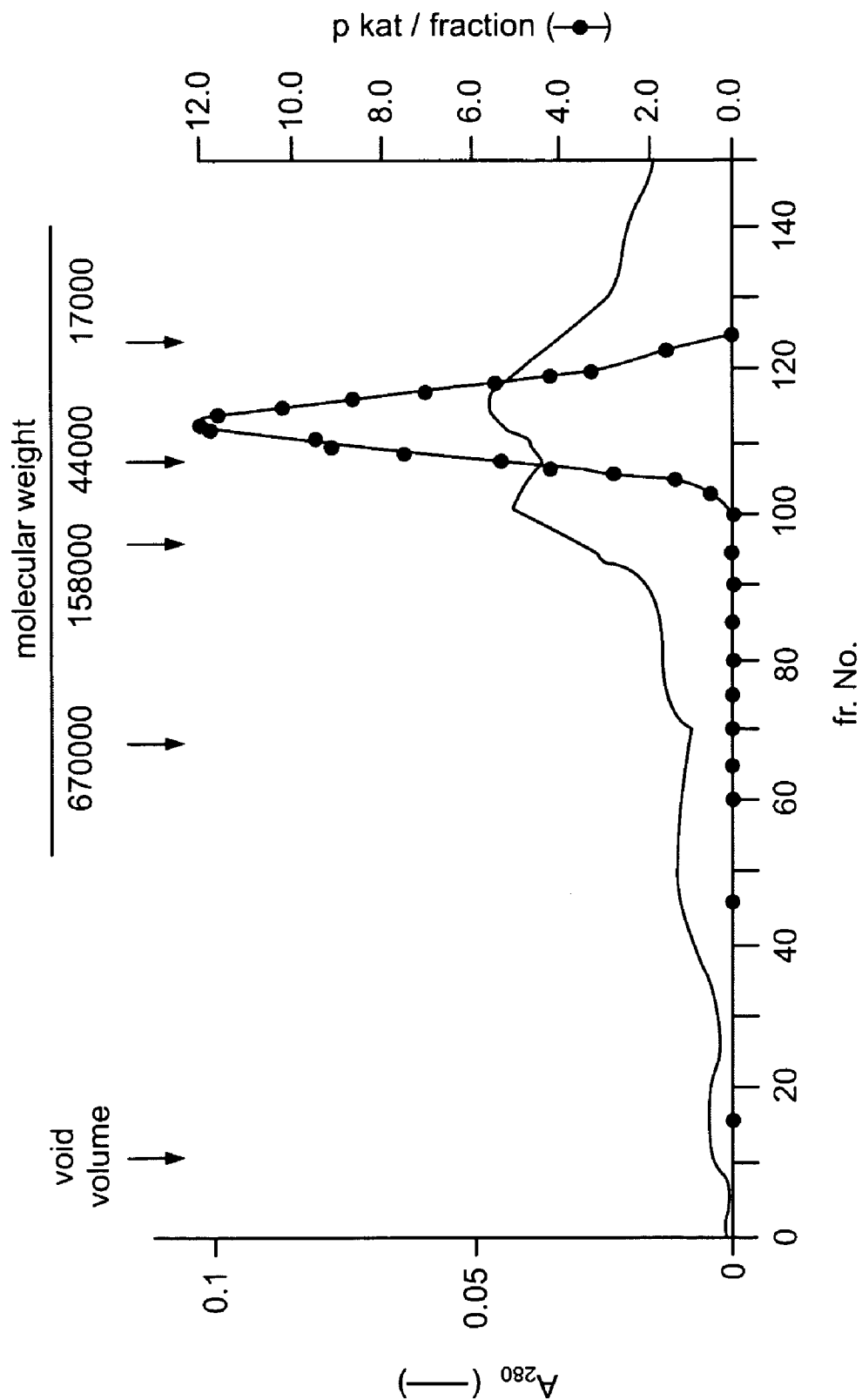


FIG. 4

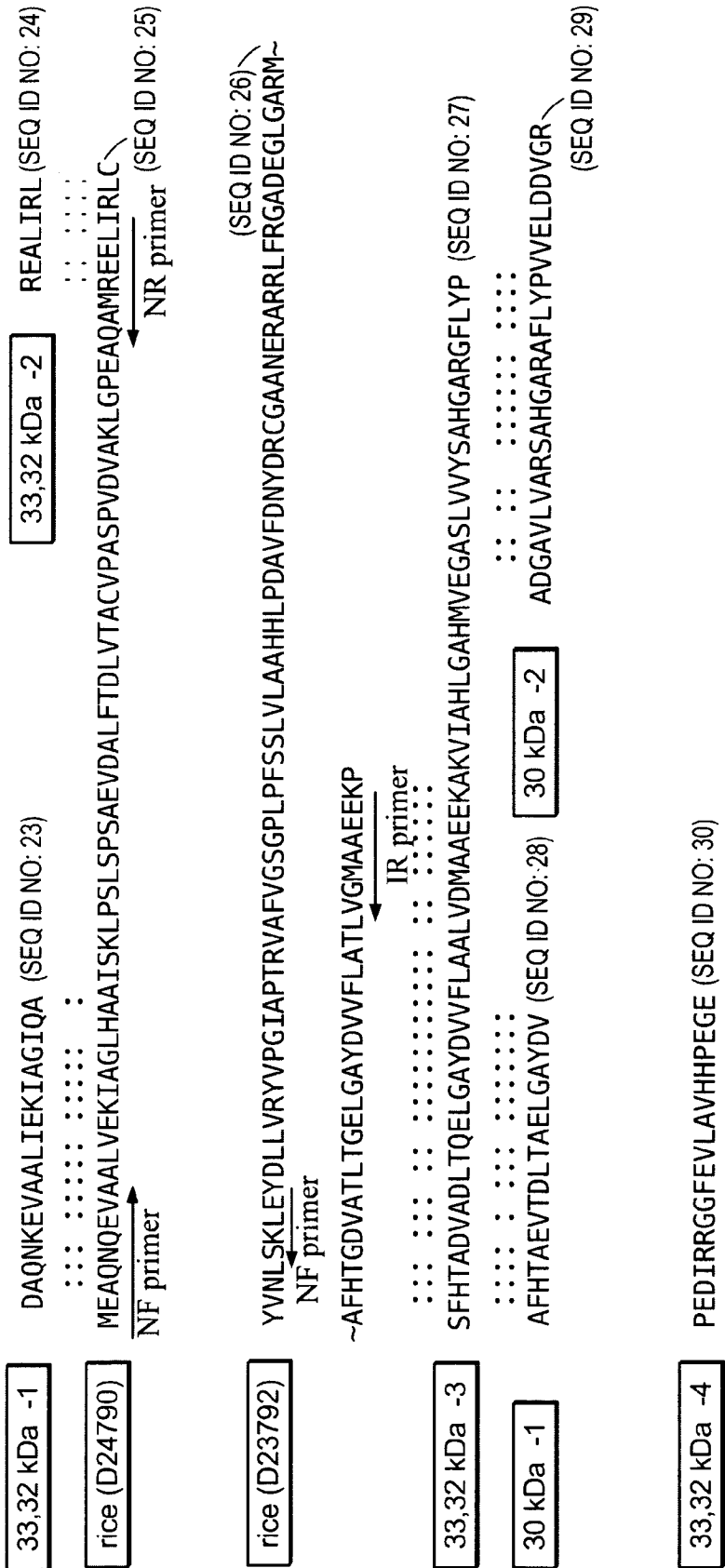


FIG. 5

(SEQ ID NO: 1) GCG TTC AGA GGC TTC CAG AGT TCT TCC GGT CAC CAA GAA GCA TTT GAT CAT AAC 54
 ATG GAT GCC CAG AAC AAG GAG GTC GCT GCT CTG ATC GAG AAG ATC GCC GGT ATC 108

(SEQ ID NO: 2) 19 M ^① D A Q N K E V A A L I E K I A G I
 CAG GCC GCC ATC GCC GAG CTG CCG TCG CTG AGC CCG TCC CCC GAG GTC GAC AGG 162
 37 Q A A I A E L P S L S P S P E V D R
 CTC TTC ACC GAC CTC GTC ACG GCC TGC GTC CCG CCG AGC CCC GTC GAC GTG ACG 216
 55 L F T D L V T A C V P P S P V D V T
 AAG CTC AGC CCG GAG CAC CAG AGG ATG CGG GAG GCT CTC ATC CGC TTG GTG TCC 270
 73 K L S P E H Q R M ^② R E A L I R L C S
 GCC GCC GAG GGG AAG CTC GAG GCG CAC TAC GCC GAC CTG CTC GCC ACC TTC GAC 324
 91 A A E G K L E A H Y A D L L A T F D
 AAC CCG CTC GAC CAC CTC GGC CTC TTC CCG TAC TAC AGC AAC TAC GTC AAC CTC 378
 109 N P L D H L G L F P Y Y S N Y V N L
 AGC AGG CTG GAG TAC GAG CTC CTG GCG CGC CAC GTG CCG GGC ATC GCG CCG GCG 432
 127 S R L E Y E L L A R H V P G I A P A
 CGC GTC GCC TTC GTC GGC TCC GGC CCG CTG CCG TTC AGC TCG CTC GTC CTC GCC 486
 145 R V A F V G S G P L P F S S L V L A
 GCG CAC CAC CTG CCC GAG ACC CAG TTC GAC AAC TAC GAC CTG TGC GGC GCG GCC 540
 163 A H H L P E T Q F D N Y D L C G A A
 AAC GAG CGC GCC AGG AAG CTG TTC GGC GCG ACG GCG GAC GGC GTC GGC GCG CGT 594
 181 N E R A R K L F G A T A D G V G A R
 ATG TCG TTC CAC ACG GCG GAC GTC GCC GAC CTC ACC CAG GAG CTC GGC GCC TAC 648
 199 M ^③ S F H T A D V A D L T Q E L G A Y
 GAC ^③ GTG GTC TTC CTC GCC GCG CTC GTC GGC ATG GCA GCC GAG GAG AAG GCC AAG 702
 217 D V V F L A A L V G M A A E E K A K
 GTG ATT GCC CAC CTG GGC GCG CAC ATG GTG GAG GGG GCG TCC CTG GTC GTG CCG 756
 235 V I A H L G A H M V E G A S L V V R
 AGC GCA CGG CCC CGC GGC TTT CTT TAC CCC ATT GTC GAC CCG GAG GAC ATC AGG 810
 253 S A R P R G F L Y P I V D P E D I R
 CGG GGT GGG TTC GAG GTG CTG GCC GTG CAC CAC CCG GAA ^④ GGT GAG GTG ATC AAC 864
 271 R G G F E V L A V H H P E G E V I N
 TCT GTC ATC GTC GCC CGT AAG GCC GTC GAA GCG CAG CTC AGT GGG CCG CAG AAC 918
 289 S V I V A R K A V E A Q L S G P Q N
 GGA GAC GCG CAC GCA CGG GGC GCG GTG CCG TTG GTC AGC CCG CCA TGC AAC TTC 972
 307 G D A H A R G A V P L V S P P C N F
 TCC ACC AAG ATG GAG GCG AGC GCG CTT GAG AAG AGC GAG GAG CTG ACC GCC AAA 1026
 325 S T K M E A S A L E K S E E L T A K
 GAG CTG GCC TTT TGA TTG AAG AGT GCG CGT GGT CAT TCT GTC GCC TGC GAT CGT 1080
 E L A F *

GGT AAC TTT CCT ACT CGT GTG TGT TTT GAT GTT TGT GCC TGT AAG AGT TAT GCT 1134
 TCC GGC CTT GTG CTG TTA ATT TAC ACG CGT TAC ATG TAG TAC TTG TAT TTA TAC 1188
 CTG GAA TAA CGG TAT GTA ACA TAA ATA TTA GTG GGA TTT GAA GTG TAA TGC TAA
 ATA ATA ATA AAA CTT GAT GCA GAC ATT CAA AAA AAA AAA AAA AAA AAA AA 1242

FIG. 6

FIG. 7A
FIG. 7B

FIG. 7

FIG. 7A

```

(SEQ ID NO: 7) HvNAS4      MDGQSE - - EVDALVQK I TGLHAA I AKLP SLSPSPD VD DALFT DLVTACVPPSPVDVTKLAP
(SEQ ID NO: 13) HvNAS7     MDAQSK - - EVDALVQK I TGLHAA I AKLP SLSPSPD VD DALFT DLVTACVPPSPVDVTKLAP
(SEQ ID NO: 1) HvNAS6     MDAQNK - - EVDALVQK I TGLHAA I AKLP SLSPSPD VD DALFT DLVTACVPPSPVDVTKLGS
(SEQ ID NO: 3) HvNAS2     MAAQNN - Q EVDALVEK I TGLHAA I AKLP SLSPSPD VD DALFT ELVTACVPPSPVDVTKLGP
(SEQ ID NO: 5) HvNAS3     MAAQNN N K DVAALVEK I TGLHAA I AKLP SLSPSPD VD DALFT ELVTACVPPSPVDVTKLGP
(SEQ ID NO: 11) HvNAS1    MDAQNK - - EVAALI EK I AG I QAA I AELP SLSPSP EVDRLFT DLVTACVPPSPVDVTKLSP
(SEQ ID NO: 9) NvNAS5     MEAENG - - EVAALVEK I TGLHAA I SKLP ALSPSPQVDALFT ELVAACVPS SPVDVTKLGP
* * * * *
HvNAS4      EAQAMREG LIRLCSEAE GKLEAHYSDML AAFDNPLDHLGVFPYYSNY I NLSKLEYE LLAR
HvNAS7     EAQAMREG LIRLCSEAE GKLEAHYSDML AAFDNPLDHLGVFPYYSNY I NLSKLEYE LLAR
HvNAS6     EAQEMREG LIRLCSEAE GKLEAHYSDML AAFDNPLDHLGMFPYYSNY I NLSKLEYE LLAR
HvNAS2     EAQEMREG LIRLCSEAE GKLEAHYSDML AAFDKPLDHLGMFPYYNNY I NLSKLEYE LLAR
HvNAS3     EAQEMREG LIRLCSEAE GKLEAHYSDML AAFDNPLDHLGI FFPYYSNY I NLSKLEYE LLAR
HvNAS1     EHQR MREALIRLCSEAE GKLEAHYADLL ATFDNPLDHLGL FFPYYSNY V NLSRLEYE LLAR
NvNAS5     EAQEMRQD LIRLCSEAE GLL EAHYSDML TALDSPLDHLGRFPYFDNYV NLSREHDE LLAG
* * * * *
HvNAS4      YVGRHRPAP VAF I GSGPLPFSSYV LAAR HLPD TVFDNYDLCGAANDRAT RLF FRAD KD - V
HvNAS7     YVPG GIAPAP VAF I GSGPLPFSSYV LAAR HLPD TVFDNYV PVAANDRAT RLF FRAD KD - V
HvNAS6     YVPG GIARPA VAF I GSGPLPFSSYV LAAR HLPD AMFDNYDLC SAANDRAS KLF FRAD KD - V
HvNAS2     YVPGYRPAR VAF I GSGPLPFSSFV LAAR HLPD TMFDNYDLC GAANDRAS KLF FRAD RD - V
HvNAS3     VVRR - HRPAR VAF I GSGPLPFSSFV LAAR HLPD TMFDNYDLC GAANDRAS KLF FRAD TD - V
HvNAS1     HVPG - I APAR VAFV GSGPLPFSSLV LAAH HLPETQFDNYDLC GAANERAR KLF FGAT ADGV
NvNAS5     HVAA - - - PAR VAF I GSGPLPFSSLF LATY HLPD TRFDNYDRCS VANGRAMKLV VGAA DEGV
* * * * *

