



US007132587B2

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

(10) **Patent No.:** **US 7,132,587 B2**
(45) **Date of Patent:** **Nov. 7, 2006**

(54) **NON-AUTONOMOUS TRANSPOSON GENE OF RICE, TRANSFORMED PLANT AND METHOD OF USE**

WO WO 01/73036 A1 10/2001

OTHER PUBLICATIONS

(75) Inventors: **Kazuhiro Kikuchi**, Okazaki (JP);
Hiroyuki Hirano, Tokyo (JP);
Masamitsu Wada, Tokyo (JP)

Yano et al. Hd1, a Major Photoperiod Sensitivity Quantitative Trait Locus in Rice, Is Closely Related to the *Arabidopsis* Flowering Time Gene CONSTANS (2000) Plant Cell vol. 12 pp. 2473-2483.*
Kikuchi et al. The Plant MITE mPING is Mobilized in Anther Culture (2003) Nature vol. 421, pp. 167-170.*

(73) Assignee: **Agency of Industrial Science and Technology**, Saitama (JP)

Jurka, J. et al. "PIFs meet Tourists and Harbingers: A superfamily Reunion", *PNAS*, vol. 98(22) pp. 12315-12316, 2001.

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

Zhang, X. et al. "P instability factor: An active maize transposon system associated with the amplification of Tourist-like MITEs and a new superfamily of transposases", *PNAS Early Edition*, pp. 1-6, 2001.

(21) Appl. No.: **10/494,944**

Scortecci, K. C. et al. "Somatic Excision of the Ac Transposable Element in Transgenic *Arabidopsis thaliana* after 5-Azacytidine Treatment", *Plant Cell Physiol.*, vol. 38(3) pp. 336-343, 1997.

(22) PCT Filed: **Nov. 6, 2002**

Sano, H. et al. "A single treatment of rice seedlings with 5-azacytidine induces heritable dwarfism and undermethylation of genomic DNA", *Mol Gen Genet.*, vol. 220 pp. 441-447, 1990.

(86) PCT No.: **PCT/JP02/11585**

Le, Q.H. et al. "Tc8, a Tourist-like Transposon in *Caenorhabditis elegans*", *Genetics*, vol. 158 pp. 1081-1088, 2001.

§ 371 (c)(1),
(2), (4) Date: **Sep. 29, 2004**

Nakazaki, T. et al. "Polymorphic insertion of transposon-like sequence in mutable slender-granule gene slg locus", Japanese Society of Breeding 100th Conference, Autumn meeting, Oct. 2001; This is the English translation of this paper, first cited in IDS filed Sep. 29, 2004 for this application.

(87) PCT Pub. No.: **WO03/040363**

Database EMBL 'Online! May 24, 2001, "Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 1, BAC clone: B1158C05." XP 002359806 (abstract).

PCT Pub. Date: **May 15, 2003**

Database EMBL 'Online! Aug. 2, 2001, "Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 6, BAC clone: OJ1057_A09, Working Draft Sequence, 1 ordered pieces." XP002359807 (abstract).

(65) **Prior Publication Data**

US 2005/0125854 A1 Jun. 9, 2005

(30) **Foreign Application Priority Data**

Nov. 8, 2001	(JP)	2001-343002
Jan. 18, 2002	(JP)	2002-009729
Jun. 7, 2002	(JP)	2002-167345
Aug. 12, 2002	(JP)	2002-234412

Database EMBL 'Online! May 31, 2001, "Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 6, PAC clone: PO656E03." XP002359808 (abstract).

(51) **Int. Cl.**

C12N 15/01 (2006.01)
C12Q 12/68 (2006.01)

Turcotte Kime et al., "Survey of transposable elements from rice genomic sequences," *Plant Journal*, vol. 25, No. 2, Jan. 2001, pp. 169-179, XP002359802, Publisher: Blackwell Sciences, Oxford, England.

(52) **U.S. Cl.** **800/276; 435/91.2**

Chang-Gyun Han et al., "New transposable elements identified as insertions in rice transposon Tnr1," *Genes Genet. Syst.*, 2000, vol. 75, pp. 69-77.

(58) **Field of Classification Search** 536/23.1,
536/23.6; 435/468, 410, 320.1; 800/278,
800/291, 295, 298

Thomas E. Bureau et al., "A computer-based systematic survey reveals the predo, onance of small inverted-repeat elements in wild-type rice genes," *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, pp. 8524-8529; Publisher: National Academy of Sciences, Washington, D.C.

See application file for complete search history.

Long Mao et al., "Rice Transposable Elements: A Survey of 73,000 Sequence-Tagged-Connectors," *Genome Research*, 2000, vol. 10, pp. 982-990.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,965,791 A 10/1999 Ebinuma 800/278

* cited by examiner

Primary Examiner—Ashwin D. Mehta

Assistant Examiner—Cathy Kingdon Worley

(74) *Attorney, Agent, or Firm*—Drinker Biddle & Reath LLP

FOREIGN PATENT DOCUMENTS

EP	716147 A1	6/1996
EP	1275719 A1	1/2003
JP	9-154580 A	6/1997
JP	2001-343002	11/2001
JP	2002-009729	1/2002
JP	2002-167345	6/2002
JP	2002-234412	8/2002
WO	WO 96/15252 A1	5/1996
WO	WO 01/32881	* 10/2001

(57) **ABSTRACT**

The invention includes a method of transposing a non-autonomous transposon gene of rice (*Oryza sativa*). The method comprises culturing anthers of rice or treating roots, seeds, leaves, and stems of axillary buds of rice, or a callus derived from them, with 5-azacytidine or 5-azadeoxycytidine. Also included in the invention is a plant or plant seeds produced by the method.

3 Claims, 11 Drawing Sheets

Figure 1

144010	144020	144030	144040	144050	144060	
TTTCAAGTAC	AATCTCAACT	TAGGGAAAGT	TGTGATTGAG	GGAGGATGTT	AGATAATGTT	
144070	144080	144090	144100	144110	144120	
AGTTAGTTTG	TTATAGAGAT	AGATTAGTTC	TGTTACCGCA	TGTACTTTCT	TGTATCTATC	
144130	144140	144150	144160	144170	144180	
TCTATATCCA	GGATTGCTCT	AGGTTGTTGA	GATTAATCCT	ATCCTTTGTA	CACGCCACGG	3' LTR
144190	144200	144210	144220	144230	144240	
TAGAGGCTCT	TTCTGCCAT	ATCAACAAG	GFGCGCCCC	GFAAAGGGT	TCAACGCTTC	
144250	144260	144270	144280	144290	144300	
TCATTCCGTT	TTACAATCCT	CCTTCTCCT	CCTGGTGTG	AAAATTGTT	GATCGAGTTG	
144310	144320	144330	144340	144350	144360	
AAACTCTCAT	CCTTCATCAT	GTGCTGCAGA	AACTAACGCG	TGCACAGATG	ATGGATGGGT	
144370	144380	144390	144400	144410	144420	
GTGGTGTGAC	ATGAAAGTGG	ATCAATGACA	CGCGGCACAT	TTAGGGGAGT	GTGTCGTGTC	
144430	144440	144450	144460	144470	144480	
TTGACTTCTT	CATGCAAAAG	TATACCAACC	CTGTATAAGG	CCAGTCACAA	TGGCTAGTGT	
144490	144500	144510	144520	144530	144540	
CATTGCACGG	CTACCCAAA	TATTATACCA	TCTTCTCTCA	AATGAAATCT	TTTATGAAAC	
144550	144560	144570	144580	144590	144600	
AATCCCCACA	GTGAGGGGT	TTCACTTTGA	CGTTTCCAAG	ACTAAGCAAA	GCATTTAATT	
144610	144620	144630	144640	144650	144660	
GATACAAGTT	GCTGGGATCA	TTTGTACCCA	AAATCCGGCG	CGGCGCGGGA	GAATGCGGAG	Inverted Repeats
144670	144680	144690	144700	144710	144720	
GTGCGACGGC	GGAGCGGAC	GCAAGAGATC	CGGTGAATGA	AACGAATCGG	CCTCAACGGG	
144730	144740	144750	144760	144770	144780	
GGTTTCACTC	TGTTACCGAG	GACITGGAAA	CGACGCTGAC	GAGTTTCACC	AGGATGAAAC	
144790	144800	144810	144820	144830	144840	
TCMTTCCTTC	TCTCTCATCC	CCATTTTCATG	CAAATAATCA	TTTTTTATTTC	AGTCTTACCC	
144850	144860	144870	144880	144890	144900	
CTATTAAATG	TGCATGACAC	ACCAGTGAAA	CCCCCATTGT	GACTGGCCTA	AGCATCTTGG	

Figure 2

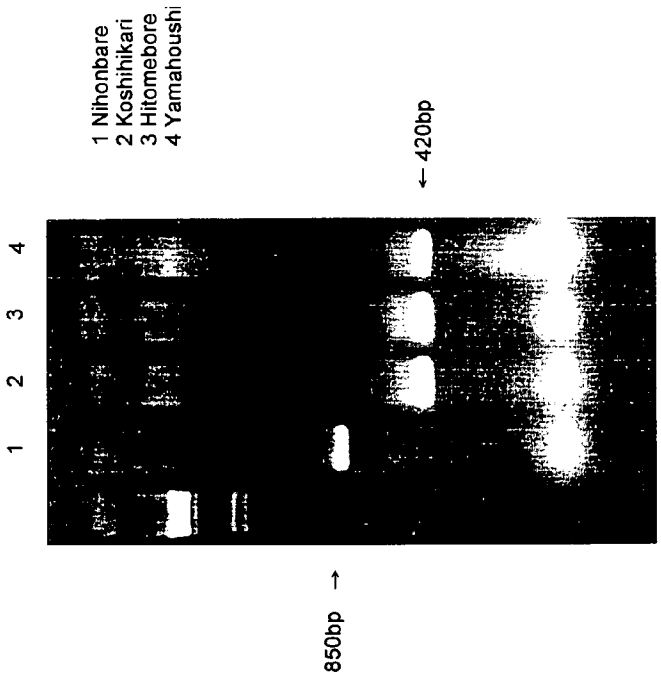


Figure 3

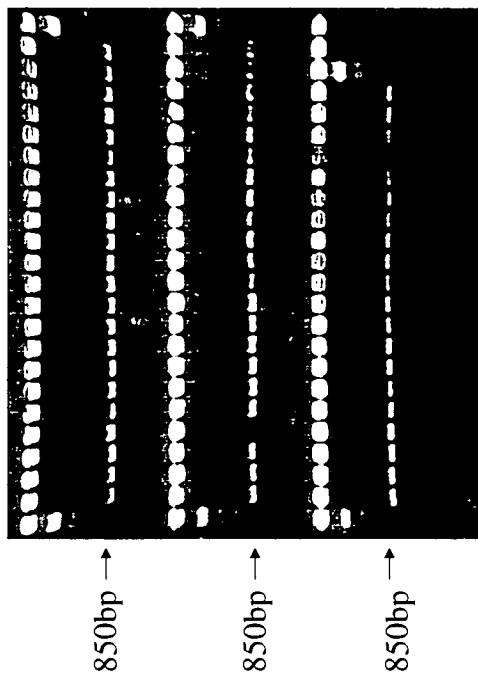


Figure 4

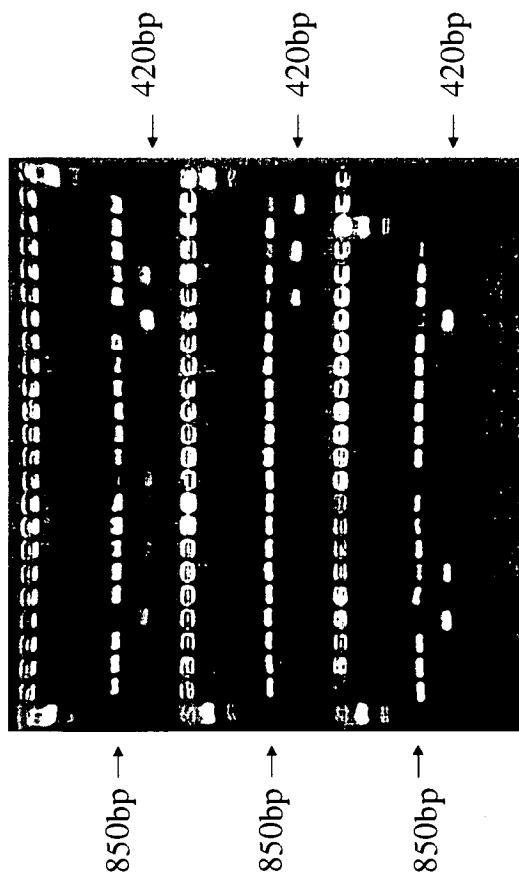


Figure 5

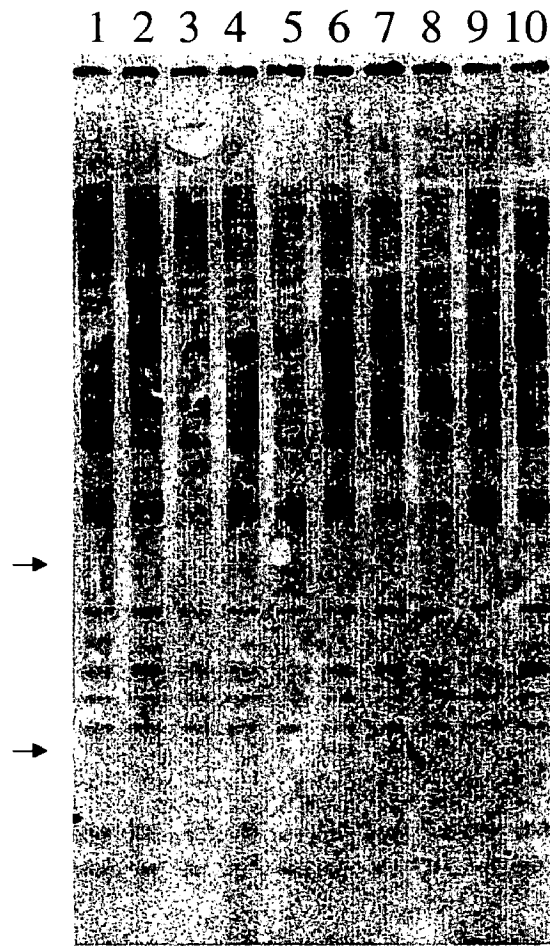


Figure 6



Figure 7

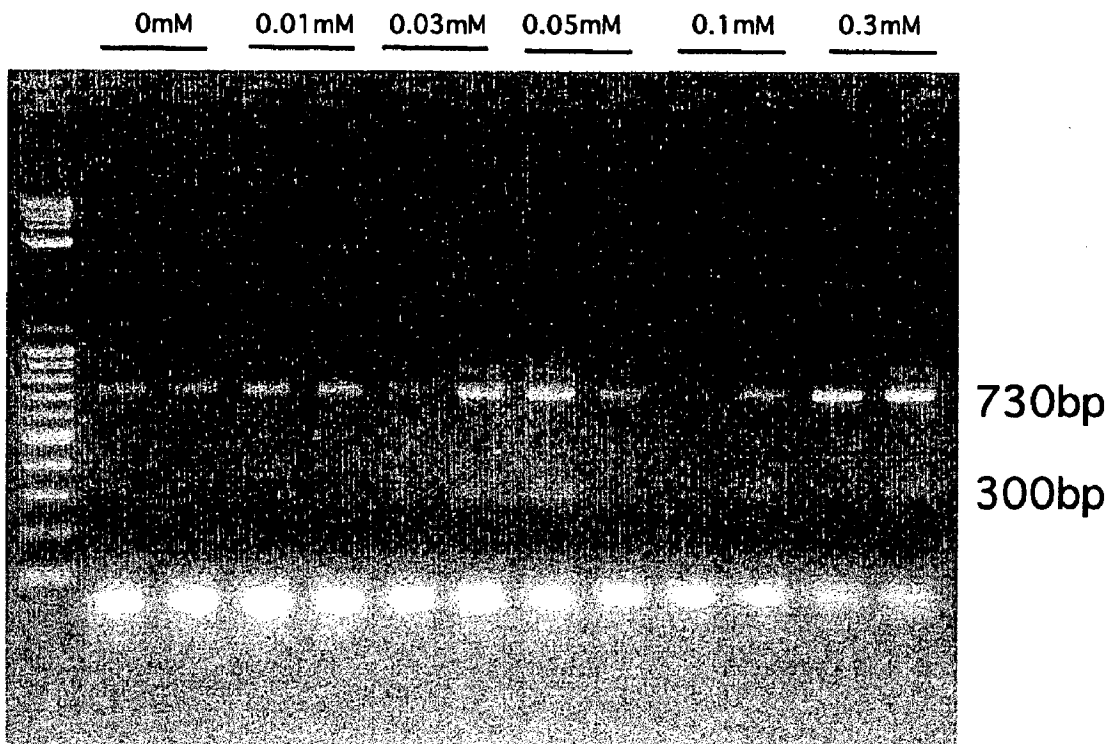
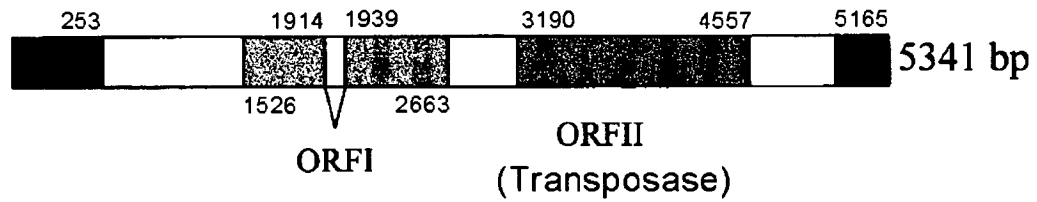