```

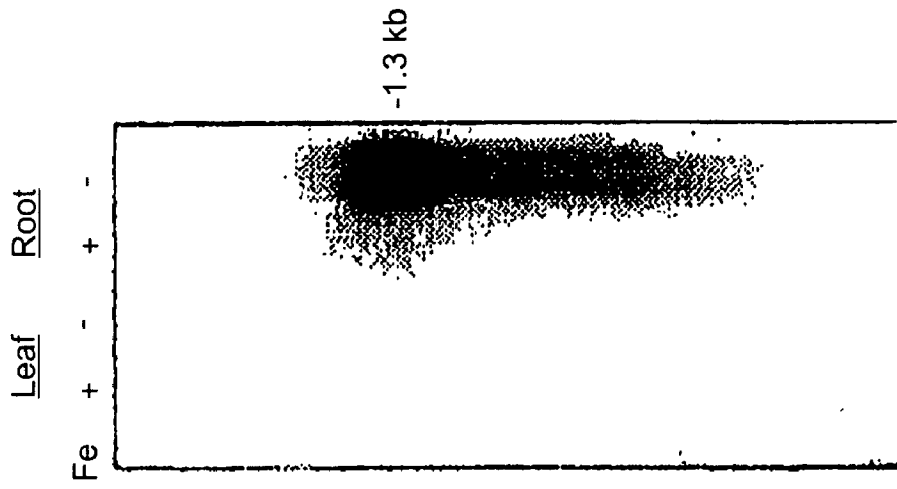



FIG. 9

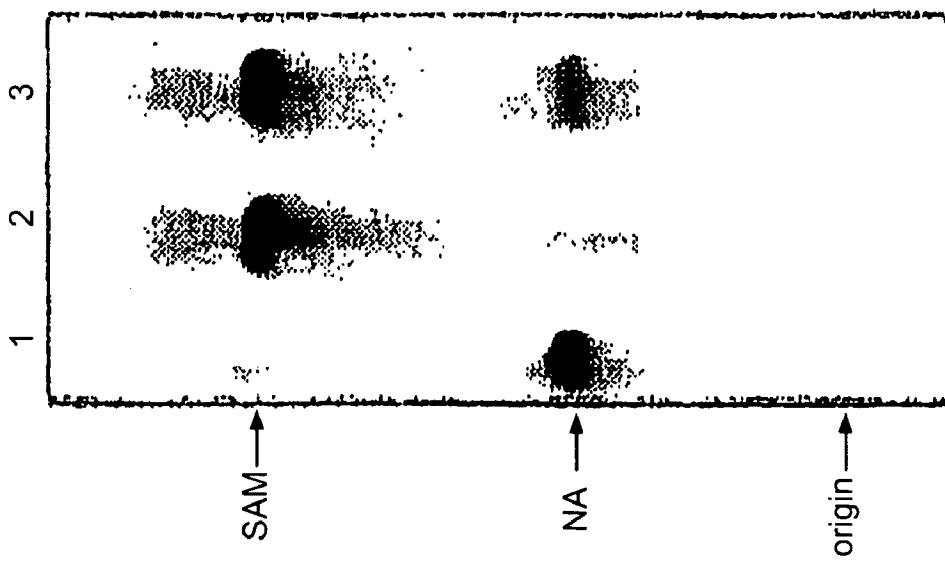


FIG. 8

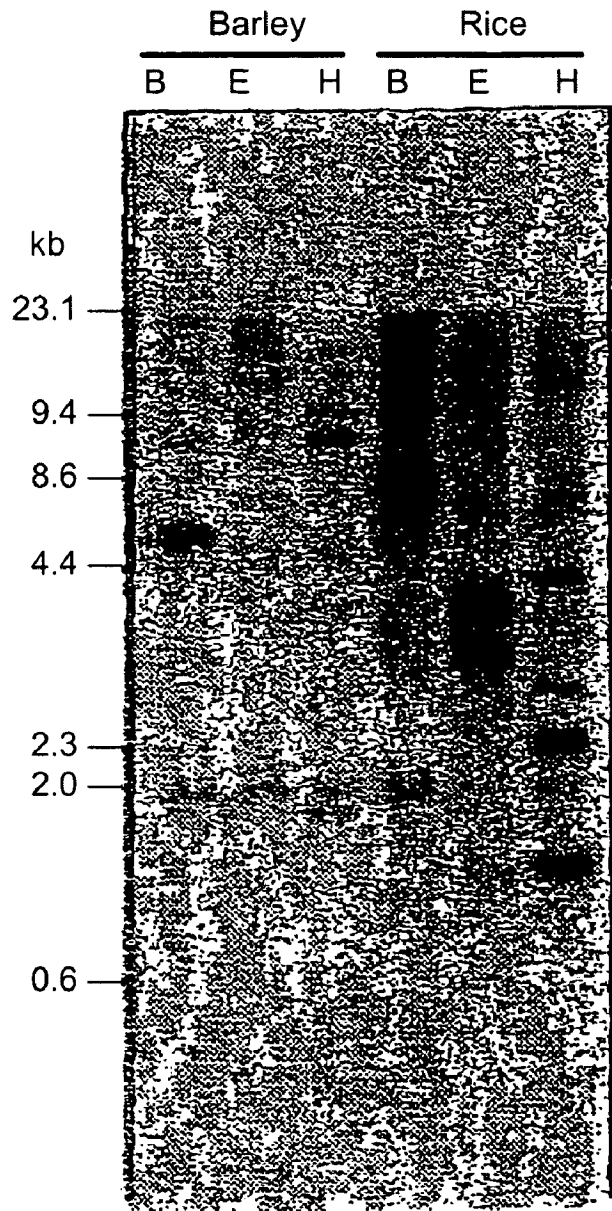


FIG. 10

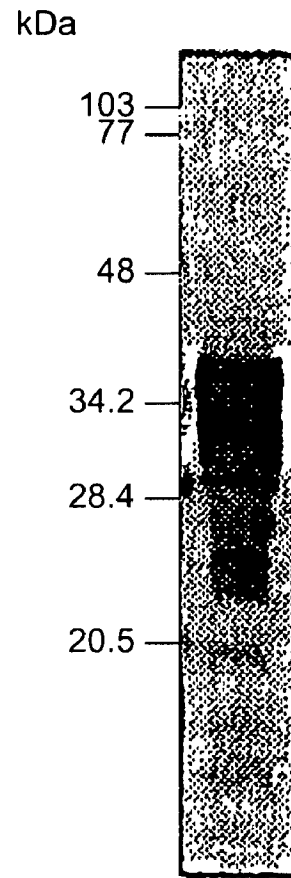


FIG. 11

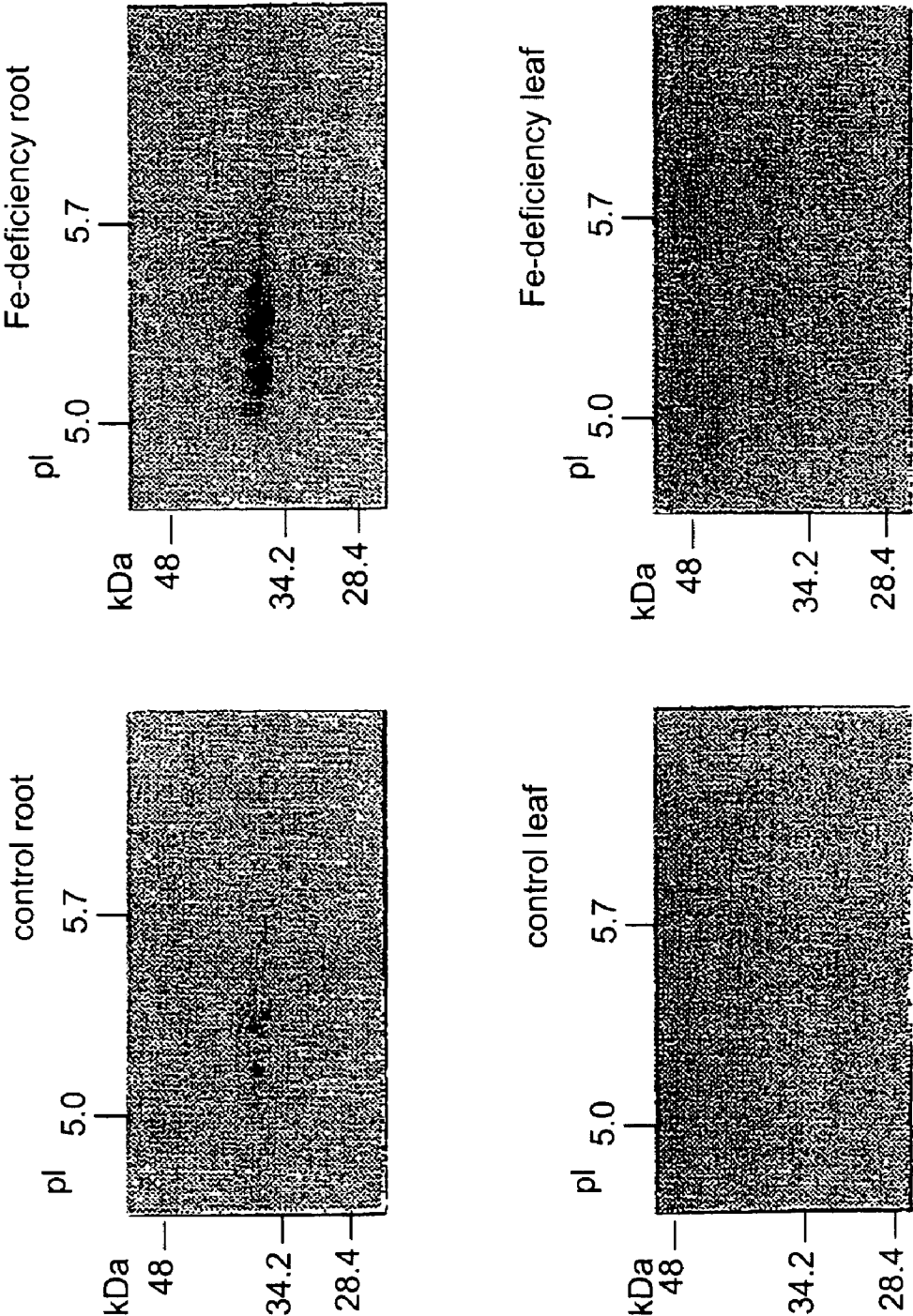


FIG. 12

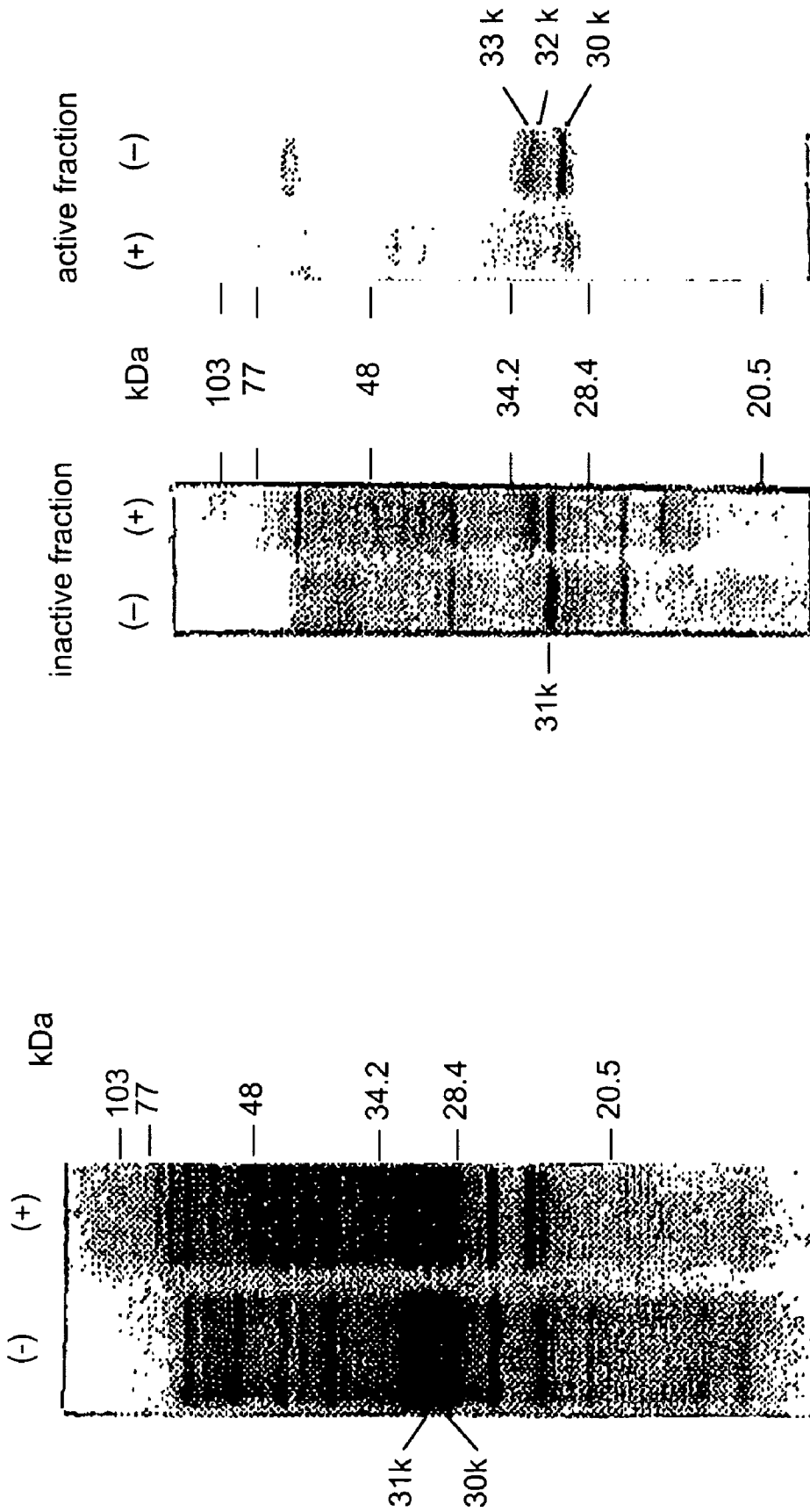


FIG. 14

FIG. 13

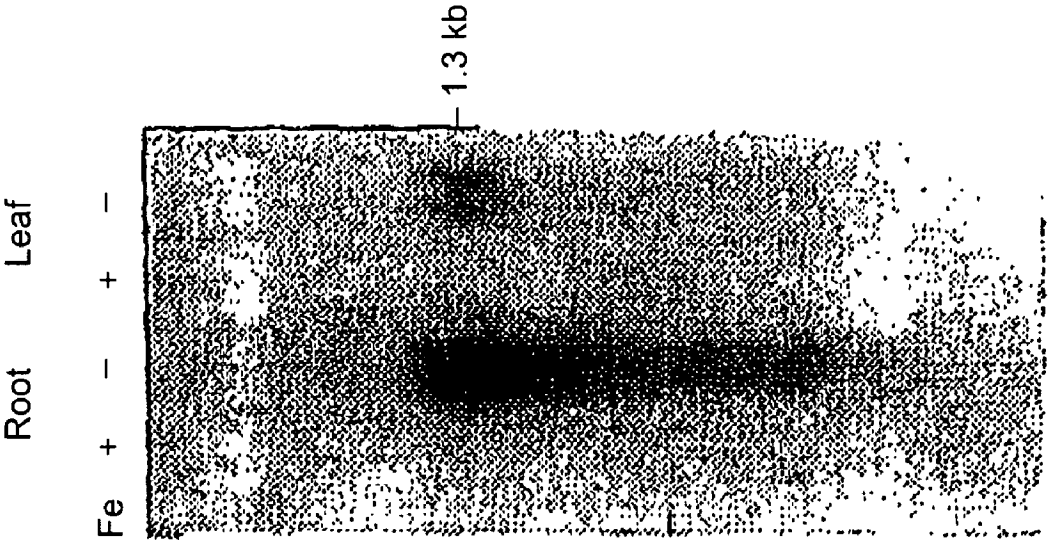


FIG. 16

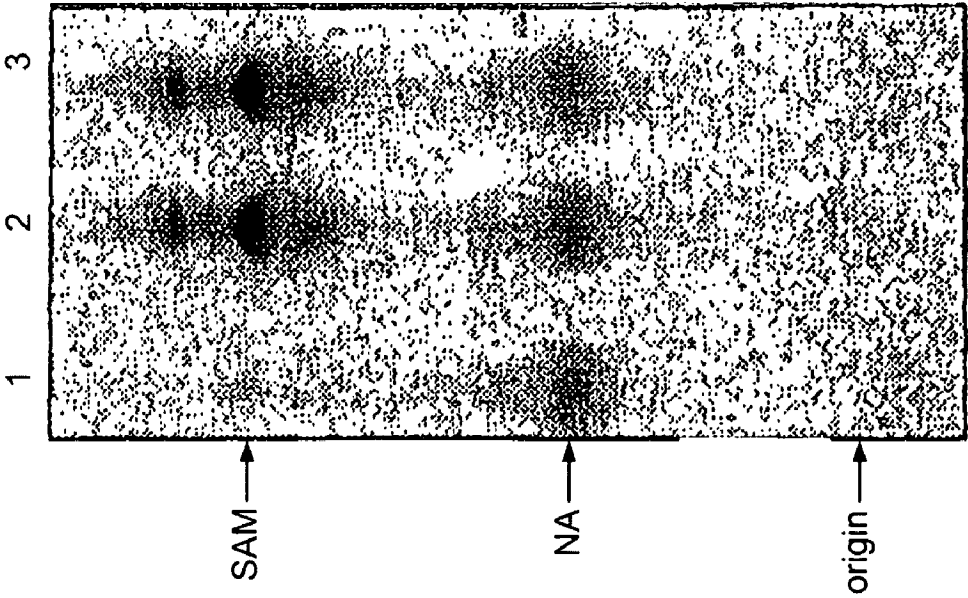


FIG. 15

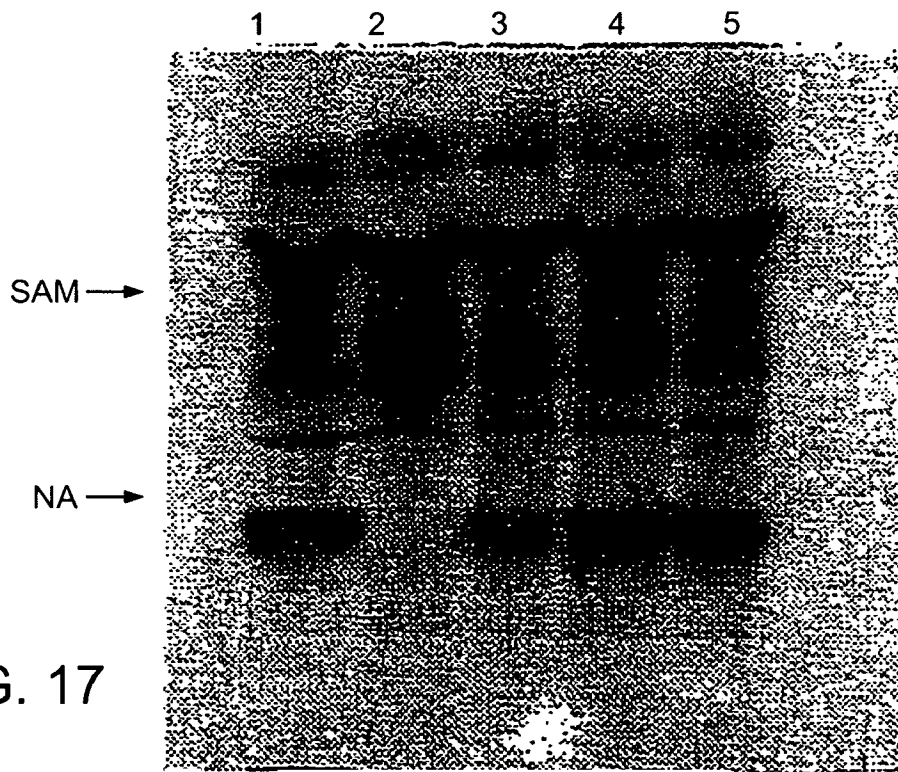


FIG. 17

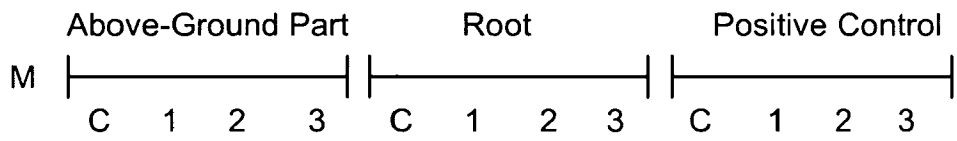


FIG. 18

1

NICOTIANAMINE SYNTHASE

RELATED APPLICATIONS

This application is a divisional application of U.S. application Ser. No. 09/674,337, filed Jul. 26, 2001, allowed, which is the U.S. National Stage Application of PCT/JP99/02305, filed Apr. 30, 1999. The entire contents of each of the aforementioned applications is hereby expressly incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to a nicotianamine synthase involved in the mugineic acid biosynthetic pathway, the amino acid sequence thereof, a gene encoding the same, a vector, a process for producing nicotianamine by using the same, plants transformed by the gene encoding the nicotianamine synthase, and an antibody against the nicotianamine synthase.

BACKGROUND ART

Graminaceous plants that absorb by chelating the insoluble state Fe(III) in soil using mugineic acid and adopt so called the Strategy-II mechanism of Fe acquisition secrete Fe chelators (phytosiderophores) from their roots to solubilize sparingly soluble Fe in the rhizosphere (Roemheld, 1987). The amount of the secreted phytosiderophores increases under Fe-deficiency stress. The mugineic acid family is the only examples of phytosiderophores known so far (Takagi, 1976). Tolerance to Fe deficiency in graminaceous plants is thought to depend on a quantity of mugineic acid family secreted by plants (Takagi et al. 1984, Roemheld and Marschner 1986, Marschner et al. 1987, Mori et al. 1987, Kawai et al. 1988, Mori et al. 1988, Mihashi and Mori 1989, and Shingh et al. 1993).

The biosynthetic pathway of mugineic acid in plants is shown in FIG. 1. S-adenosylmethionine is synthesized from methionine by S-adenosylmethionine synthase. Subsequently, three molecules of S-adenosylmethionine are combined to form one molecule of nicotianamine by nicotianamine synthase. The generated nicotianamine is then converted to 3"-keto acid by nicotianamine aminotransferase, and 2'-deoxymugineic acid is synthesized by the subsequent action of a reductase. A further series of hydroxylation steps produces the other mugineic acid derivatives including mugineic acid from the deoxymugineic acid (Mori and Nishizawa 1987, Shojima et al. 1989, Shojima et al. 1990 and Ma and Nomoto 1993).

A compound in FIG. 1, a compound in the lower right, wherein R₁ and R₂ are hydrogen and R₃ is hydroxyl, is mugineic acid. A compound wherein R₁ is hydrogen and R₂ and R₃ are hydroxyl, is 3-hydroxymugineic acid. Also a compound wherein R₂ is hydrogen and R₁ and R₃ are hydroxyl, is 3-epi-hydroxymugineic acid.

Three S-adenosylmethionine synthase genes were isolated from barley roots, but these genes were not induced by Fe deficiency (Takizawa et al. 1996). A gene *Ids3*, which is obtained from the barley by differential screening, is suspected to be a gene, which converts deoxymugineic acid to mugineic acid by hydroxylation and is strongly induced by Fe-deficiency (Nakanishi et al. 1993). Further, nicotianamine aminotransferase was purified and isolated from Fe-deficient barley roots, and two nicotianamine aminotransferase genes, Naat-A and Naat-B, were isolated (Takahashi et al. 1997). Naat-A expression was induced in Fe-deficient roots.

2

The synthesis of nicotianamine from S-adenosylmethionine is similar to polyamine synthesis from decarboxy-S-adenosylmethionine. In contrast to polyamine synthase, however, nicotianamine synthase catalyzes the combination of three S-adenosylmethionine molecules and the azetidide ring formation at the same time (FIG. 1). Such the nicotianamine synthase is a novel type of enzyme. Previously, we reported the partial purification of nicotianamine synthase from the roots of Fe-deficient barley and expression pattern of the activity (Higuchi et al. 1994, Higuchi et al. 1995, Kanazawa et al. 1995, Higuchi et al. 1996a and Higuchi et al. 1996b). Since nicotianamine synthase is easily decomposed during extraction and purification, it has been difficult to purify sufficient quantities for amino acid sequencing.

The present invention has an object to provide a plant, especially graminaceous plant, highly tolerant to Fe-deficiency, as a result of isolating and purifying a nicotianamine synthase, being cloned the gene of this enzyme, determining the base sequence and amino acid sequence thereof, and using said enzyme.

DISCLOSURE OF INVENTION

The present invention relates to a nicotianamine synthase shown in SEQ ID NO: 1 comprising amino acid sequence shown in SEQ ID NO: 1, or amino acid sequence having deletion in a part thereof, being substituted by the other amino acids or being added with the other amino acids.

The present invention relates to the gene encoding said amino acid sequence of nicotianamine synthase.

The present invention also relates to a vector comprising containing said gene, and a transformant transformed by the said vector.

The present invention relates to a process for production of nicotianamine using the said transformant.

The present invention further relates to plants, especially graminaceous plants, to which said gene is introduced, and fruits obtained by growing said plants.

The present invention relates to a process for extraction of said nicotianamine synthase in the presence of thiol protease inhibitor, preferably E-64.

Further, the present invention relates to an antibody against said nicotianamine synthase.

BRIEF DESCRIPTION OF DRAWING

FIG. 1 shows the biosynthetic pathway of mugineic acid family.

FIG. 2 shows a comparison of nicotianamine synthase purification from Fe-dependent and control barley roots.

FIG. 3 shows a preparative SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis, hereinafter designated as SDS-PAGE) around 30-35 kDa. The horizontal bar indicates relative enzyme activity detected from the gels.

FIG. 4 shows elution pattern of nicotianamine synthase activity from the gel-filtration column.

The large closed circles (●) indicates enzyme activity.

FIG. 5 shows a comparison with a six partial amino acid sequence determined by nicotianamine synthase originated from barley and similar sequence of graminaceous plants obtained by computer search of the database. Identical amino acid residue is shown in a “.”.

FIG. 6 shows full length of HvNAS1 cDNA and amino acid sequence deduced therefrom. The underlined sequences indicate the identical partial amino acid sequences of fragments in the above FIG. 5. Numbers of the nucleotide sequence are

3

indicated to the right of each row. Amino acid numbers are indicated on the left of each row.

FIG. 7 shows comparison of the deduced amino acid sequences of the above 7 cDNA obtained from barley. Asterisks "*" indicates identical amino acid residues in all sequences.

FIG. 8 shows results of thin layer chromatographic (TLC) analysis of nicotianamine synthase activity obtained from *E. coli* crude extract expressing a fused protein of maltose binding protein-HvNAS1.

FIG. 9 shows Northern-hybridization analysis of HvNAS1 as a probe.

FIG. 10 shows Southern-hybridization analysis of HvNAS1 as a probe.

FIG. 11 shows Western-blot analysis of crude enzyme used for detection of nicotianamine synthase activity.

FIG. 12 shows Western-blot analysis of total protein extracted by trichloroacetic acid/acetone.

FIG. 13 shows comparison of nicotianamine synthase purification from Fe-deficient barley and control barley after DEAE-Sepharose FF.

FIG. 14 shows comparison of nicotianamine synthase purification from Fe-deficient barley and control barley after Ether Toyopearl 650M.

FIG. 15 shows results of thin layer chromatographic (TLC) analysis of nicotianamine synthase activity obtained from *E. coli* crude extract expressing a fused protein of maltose binding protein-OsNAS1.

FIG. 16 shows Northern-hybridization analysis of OsNAS1 as a probe.

FIG. 17 shows results of thin layer chromatographic (TLC) analysis of nicotianamine synthase activity obtained from *E. coli* crude extract expressing a fused proteins of maltose binding protein-AtNAS1, AtNAS2 or AtNAS3.