Figure 8

L05	ATGTAGTTTG	TCGGTAAGTT	TGATTATCAC	GGTAACCACA	AGTCAAGGGA
AzaC-callus1	ATGTAGTTTG	TCGGTAAGTT	TGATTATCAC	GGTAACCACA	AGTCAAGGGA
AzaC-callus2	ATGTAGTTTG	TCGGTAAGTT	TGATTATCAC	GGTAACCACA	AGTCAAGGGA
AzaC-callus3	ATGTAGTTTG	TCGGTAAGTT	TGATTATCAC	GGTAACCACA	-----
AzaC-callus4	ATGTAGTTTG	TCGGTAAGTT	TGATTATCaC	GGTAAC----	-----
L05	AAGATATGGA	CTCCTTAATA	AGGCCAGTCA	CAATGGGGGT	TTCACTGGTG
AzaC-callus1	AAGATATGGA	CTCCTTAA--	-----	-----	-----
AzaC-callus2	AAGATATGGA	CTCCTTAATA	A-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	TGTCATGCAC	ATTTAATAGG	GGTAAGACTG	AATAAAAAAT	GATTATTTGC
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	ATGAAATGGG	GATGAGAGAG	AAGGAAAGAG	TTTCATCCTG	GTGAAACTCG
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	TCAGCGTCGT	TTCCAAGTCC	TCGGTAACAG	AGTGAAACCC	CCGTTGAGGC
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	CGATTCGTTT	CATTCACCGG	ATCTCTTGCG	TCCGCCTCCG	CCGTGCGACC
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	TCCGCATTCT	CCCGCGCCGC	GCCGGATTTT	GGGTACAAAT	GATCCCAGCA
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	ACTTGTATCA	ATTAAATGCT	TTGCTTAGTC	TTGGAAACGT	CAAAGTGAAA
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	CCCCTCCACT	GTGGGGATTG	TTTCATAAAA	GATTTCATT	GAGAGAAGAT
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	GGTATAATAT	TTTGGGTAGC	CGTGCAATGA	CACTAGCCAT	TGTGACTGGC
AzaC-callus1	-----	-----	-----	-----	-----
AzaC-callus2	-----	-----	-----	-----	-----
AzaC-callus3	-----	-----	-----	-----	-----
AzaC-callus4	-----	-----	-----	-----	-----
L05	CTAACACTGA	ACTGATCAAA	GAGCATTAT	TATGGAAAGA	TGATTGTGTC
AzaC-callus1	----CACTGA	ACTGATCAAA	GAGCATTAT	TATGGAAAGA	TGATTGTGTC
AzaC-callus2	----CACTGA	ACTGATCAAA	GAGCATTAT	TATGGAAAGA	TGATTGTGTC
AzaC-callus3	-----CTGA	ACTGATCAAA	GAGCATTAT	TATGGAAAGA	TGATTGTGTC
AzaC-callus4	----ACTGA	ACTGATCAAA	GAGCATTAT	TATGGAAAGA	TGATTGTGTC

Figure 9

Sequence number 2



Sequence number 3

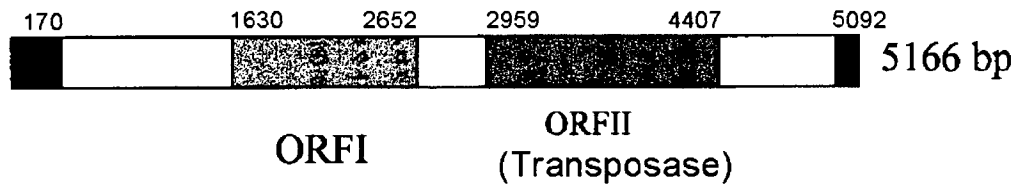


Figure 10

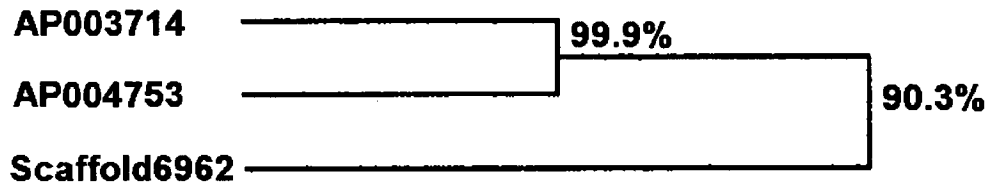


Figure 11

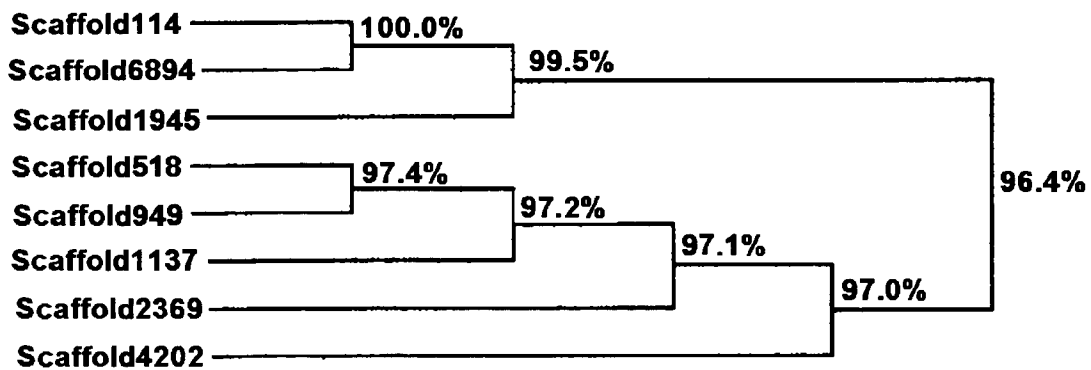


Figure 12

```

MS GNENQIPVSL
MQSLAISLLL SETHSLFSHT KTSSLLSLLF LSSSKMSEQN TDGSQVPVNL
* * * *
LDEFLAEDEI MDEIMDDVLH EMMVLLQSSI GDLEREAADH RLHPRKHIKR
LDEFLAEDEI ID----DLLT EATVVVQSTI EGLQNEASDH RHHPRKHIKR
***** * * * * * * * * * * * * * * * * * * * * * *
PREEAHQNLV NDYFSENPLY PSNIFRRRFR MYRPLFLRIV DALGQWSDYF
PREEAHQQLV NDYFSENPLY PSKIFRRRFR MSRPLFLRIV EALGQWSVYF
***** * * * * * * * * * * * * * * * * * * * * * *
TQRVDAAGRQ GLSPLQKCTA AIRQLATGSG ADELDEYLKI GETTAMDAMK
TQRVDAVNRK GLSPLQKCTA AIRQLATGSG ADELDEYLKI GETTAMEAMK
***** * * * * * * * * * * * * * * * * * * * * * *
NFVKGIREVF GERYLRRPTV EDTERLLELG ERGFPGMFG SIDCMHWQWE
NFVKGQLQDVF GERYLRRPTM EDTERLLQLG EKRGFPGMFG SIDCMHWHWE
***** * * * * * * * * * * * * * * * * * * * * * *
RCPTAWKGQF TRGDQKVPTL ILEAVASHDL WIWHAFFGVA GSNNDINVLS
RCPVAWKGQF TRGDQKVPTL ILEAVASHDL WIWHAFFGAA GSNNDINVLN
*** * * * * * * * * * * * * * * * * * * * * * *
RSTVFINELK GQAPRVQYMV NGNQYNEGYF LADGIYPEWK VFAKSYRLPI
QSTVFIKELK GQAPRVQYMV NGNQYNTGYF LADGIYPEWA VFKSIRLPN
***** * * * * * * * * * * * * * * * * * * * * * *
DXG/AF/F motif
TEKEKLYAQH QEGARKDIER AFGVLQRRFC ILKRPARLYD RGVLRDVVLG
TEKEKLYADM QEGARKDIER AFGVLQRRFC ILKRPARLYD RGVLRDVVLA
***** * * * * * * * * * * * * * * * * * * * * * *
YREK motif
CIILHNMIVE DEKEARLIEE NLDLNEPASS STVQAPEFSP DQHVPLERIL
CIILHNMIVE DEKETRIIEE DLDLNVPPSS STVQPEFSP EQNTPFDRVL
***** * * * * * * * * * * * * * * * * * * * * * *
EKDTSMRDRL AHRRLKNDLV EHIWNKFGGG AHSSGNYVFI LHY
EKDISIRDRA AHNRLKKDLV EHIWNKFGGA AHRTGN
*** * * * * * * * * * * * * * * * * * * * * * *