FIG. 18 shows results of RT-PCR of total RNA extracted from the aboveground parts and roots of *Arabidopsis thaliana*. Right group indicates positive control.

BEST MODE FOR CARRYING OUT THE INVENTION

We have tried to isolate nicotianamine synthase (Higuchi et al. Plant & Soil, Vol. 165, p. 173-179, 1994), and since nicotianamine synthase was easily decomposed and was difficult to isolate and purify, we were unable to obtain sufficient amounts of protein to determine its partial amino acid sequence. Subsequently, it was found that a thiol protease inhibitor E-64 (hereinafter designates as E-64) was very effective in suppressing degradation of nicotianamine synthase (Higuchi et al. Plant & Soil, Vol. 178, p. 171-177, 1996 a).

In the present invention, as a result that frozen roots were crushed to a fine powder in liquid N₂ and then rapidly homogenized with buffer containing 0.1 mM thiol protease inhibitor E-64, nicotianamine synthase protein could be isolated and its gene could also be isolated.

Further, the enzyme of the present invention recovered its activity by removal of SDS after SDS-PAGE treatment, but the rate of recovery was very low (Higuchi et al. Plant & Soil, Vol. 165, p. 173-179, 1994). Consequently, degree of purification should be increased up before treatment of SDS-PAGE. Then the column chromatography procedures were further improved.

We have also found that the enzyme of the present invention is relatively hydrophobic and a buffer containing a mild surface active agent CHAPS increased the rate of recovery. Several ion-exchange chromatography carriers were tested,

4

and DEAE-Sepharose FF and DEAE Sephacel were found to be the most effective. In addition to TSK gel Butyl Toyopearl, another hydrophobic chromatography carrier, TSK gel Ether Toyopearl 650M, effectively removed impurities of the 30-35 kDa.

The enzyme of the present invention has been reported that it was the peptide of 30-35 kDa, the activity of which was recovered by removing SDS after SDS-PAGE treatment, and the activity was detected as a broad molecular weight range of 30-35 kDa (refer to FIG. 3). FIG. 3 shows a result of preparative SDS-PAGE in the fractions showing enzyme activity. SDS-PAGE was carried out using 11% acrylamide slab gels. A portion of the gel was stained with Coomassie brilliant blue and the rest of the gel was stained with Cu. The gel containing proteins between 30-35 kDa in size was cut into seven fragments (indicated by the short lines). The thick bars in FIG. 3 indicate relative enzymatic activities detected from each gel fragment.

In order to identify nicotianamine synthase peptide from the proteins having these molecular weights, the peptides, which were contained in the nicotianamine synthase fractions, purified from Fe-deficient and control barley roots were compared using SDS-PAGE. From each barley root 200 g, the present enzyme was purified according to the method described in example 3 hereinbelow.

The enzyme activity of the control was a quarter of the Fe-deficient roots.

The peptide composition of the active enzyme fraction from each purification step of the present enzyme was analyzed and compared by SDS-PAGE, and results are shown in FIG. 2, FIG. 13 and FIG. 14. FIG. 2, FIG. 13 and FIG. 14 show comparison with the active fraction from the purification step of Fe-deficient barley roots 200 g [in the figure, shown with (-)], and the active fraction from the purification step of the control barley roots 200 g [in the figure, shown with (+)]. SDS-PAGE was carried out using 12.5% acrylamide slab gels (Laemmli, Nature Vol. 227, p. 680-685, 1970). Gels were stained with Coomassie brilliant blue. FIG. 2 shows a step before DEAE-Sepharose. The upper row shows enzyme from Fe-deficient barley roots and the lower row shows enzyme from control roots. In each lane, lanes 1, crude extract, 200 µg of protein; lanes 2, after Butyl Toyopearl 650M, 100 µg of protein; lanes 3, after hydroxyapatite, 20 µg of protein; and lanes 4, after Butyl Toyopearl 650M, 15 µg of protein, are shown.

FIG. 13 shows after DEAE-Sepharose FF, each lane, 25 µg of protein. FIG. 14 shows after Ether Toyopearl 650M; in which left shows inactive fraction, and right shows active fraction, and 1/2s of each fraction is electrophoresed.

As a result, almost no difference was observed in both Fe-deficient and control roots before DEAE-Sepharose step (refer to FIG. 2). After the DEAE-Sepharose step it became clear that the 30- and 31-kDa peptides were induced by Fe-deficiency (refer to FIG. 13). After the Ether Toyopearl step, the 31 kDa peptide was eliminated from the active fraction. The 32 and 33 kDa peptides were found to be newly induced by Fe-deficiency (refer to FIG. 14). Activities were detected from the 32 and 33 kDa peptides, but no activity was detected from 30 kDa peptide (refer to FIG. 3).

Molecular weight of the enzyme of the present invention was determined by gel-filtration.

Estimated molecular weight of nicotianamine synthase by gel-filtration was reported to be 40,000-50,000 (Higuchi et al. Plant & Soil, Vol. 165, p. 173-179, 1994). But this did not correspond with the value estimated by SDS-PAGE.

In the present study, the buffer containing CHAPS effectively increased the resolution and molecular weight of the

5

present enzyme was estimated to be 35,000 (refer to FIG. 4). this corresponds well to the value estimated by SDS-PAGE.

FIG. 4 shows elution pattern of nicotianamine synthase from the gel-filtration column. The black circles (●) indicate the enzyme activity and the solid line indicates absorption at 280 nm. The active fraction after hydroxyapatite chromatography was applied to a Sephacryl S300HR (Pharmacia) column (1.5 cm×71 cm, 125 ml), equilibrated with developing buffer (50 mM Tris, 1 mM EDTA, 0.1 M KCl, 0.05% CHAPS, 0.1 mM p-APMSF and 3 mM DTT, pH 8.0). Molecular weight markers used were thyroglobulin (Mr 670,000), γ -globulin (Mr 158,000), ovalbumin (Mr 44,000), and myoglobin (Mr 17,000). The linear flow was 10 cm/hour.

Partial amino acid sequence was determined from purified nicotianamine synthase.

The above explained 30 kDa, 32 kDa and 33 kDa peptides were purified from 1 kg of Fe-deficient barley roots by using a method in example 3 hereinbelow. These were partially degraded using a method in example 4 hereinbelow. Although 32- and 33-kDa peptides could not be completely separated from each other, these might have similar sequence or 32 kDa peptide was presumed to be the degradation product of 33 kDa peptide, and both of them were degraded in together.

The determined partial amino acid sequences indicated that these peptides were very similar in each other (FIG. 5). Further, since the molecular weights of the 33 kDa and 32 kDa (1) fragments had almost unchanged-molecular weight as compared with before degradation, this sequence might be N-terminal region of the present enzyme. As a result of computer search of the database, a gene of unknown function having very similar sequence to these sequences was found to exist in *Oryza sativa* and *Alabidopsis thaliana*. Especially, EST-cDNA clones D23792 and D24790 of *Oryza sativa* were very similar with 80.0% identity in a 33-amino acid overlap in the former and 68.4% identity in a 19-amino acid overlap in the latter (FIG. 5).

FIG. 5 shows a comparison with a six partial amino acid sequence determined by nicotianamine synthase originated from barley and similar sequence of graminaceous plants obtained by computer search of the database. Identical amino acid residue is shown in “:”. The part of nucleotide sequences indicated by the arrows was applied for the sequences of primer used in PCR.

Cloning and nucleotide sequences of cDNA clones encoding nicotianamine synthase were performed and determined.

PCR amplification of total cDNA prepared from Fe-deficient barley roots using degenerate primers designed from the partial amino acid sequence obtained from the method explained hereinbefore was performed, but the objective DNA could not amplified. Then the primers having single nucleotide sequence (shown by arrows in FIG. 5) from sequences of *Oryza sativa*, D23792 and D24790, were synthesized and PCR amplification was performed. The 205 bp fragment was amplified by PCR using NF and NR primers and the 274 bp fragment was amplified by PCR using IF and IR primers, and these contained the objective sequences. A cDNA library prepared using poly (A)⁺ RNA from Fe-deficient barley roots was screened and 19 positive clones using the 205 bp fragment probe and 88 positive clones using the 274 fragment bp probe were obtained.