```


Figure 13

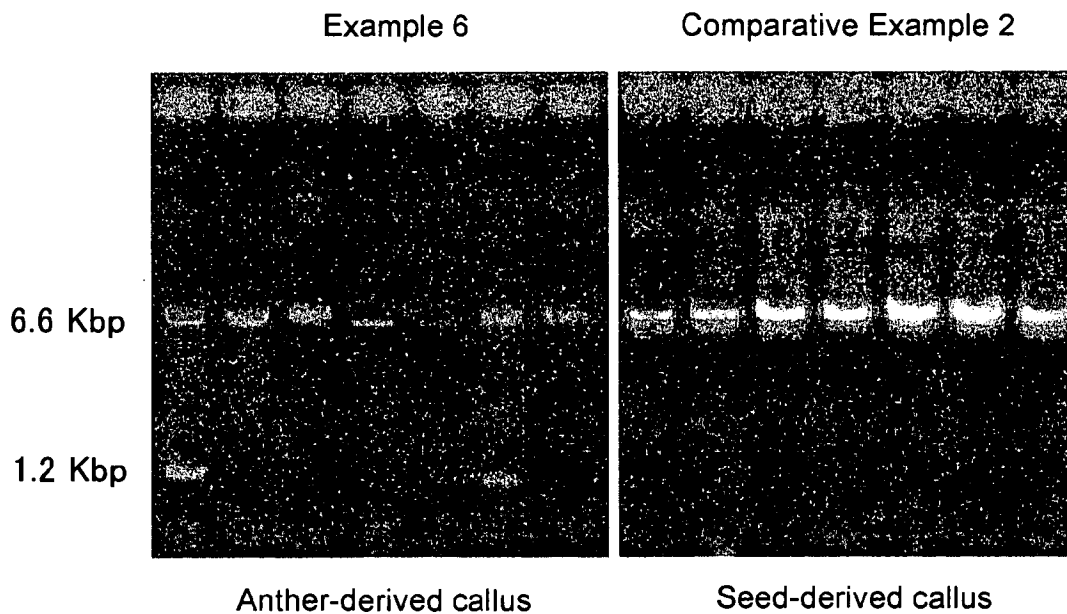


Figure 14

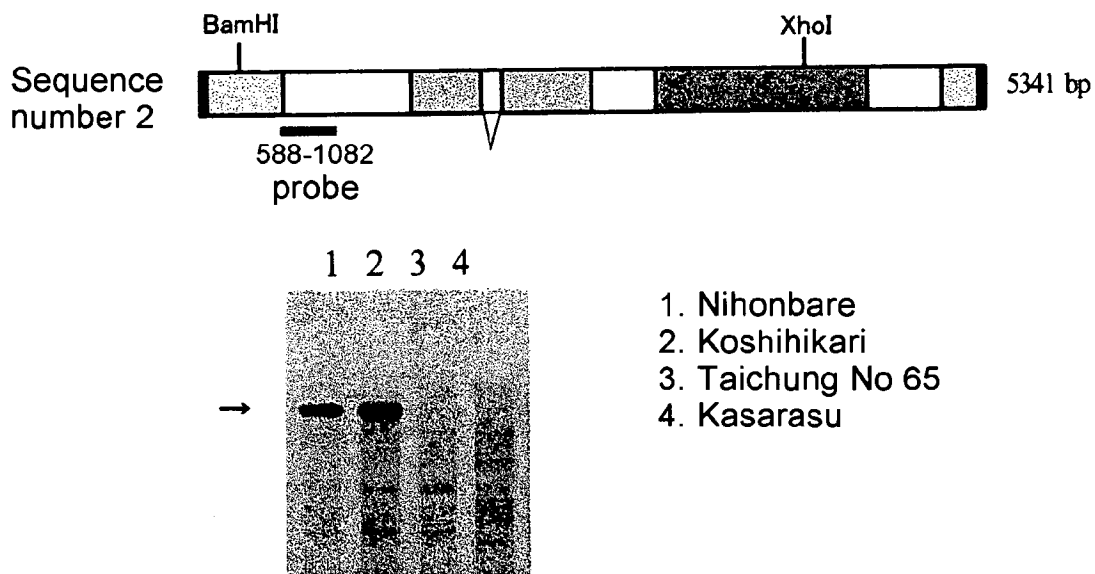


Figure 15

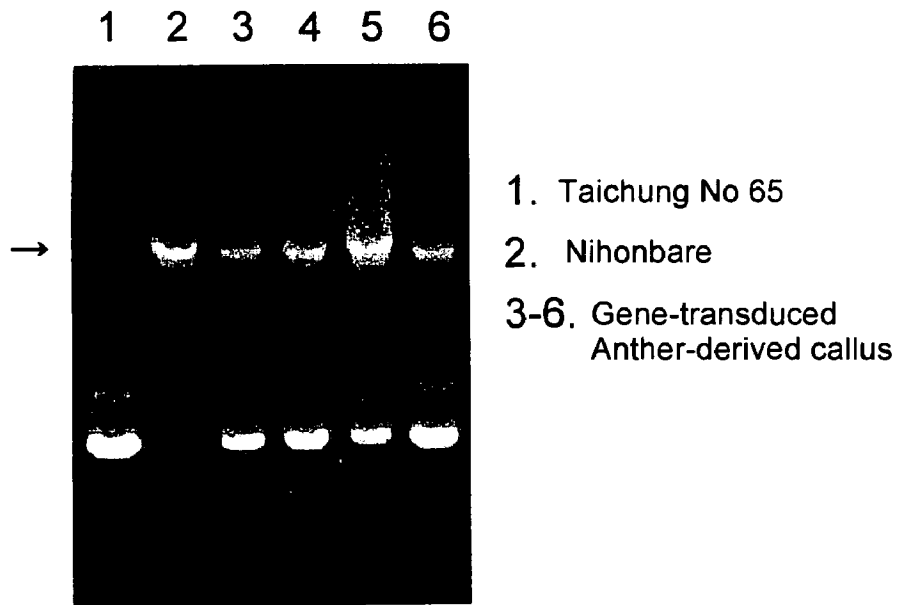


Figure 16

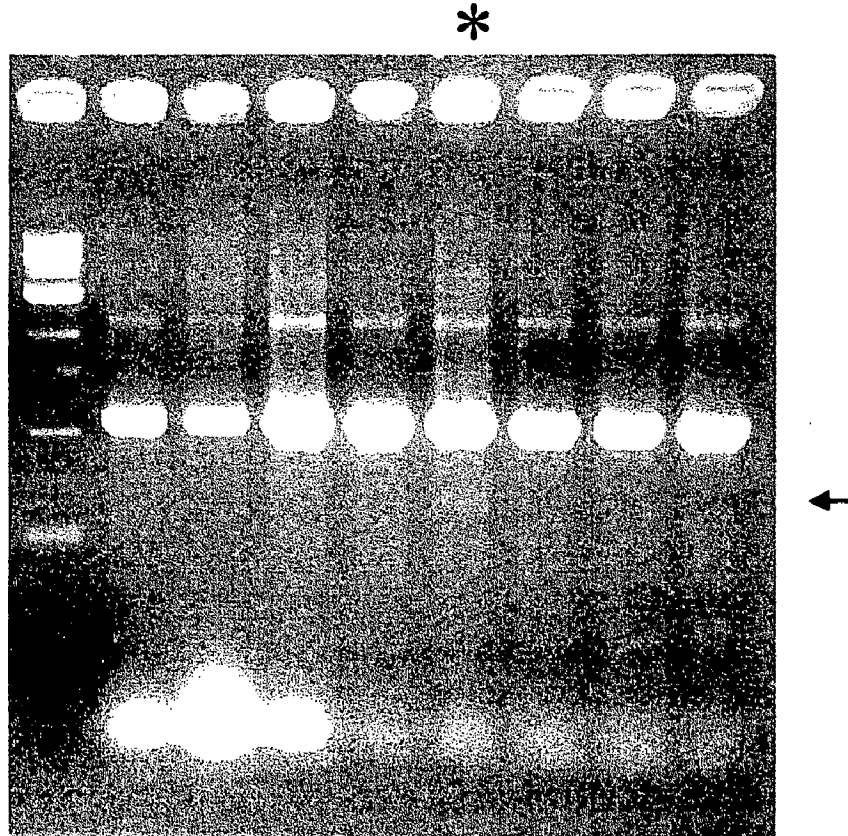


Figure 17

L02 GCGTGGACAC ACTGATTGGC CTGACAAAAC ATAGTTAGCA ATTTGCATTA
Seq GCGTGGACAC ACTGATTGGC CTGACAAAAC ATAGTTAGCA ATT——

L02 GGCCAGTCAC AATGGCTAGT GTCATTGCAC GGCTACCCAA AATATTATAC
Seq —————

L02 CATCTTCTCT CAAATGAAAT CTTTTATGAA ACAATCCCA CAGTGGAGGG
Seq —————

L02 GTTTCAC TTT GACGTTTCCA AGACTAAGCA AAGCATTTAA TTGATACAAG
Seq —————

L02 TTGCTGGGAT CATTGTACC CAAAATCCGG CGCGGCGCGG GAGAATGCGG
Seq —————

L02 AGGTCGCACG GCGGAGGCGG ACGCAAGAGA TCCGGTGAAT GAAACGAATC
Seq —————

L02 GGCCCTCAACG GGGGTTTAC TCTGTTACCG AGGACTTGA AACGACGCTG
Seq —————

L02 ACGAGTTTCA CCAGGATGAA ACTCTTTCCT TCTCTCTCAT CCCCATTTCA
Seq —————

L02 TGCAAATAAT CATT TTTTAT TCAGTCTTAC CCCTATTAAA TGTGCATGAC
Seq —————

L02 ACACCAGTGA AACCCCATTT GTGACTGGCC TTACGGCAAC ATTTGGATAT
Seq —————

L02 CGAATTATGT CCAAAGAGCG AAGGTATCTG TTAGCTAATC ATCCGATCGG
Seq —ATGT CCAAAGAGCG AAGGTATCTG TTAGCTAATC ATCCGATCGG

Figure 18

L06 TGGTCCTCGA TACTGTTGCC TGTTGGTACG GCACCACACC ACTCTGTTTT
Seq TGGTCCTCGA TACTGTTGCC TGTTGGTACG GCACCACACC ACTCTGTTTT

L06 TATTAGGCCA GTCACAATGG CTAGTGTCAT TGCACGGCTA CCCAAAATAT
Seq TATTAG_____

L06 TATACCATCT TCTCTCAAAT GAAATCTTTT ATGAAACAAT CCCACAGTG
Seq _____

L06 GAGGGGTTTC ACTTTGACGT TTCCAAGACT AAGCAAAGCA TTTAATTGAT
Seq _____

L06 ACAAGTTGCT GGGATCATTT GTACCCAAAA TCCGGCGCGG CGCGGGAGAA
Seq _____

L06 TGCGGAGGTC GCACGGCGGA GGCGGACGCA AGAGATCCGG TGAATGAAAC
Seq _____

L06 GAATCGGCCT CAACGGGGGT TTCACTCTGT TACCGAGGAC TTGAAACGA
Seq _____

L06 CGCTGACGAG TTTCACCAGG ATGAAACTCT TTCCTTCTCT CTCATCCCCA
Seq _____

L06 TTTCATGCAA ATAATCATTT TTTATTCAGT CTTACCCCTA TTAAATGTGC
Seq _____

L06 ATGACACACC AGTGAAACCC CCATTGTGAC TGGCCTTAGA GGTAAGTTTG
Seq _____A GGTAAGTTTG

L06 ATAGTACAGC CCACTACCAG CTCTAAATCA GTCAATGTAG TAGCTAATTC
Seq ATAGTACAGC CCACTACCAG CTCTAAATCA GTCAATGTAG TAGCTAATTC

**NON-AUTONOMOUS TRANSPOSON GENE
OF RICE, TRANSFORMED PLANT AND
METHOD OF USE**

RELATED APPLICATIONS

This application claims priority to PCT JP02/11585, and to Japanese Patent Application Nos. 2001-343002, 2002-9729, 2002-167345.

FIELD OF THE INVENTION

The present invention relates to a nonautonomous transposon gene, autonomous transposon gene and transposase gene of rice (*Oryza sativa*); and a method to transpose the transposon gene; and transformed plants by the transposition.

DESCRIPTION OF THE BACKGROUND

A gene disruption method has been used as a tool to analyze genome of rice (*Oryza sativa*). To disrupt genomes of rice, a method to activate a segregation factor by mating an individual, wherein a transposase gene (transposition enzyme) is transduced as an activator, with an individual, wherein a segregation factor is introduced; a method to use T-DNA; and a method to use retrotransposon have been known. However, analysis of spontaneous mutants of rice has not served to find active transposons (Supplementary volume of Cell Engineering, Plant cell engineering series 14, "Plant genome research protocols" 2000, February, Shujun press Co.).

On the other hand, a genome sequencing project is determining the nucleotide sequences of many plants as well

posons. However, enough elucidation has not been demonstrated. Additionally, a nucleotide sequence, assigned to a putative transposon gene has been found in a mutated rice induced by γ -irradiation (Tetsuya Nakazaki et al., "Polymorphic insertion of transposon-like sequence in mutable slender-granule gene *slg* locus" Japanese Society of Breeding 100th Conference, Autumn meeting, 2001, October).

Problems to be Solved by the Invention:

The inventors discovered an inverted repeated sequence characteristic to transposon genes in rice genome nucleotide sequence under investigation. Examining various tests on the possible transposon nucleotide sequence, the inventors confirmed that the nucleotide sequence is ascribed to a transposon gene (nonautonomous transposon).

Furthermore, the inventors discovered autonomous transposon genes on the basis of the nonautonomous transposon gene. Moreover, the inventors identified transposase genes, which enable to transpose the transposon gene.

Means of Solving the Problems:

The inventors, starting from chromosome No. 1, examined long terminal repeats (LTR) in rice genome sequence, which is registered seriatim in database. The inventors noticed a LTR on the clone, accession number AP002843, as shown in Table 1, investigated the sequence in detail and discovered the inverted repeat sequence characteristic to a transposon gene at the site of 144459th-144473rd and 144874th-144888th bases at adjacent downstream of LTR (FIG. 1, SEQ ID NO: 6). As disclosing in the example shown later, the inventors confirmed that the nucleotide sequence (SEQ ID NO: 1) located between the inverted repeats is a nonautonomous transposon gene, markedly transposable by such artificial treatment as anther culture.

TABLE 1

AP002843	<i>Oryza sativa</i>	genomic DNA, chromosome 1, PA			
ACCESSION	AP002843	NCBI SRS Genome-Net			
ORGANISM	<i>Oryza sativa</i>	NCBI SRS			
LOCUS	AP002843	148762 bp	DNA	PLN	26-JAN-2001
FEATURES		Location/Qualifiers			
source		1..148762			
		/chromosome="1"			
		/clone="P0407B12"			
		/cultivar="Nipponbare"			
		/organism="Oryza sativa"			
		/sequenced_mol="DNA"			
LTR		139482..139690			
		/note="5' LTR"			
CDS		139739..144052			
		/gene="P0407B12.28"			
		/note="probably inactive due to frameshifts in CDS"			
		/note="pseudogene, similar to <i>Oryza longistaminata</i> probable gag/pol polyprotein U72725"			
		/pseudo			
LTR		144047..144255			
		/note="3' LTR"			
CDS		join(144653..144692, 145148..145311)			
		/codon_start=1			
		/gene="P0407B12.29"			
		/note="hypothetical protein"			
		/protein_id="BAB17191.1"			
		/translation="MRRSHGGGGRKRSVPSSSHPEKKAIDRIKREDAGRRAGRVSIVVPLAAFPATDGGGGGLARLLRWW"			
		(SEQ. ID NO: 28)			

as rice and the results are databanked. Furthermore, transposon genes, mobile genes in animal and plant, have, to some extent, unique nucleotide sequences, whose information has enabled research on wider applications of trans-

Then, the inventors searched for homologous sequences using the nonautonomous transposon gene (SEQ ID NO: 1) by Blast search. Most of the results of the search lead to the nonautonomous transposon gene (SEQ ID NO: 1) itself, but

3

additionally, accession numbers AP004236 and AP003968, which were expected as transposon genes, were found. Comparing the nucleotide sequence of AP004236 and AP003968, the inventors found that these are cloned on chromosome No. 6 and are the sequence of the identical overlapped region.