Among the thus obtained clones, the clone designated as HvNAS1, contained a translated region of 985 bp and amino acid sequence deduced therefrom was 328 amino acids residue, with deduced molecular weight of 35,144. This corresponded well with the value estimated by SDS-PAGE and

6

gel-filtration. The partial amino acid sequences of the 32 kDa and 33 kDa peptides were included totally in HvNAS1 (FIG. 6).

FIG. 6 shows full length of HvNAS1 cDNA and amino acid sequence deduced therefrom. The underlined sequences indicate the identical partial amino acid sequences of fragments in the above FIG. 5. Numbers of the nucleotide sequence are indicated to the right of each row. Amino acid numbers are indicated on the left of each row.

The predicted pI of 5.2 matched the value estimated by native isoelectric focusing electrophoresis well. The six clones having very similar sequence other than HvNAS1, i.e. HvNAS2, HvNAS3, HvNAS4, HvNAS5, HvNAS6 and HvNAS7, were also obtained (Table 1, FIG. 7).

FIG. 7 shows comparison of the deduced amino acid sequences of the above 7 cDNA obtained from barley. Asterisks “*” indicates identical amino acid residues in all sequences.

The nucleotide sequences of these clones are shown in SEQ ID NO: 2 (HvNAS1), SEQ ID NO: 4 (HvNAS2), SEQ ID NO: 6 (HvNAS3), SEQ ID NO: 8 (HvNAS4), SEQ ID NO: 10 (HvNAS5), SEQ ID NO: 12 (HvNAS6) and SEQ ID NO: 14 (HvNAS7), respectively. The amino acid sequences of these amino acid sequences are shown in. SEQ ID NO: 1 (HvNAS1), SEQ ID NO: 3 (HvNAS2), SEQ ID NO: 5 (HvNAS3), SEQ ID NO: 7 (HvNAS4), SEQ ID NO: 9 (HvNAS5), SEQ ID NO: 11 (HvNAS6) and SEQ ID NO: 13 (HvNAS7), respectively.

TABLE 1

Clone	Properties of nas clones					
	Number of Amino Acid Residues	Molecular Weight	pI	Identity to nas 1 (%)	Identity to nas 2 (%)	Identity to nas 4 (%)
HvNAS 1	328	35144	5.20	—	—	—
HvNAS 2	336	35839	5.07	72	—	—
HvNAS 3	336	36013	5.47	72	95	—
HvNAS 4	330	35396	4.91	73	89	—
HvNAS 5	283	30148	5.22	61	61	59
HvNAS 6	329	35350	5.07	74	89	88
HvNAS 7	330	35244	4.98	70	86	91

The partial amino acid sequences determined from the 30 kDa peptide were all included in HvNAS5. The 5'- and 3'-non-translated regions of these clones were not similar with each other.

D23792 and D24790 similar to nicotianamine synthase of *Oryza sativa* were found with about 80% identity to HvNAS1. AC003114 and AB005245 of *Arbidopsis thaliana* were found with about 45% identity to HvNAS1.

The obtained HvNAS1 protein was expressed in *E. coli*.

The PCR amplification of HvNAS1 ORF was cloned with vector pMAL-c2 to express HvNAS1 fused with C-terminal of maltose binding protein. The expression of fused protein is strongly induced by IPTG.

The crude extract was obtained from the transformed *E. coli*, and nicotianamine synthase activity was assayed in the state of the fused protein. The crude extract from the strain transformed with only the vector could not be detected the activity, whereas in case of inserted with HvNAS1 ORF, the activity was detected. Result is shown in FIG. 8.

FIG. 8 shows results of thin layer chromatographic (TLC) analysis of nicotianamine synthase obtained from *E. coli* crude extract expressing a fused protein of maltose binding protein-HvNAS1. In FIG. 8, lane 1: a standard nicotianamine

7

synthase; lane 2: *E. coli* expressing maltose binding protein (SAM); and lane 3: *E. coli* expressing maltose binding protein-HvNAS1 fused protein.

Northern hybridization analysis conducted by the method described in example 7 hereinbelow indicated that this gene was strongly induced in Fe-deficient roots (FIG. 9). This coincides with expression pattern of the present enzyme activity (Higuchi et al. 1994). FIG. 9 shows a result of Northern hybridization analysis using HvNAS1 as a probe. Total RNA was extracted from after one week of Fe-deficient-treatment and control barley leaves and roots, and in each lane, 5 µg of RNA were electrophoresed.

Southern hybridization analysis of the barley genome DNA was performed according to the method described in example 8 hereinafter mentioned. Cutting of DNA with BamHI, EcoRI or HindIII produced plurality of fragments, however none of clones obtained at present could be digested by BamHI and EcoRI, consequently nicotianamine synthase gene might exist with multiple copies in genomes of barley and rice (FIG. 10).

FIG. 10 shows Southern-hybridization analysis of HvNAS1 as a probe.

Genomic DNAs from barley and rice were digested with BamHI (lanes B), EcoRI (lanes R) and HindIII (lanes H) and 10 µg thereof were electrophoresed in each lane.

Further, using antigen prepared by the method described in example 9 hereinbelow, Western-blot analysis was performed according to the method described in example 10. It was found that the present enzyme protein was rapidly decomposed during the operation in the crude extract prepared for detecting the present enzyme activity (FIG. 11). The staining patterns coincided with the fact that the present enzyme activity was detected on the broad range between 30-35 kDa after SDS-PAGE (refer to FIG. 3).

FIG. 11 shows Western-blot analysis of crude enzyme used for detection of activity. SDS-PAGE was performed using 12.5% acrylamide slab gel. Protein 100 µg was electrophoresed.

The crude extract obtained from denatured protein according to the method described in example 10 hereinbelow was detected as almost single band with 35-36 kDa (FIG. 12). This value coincided with the deduced value from the amino acid sequence.

FIG. 12 shows Western-blot analysis of total protein extracted by trichloroacetic acid/acetone. SDS-PAGE was performed using 12.5% acrylamide slab gel. Protein 100 µg was electrophoresed. Proteins 200 µg extracted from roots and proteins 500 µg extracted from leaves were electrophoresed.

Western-blot analysis after 2-dimension electrophoresis reveals to detect several spots. This coincided with the fact of obtaining plurality of nicotianamine synthase gene. All spots were induced in Fe-deficient roots.

As a result that cDNA library from Fe-deficient rice roots poly (A)+RNA was screened using probes prepared by cutting HvNAS1 with restriction enzymes ApaI and XhoI, 20 clones were obtained. These clones were divided into 3 types of clones according to their sequences, and among them, only one type contains ORF full length, which was designated as OsNAS1. Nucleotide sequence of OsNAS1 is shown in SEQ ID NO: 16 and amino acid sequence is shown in SEQ ID NO: 15.

PCR amplification of OsNAS1 ORF was cloned with a vector pMAL-c2 to express a form fused with maltose binding protein C-terminal. The fused protein is strongly induced its expression by IPTG.

8

Crude extract from the transformed *E. coli* with the fused protein was obtained and nicotianamine synthase activity was assayed in the state of the fused protein. The same activity with HvNAS1 was detected. Result is shown in FIG. 15. FIG. 15 shows results of thin layer chromatographic (TLC) analysis of nicotianamine synthase obtained from *E. coli* crude extract expressing a fused protein of maltose binding protein-OsNAS1. In FIG. 15, lane 1: a standard nicotianamine (NA); lane 2: an extract from *E. coli* expressing maltose binding protein-OsNAS1 fused protein; and lane 3: an extract from *E. coli* expressing maltose binding protein-HvNAS1 fused protein.

Northern hybridization analysis conducted by the method described in example 7 hereinbelow indicated that in contrast to barley, the expression was induced in rice by Fe-deficient treatment not only in roots but also in leaves (FIG. 16). FIG. 16 shows a result of Northern hybridization analysis using OsNAS1 ORF as a probe. Total RNA was extracted from after two weeks of Fe-deficient treatment and control rice leaves and roots, and in each lane, 5 µg of RNA were electrophoresed.

Nucleotide sequence of *Arabidopsis thaliana* similar to HvNAS1 obtained by computer search of the database was used as a primer. PCR amplification for genome DNA of *Arabidopsis thaliana* resulted to obtain three nicotianamine synthase genes.

These were designated as AtNAS1, AtNAS2 and AtNAS3.

Nucleotide sequence of these genes are shown in SEQ ID NO: 18 (AtNAS1), SEQ ID NO: 20 (AtNAS2) and SEQ ID NO: 22 (AtNAS3). These amino acid sequences are shown in SEQ ID NO: 17 (AtNAS1), SEQ ID NO: 19 (AtNAS2) and SEQ ID NO: 21 (AtNAS3).

AtNAS1, AtNAS2 and AtNAS3 ORF were amplified with PCR and were cloned with a vector pMAL-c2. Each of them was tried to be expressed in the form of fusing with maltose binding protein C-terminal. The expression of the fused protein was strongly induced by IPTG.

Crude extract from the transformed *E. coli* with the fused protein was obtained and nicotianamine synthase activity was assayed in the state of the fused protein. The activity was detected. Result is shown in FIG. 17. FIG. 17 shows results of TLC analysis of nicotianamine synthase activity obtained from *E. coli* crude extract expressing a fused protein of maltose binding protein-AtNAS. In FIG. 17, lanes 1: a standard nicotianamine (NA) and S-adenosylmethionine; lanes 2: an extract from *E. coli* expressing only maltose binding protein; lanes 3: an extract from *E. coli* expressing maltose binding protein-AtNAS1 fused protein; lanes 4: an extract from *E. coli* expressing maltose binding protein-AtNAS2 fused protein; and lanes 5: an extract from *E. coli* expressing maltose binding protein-AtNAS3 fused protein.

RT-PCR was conducted according to the method described in example 11 hereinbelow. It was found that AtNAS1 was expressed in the roots and the aboveground parts of *Arabidopsis thaliana*, whereas AtNAS2 was expressed neither in the roots nor in the aboveground parts, and AtNAS3 was expressed only in the roots (FIG. 18). In FIG. 18, lane M shows molecular weight marker. Gene expression was conducted in the aboveground parts, roots and positive controls. In the figure, lanes C: AtNAS1 and AtNAS2 ORF full length were amplified; lanes 1: AtNAS1 specific amplification fragments; lanes 2: AtNAS2 specific amplification fragments; and lanes 3: AtNAS3 specific amplification fragments.

The amount of secreted mugineic acid is reported increased up to 20 mg mugineic acid/g roots dry weight/day (Takagi, 1993). Crude nicotianamine synthase activity detected by the present inventors was sufficient to fulfill it.

Since the present enzyme proteins exist in more than several types and 30 kDa peptide without activity exists, it can be speculated that as a result of aggregation of these peptides, the constructed structure, which is preferable for binding with 3 molecules of S-adenosylmethionine, reveals maximum activity. The molecular weight estimated by gel-filtration was 35,000 (FIG. 4).

Increase in activity by re-aggregation of subunits has not been observed at present. Since the fused protein with maltose binding protein and subunits showed its activity, we have at present an idea that the present enzyme might be a monomer. However, the possibility that large activity can be revealed by constructing multimer, can not completely denied.

The reaction mechanism synthesizing nicotianamine from S-adenosylmethionine may be similar to methyl transfer reaction using S-adenosylmethionine as a methyl donor, and a reaction synthesizing spermidine and spermine from decarboxylated S-adenosylmethionine. The common catalytic domain of these enzymes has been discussed in relation to equivalent amino acids configuration occupying similar positions in higher-order structures (Hashimoto et al. 1998 and Schluckebier et al. 1995).

In future, catalytic domain may be elucidated as the results of comparison with nicotianamine synthase from other plant species or X-ray crystallography.

Induction of nicotianamine synthase activity by Fe-deficiency is a specific phenomenon in graminaceous plants, and is essential for mass production of mugineic acid family. *Oryza sativa* is a plant, in which secretion of mugineic acid family is the least among major graminaceous plants, consequently it is very weak for Fe-deficiency in calcareous soil.

Consequently, as a result of creating transformant *Oryza sativa* having tolerance to Fe-deficiency by introducing nicotianamine synthase gene of the present invention into the graminaceous plants, especially *Oryza sativa*, and expressing large amount at the Fe-deficiency, cultivation of rice in the calcareous soil can be possible.

Heretofore, in the graminaceous plants, nicotianamine has been thought to have only a role as a precursor for synthesis of mugineic acid family. However, since the present invention has elucidated that nicotianamine synthase gene constituted the multiple gene family, it may play other important roles in the graminaceous plants.

In plants, which lack the ability to secrete mugineic acid family, except for graminaceous plants, it has been proposed that nicotianamine plays a key role as an endogenous chelator of divalent metal cations, such as Fe^{2+} , Cu^{2+} , Zn^{2+} and Mn^{2+} , and that it contributes to the homeostasis of those metals (Stephan et al. 1994). Consequently, it may play the same role in the graminaceous plants.

Nicotianamine synthase activity is not induced in dicots, and expression of gene of the present invention may not be induced by Fe-deficiency. We have cloned nicotianamine synthase genes of *Arabidopsis thaliana*. Composition of promoter regions in these genes can elucidate the mechanism of gene expression caused by Fe-deficiency, and the gene of the present invention may play important function not only in the graminaceous plants but also in the dicots.

SEQ ID NO: 1 shows amino acid sequence of nicotianamine synthase of the present invention.

The present invention includes nicotianamine synthase having amino acid sequence shown in SEQ ID NO: 1. However, the present invention is not limited within the above nicotianamine synthase. The nicotianamine synthase of the present invention includes, unless it loses nicotianamine synthase activity, the peptides, in which a part of the amino acid

sequence of said peptide is deleted, preferably 50% or less, more preferably 30% or less, or more further preferably 10% or less in the total amino acids, or is substituted by other amino acids, or to which other amino acids are further added, or in which these deletion, substitution and addition may be combined.

Nucleotide sequence coding nicotianamine synthase of the present invention is shown in SEQ ID NO: 2.

The present invention also includes not only a gene coding nicotianamine synthase shown in SEQ ID NO: 2 but also genes coding nicotianamine synthase mentioned herein-above.

The vector of the present invention introducing the above gene is not specifically limited, and various vectors can be introduced. Preferable vector is the expression vector.

Various cells can be transformed conventionally by using recombinant vector of the present invention. Mass production of nicotianamide can be performed by using the thus obtained transformant. These methods are well known in the person skilled in the art.

Examples of hosts for introducing the gene of the present invention are bacteria, yeasts and cells. Preferable host is plants, especially the graminaceous plant.

Method for introducing gene is not limited. It can be made by using vector or can be directly introduce in genome.

Antibody of the present invention against nicotianamine synthase can be prepared conventionally by using nicotianamine synthase of the present invention. Antibody can be a polyclonal antibody or, if necessary, monoclonal antibody.

Further, a selective breeding of plants, preferably graminaceous plants, can be made by using gene of the present invention. Especially, the gene of the present invention can be applied for improvement of varieties, which can grow even in Fe-deficient soil.

EXAMPLES

The following examples illustrate the present invention, but are not construed as limiting the present invention.

Example 1

Preparation of Plant Material

Seeds of barley (*Hordeum vulgare* L. cv Ehimehadakamugi No. 1) were germinated on wet filter paper and transferred into the standard hydroponic culture solution (Mori and Nishizawa, 1987) in a glass house at natural temperature under natural light. The pH of the hydroponic culture solution was adjusted at 5.5 by 0.5 N HCl everyday. When the third leaves developed, the plants were transferred to the hydroponic culture solution without containing Fe. The pH was maintained at 7.0 by 0.5 N NaOH everyday. The control plants were also cultured in the standard culture solution continuously. The culture solution was renewed once in every week. Two weeks after starting Fe-deficient treatment, when severe iron chlorosis significantly appeared on the 4th and 5th leaves, roots were harvested and frozen in liquid N_2 and stored at $-80^\circ C$. until use.

Example 2

Assay of Nicotianamine Synthase Activity

Modified assay method reported previously by the present inventors (Higuchi et al. 1996a) was used. Enzyme solutions were equilibrated with reaction buffer [50 mM Tris, 1 mM

11

EDTA, 3 mM dithiothreitol (hereinafter designates as DTT), 10 μ M (p-aminodiphenyl) methanesulfonyl fluoride (hereinafter designates as p-APMSF) and 10 μ M trans-epoxysuccinyl-leucylamido-(4-guanidino) butane (hereinafter designates, as E-64), pH 8.7]. Buffer exchange was performed by using ultrafiltration unit, Ultrafree C3LGC NMWL10000 (Millipore Co.). S-adenosylmethionine labeled with 14 C in carboxyl group (Amersham Inc.) was added to the enzyme solution at the final concentration of 20 μ M and kept at 25° C. for 15 minutes. The reaction products were separated by thin layer chromatography on silica gel LK6 (Whatman Inc.) using developer (phenol: butanol: formic/acid: water=12:3:2:3). Radioactivity of the reaction products was detected by image Analyzer BAS-2000 (Fuji Film Co.). The protein content was assayed by Bradford method using Protein Assay Kit (Bio Rad Inc.).

Example 3

Purification of Nicotianamine Synthase

The following operations were performed at 4° C. and E-64 was added to fractions containing nicotianamine synthase at the final concentration of 10 μ M.

The frozen roots were crushed into a fine powder in liquid N₂ and homogenized in a household juicer with 200 ml of extraction buffer [0.2 M Tris, 10 mM EDTA, 5% (v/v) glycerol, 10 mM DTT, 0.1 mM E-64, 0.1 mM p-APMSF and 5% (w/v) insoluble polyvinylpyrrolidone (PVP), pH 8.0] per 100 g of roots. The homogenate was centrifuged for 30 minutes at 22,500 \times g to obtain supernatant. Ammonium sulfate was added to the supernatant to yield a final concentration of 0.4 M and allowed to stand for 1 hour. Again, the mixture was centrifuged for 30 minutes at 22,500 \times g to obtain supernatant.

The supernatant was loaded onto a TSK gel Butyl Toyopearl 650M column (10 ml bed volume per 100 g of roots), equilibrated with the adsorption buffer [20 mM Tris, 1 mM EDTA, 3 mM DTT, 0.4 M (NH₄)₂SO₄ and 0.1 mM p-APMSF, pH 8.0] and eluted with elution buffer [10 mM Tris, 1 mM EDTA, 3 mM DTT, 0.1 mM p-APMSF, 5% glycerol and 0.05% 3-[(3-chloramidopropyl) dimethyl-ammonio] propanesulfonic acid (hereinafter designates as CHAPS), pH 8.0].

KCl was added to the active fraction to give a final concentration of 0.4 M, and 1 M potassium phosphate buffer (pH 8.0) was added to a final concentration of 1 mM of KCl. A hydroxyapatite 100-350 mesh (Nacalai Tesque), equilibrated with the adsorption buffer (1 mM K-P, 10 mM KCl, 3 mM DTT and 0.1 mM p-APMSF, pH 8.0), was prepared at 10 ml per protein 100 mg and the fractions containing nicotianamine synthase were loaded. Nicotianamine synthase was passed through without adsorption. The passed through fraction was loaded onto TSK gel Butyl Toyopearl 650M column (1 ml bed volume per 10 mg of protein), and nicotianamine synthase was eluted in the manner described above.

The active fraction was loaded onto a DEAE-Sepharose FF column (5 ml bed volume per 25 mg of protein, Pharmacia) equilibrated with the adsorption buffer (20 mM Tris, 1 mM EDTA, 3 mM DTT, 0.1 mM p-APMSF and 0.05% CHAPS, pH 8.0) and eluted with stepwise gradient elution of potassium chloride concentration of 0.05 M, 0.1 M, 0.15 M and 0.2 M. Nicotianamine synthase was eluted at 0.15 M of KCl concentration.

The active fraction was loaded onto the Ether Toyopearl 650M column (10 ml bed volume per 100 g of roots), equilibrated with adsorption buffer [20 mM Tris, 1 mM EDTA, 3 mM DTT, 1.2 M (NH₄)₂SO₄ and 0.1 mM p-APMSF, pH 8.0].

12

Nicotianamine synthase was not adsorbed and passed through from the column. The passed through fraction was loaded onto TSK gel Butyl Toyopearl 650M column and fractions containing nicotianamine synthase was eluted. The peptides in the active fraction containing nicotianamine synthase, which was purified by the above column chromatographic treatments, were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (hereinafter designates as SDS-PAGE) using 11% acrylamide slab gels. After SDS-PAGE the gel was stained with 0.3 M copper chloride (Dzandu et al. 1988), and the separated bands were cut out. The gel fragments were destained with 0.25 M EDTA/0.25 M Tris (pH 9.0) and homogenized, with the extraction buffer (1% SDS, 25 mM Tris and 192 mM glycine). Each homogenate was electroeluted with SDS-free buffer (25 mM Tris and 192 mM glycine) and peptide was recovered.

Example 4

Determination of Partial Amino Acid Sequence

The isolated nicotianamine synthase was digested chemically with cyanogen bromide (Gross 1967).

After SDS-PAGE treatment, 10-fold volume of 70% (v/v) formic acid and 1% (w/v) cyanogen bromide were added to gel fragments containing nicotianamine synthase and decomposed at 4° C. for overnight. After completion of digestion, the liquid part was collected and dried in vacuo. The dried substance was dissolved in SDS-PAGE sample buffer, and allowed to stand at room temperature for overnight, then the digested product was separated by SDS-PAGE using 16.5% acrylamide gel containing Tricine (Schagger and Jagow, 1987). The peptides were transferred onto a PVDF membrane by electroblotting (Towbin et al. 1979) and stained with amido black. The stained bands were cut out and the amino acid sequence was determined from N-terminal side of each peptide by Edman degradation in gas-phase sequencer (model 492A protein sequencer, Applied Biosystems Inc.).

Example 5

Cloning of Nicotianamine Synthase Genes

PCR amplification was conducted for cDNA originated from Fe-deficient barley roots using primers, which were synthesized based on the obtained partial amino acid sequence. A pYH23 cDNA library prepared from the poly (A)⁺RNA of Fe-deficient barley roots was screened with the thus obtained DNA fragments of PCR product, which was labeled with [α -³²P]dATP using the random primer kit (Takara Shuzo Co.), as the primers. The isolated cDNA clones were sequenced by cycle sequencing kit (Shimadzu Bunko Co.) using Shimadzu DNA sequencer DSQ-2000L.

As a result that cDNA library from Fe-deficient rice roots poly (A)⁺RNA was screened using probes prepared by cutting HvNAS 1 with restriction enzymes ApaI and XhoI. The isolated cDNA clones were sequenced by cycle sequencing kit (Shimadzu Bunko Co.) using Shimadzu DNA sequencer DSQ-2000L.

PCR amplification was conducted for genomic DNA of *Arabidopsis thaliana* using primers, which were synthesized based on nucleotide sequences of AC003114 and AB005245 of *Arabidopsis thaliana*. The thus obtained DNA fragments were sequenced by cycle sequencing kit (Shimadzu Bunko Co.) using Shimadzu DNA sequencer DSQ-1000L.

The determined nucleotide sequence is shown in SEQ ID NO: 2.

13

Example 6

Expression of NAS1 Protein in *E. coli*

A fragment, in which EcoRI site was introduced into the upstream of the first ATG of the HvNAS1 cDNA and PstI and BamHI sites were introduced into the downstream of the stop codon of the HvNAS1 cDNA, was amplified by PCR. The thus obtained amplified product was subcloned in the pBluescriptII SK— using EcoRI site and BamHI site, and the correct nucleotide sequence was confirmed. The fragment between EcoRI site and PstI site was cloned into pMAL-c2 to make expression in the form of fusing the HvNAS1 to the C-terminal of maltose binding protein.

A fragment, in which EcoRI site was introduced into the upstream of the first ATG of the OsNAS1 and HindIII site was introduced into the downstream of the stop codon of the OsNAS1, was amplified by PCR. The thus obtained amplified product was subcloned in the pBluescriptII SK— using EcoRI site and HindIII site, and the correct nucleotide sequence was confirmed. The fragment between EcoRI site and HindIII site was cloned into pMAL-c2 to make expression in the form of fusing the OsNAS1 to the C-terminal of maltose binding protein.

A fragment, in which EcoRI site was introduced into the upstream of the first ATG of the AtNAS1, AtNAS2 and AtNAS3 and XbaI site was introduced into the downstream of the stop codon of the AtNAS1, AtNAS2 and AtNAS3, was amplified by PCR. The thus obtained amplified products were subcloned in the pBluescriptII SK—, and the correct nucleotide sequences were confirmed. The fragment between EcoRI site and XbaI site was cloned into pMAL-c2 to make expression in the form of fusing the AtNAS1, AtNAS2 and AtNAS3 to the C-terminal of maltose binding proteins, respectively.

E. coli strain XL1-Blue was used as a host for expressing the said fused protein. pMAL-c2-HvNAS1 and pMAL-c2, respectively, were introduced into XL1-Blue. The thus obtained recombinant bacteria were cultured in LB medium containing ampicillin and tetracycline, each 50 µg/ml, at 37° C. until the OD 600 of the culture reached 0.5. Isopropyl 62-D-thiogalactopyranoside (IPTG) was added to the final concentration of 0.3 mM, and continuously cultured at 37° C. for 3 hours, and collected bacterial cells. Cells were suspended in 10 mM Tris buffer containing 0.2 M NaCl, 1 mM EDTA, 3 mM DTT and 0.1 mM E-64, pH 7.4 and frozen with liquid nitrogen. This was melted in ice water and ultrasonication for 15 seconds was repeated for 10 times. Nicotianamine synthase activity of the thus obtained crude extract was assayed according to the method described in example 2 and the enzyme activity was confirmed.

Example 7

Northern Hybridization

Northern hybridization of barley RNA was performed using DNA fragment, which was prepared by cutting HvNAS1 cDNA with HindIII and NotI and labeled with [α -³²P] dATP, as a probe. Total RNA was extracted from barley (Naito et al. 1988). The extracted RNA was separated by 1.4% agarose gel electrophoresis, and blotted onto Hybond-N⁺ membranes (Amersham). Northern hybridization of rice RNA was performed using OsNAS1 ORF, which was labeled with [α -³²P]dATP, as a probe. Total RNA was extracted from rice. The extracted RNA was separated by 1.4% agarose gel electrophoresis, and blotted onto Hybond-

14

N⁺ membranes (Amersham). The membrane was hybridized with the probe in 0.5 M Church phosphate buffer (Church and Gilbert 1984), 1 mM EDTA, 7% (w/v) SDS with 100 µg/ml salmon sperm DNA at 65° C. for overnight. The membrane was washed with buffer containing 40 mM Church phosphate buffer and 1% (w/v) SDS at 65° C. for 10 minutes. After the washing was repeated once again, the membrane was washed with buffer containing 0.2×SSPE and 0.1% (w/v) SDS at 65° C. for 10 minutes. Radioactivity was detected using the image analyzer BAS-2000.

Results are shown in FIG. 9 and FIG. 16.

Example 8

Southern Hybridization

Genomic DNA was extracted from leaves of barley and rice. The extract was digested with BamHI, EcoRI or HindIII, separated on a 0.8% (w/v) agarose gel electrophoresis, and transferred onto Hybond-N⁺ membranes (Amersham). The hybridization was performed according to the method described in example 7 and radioactivity was detected.

Result is shown in FIG. 10.

Example 9

Preparation of Polyclonal Antibody

Total protein was extracted using trichloroacetic acid and acetone (Damerval et al. 1986). The plants were crashed in the liquid nitrogen until powder was obtained, and mixed with acetone containing 0.1% (v/v) 2-mercaptoethanol. The protein was precipitated by allowing to stand at -20° C. for 1 hour, and the precipitate was collected by centrifugation at 16,000×g for 30 minutes. The precipitate was suspended in acetone containing 0.1% (v/v) 2-mercaptoethanol and allowed to stand at -20° C. for 1 hour, then collected the precipitate by centrifugation at 16,000×g for 30 minutes. The precipitate was dried in vacuo, and dissolved in the sample buffer [9.5 M urea, 2% (w/v) Triton X-100 and 5% (v/v) 2-ME], then centrifuged at 16,000×g for 10 minutes to obtain the supernatant. The proteins contained in the supernatant were separated by SDS-PAGE or the denaturing two-dimensional electrophoresis (O'Farrell 1975) and transferred onto PVDF membrane. Western blotting analysis was performed by applying the primary antibody containing anti-nicotianamine synthase antibody prepared in example 1 and the secondary antibody containing horse radish binding anti-mouse IgG (H+L) goat antibody (Wako Pure Chemicals Co.) on the membrane and coloring with diaminobenzidine.

Result is shown in FIG. 12. SDS-PAGE was performed using 12.5% acrylamide slab gel. Protein 100 µg was electrophoresed. Proteins of roots 200 µg and leaves 500 µg were electrophoresed.

Example 11

RT-PCR

Total RNA was extracted from *Arabidopsis thaliana*. RT-PCR was performed with 1 µg RNA as a template by using the EZ rTth RNA PCR kit (Parkin Elmer Inc.). Specific primers for AtNAS1, AtNAS2 and AtNAS3, respectively, were used. Result is shown in FIG. 18.

INDUSTRIAL APPLICABILITY

Various cells are transformed according to the conventional method by using recombinant vectors of the present

invention. Mass production of nicotianamine can be performed by using the obtained transformant. These methods can be performed according to the method known in the person skilled in the art.

Selective breeding of plants, preferably graminaceous plants can also be performed using genes of the present invention. Especially, genes of the present invention can be applied for improving varieties, which can grow on Fe-deficient soil.

 SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 37

<210> SEQ ID NO 1

<211> LENGTH: 328

<212> TYPE: PRT

<213> ORGANISM: *Hordeum vulgare*

<400> SEQUENCE: 1

```

Met Asp Ala Gln Asn Lys Glu Val Ala Ala Leu Ile Glu Lys Ile Ala
1          5          10          15

Gly Ile Gln Ala Ala Ile Ala Glu Leu Pro Ser Leu Ser Pro Ser Pro
20          25          30

Glu Val Asp Arg Leu Phe Thr Asp Leu Val Thr Ala Cys Val Pro Pro
35          40          45

Ser Pro Val Asp Val Thr Lys Leu Ser Pro Glu His Gln Arg Met Arg
50          55          60

Glu Ala Leu Ile Arg Leu Cys Ser Ala Ala Glu Gly Lys Leu Glu Ala
65          70          75          80

His Tyr Ala Asp Leu Leu Ala Thr Phe Asp Asn Pro Leu Asp His Leu
85          90          95

Gly Leu Phe Pro Tyr Tyr Ser Asn Tyr Val Asn Leu Ser Arg Leu Glu
100         105         110

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

Ala Phe Val Gly Ser Gly Pro Leu Pro Phe Ser Ser Leu Val Leu Ala
130         135         140

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

Ala Ala Asn Glu Arg Ala Arg Lys Leu Phe Gly Ala Thr Ala Asp Gly
165         170         175

Val Gly Ala Arg Met Ser Phe His Thr Ala Asp Val Ala Asp Leu Thr
180         185         190

Gln Glu Leu Gly Ala Tyr Asp Val Val Phe Leu Ala Ala Leu Val Gly
195         200         205

Met Ala Ala Glu Glu Lys Ala Lys Val Ile Ala His Leu Gly Ala His
210         215         220

Met Val Glu Gly Ala Ser Leu Val Val Arg Ser Ala Arg Pro Arg Gly
225         230         235         240

Phe Leu Tyr Pro Ile Val Asp Pro Glu Asp Ile Arg Arg Gly Gly Phe
245         250         255

Glu Val Leu Ala Val His His Pro Glu Gly Glu Val Ile Asn Ser Val
260         265         270

Ile Val Ala Arg Lys Ala Val Glu Ala Gln Leu Ser Gly Pro Gln Asn
275         280         285

Gly Asp Ala His Ala Arg Gly Ala Val Pro Leu Val Ser Pro Pro Cys
290         295         300

Asn Phe Ser Thr Lys Met Glu Ala Ser Ala Leu Glu Lys Ser Glu Glu
305         310         315         320

Leu Thr Ala Lys Glu Leu Ala Phe