Therefore, the homology between 1st-253rd bases of the nonautonomous transposon gene (SEQ ID NO: 1) and 89360th-89612nd bases of AP004236 was 252/253 (99%) and that between 254th-430th bases of the nonautonomous transposon gene (SEQ ID NO: 1) and 94524th-94700th bases of AP004236 was 177/177 (100%). They are well-conserved sequences.

Both nonautonomous transposon gene (SEQ ID NO: 1, 430 bp) and the transposon (SEQ ID NO: 2, 5341 bp) have inverted repeats of 15 bp and TTA and TAA are recognized and inserted. Open Reading Frame (ORF) was searched on the basis of SEQ ID NO: 2 (5341 bp) and two kinds of putative ORFI and ORFII were obtained.

Open Reading Frame (ORF) was searched on the basis of Sequence Number 2 (5341 bp) and two kinds of putative ORFI and ORFII were obtained.

The structure of the transposon gene comprising nucleotide sequence of SEQ ID NO: 2 is shown in the upper diagram of FIG. 9. The nonautonomous transposon gene (SEQ ID NO: 1, 430 bp) is located at 1st-253rd and at 5165th-5341st bases, ORFI is located at 1526th-1914th bases and at 1939th-2663rd bases and ORFII is located at 3190th-4557th bases.

Furthermore, to obtain similar genes to the gene comprising nucleotide sequence of SEQ ID NO: 2, the inventors performed homological searches using nucleotide sequence of SEQ ID NO: 2 as Query (DNA for homological search) of Blast search and found accession numbers AP004753 (chromosome No. 2) and AP003714 (chromosome No. 6) as well as the sequence of SEQ ID NO: 2 itself. These two clones have the identical nucleotide sequence (SEQ ID NO: 3) and located on different chromosomes (several copies). Since the nucleotide sequence is present also in indica (a cultivar of rice), the gene must be conserved in many cultivars, from japonica to indica. The nucleotide sequence of SEQ ID NO: 3 (5166 bp) has inverted repeats, as the sequence of SEQ ID NO: 2 has, and TAA (3 bp) was also recognized and inserted.

Open Reading Frame (ORF) was searched on the basis of the sequence of SEQ ID NO: 3 and ORFI and ORFII were obtained which may code two kinds of proteins. The structure of transposon gene comprising nucleotide sequence of SEQ ID NO: 3 is shown in the lower diagram of FIG. 9. The nonautonomous transposon gene (SEQ ID NO: 1, 430 bp) is located at 1st-170th and at 5092nd-5166th bases, however, the homological nucleotide sequence to the nonautonomous transposon gene (SEQ ID NO: 1, 430 bp) is disappeared in the middle. ORFI is located at 1630th-2652nd bases and ORFII is located at 2959th-4407th bases. The nucleotide sequence of SEQ ID NO: 3 was compared between japonica (AP004753 and AP003714) and indica (Scaffold6962) and the homology of more than 90% was confirmed as shown in FIG. 10. Additionally, the inventors examined the mutation frequency of nonautonomous transposon gene (SEQ ID NO: 1, 430 bp) in indica, and confirmed that the sequence homology was more than 95% as shown in FIG. 11.

The inventors have no idea on the function of ORFI in SEQ ID NOS 2 and 3, for the moment.

To examine whether ORFII encodes transposase (transposition enzyme) or not, the inventors checked whether the amino acid sequence of ORFII shares a conservative region

4

with that of known transposase gene. The amino acid sequences of ORFII in SEQ ID NO: 2 and in SEQ ID NO: 3 are shown as SEQ ID NOS 4 and 5, respectively. The alignment of amino acid sequences of these two ORFII (SEQ ID NOS 4 and 5) is shown in FIG. 12 and the homology of these sequences was more than 75% (77%). These two amino acid sequences have DXG/AF/F motif and YREK motif (SEQ ID NO: 39) (Q. H. Le, K. Turcotte and T. Bureau, Genetics 158: 1081-1088 (2001)), then it is concluded that these belong to IS transposase family.

Also, the homology of the nucleotide sequences of ORFII in SEQ ID NO: 2 and that in SEQ ID NO: 3 was more than 75% (79.3%).

Therefore, the present invention is a transposon gene of rice consisting of a nucleotide sequence which is at least 95% homological to SEQ ID NO: 1. The DNA being at least 95% homological to SEQ ID NO: 1 is considered to be functional as nonautonomous transposon, which is transposable by anther culture or by the treatment with chemical agents. Also, the present invention is the transposon gene of rice, wherein enhancers or promoters are inserted.

Furthermore, the present invention is the transposon gene of rice, whose nucleotide sequence is at least 90% homological to the nucleotide sequence of SEQ ID NO: 2 or 3. The DNA being at least 90% homological to the nucleotide sequence of SEQ ID NO: 2 or 3 is considered to be functional as an autonomous transposon gene, which is transposable by anther culture or by the treatment with chemical agents.

Also, the present invention is the transposase gene of rice, whose DNA being at least 75% homological to the nucleotide sequence of 3190th-4557th bases off SEQ ID NO: 2 or the nucleotide sequence of 2959th-4407th bases of SEQ ID NO: 3. The DNA being at least 75% homological to these nucleotide sequences is considered to be functional as the gene, which enables transposition of the transposons.

Also, the present invention is the transposase gene encoding a protein consisting of an amino acid sequence of SEQ ID NO: 4 or 5 or an amino acid sequence wherein one or several amino acids are deleted, substituted or added in said amino acid sequence. Also, the transposase could be a transposon gene of rice, whose amino acid sequence is at least 75% homological to SEQ ID NO: 4 or 5.

Moreover, the present invention is the transposase gene encoding this protein. Also, the present invention is the plasmid containing any one of said transposon genes. Still furthermore, the present invention is the plasmid containing promoters and any one of said transposase genes. Such binary vector as Ti plasmid and pBI-121 plasmid can be used for the purpose. 35S promoter of cauliflower mosaic virus, heat shock promoter, chemotaxis promoter and others can be used for the purpose of this invention. There are no restrictions on the method of incorporation of promoters and said genomes and general method of genetic engineering can be applied.

Also, the present invention is the transformants, wherein any of said transposon genes are transduced. Preferably, plants, especially, rice, barley, wheat or maize are used as the host. To transform these plants, using general method of genetic engineering, we can insert these genomes into said plasmid and transform the plants.

Still moreover, the present invention is the transformants, wherein promoters and said transposase genes are transduced. Other transposon genes can be transduced, if necessary. Said promoters can be used for the present purpose. Preferably, plants, especially, barely, wheat or maize are used as the host. To transform these plants, using general

method of genetic engineering, we can insert said genomes into said plasmid and transform the plants.

Also, the present invention is the method for transposing any of said transposon genes, comprising subjecting said transformants to anther culture or treating any of the transformants with a chemical agent.

Furthermore, the present invention is the plant or the seed, which is transformed by the transposition of said transposon genes by any one of said methods. Preferably, rice or barley, a vicinal species of rice, wheat or maize is used as the plant.

Also, the present invention is a method for determining the integrated region of transposon gene, which comprises the steps of transposing any one of said transposon gene by any one of the above methods, extracting DNA from the plant obtained by the previous step, digesting said DNA by a restriction enzyme with no cutting sites inside the transposon gene, ligating said DNA fragments obtained by the previous step, conducting PCR for said DNA fragments obtained by the previous step, and determining the nucleotide sequence of said PCR products obtained by the previous steps. The primers of said PCR involve the oligonucleotides, which comprises 10 consecutive bases, preferably 10~20 consecutive bases, more preferably 10~15 consecutive bases in the nucleotide sequence from the 5'-end of SEQ ID NO: 1; and the oligonucleotide, which comprises 10 consecutive bases, preferably 10~20 consecutive bases, more preferably 10~15 consecutive bases in the nucleotide sequence from the 3'-end of SEQ ID NO: 1, or the oligonucleotides, comprising the nucleotide sequences complementary to said sequences. Since the oligonucleotide comprising 10~15 consecutive bases from the 5'-end of the nucleotide sequence overlaps with that comprising 10~15 consecutive bases in the nucleotide sequence from the 3'-end of that of SEQ ID NO: 1, we can use single kind of primer, if the oligonucleotide bases comprises less than 15 consecutive bases. In other words, in this case, we can use the oligonucleotide comprising 10~15 consecutive bases in the nucleotide sequence from the 5'-end of SEQ ID NO: 1 or the oligonucleotides complementary to said nucleotide sequences as the PCR primer. In this way, identification of the integration site of the transposon enables to find the disrupted genomes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a part (SEQ. ID NO: 6) of genomic nucleotide sequence of accession number AP002843. The inverted repeated sequences characteristic to the transposon gene are located at the positions of 144459 to 144473 and 144874 to 144888, immediately after LTR.