```

-continued

325

```

<210> SEQ ID NO 2
<211> LENGTH: 1295
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (55)..(1041)

<400> SEQUENCE: 2

gcggttcagag gcttcacagag ttcttcggt caccaagaag catttgatca taac atg      57
Met
1

gat gcc cag aac aag gag gtc gct gct ctg atc gag aag atc gcc ggt      105
Asp Ala Gln Asn Lys Glu Val Ala Ala Leu Ile Glu Lys Ile Ala Gly
5          10          15

atc cag gcc gcc atc gcc gag ctg ccg tcg ctg agc ccg tcc ccc gag      153
Ile Gln Ala Ala Ile Ala Glu Leu Pro Ser Leu Ser Pro Ser Pro Glu
20          25          30

gtc gac agg ctc ttc acc gac ctc gtc acg gcc tgc gtc ccg ccg agc      201
Val Asp Arg Leu Phe Thr Asp Leu Val Thr Ala Cys Val Pro Pro Ser
35          40          45

ccc gtc gac gtg acg aag ctc agc ccg gag cac cag agg atg cgg gag      249
Pro Val Asp Val Thr Lys Leu Ser Pro Glu His Gln Arg Met Arg Glu
50          55          60          65

gct ctc atc cgc ttg tgc tcc gcc gcc gag ggg aag ctc gag gcg cac      297
Ala Leu Ile Arg Leu Cys Ser Ala Ala Glu Gly Lys Leu Glu Ala His
70          75          80

tac gcc gac ctg ctc gcc acc ttc gac aac ccg ctc gac cac ctc ggc      345
Tyr Ala Asp Leu Leu Ala Thr Phe Asp Asn Pro Leu Asp His Leu Gly
85          90          95

ctc ttc ccg tac tac agc aac tac gtc aac ctc agc agg ctg gag tac      393
Leu Phe Pro Tyr Tyr Ser Asn Tyr Val Asn Leu Ser Arg Leu Glu Tyr
100         105         110

gag ctc ctg gcg cgc cac gtg ccg ggc atc gcg ccg gcg cgc gtc gcc      441
Glu Leu Leu Ala Arg His Val Pro Gly Ile Ala Pro Ala Arg Val Ala
115         120         125

ttc gtc ggc tcc gcc ccg ctg ccg ttc agc tcg ctc gtc ctc gcc gcg      489
Phe Val Gly Ser Gly Pro Leu Pro Phe Ser Ser Leu Val Leu Ala Ala
130         135         140         145

cac cac ctg ccc gag acc cag ttc gac aac tac gac ctg tgc ggc gcg      537
His His Leu Pro Glu Thr Gln Phe Asp Asn Tyr Asp Leu Cys Gly Ala
150         155         160

gcc aac gag cgc gcc agg aag ctg ttc ggc gcg acg gcg gac ggc gtc      585
Ala Asn Glu Arg Ala Arg Lys Leu Phe Gly Ala Thr Ala Asp Gly Val
165         170         175

ggc gcg cgt atg tcg ttc cac acg gcg gac gtc gcc gac ctc acc cag      633
Gly Ala Arg Met Ser Phe His Thr Ala Asp Val Ala Asp Leu Thr Gln
180         185         190

gag ctc ggc gcc tac gac gtg gtc ttc ctc gcc gcg ctc gtc ggc atg      681
Glu Leu Gly Ala Tyr Asp Val Val Phe Leu Ala Ala Leu Val Gly Met
195         200         205

gca gcc gag gag aag gcc aag gtg att gcc cac ctg ggc gcg cac atg      729
Ala Ala Glu Glu Lys Ala Lys Val Ile Ala His Leu Gly Ala His Met
210         215         220         225

gtg gag ggg gcg tcc ctg gtc gtg cgg agc gca cgg ccc cgc ggc ttt      777
Val Glu Gly Ala Ser Leu Val Val Arg Ser Ala Arg Pro Arg Gly Phe
230         235         240

ctt tac ccc att gtc gac ccg gag gac atc agg cgg ggt ggg ttc gag      825
Leu Tyr Pro Ile Val Asp Pro Glu Asp Ile Arg Arg Gly Gly Phe Glu

```


-continued

245	250	255	
gtg ctg gcc gtg cac cac ccg gaa ggt gag gtg atc aac tct gtc atc			873
Val Leu Ala Val His His Pro Glu Gly Glu Val Ile Asn Ser Val Ile			
260	265	270	
gtc gcc cgt aag gcc gtc gaa gcg cag ctc agt ggg ccg cag aac gga			921
Val Ala Arg Lys Ala Val Glu Ala Gln Leu Ser Gly Pro Gln Asn Gly			
275	280	285	
gac gcg cac gca cgg ggc gcg gtg ccg ttg gtc agc ccg cca tgc aac			969
Asp Ala His Ala Arg Gly Ala Val Pro Leu Val Ser Pro Pro Cys Asn			
290	295	300	305
ttc tcc acc aag atg gag gcg agc gcg ctt gag aag agc gag gag ctg			1017
Phe Ser Thr Lys Met Glu Ala Ser Ala Leu Glu Lys Ser Glu Glu Leu			
310	315	320	
acc gcc aaa gag ctg gcc ttt tga ttgaagagtg cgcgtggtca ttctgtcgcc			1071
Thr Ala Lys Glu Leu Ala Phe			
325			
tgcgatcgtg gtaactttcc tactcgtgtg tgttttgatg tttgtgectg taagagttat			1131
gcttccggcc ttgtgctggt aatttacacg cgttacatgt agtacttgta tttatactg			1191
gaataacggt atgtaacata aatattagtg ggatttgaag tgtaatgcta aataataaga			1251
aaacttgatg cagacattca aaaaaaaaaa aaaaaaaaaa aaaa			1295

<210> SEQ ID NO 3

<211> LENGTH: 335

<212> TYPE: PRT

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 3

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

-continued

His Met Ala Asp Gly Ala Ala Leu Val Val Arg Ser Ala His Gly Ala
 225 230 235 240
 Arg Gly Phe Leu Tyr Pro Ile Val Asp Pro Gln Asp Ile Gly Arg Gly
 245 250 255
 Gly Phe Glu Val Leu Ala Val Cys His Pro Asp Asp Asp Val Val Asn
 260 265 270
 Ser Val Ile Ile Ala Gln Lys Ser Lys Asp Val His Ala Asp Gly Leu
 275 280 285
 Gly Ser Gly Arg Gly Ala Gly Gly Gln Tyr Ala Arg Gly Thr Val Pro
 290 295 300
 Val Val Ser Pro Pro Cys Arg Phe Gly Glu Met Val Ala Asp Val Thr
 305 310 315 320
 Gln Asn His Lys Arg Asp Glu Phe Ala Asn Ala Glu Val Ala Phe
 325 330 335

<210> SEQ ID NO 4

<211> LENGTH: 1342

<212> TYPE: DNA

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 4

```

ctcctgtgcc tgtcctgagg taccaagaac accagtgaat tggctgcccc gaacaaccag    60
gaggtggatg ccctgggtga gaagatcacc gggctccatg cgcgaatcgc caagctgccg    120
tgctcagcc catccccga cgtcgacgcg ctcttcacgg agctgggtcac ggcgtgcggt    180
ccaccgagtc cagtggacgt gaccaagctc gggccggagg cgcaggagat gcgggagggc    240
ctcatccgcc tatgtccga ggcgagggg aagctggagg cgcactactc cgacatgctc    300
gcgcctctcg acaagccgct ggatcacctc ggcattgtcc cctactacaa caactacatc    360
aacctcagca agctcgagta cgagctctcg gcccgctacg tgctggcggg ctatcgcccc    420
gcgcgcgctg cgttcacggt ctccggcccg ctgcccgtca gctcctttgt cctggccgcg    480
cgccacctgc cgcacacat gttcgacaac tatgacctgt gcggtgcggc caacgatcgc    540
gccagcaagc tcttcgcgcg ggatcgcgac gtgggtgccc gcattgctgt ccacacggcc    600
gacgtcgcgg acctcgccgg cgagctcgcc aagtaacgac ttgtcttctt ggccgcactc    660
gtcggcatgg ccgcccagga caaggcgaag gtgatcgcgc acctcgccgc acacatggca    720
gacggggcgg ccctcgtcgt gcgcagcgca cacggagcgc gggggttctt gtaccgatc    780
gtcgaccccc aggacatcgg ccgagccggg ttcgaggtgc tggccgtgtg ccatccccac    840
gacgacgtgg tgaactccgt catcatcgca cagaagtcca aggacgtgca tgcgatgga    900
cttggcagcg ggcgtggtgc cgggtgacag tacgcgcccg gcacgggtgcc tgttgtcagc    960
ccccctgca ggttcggcga gatggtggcg gacgtgaccc agaaccacaa gagagacgag   1020
tttgccaaag ccgaagtggc cttttgatcg ttcgctgcga ggggtgtgcat ccatgatcca   1080
tccatacctc gttctgtgat tgcacaaagc ttgcaatcgt atgcatttca agtcacgtgt   1140
tgcttctatc caataatgta cgtgtggtgt ttacacgcga atgtcttcta gacctttgta   1200
tgtgtacaag tgaattttaa ttcacaagta catataatgg tcaccattga aaagatgttt   1260
agtgtgtgtt ttccaatata tgtttgtgta aggttcatca tctaataaaa tatgtttgga   1320
acccaaaaaa aaaaaaaaaa aa                                           1342

```

<210> SEQ ID NO 5

<211> LENGTH: 335

-continued

```

<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 5

Met Ala Ala Gln Asn Asn Asn Lys Asp Val Ala Ala Leu Val Glu Lys
1          5          10          15

Ile Thr Gly Leu His Ala Ala Ile Ala Lys Leu Pro Ser Leu Ser Pro
20        25        30

Ser Pro Asp Val Asp Ala Leu Phe Thr Glu Leu Val Thr Ala Cys Val
35        40        45

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

Met Arg Glu Gly Leu Ile Arg Leu Cys Ser Glu Ala Glu Gly Lys Leu
65        70        75        80

Glu Ala His Tyr Ser Asp Met Leu Ala Ala Phe Asp Asn Pro Leu Asp
85        90        95

His Leu Gly Ile Phe Pro Tyr Tyr Ser Asn Tyr Ile Asn Leu Ser Lys
100       105       110

Leu Glu Tyr Glu Leu Leu Ala Arg Tyr Val Arg Arg His Arg Pro Ala
115       120       125

Arg Val Ala Phe Ile Gly Ser Gly Pro Leu Pro Phe Ser Ser Phe Val
130       135       140

Leu Ala Ala Arg His Leu Pro Asp Thr Met Phe Asp Asn Tyr Asp Leu
145       150       155       160

Cys Gly Ala Ala Asn Asp Arg Ala Ser Lys Leu Phe Arg Ala Asp Thr
165       170       175

Asp Val Gly Ala Arg Met Ser Phe His Thr Ala Asp Val Ala Asp Leu
180       185       190

Ala Ser Glu Leu Ala Lys Tyr Asp Val Val Phe Leu Ala Ala Leu Val
195       200       205

Gly Met Ala Ala Glu Asp Lys Ala Lys Val Ile Ala His Leu Gly Ala
210       215       220

His Met Ala Asp Gly Ala Ala Leu Val Val Arg Ser Ala His Gly Ala
225       230       235       240

Arg Gly Phe Leu Tyr Pro Ile Val Asp Pro Gln Asp Ile Gly Arg Gly
245       250       255

Gly Phe Glu Val Leu Ala Val Cys His Pro Asp Asp Asp Val Val Asn
260       265       270

Ser Val Ile Ile Ala Gln Lys Ser Lys Glu Val His Ala Asp Gly Leu
275       280       285

Gly Ser Ala Arg Gly Ala Gly Arg Gln Tyr Ala Arg Gly Thr Val Pro
290       295       300

Val Val Ser Pro Pro Cys Arg Phe Gly Glu Met Val Ala Asp Val Thr
305       310       315       320

Gln Asn His Lys Arg Asp Glu Phe Ala Asn Ala Glu Val Ala Phe
325       330       335

```

```

<210> SEQ ID NO 6
<211> LENGTH: 1314
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare

```

```

<400> SEQUENCE: 6

```

```

ctacttcact cacactagtg cccagaaaga aggctgcaat ggctgcccag aacaacaaca    60
aggatgtcgc tgccctggtg gagaagatca ccgggtcca cgccgccatc gccaaagtgc    120

```

- continued

```

cgctcgctcag cccatccccg gacgtcgacg cgctcttcac cgagctggtc acggcgtgcg 180
ttcccccgag ccccggtggac gtgaccaagc tcggccccga ggcgcaggag atgcggggagg 240
gcctcaatccg cctctgctcc gaggccgagg ggaagctgga ggcgcactac tccgacatgc 300
tcgcccgcctt cgacaacccg ctggatcacc tcggcatctt cccctactac agcaactaca 360
tcaacctcag caagctggag tacgagctcc tggcacgcta cgtccggcgg catcgcccgg 420
cccgcgtcgc gttcatcggc tccggcccgc tgccgttcag ctccctttgtc ctggcccgcg 480
gccacctgcc cgacacatg tttgacaact acgacctttg cggcgcggcc aacgatcgcg 540
ccagcaagct cttccgcgcg gacacggacg tgggtgcccg catgtcgttc cacacggccg 600
acgtcgcgga cctcgcacg gagctcgcca agtacgacgt cgtcttctcg gccgcgctcg 660
tcggcatggc cgccgaggac aaggccaagg tgatcgcgca cctcggcgca cacatggcag 720
acggggggcg cctcgtcgtg cgcagcgcac acggagcgcg cgggttctcg tacccgattg 780
tcgacccccg ggacatcggc cgcggcgggt tcgaggtgct ggccgtgtgc cacccegcag 840
acgacgtggt gaactccgtc atcatcgcac agaagtcaa ggaggtgcat gccgatggac 900
ttggcagcgc gcgtggtgcc ggtcgacagt acgcgcgcgg cacggtgccg gttgtcagcc 960
ccccgtgcag gttcggtgag atggtggcgg atgtgaccca gaaccacaag agagacgagt 1020
ttgccaacgc cgaagtggcc ttttgatcga tcgtcgccaa gggacaataa atgaactggtg 1080
atgtggtagg gtaatttgc tacctcgtg cttgatcgct tgcaatatgt gcacattttc 1140
ctactaccgc tgcttatgca tttcaagcca tgtgatgtg gtatccaata aagtatgtgt 1200
agggtttaca cgaaatgtc tttacacctt gtacgtgtaa gtgttgacaa cgatgaattt 1260
cagttcacia ttaataaata gtataatgga ttcaaaaaaa aaaaaaaaaa aaaa 1314

```

<210> SEQ ID NO 7

<211> LENGTH: 329

<212> TYPE: PRT

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 7

```

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

```

-continued

Gly Ala Ala Asn Asp Arg Ala Thr Arg Leu Phe Arg Ala Asp Lys Asp
 165 170 175

Val Gly Ala Arg Met Ser Phe His Thr Ala Asp Val Ala Asp Leu Thr
 180 185 190

Asp Glu Leu Ala Thr Tyr Asp Val Val Phe Leu Ala Ala Leu Val Gly
 195 200 205

Met Ala Ala Glu Asp Lys Ala Lys Val Ile Ala His Leu Gly Ala His
 210 215 220

Met Ala Asp Gly Ala Ala Leu Val Ala Arg His Gly Ala Arg Gly Phe
 225 230 235 240

Leu Tyr Pro Ile Val Asp Pro Gln Asp Ile Gly Arg Gly Gly Phe Glu
 245 250 255

Val Leu Ala Val Cys His Pro Asp Asp Asp Val Val Asn Ser Val Ile
 260 265 270

Ile Ala Gln Lys Ser Asn Asp Val His Glu Tyr Gly Leu Gly Ser Gly
 275 280 285

Arg Gly Gly Arg Tyr Ala Arg Gly Thr Val Val Pro Val Val Ser Pro
 290 295 300

Pro Cys Arg Phe Gly Glu Met Val Ala Asp Val Thr Gln Lys Arg Glu
 305 310 315 320

Glu Phe Ala Asn Ala Glu Val Ala Phe
 325

<210> SEQ ID NO 8

<211> LENGTH: 1249

<212> TYPE: DNA

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 8

```

ccactaccga ctaccgtagt accgtgctc agagctcatc actggtcagg taccaagaag    60
acataaaaat ggacggccag agcgaggagg tcgacgcctt tgtccagaag atcaccggcc    120
tccacgccgc catcgccaag ctgccctcgc tcagcccgtc cccggacgtc gacgcgctct    180
tcaccgacct ggtcaccgcy tgcgtgcccc cgagccccgt ggacgtgacc aagctcgccc    240
cggaggcgca ggcgatgcyg gagggcctca tccgcctctg ctccgaggcc gagggcaagc    300
tggaggcgca ctactccgac atgctcgccg ccttcgacaa cccgctcgac cacctcggcg    360
tcttccccta ctacagcaac tacatcaacc tcagcaagct tgagtacgag ctctcgcgcc    420
gctacgtgcc eggaggeat cgcgccgccc gcgtgcctt catcggtccc ggcgccgtgc    480
cgttcagctc ctacgtctc gccgcgccc acctgcccga caccgtgttc gacaactacg    540
acctgtgcyg cgcggccaac gaccgcgcyg ccaggctgtt ccgcgcggac aaggacgtcg    600
gcccgcgat gtcgttccac accgccgacg tcgcygacct caccgacgag ctcgctacgt    660
acgacgtcgt cttcctggcc gcgctcgtgg gcatggcccg cgaggacaag gccaaagtga    720
tcgcgcacct tggcgcgcac atggcggacg gggcgcccct cgttgccgcyg cacggcgcyg    780
gtgggttctt ctacccgatc gtcgatcccc aggacatcgg tcgaggcggg ttcgagggtgc    840
tcgcccgtgtg tcaccccgac gacgacgtgg tgaactccgt catcatcgca caaaagagca    900
acgacgtgca cgagtatgga cttggcagcy ggcgtggtgg acggtacgcy cgaggcacgy    960
tggtgccggt ggtagccca cctgcaggt tcggcgagat ggtggcagac gtgaccacga    1020
agagagagga gtttcccaac gcggaagtgg ccttctgatt gctgctgaat cgcttctgat    1080
cgtacgtggt aatcttctta ctactctctc tctaccacc acctatcacc tatgtatgca    1140

```

-continued

```

tttcaagtcg tgtgttggtt gttccaata atgtaagtga gatggtttaca cgcgcaaaaa 1200
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1249

```

```

<210> SEQ ID NO 9
<211> LENGTH: 282
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

```

```

<400> SEQUENCE: 9

```

```

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

```

```

<210> SEQ ID NO 10
<211> LENGTH: 1044
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare

```

```

<400> SEQUENCE: 10

```

```

gtgacatgga ggccgaaaac ggcgaggtgg ctgctctggt cgagaagatc accggtctcc 60
acgccgccat ctccaagtc cccgactaa gcccgctctcc tcaagtcgac gcgctcttca 120
ccgagctggt tgcggcgtgc gtcccatcaa gcccggtgga cgtgaccaag ctcggcccg 180

```

-continued

```

aggcgcagga gatgcggcag gacctcatcc gtctctgctc ggccgcccag gggctgctcg 240
aggcgcacta ctccgacatg ctcaccgctg tggacagccc gctcgaccac ctggcccgtc 300
tcccttactt cgacaactac gtcaacctca gcaagctcga gcacgatctt ctggcaggtc 360
acgtggcggc cccggcccgc gtggcggttc tgggctcggg gccactgccc ttcagctcgc 420
tcttccttgc gacgtaccac ctgccggaca cccggttcga caactacgac cgggtgcagcg 480
tggcgaatgg ccggggcagc aagctggctg gcgcggcggc cgagggcgtg cgatcacgca 540
tggcggtcca cacggcccga gtcacggacc tcacggctga gctcggcgct tacgacgtgg 600
tcttcctggc cgcgctcgtg ggaatgacgt ccaaggagaa ggccgacgcc atagcgcact 660
tggggaagca catggcagat gggggcgtgc tcgtgcgcga agcgcctgcac ggggcgcgag 720
cgttcctgta tcctgtcgtg gagctggacg atgtcggcgg tggtgggttc caagtgtcgg 780
ccgtgcacca cctcgcaggc gatgaggtgt tcaactcatt catagttgcc cggaagtgta 840
aaatgagtgc ttaaattaag aaaaggggta gcctgtctgc ttgtgcaaat ggtgtctcac 900
attgataata accagatgat acctgcaca ttgatggggg tactgcagta tgtttcaatg 960
aggtctggtt gtatcaata tgagtatttg gcttaataat atcagcgaat atgtttcgat 1020
taaaaaaaaa aaaaaaaaaa aaaa 1044

```

<210> SEQ ID NO 11

<211> LENGTH: 328

<212> TYPE: PRT

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 11

```

Met Asp Ala Gln Asn Lys Glu Val Asp Ala Leu Val Gln Lys Ile Thr
 1             5             10             15

Gly Leu His Ala Ala Ile Ala Lys Leu Pro Ser Leu Ser Pro Ser Pro
20             25             30

Asp Val Asp Ala Leu Phe Thr Asp Leu Val Thr Ala Cys Val Pro Pro
35             40             45

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

Glu Gly Leu Ile Arg Leu Cys Ser Glu Ala Glu Gly Lys Leu Glu Ala
65             70             75             80

His Tyr Ser Asp Met Leu Ala Ala Phe Asp Asn Pro Leu Asp His Leu
85             90             95

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

Tyr Glu Leu Leu Ala Arg Tyr Val Pro Gly Gly Ile Ala Arg Pro Ala
115            120            125

Val Ala Phe Ile Gly Ser Gly Pro Leu Pro Phe Ser Ser Tyr Val Leu
130            135            140

Ala Ala Arg His Leu Pro Asp Ala Met Phe Asp Asn Tyr Asp Leu Cys
145            150            155            160

Ser Ala Ala Asn Asp Arg Ala Ser Lys Leu Phe Arg Ala Asp Lys Asp
165            170            175

Val Gly Ala Arg Met Ser Phe His Thr Ala Asp Val Ala Asp Leu Thr
180            185            190

Arg Glu Leu Ala Ala Tyr Asp Val Val Phe Leu Ala Ala Leu Val Gly
195            200            205

Met Ala Ala Glu Asp Lys Ala Lys Val Ile Pro His Leu Gly Ala His

```

-continued

210	215	220													
Met	Ala	Asp	Gly	Ala	Ala	Leu	Val	Val	Arg	Ser	Ala	Gln	Ala	Arg	Gly
225				230						235					240
Phe	Leu	Tyr	Pro	Ile	Val	Asp	Pro	Gln	Asp	Ile	Gly	Arg	Gly	Gly	Phe
245				250						255					
Glu	Val	Leu	Ala	Val	Cys	His	Pro	Asp	Asp	Asp	Val	Val	Asn	Ser	Val
260				265						270					
Ile	Ile	Ala	His	Lys	Ser	Lys	Asp	Val	His	Ala	Asn	Glu	Arg	Pro	Asn
275				280						285					
Gly	Arg	Gly	Gly	Gln	Tyr	Arg	Gly	Ala	Val	Pro	Val	Val	Ser	Pro	Pro
290				295						300					
Cys	Arg	Phe	Gly	Glu	Met	Val	Ala	Asp	Val	Thr	His	Lys	Arg	Glu	Glu
305				310						315					320
Phe	Thr	Asn	Ala	Glu	Val	Ala	Phe								
325															

<210> SEQ ID NO 12

<211> LENGTH: 1352

<212> TYPE: DNA

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 12

```

ctccacttcg ctccctgtgc tcaggtagcc acaacataca gtattaaaat ggatgccccag    60
aacaaggagg ttgatgccct ggtccagaag atcaccggcc tccacgccgc catcgccaag    120
ctgcccgtccc tcagcccata acccgacgtc gacgcgctct tcaccgacct ggtcaccgcg    180
tgcgcccccc cgagccccct ggacgtgacc aagctcgggt cggaggcgca ggagatgcgg    240
gagggcctca tccgctctgt ctccgaggcc gaggggaagc tggaggcgca ctactccgac    300
atgctggccg ccttcgacaa cccgctcgac cacctcggca tggttccccta ctacagcaac    360
tacatcaacc tcagcaagct ggagtagcag ctccctgggc gctacgtgcc gggcgccatc    420
gcccggcccc ctgtcgcggt catcggtccc gggccgctgc cgttcagctc ctacgtctc    480
gcccgtcgcc acctgcccga cgccatgttc gacaactacg acctgtgtag cgcggccaac    540
gaccgtgcga gcaagctgtt ccgcgcggac aaggacgtgg gcgcccgcac gtctttccac    600
accgcccagc tagcggacct caccgcgag ctgcgcgctg acgaogctgt ctctctggcc    660
gcgctcgtgg gcatggctgc cgaggacaag gccaaagtga ttccgcacct cggcgcgcac    720
atggcggaag gggcgccct cgtcgtgccc agtgccgagg caegtggggt cctctacccc    780
atcgtcgatc cccaggacat cggctcaggg gggtttgagg tgetggccgt gtgtcacccc    840
gacgatgacg tggatgaact cgtcatcacc gcacacaagt ccaaggacct gcatgccaat    900
gaacgtccca acggcgctgg tggacagtac cggggcgcgg taccgggtgt cagcccgcgg    960
tgcaggttcg gtgagatggt ggcggacgtg acccacaaga gagaggagtt caccaacgcg    1020
gaagtggcct tctgatcgtt gcgaggggat gaaaatgaag gtggacgtgt gtggtcagca    1080
tccatcagtg gctgctgct tcacgcttg caatcgtact actacctacc tatgcagttc    1140
aagtcatgtg ttgtcaatgt aagtgtgatg ttacactag tctatgaaag gcagggcaga    1200
cgagggtagt gtgccaagta acagtgtgtc attataggtg taagtgttga gaataagacc    1260
atTTTTgttc acaaatagta tgatgtaate ggtgtcatat tcgtattgag tacatttgtc    1320
aagttggttg ctaaaaaaaaa aaaaaaaaaa aa                                1352

```

<210> SEQ ID NO 13

-continued

```

<211> LENGTH: 329
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 13

Met Asp Ala Gln Ser Lys Glu Val Asp Ala Leu Val Gln Lys Ile Thr
 1           5           10           15

Gly Leu His Ala Ala Ile Ala Lys Leu Pro Ser Leu Ser Pro Ser Pro
20           25           30

Asp Val Asp Ala Leu Phe Thr Asp Leu Val Thr Ala Cys Val Pro Pro
35           40           45

Ser Pro Val Asp Val Thr Lys Leu Ala Pro Glu Ala Gln Ala Met Arg
50           55           60

Glu Gly Leu Ile Arg Leu Cys Ser Glu Ala Glu Gly Lys Leu Glu Ala
65           70           75           80

His Tyr Ser Asp Met Leu Ala Ala Phe Asp Asn Pro Leu Asp His Leu
85           90           95

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

Tyr Glu Leu Leu Ala Arg Tyr Val Pro Gly Gly Ile Ala Pro Ala Arg
115          120          125

Val Ala Phe Ile Gly Ser Gly Pro Leu Pro Phe Ser Ser Tyr Val Leu
130          135          140

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

Arg Ala Ala Asn Asp Arg Ala Thr Arg Leu Phe Arg Ala Asp Lys Asp
165          170          175

Val Gly Ala Arg Met Ser Phe His Thr Ala Asp Val Ala Asp Leu Thr
180          185          190

Asp Glu Leu Ala Thr Tyr Asp Val Val Phe Leu Ala Ala Leu Val Gly
195          200          205

Met Ala Ala Glu Asp Lys Gly Gln Gly Asp Pro His Leu Gly Ala His
210          215          220

Met Ala Asp Gly Ala Ala Leu Val Arg Ser Ala His Gly Ala Arg Gly
225          230          235          240

Phe Leu Tyr Pro Ile Val Asp Pro Gln Asp Ile Gly Arg Gly Gly Phe
245          250          255

Glu Val Leu Ala Val Cys His Pro Asp Asp Asp Val Val Asn Ser Val
260          265          270

Ile Ile Ala Gln Lys Ser Lys Asp Met Phe Ala Asn Gly Pro Arg Asn
275          280          285

Gly Cys Gly Gly Arg Tyr Ala Arg Gly Thr Val Pro Val Val Ser Pro
290          295          300

Pro Cys Arg Phe Gly Glu Met Val Ala Asp Val Thr Gln Lys Arg Glu
305          310          315          320

Glu Phe Ala Lys Ala Glu Val Ala Phe
325

```

```

<210> SEQ ID NO 14
<211> LENGTH: 1371
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (8)
<223> OTHER INFORMATION: a, c, g, t, unknown or other

```

-continued

<400> SEQUENCE: 14

```

ggagcggnac gcgtggcgga ggtgggcact accgtagtac cgtgcctcag agctcatcac    60
tggtcaggta ccaagaagac ataaaaatgg acgcccagag caaggaggtc gacgccccttg    120
tccagaagat caccggcctc cacgccgcca tcgccaagct gccctcgctc agcccgtccc    180
cggacgtcga cgcgctcttc accgacctgg tcaccgctgt cgtgcccccg agcccctgtg    240
acgtgaccaa gctcgccccg gaggcgcagg cgatgcggga gggcctcacc cgctctgct    300
ccgaggccga gggcaagctg gaggcgcaact actccgacat gctcgccgcc ttcgacaacc    360
cgctcgacca cctcgcgctc tccccctact acagcaacta catcaacctc agcaagctcg    420
agtaagagct cctcgcgctc taegtgcctg gggcctcgc cccggcccgc gtgccttca    480
tcggctccgg cccgctcccg ttcagctcct acgtcctcgc cgcgcccacc ctgcccgaca    540
ccgtgttcga caactacgta cctgtgcgcg cggccaacga ccgcgcgacc aggtgttcc    600
gcgcggaaca ggacgtcggc gcccgcatgt cgttccacac cgccgacgtc ggggacctca    660
ccgacgagct cgctacgtac gacgtcgtct tcctggccgc gctcgtgggc atggccgccc    720
aggacaaggg ccaaggtgat ccgcaacctg gcgcgcacat ggcggacggg gcggccctcg    780
tccgagcgcg gcacggggcg cgtgggttcc tctaccgat cgtegatccc caagacattg    840
gtcgagggcg gttcgaggtg ctcgccgtgt gtcaccccga cgacgacgtg gtgaactccg    900
tcatcatcgc gcagaagtct aaggacatgt ttgccaatgg acctcgcaac ggggtgtggtg    960
gacggtacgc gcgaggcacg gtgccggtgg tcagcccgcc ctgcaggttc ggcgagatgg    1020
tggcagacgt gaccagaag agagaggagt ttgccaaggc ggaagtggcc ttctgattgc    1080
tgcgaggtea ccctccgtat gccgctgcta cctttcaata tcttgcaatc gtaggtggcg    1140
atcttccctac tcttgttacg acctttcaaa tcatatgttg tttgtacca ataataatgtaag    1200
tgtgttgctt acacgcgcat gtctgtgaca ctcggtctct agaaggcagg gcagatcaag    1260
agactgtgca aaggaaaaga aatgtgtgtt gttgtagggt tatgagttgg gagtaagatg    1320
attctagttc acaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a          1371

```

<210> SEQ ID NO 15

<211> LENGTH: 324

<212> TYPE: PRT

<213> ORGANISM: *Oryza sativa*

<400> SEQUENCE: 15

```

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

```

-continued

Ser Gly Pro Leu Pro Phe Ser Ser Leu Val Leu Ala Ala His His Leu
130 135 140

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

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

Met Ala Phe His Thr Ala Asp Val Ala Thr Leu Thr Gly Glu Leu Gly
180 185 190

Ala Tyr Asp Val Val Phe Leu Ala Ala Leu Val Gly Met Ala Ala Glu
195 200 205

Glu Lys Ala Gly Val Ala His Leu Gly Ala His Met Ala Asp Gly Ala
210 215 220

Ala Leu Val Val Arg Thr Ala His Gly Ala Arg Gly Phe Leu Tyr Pro
225 230 235 240

Val Asp Pro Glu Asp Val Arg Arg Gly Gly Phe Asp Val Leu Ala Val
245 250 255

Cys His Pro Glu Asp Glu Val Asn Ser Val Val Ala Arg Lys Val Gly
260 265 270

Ala Ala Ala Ala Ala Ala Ala Ala Ala Arg Arg Asp Glu Leu Ala Asp Ser
275 280 285

Arg Gly Val Val Leu Pro Val Val Gly Pro Pro Ser Thr Cys Cys Lys
290 295 300

Val Glu Ala Ser Ala Val Glu Lys Ala Glu Glu Phe Ala Ala Asn Lys
305 310 315 320

Glu Leu Ser Val

<210> SEQ ID NO 16
<211> LENGTH: 1372
<212> TYPE: DNA
<213> ORGANISM: *Oryza sativa*

<400> SEQUENCE: 16

ctccatttgg ttgtcatttt caactataat ccaccacaac tcgtgcaaca tcagctcact 60
cgtgttccca accgcgacaa agcttcacag atggaggctc agaaccaaga ggctcgtgcc 120
ctggtcgaga agatgcgccg cctccacgcc gccatctcca agctgcccgc gctgagccca 180
tccgcccagg tggaocgcct cttcacccac ctgcgcacgg cgtgcgtccc ggcgagcccc 240
gtcgacgtgg ccaagctcgg cccggaggcg caggcgtatg gggaggagct catcccctc 300
tgctccgccg ccgagggcca cctcgaggcg cactacgccg acatgctcgc cgccttcgac 360
aaccgcctcg accacctcgc ccgcttcccg tactacggca actacgtcaa cctgagcaag 420
ctggagtaag acctcctcgt ccgctacgtc cccggcattg cccccaccg cgtcgccttc 480
gtcgggtcgg gcccgctgcc gttcagctcc ctctgctcgc ctgcgcacca cctgcccggac 540
gcggtgttcg acaactacga ccgggtcggc gcggccaacg agcgggcgag gaggtgttc 600
cgcggcgcgg acgagggcct cggcgcgcgc atggcgcttc acaccgcga cgtggcgacc 660
ctgacggggg agctcggcgc gtaacagctc gtgttcctgg cggcgtcgt gggcatggcg 720
gccgaggaga aggcgggggt gatcgcgcac ctgggcgcgc acatggcgga cggcgcggcg 780
ctcgtcgtgc ggacggcgca cggggcgcgc gggttcctgt acccgatcgt cgatcccag 840
gacgtcaggc gtggcgggtt cgacgttctg gcggtgtgcc acccgaggga cgaggtgatc 900
aactccgtca tcgtgcgccg caaggtcggg gccgcgcgcg ccgcgcgcgc ggcgcgcaga 960

-continued

```

gacgagctcg cggactcgcg cggcgtgggt ctgccggtgg tggggccgccc gtcacagtgc 1020
tgcaaggtgg aggcgagcgc ggttgagaag gcagaagagt ttgccgcca caaggagctg 1080
tccgtctaac agccggacga tcgaaaggcg cactatatta tggcaataaa tcatttgatt 1140
atacttatgc tgcatttgcg aagctaaggt atactatgca agccatatgt ttgtgttctg 1200
acgtgttgtt tgggacgtac agttgtgttg ttgtacgtcg tgaagtactg aagtgttcac 1260
agtagatcac aagttcacag caatcaatga ggaccctgta agccagtgta aacgaggaac 1320
atgccatctg tgtatgacag tgagaaatta tataagaaaa acattttgtg ac 1372

```

```

<210> SEQ ID NO 17
<211> LENGTH: 320
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