FIG. 2 shows the result of agarose gel electrophoresis of PCR products of the DNA region (Accession Number AP002843) containing the transposon DNA from four cultivars of rice mature leaves (Example 2). An approximately 850 bp band in Nihonbare shows that a transposon gene (430 bp) is inserted in the region. While, approximately 420 bp bands for Koshihikari, Hitomebore and Yamahoushi show that transposon genes are not inserted.

FIG. 3 shows the result of agarose gel electrophoresis of PCR products of the DNA region (Accession Number AP002843) containing transposon DNA in calli derived from Nihonbare seeds (Comparative Example 1).

FIG. 4 shows the result of agarose gel electrophoresis of PCR products of the DNA region (Accession Number AP002843) containing transposon DNA in various calli

derived from Nihonbare anthers (Example 3). The DNA bands of approximately 420 bp demonstrate that the transposon gene is deleted.

FIG. 5 shows the result of agarose gel electrophoresis of DNA from various calli derived from Nihonbare anthers (Example 4). Lane 1 shows control Nihonbare and Lanes 2~10 show plant regenerated from anther derived-calli. DNA bands were detected using a probe comprising a part of the transposon nucleotide sequence. Lanes 2 and 6 show new bands (indicated by arrowheads), which were not found in control Nihonbare.

FIG. 6 is a photograph of a phenotypic transformer found in regenerated rice in example 4. The leaves of the rice are curly and short.

FIG. 7 shows the result of agarose gel electrophoresis of PCR products of the DNA region containing the transposon DNA from various calli derived from Nihonbare seeds (Example 5). The numbers on the upper margin indicate concentrations of 5-azacytidine. The DNA bands of approximately 300 bp demonstrate that the transposon gene (430 bp) is deleted.

FIG. 8 shows the base sequence of 4 clones, from which the transposon gene (430 bp) is deleted (SEQ. ID NOS.: 36, 30, 31, 32 and 33, respectively in order of appearance).

FIG. 9 shows the structure of the transposon genes with nucleotide sequences of SEQ. ID NOS.: 2 and 3.

FIG. 10 shows the homology of nucleotide sequence between japonica (AP004753 and AP003714) and indica (Scaffold6962) in the region of SEQ. ID NO.: 3.

FIG. 11 shows the mutation frequency of transposon gene (SEQ. ID NO: 1, 430 bp) in indica.

FIG. 12 shows the alignment of amino acid sequence of ORFII in SEQ. ID NO.: 2 and in SEQ. ID NO.: 3. The homology of their sequence is more than 75% (77.8%; in the figure, the homological amino acids are shown by *) and both sequences have DXG/AF/F motif and YREK motif (SEQ ID NO: 39). The upper line shows the amino acid sequence of SEQ ID NO: 4 (correspond to ORF II of SEQ. ID NO.: 2), and the lower line shows that of SEQ. ID NO.: 5 (correspond to ORF II of SEQ. ID NO.: 3).

FIG. 13—left shows the result of agarose gel electrophoresis of PCR products of the DNA region (accession number AP004236) containing transposon DNA in various calli derived from anthers of Nihonbare (Example 6). The DNA bands of 1.2 kbp indicate deletion of the transposon gene. FIG. 13—right shows the result of agarose gel electrophoresis of the DNA region (accession number AP004236) containing the transposon DNA in various calli derived from seeds of Nihonbare (Comparative Example 2).

FIG. 14 is the result of gel electrophoresis of DNA, which shows presence or absence of the autonomous transposon gene (SEQ ID NO: 2; shown on the upper margin of the photograph) in various rice cultivars (Example 7). A DNA band (shown by an arrow) indicating the presence of the autonomous transposon gene (SEQ. ID NO.: 2) was found in Nihonbare (lane 1) and in Koshihikari (lane 2), while, the DNA band was absent in Taichung No. 65 (lane 3) and in Kasararu (lane 4).

FIG. 15 is the result of gel electrophoresis of DNA from calli derived from anthers of Taichung No. 65, wherein the autonomous transposon gene from Nihonbare (SEQ. ID NO.: 2) was transduced (example 8). The DNA bands (shown by an arrow) indicating that the autonomous transposon gene (SEQ. ID NO.: 2) was absent in original Taichung No. 65 (lane 2), but present in calli derived from

anthers of Taichung No. 65 (lanes 3 to 6), wherein the autonomous transposon gene (SEQ. ID NO.: 2) was transduced.

FIG. 16 is the result of gel electrophoresis of PCR products of the DNA region containing the nonautonomous transposon gene of calli derived from anthers of Taichung No. 65, wherein the autonomous transposon gene from Nihonbare (SEQ. ID NO.: 2, Example 8) was transduced. On L06 gene locus, such size of DNA band (shown by an arrow) that is suggestive of deletion of the nonautonomous transposon gene, was observed.

FIG. 17 shows the nucleotide sequence of such size of DNA fragment SEQ. ID NO.: 34 that is suggestive of deletion of the nonautonomous transposon gene located in L02 gene locus (SEQ ID NOS 37). The nucleotide sequence of the nonautonomous transposon gene (SEQ. ID NO.: 1) is deleted.

FIG. 18 shows the nucleotide sequence of such size of DNA fragment SEQ. ID NO.: 35 that is suggestive of deletion of the nonautonomous transposon gene located in L06 gene locus (SEQ ID NO: 38). The nucleotide sequence of the nonautonomous transposon gene (SEQ. ID NO.: 1) is deleted.

FEATURE OF EMBODIMENT

First of all, this invention is the nonautonomous transposon gene (SEQ. ID NO.: 1) of rice with the DNA size of 430 bp. This transposon gene has terminal inverted repeats with the size of 15 bp and has a symmetric structure with 215 bp (CT).

Then, two kinds of transposon genes (SEQ. ID NOS.: 2 and 3) encode transposases (SEQ ID NOS 4 and 5) and are autonomous transposon genes.

An autonomous transposon gene is characteristic in encoding transposases, is mobile and induces transposition of a nonautonomous transposon gene. Whereas, a nonautonomous transposon gene is lacking a transposase, is not mobile and needs a help of autonomous transposon gene to transpose. The comparison structurally of autonomous transposon gene with nonautonomous gene, demonstrated the characteristics that the nucleotide sequences of both genes are homological and are well conserved except the DNA region deleted.

The plants carrying the transposon of this invention; or the plants transformed by the transposon of this invention; or the plants carrying the transposase gene of this invention; or the plants transformed by the transposase gene of this invention enable to transpose the transposons of this invention by activation, induced by irradiation; induced by treatment with chemical agents; or induced by anther culture. Since transposons can be greatly activated by these methods, transposition of an artificial transposon takes place easily.

Treatment with chemical agents is carried out by treating seeds, leaves, roots, and stems of axillary buds of a plant such as rice; or by treating calli derived from them, with chemical agents. For example, the treatment of these plants with 5-azacytidine or 5-azadeoxycytidine is generally carried out by transplanting them on solid or liquid medium containing 0.01~5 mM, preferably 0.05~2 mM of these chemical agents. As used therein, callus means a cellular mass, formed by dedifferentiation of differentiated plant organs, obtained by culturing plant organs such as roots, leaves or stems in an appropriate medium supplemented with appropriate concentrations of auxin or cytokinin. This cellular mass is not differentiated and has totipotency in differentiation. Totipotency of differentiation means that the

cellular mass can regenerate new organs such as buds or roots. For example, a single leaf can produce hundreds of clones, mediated by callus.

Anther culture is a kind of method to produce doubled haploid breeding. Anthers are taken out from the tip of stamens of rice and are cultured in a medium supplemented mainly with such hormone as auxin together with such polyploidy-inducing chemicals as colchicine and others. Since haploid cells can easily turn to diploid, it is easy to obtain diploid cells from anthers, i.e. haploid cells with a set of genes. Anther-derived callus of rice spontaneously doubles the number of chromosome, resulting in doubled haploid breeds, during prolonged culture period. A homozygote seed, a mutant plant, can be obtained by regeneration culture of the callus. We mainly, for regeneration culture, used the medium supplemented with such hormone as cytokinin.