```

```

<400> SEQUENCE: 17

```

```

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

```

-continued

Asn Asn Arg Gly Lys Lys Asn Met Ile Glu Glu Phe Ser Thr Ile Glu
305 310 315 320

<210> SEQ ID NO 18
<211> LENGTH: 963
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 18

```

atggcttgcc aaaacaatct cgttgtgaag caaatcatcg acttgtagca ccaaattcca    60
aagctcaaga gcttaaaacc ttccaaaat gtcgacactt tggtcggaca actcgtgtcc    120
acgtgcttac ccacggatag aaacatcgat gtcacaaata tgtgtgaaga agtcaaagac    180
atgagagcta atctcatcaa gctttgtggt gaagccgaag gttatttggg gcaacacttc    240
tccacaattt tgggatcttt acaagaagac caaaacccac ttgaccattt acacatcttt    300
ccttactact ccaactacct caagctagge aagctcgagt tcgatctcct gagccaacac    360
tcaagccatg tccccaccaa gattgccttc gtgggttcgg gtccgatgcc tctcacatcc    420
atcgtattgg ccaagtttca cctcccacac acgacgttcc acaactttga catcgactca    480
cacgcaaaca cactcgcttc aaacctcgtc tctcgcgacc cggacctctc aaaacgcatg    540
atcttcacac caacggacgt actaaacgca accgaagccc ttgaccaata tgacgtcgtt    600
ttcttagcgg cgcttgtagg gatggacaaa gagtcaaagg tcaaagccat cgagcacttg    660
gagaaacaca tggtcctcgg agctgttctt atgctaagga gggctcatgc tctcagagct    720
ttcttatatc caatcgttga ctcgtctgat ctcaaaggct ttcaactctt gaccatctat    780
catccaacgg atgacgtggt taactcgggt gtgatcgcac gtaagctcgg tggtcggacc    840
acgcccgggg ttaatggtac tcgtggatgc atgtttatgc cttgtaactg ctccaagatt    900
cacgcatgca tgaacaacgg tgtaagaag aatatgatcg aggagttag taccatcgag    960
taa

```

<210> SEQ ID NO 19
<211> LENGTH: 320
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 19

```

Met Ala Cys Gln Asn Asn Leu Val Val Lys Gln Ile Met Asp Leu Tyr
1          5          10          15

Asn Gln Ile Ser Asn Leu Glu Ser Leu Lys Pro Ser Lys Asn Val Asp
20          25          30

Thr Leu Phe Arg Gln Leu Val Ser Thr Cys Leu Pro Thr Asp Thr Asn
35          40          45

Ile Asp Val Thr Glu Ile His Asp Glu Lys Val Lys Asp Met Arg Ser
50          55          60

His Leu Ile Lys Leu Cys Gly Glu Ala Glu Gly Tyr Leu Glu Gln His
65          70          75          80

Phe Ser Ala Ile Leu Gly Ser Phe Glu Asp Asn Pro Leu Asn His Leu
85          90          95

His Ile Phe Pro Tyr Tyr Asn Asn Tyr Leu Lys Leu Gly Lys Leu Glu
100         105         110

Phe Asp Leu Leu Ser Gln His Thr Thr His Val Pro Thr Lys Val Ala
115         120         125

Phe Ile Gly Ser Gly Pro Met Pro Leu Thr Ser Ile Val Leu Ala Lys
130         135         140

```

-continued

Phe His Leu Pro Asn Thr Thr Phe His Asn Phe Asp Ile Asp Ser His
 145 150 155 160
 Ala Asn Thr Leu Ala Ser Asn Leu Val Ser Arg Asp Ser Asp Leu Ser
 165 170 175
 Lys Arg Met Ile Phe His Thr Thr Asp Val Leu Asn Ala Lys Glu Gly
 180 185 190
 Leu Asp Gln Tyr Asp Val Val Phe Leu Ala Ala Leu Val Gly Met Asp
 195 200 205
 Lys Glu Ser Lys Val Lys Ala Ile Glu His Leu Glu Lys His Met Ala
 210 215 220
 Pro Gly Ala Val Val Met Leu Arg Ser Ala His Gly Leu Arg Ala Phe
 225 230 235 240
 Leu Tyr Pro Ile Val Asp Ser Cys Asp Leu Lys Gly Phe Glu Val Leu
 245 250 255
 Thr Ile Tyr His Pro Ser Asp Asp Val Val Asn Ser Val Val Ile Ala
 260 265 270
 Arg Lys Leu Gly Gly Ser Asn Gly Ala Arg Gly Ser Gln Ile Gly Arg
 275 280 285
 Cys Val Val Met Pro Cys Asn Cys Ser Lys Val His Ala Ile Leu Asn
 290 295 300
 Asn Arg Gly Met Glu Lys Asn Leu Ile Glu Glu Phe Ser Ala Ile Glu
 305 310 315 320

<210> SEQ ID NO 20

<211> LENGTH: 963

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 20

```

atggcttgcc aaaacaatct cgttgtgaag caaatcatgg acttatacaa ccaaatctca    60
aacctcgaga gcttaaaacc atccaagaat gtcgacactt tgttcagaca acttgtgtcc    120
acgtgcttac caacggacac gaacatcgat gtcacagaga tacacgatga aaaagtcaaa    180
gacatgagat ctcatctcat caagctttgt ggtgaagccg aaggttattt agagcaaac    240
tttcagcaa tcttaggtct tttgaagac aacctctaa accatttaca catcttcccc    300
tattacaaca actatctcaa actaggcaaa ctgaattcg atctccttc tcagcacaca    360
acctatgtcc cgacaaaagt cgcctttatt ggttcgggtc cgatgccact tacttccatc    420
gtcttgcca agttccacct cccaacaca acgttcacac acttogacat cgactcacac    480
gccaacacac tcgcttcaaa cctcgtttct cgtgattctg acctttccaa acgcatgatt    540
ttccacacaa ctgatgtatt aaacgctaag gaggggtag accaatacga tgtgttttc    600
ttggcagctc ttgttgggat ggataaagag tcaaaggtea aagctattga gcatttagag    660
aagcatatgg cccctggagc tgtggtgatg ctaagaagtg ctcatggtct tagagctttc    720
ttgtatccaa tcgttgactc ttgtgatctt aaagggtttg aggtgttaac catttatcat    780
cgtctgacg acgtgggtaa ttgggtggtc atcgcacgta agcttgggtg ttcaaatgga    840
gctcgaggca gccagatcgg acggtgtgtg gttatgcctt gtaattgctc taaggtcac    900
gcatcttga acaatcgtgg tatggagaag aattgatcg aggagttag tgccatcgag    960
taa

```

<210> SEQ ID NO 21

<211> LENGTH: 320

-continued

```

<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 21

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

```

```

<210> SEQ ID NO 22
<211> LENGTH: 963
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

```

```

<400> SEQUENCE: 22

```

```

atgggttgcc aagacgaaca attggtgcaa acaatatgag atctctacga aaagatctca    60
aagcttgaga gtctaaaacc atccgaagat gtcaacattc tcttcaagca gctcgtttcc    120
acatgcatac caccaaaccc taacatcgat gtcaccaaga tgtgtgacag agtccaagag    180

```

-continued

```

attcgactta atctcatcaa gatttgtggt ctagccgaag gtcacttaga aaaccatttc 240
tcttcgatct tgacctctta ccaagacaac ccacttcac atttaaacat ttcccttat 300
tacaacaact atttgaaact cggaaagctc gagttcgacc tcctogaaca aaacctaaat 360
ggctttgtcc caaagagtgt ggctttcatt ggatctggtc ctcttctct cacttccatc 420
gttcttgctt cattccatct caaagacaca atctttcaca actttgacat cgacctatca 480
gcgaactcac tcgcttctct tctggtttcc tctgatccag acatctctca acgcatgttc 540
ttccacaccg ttgatataat ggacgtgaca gagagcttaa agagcttga tgtcgtgttt 600
ctagctgctc ttgttgaat gaacaaagag gagaaagta aagtgatcga gcatctgcag 660
aaacacatgg ctctgtgtgc tgtgtcatg cttaggagtg ctcatggtc gagagcgttt 720
ctttatccga tcgttgagcc gtgtgatctt caggggttcg aggttttgc tatttatcac 780
ccaacagatg atgttatcaa ctccgtggtg atctctaaaa agcatccagt tgtttcaatt 840
gggaatgttg gtggctctaa ttcatgcttg ctcaagcctt gcaactgttc caagaccac 900
gcgaaaatga acaagaacat gatgatcgag gagttcggag ctaggaggga acagttgtct 960
taa 963

```

```

<210> SEQ ID NO 23
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
consensus sequence
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (16)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (19)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (24)
<223> OTHER INFORMATION: Variable amino acid residue

```

```

<400> SEQUENCE: 23

```

```

Leu Pro Xaa Leu Ser Pro Ser Pro Xaa Val Asp Arg Leu Phe Thr Xaa
1           5           10           15

```

```

Leu Val Xaa Ala Cys Val Pro Xaa Ser Pro Val Asp Val Thr Lys Leu
20           25           30

```

```

<210> SEQ ID NO 24
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
consensus sequence
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES

```


-continued

<222> LOCATION: (11)

<223> OTHER INFORMATION: Variable amino acid residue

<400> SEQUENCE: 24

Leu Ile Arg Leu Cys Ser Xaa Ala Glu Gly Xaa Leu Glu Ala His Tyr
 1 5 10 15

<210> SEQ ID NO 25

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic consensus sequence

<220> FEATURE:

<221> NAME/KEY: MOD_RES

<222> LOCATION: (7)

<223> OTHER INFORMATION: Variable amino acid residue

<400> SEQUENCE: 25

Pro Leu Asp His Leu Gly Xaa Phe Pro Tyr
 1 5 10

<210> SEQ ID NO 26

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic consensus sequence

<220> FEATURE:

<221> NAME/KEY: MOD_RES

<222> LOCATION: (4)

<223> OTHER INFORMATION: Variable amino acid residue

<400> SEQUENCE: 26

Val Ala Phe Xaa Gly Ser Gly Pro Leu Pro Phe Ser Ser
 1 5 10

<210> SEQ ID NO 27

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic consensus sequence

<400> SEQUENCE: 27

Asp Val Val Phe Leu Ala Ala Leu Val Gly Met
 1 5 10

<210> SEQ ID NO 28

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic consensus sequence

<220> FEATURE:

<221> NAME/KEY: MOD_RES

<222> LOCATION: (5)

<223> OTHER INFORMATION: Variable amino acid residue

<220> FEATURE:

<221> NAME/KEY: MOD_RES

<222> LOCATION: (10)

<223> OTHER INFORMATION: Variable amino acid residue

<400> SEQUENCE: 28

Arg Gly Gly Phe Xaa Val Leu Ala Val Xaa His Pro
 1 5 10

-continued

<210> SEQ ID NO 29
<211> LENGTH: 328
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
amino acid sequence of nicotianamine synthase
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(10)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (12)..(13)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (16)..(21)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (23)..(24)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (26)..(27)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (31)..(36)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (39)..(40)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (43)..(44)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (46)..(46)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (48)..(51)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (54)..(60)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (62)..(62)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (65)..(66)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (69)..(70)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (72)..(73)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (76)..(77)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES

-continued

<222> LOCATION: (80)..(80)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (82)..(85)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (87)..(89)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (91)..(91)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (94)..(94)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (97)..(98)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (102)..(103)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (106)..(107)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (109)..(110)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (113)..(114)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (117)..(128)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (131)..(131)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (138)..(139)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (141)..(142)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (145)..(146)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (149)..(152)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (154)..(154)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (156)..(161)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (164)..(165)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (167)..(168)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:

-continued

<221> NAME/KEY: MOD_RES
<222> LOCATION: (170)..(179)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (182)..(182)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (184)..(184)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (186)..(194)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (196)..(198)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (206)..(206)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (210)..(213)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (215)..(219)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (222)..(223)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (226)..(227)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (230)..(230)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (232)..(238)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (240)..(240)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (245)..(245)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (247)..(254)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (257)..(257)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (260)..(260)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (262)..(262)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (265)..(267)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (269)..(269)
<223> OTHER INFORMATION: Variable amino acid residue

-continued

```

<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (272)..(276)
<223> OTHER INFORMATION: Variable amino acid residue
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (278)..(328)
<223> OTHER INFORMATION: Variable amino acid residue

<400> SEQUENCE: 29

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Lys Ile Xaa
1           5           10           15

Xaa Xaa Xaa Xaa Xaa Ile Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Xaa Xaa
20          25          30

Xaa Xaa Xaa Xaa Leu Phe Xaa Xaa Leu Val Xaa Xaa Cys Xaa Pro Xaa
35          40          45

Xaa Xaa Xaa Asp Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gln Xaa Met Arg
50          55          60

Xaa Xaa Leu Ile Xaa Xaa Cys Xaa Xaa Ala Glu Xaa Xaa Leu Glu Xaa
65          70          75          80

His Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Asp Xaa Pro Leu Xaa His Leu
85          90          95

Xaa Xaa Phe Pro Tyr Xaa Xaa Asn Tyr Xaa Xaa Leu Xaa Xaa Leu Glu
100         105         110

Xaa Xaa Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
115         120         125

Ala Phe Xaa Gly Ser Gly Pro Leu Pro Xaa Xaa Ser Xaa Xaa Leu Ala
130         135         140

Xaa Xaa His Leu Xaa Xaa Xaa Xaa Phe Xaa Asn Xaa Xaa Xaa Xaa Xaa
145         150         155         160

Xaa Ala Asn Xaa Xaa Ala Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
165         170         175

Xaa Xaa Xaa Arg Met Xaa Phe Xaa Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa
180         185         190

Xaa Xaa Leu Xaa Xaa Xaa Asp Val Val Phe Leu Ala Ala Xaa Val Gly
195         200         205

Met Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa His Leu Xaa Xaa His
210         215         220

Met Xaa Xaa Gly Ala Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Arg Xaa
225         230         235         240

Phe Leu Tyr Pro Xaa Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Phe
245         250         255

Xaa Val Leu Xaa Val Xaa His Pro Xaa Xaa Xaa Xaa Val Xaa Asn Ser Xaa
260         265         270

Xaa Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
275         280         285

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
290         295         300

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
305         310         315         320

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
325

<210> SEQ ID NO 30
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

```

-continued

<400> SEQUENCE: 30

Asp Ala Gln Asn Lys Glu Val Ala Ala Leu Ile Glu Lys Ile Ala Gly
 1 5 10 15

Ile Gln Ala

<210> SEQ ID NO 31

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 31

Arg Glu Ala Leu Ile Arg Leu
 1 5

<210> SEQ ID NO 32

<211> LENGTH: 71

<212> TYPE: PRT

<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 32

Met Glu Ala Gln Asn Gln Glu Val Ala Ala Leu Val Glu Lys Ile Ala
 1 5 10 15

Gly Leu His Ala Ala Ile Ser Lys Leu Pro Ser Leu Ser Pro Ser Ala
 20 25 30

Glu Val Asp Ala Leu Phe Thr Asp Leu Val Thr Ala Cys Val Pro Ala
 35 40 45

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

Glu Glu Leu Ile Arg Leu Cys
 65 70

<210> SEQ ID NO 33

<211> LENGTH: 111

<212> TYPE: PRT

<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 33

Tyr Val Asn Leu Ser Lys Leu Glu Tyr Asp Leu Leu Val Arg Tyr Val
 1 5 10 15

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

Pro Phe Ser Ser Leu Val Leu Ala Ala His His Leu Pro Asp Ala Val
 35 40 45

Phe Asp Asn Tyr Asp Arg Cys Gly Ala Ala Asn Glu Arg Ala Arg Arg
 50 55 60

Leu Phe Arg Gly Ala Asp Glu Gly Leu Gly Ala Arg Met Ala Phe His
 65 70 75 80

Thr Gly Asp Val Ala Thr Leu Thr Gly Glu Leu Gly Ala Tyr Asp Val
 85 90 95

Val Phe Leu Ala Thr Leu Val Gly Met Ala Ala Glu Glu Lys Pro
 100 105 110

<210> SEQ ID NO 34

<211> LENGTH: 64

<212> TYPE: PRT

<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 34

-continued

```

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

```

```

<210> SEQ ID NO 35
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

```

```

<400> SEQUENCE: 35

```

```

Ala Phe His Thr Ala Glu Val Thr Asp Leu Thr Ala Glu Leu Gly Ala
1           5           10           15

```

```

Tyr Asp Val

```

```

<210> SEQ ID NO 36
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

```

```

<400> SEQUENCE: 36

```

```

Ala Asp Gly Ala Val Leu Val Ala Arg Ser Ala His Gly Ala Arg Ala
1           5           10           15

```

```

Phe Leu Tyr Pro Val Val Glu Leu Asp Asp Val Gly Arg
20          25

```

```

<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

```

```

<400> SEQUENCE: 37

```

```

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

```

```

Pro Glu Gly Glu
20

```

The invention claimed is:

1. An isolated or purified polypeptide comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:15, wherein the polypeptide has nicotianamine synthase activity. 50

2. The isolated or purified polypeptide of claim 1 comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:15, wherein the polypeptide has nicotianamine synthase activity. 55

3. An isolated or purified *Oryza* enzyme exhibiting nicotianamine synthase activity, wherein:

- a. the enzyme is;
 - i. isolated or purified from *Oryza*; or
 - ii. expressed from a nucleic acid isolated or purified from *Oryza*; or

iii. expressed from a chimeric nucleic acid at least partially isolated or purified from *Oryza*;

b. the enzyme comprises an amino acid sequence having at least 90% identity with an amino acid sequence of SEQ ID NO: 15,

c. the enzyme has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:15.

4. An isolated polypeptide consisting of the amino acid set forth as SEQ ID NO:15.

* * * * *