The disrupted transposon gene, due to the insertion of transposed transposon gene, could be identified using the probe prepared by PCR using a primer set derived from appropriate two different nucleotide sequences in the transposon gene. An examination of correlation between transformed rice mutant and its gene enables to clarify the function of the gene.

It is a challenging object to identify easily a disrupted gene by a transposon tagging system. A lot of methods are known to determine the exact integration site of a transposon such as transposon display. However, they usually accompany complicated handlings. We inventors established a simple method using inverse PCR. In this method, an important key step is the design of PCR primers in the interior of the transposon gene. DNA is isolated from plants, digested by a restriction enzyme (we used AluI in this example), which has no cutting sites in the internal region of the transposon gene, and ligated intramolecularly to circularize the DNA by a ligase. On the basis of the obtained circular DNA as a template, we performed a PCR reaction using a primer set, which originally pointed away each other but which, after ligation, will prime towards one another around the circular DNA. The designed primer set, which consists of the oligonucleotide comprising at least 10 consecutive bases from the 5'-end of the nucleotide sequence of SEQ. ID NO.: 1, and the oligonucleotide comprising at least 10 consecutive bases from the 3'-end of the nucleotide sequence of SEQ. ID NO.: 1, preferably in the internal region of terminal inverted repeats (15 consecutive bases from both the 5'- and 3'-end of SEQ. ID NO.: 1); or oligonucleotides comprising the nucleotide sequence complementary to them, can be used for the inverse PCR. DNA sequencing of the obtained PCR products clarifies the integration site of the transposon gene. As an example of application of the nonautonomous transposon (SEQ. ID NO.: 1), integration of enhancers or promoters into the internal region of the nonautonomous transposon enables to induce transposition of these enhancers or promoters together with the nonautonomous transposon. More specifically, transducing the genome, wherein enhancers or promoters are inserted, into rice or other plants; or culturing of anthers; or treating them with chemical agents; or inducing the transposition of the genome, we can demonstrate active expression other genomes flanking to the integration site of the transposed genome and, as a result, we can get a lot of mutants with gain-of-function.

As promoters and enhancers for said example, we can use the 35S promoter of cauliflower mosaic virus and four sets in series of enhancer region (at positions -90 to -440 in the sequence) in the 35S promoter, respectively. There are no

restrictions on the integration site for the enhancer except the internal region of the inverted repeats of the genome with SEQ. ID NO.: 1. Also on the integration site of the promoters, there are no restrictions except the internal region of the inverted repeats of the genome with SEQ. ID NO.: 1 and except nonexistence of methionine in the adjacent downstream region of the integrated site. Preferably, both enhancers and promoters are integrated at position around 250 of SEQ. ID NO.: 1, if there are no troubles in the transposition of the transposon. To integrate enhancers and promoters, we can use the restriction enzyme site, which divides in two the nucleotide sequence of the genome of SEQ. ID NO.: 1 or use the cloning sites prepared by PCR.

EFFECTS OF THE INVENTION

The present invention, for the first time, demonstrated mobile transposon genes of rice, since previously these rice genes have not been known. Furthermore, inventors confirmed, by such simple method as anther culture and others, that nonautonomous transposon genes are transposable. In other words, we succeeded to provide a new method to transpose artificially a nonautonomous transposon gene.

Also in the examples, we demonstrated that nonautonomous transposon genes are deletable by such artificial treatment as anther culture and others. Furthermore, we directly confirmed the genetic locus, wherein nonautonomous transposon genes are inserted. Since we found artificially transposable transposon genes, we succeeded, for the first time, in preparing an artificial system of transposon tagging in rice.

Furthermore, the present invention demonstrated autonomous transposon genes (SEQ. ID NOS.: 2 and 3) of rice and transposase genes included in the genomes. The inventors confirmed, by such simple method as anther culture or drug treatment, that the transposon gene could be transposed in rice, wherein the autonomous transposon gene was transduced. In other words, the present invention provides a method to transform these plants easily and artificially, whereby the autonomous transposon genes or transposase genes are transduced artificially into rice and other plants.

The autonomous transposon genes of this invention can be used as a source of random mutagens and can produce a system of transposon tagging in rice and other plants. This invention can be used to produce several dozen of plant breeds, wherein the transposons are randomly transposed. Since spontaneous transposition in growing natural plants are very rare, efficient induction of transposition can be achieved in callus derived from induced anthers in plant tissue culture or callus derived from seeds treated with 5-azacytidine. It is possible to induce efficient transposition in plants other than rice, wherein the autonomous transposon genes of this invention are transduced by a transformation method. The mutated plants thus obtained can be analyzed by genetic analysis or by reverse genetic analysis.

Genetic analysis is a method to isolate a causal gene from phenotypes of mutants. If a transposon gene of this invention is linked to a phenotype of mutants, the causal gene of the phenotype can easily be isolated by the help of tagging (the transposon).

For example, if one wishes to look for a rice breed tolerant to salt, one may examine the tolerance of rice grown from seeds from the system of transposon tagging and may find the desired rice.

Reverse genetic analysis is a method to isolate a mutant, wherein genetic function is lost, from a pool of wild host. If a desired gene of a plant is tagged by the transposon, the

phenotype related to the gene should be mutated. DNA is isolated from various mutants and a genomic library of a tagged genome is prepared. We can request a transposon tagging system from a public stock centers. Screening the transposon tagging system, we can isolate tagged desired gene-transposon ensemble by PCR.

Recent progress of genome project made it cyclopaedically possible to prepare a set of mutants corresponding to entire genomes of rice and to prepare a database for the site of insertion of transposon corresponding to the mutants. User can order seeds of desired mutant by searching the database.

The following examples illustrate this invention, however, it is not intended to limit the scope of the invention.

EXAMPLE 1

DNA was extracted from mature leaves of Nihonbare, a rice cultivar (Kikuchi et al. (1998) Plant Biotechnology 15: 45-48). To amplify the central DNA region between both inverted terminal repeats located on both ends of transposon DNA, we used the oligonucleotide comprising the sequence of SEQ. ID NO.: 7 as PCR primers. We carried out PCR using GENEAMP9600® system (ABI Co.). Each reaction mixture (100 µl) contained 200 ng of DNA, 2.5 units of TAKARA EX TAQ® (Takara Co.), 10 µl of 10×Ex Taq buffer, 8 µl of dNTP mixture (2.5 mM each dNTP) and 200 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 94° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 12 min. This cycle was repeated 35 times. After the reaction, DNA was separated on 1% SEAKEM GTG agarose® (FMC Co.). The amplified DNA fragment with approximately 450 bp was recovered from the gel and it was subcloned in plasmid pCRII-TOPO using TA CLONING KIT® (In Vitrogen). The nucleotide sequence of the obtained clone was determined using 310 DNA SEQUENCER® (ABI Co.). The nucleotide sequence thus determined was shown as SEQ. ID NO.: 1, consisted of 430 bp.

EXAMPLE 2

DNA was isolated from leaves of 4 cultivars of rice, Nihonbare, Koshihikari, Hitomebore and Yamahoushi. To amplify the DNA region containing the transposon DNA (accession number AP002843) by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 8 and 9 as PCR primers. Each reaction mixture (100 µl) contained 200 ng of DNA, 2.5 units of AMPLITAQ GOLD® (ABICo.), 10 µl of GENEAMP10®xPCR buffer (contains 15 mM MgCl₂), 10 µl of GENE AMP MIXTURE® (2 mM each dNTP) and 200 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 1 min. This cycle was repeated 35 times. After the reaction, DNA was separated on 2% LO3 AGAROSE® (Takara Co.). FIG. 2 shows the result of the agarose gel electrophoresis. The DNA band of approximately 850 bp was found only for Nihonbare (lane 2). The DNA band of approximately 850 bp indicates the DNA fragment, including the transposon gene (430 bp) of SEQ. ID NO.: 1 as described in example 1. While, DNA fragments of 420 bp, not including the transposon gene, were found for Koshihikari, Hitomebore and Yamahoushi. The fact that the gene comprising the sequence of SEQ. ID

NO.: 1 was found only for Nihonbare among these rice cultivars suggests that the gene may function as a transposable element.

COMPARATIVE EXAMPLE 1

The seeds of Nihonbare were sterilized in 3% sodium hypochlorite solution for 15 to 30 min, washed with sterilized water, inoculated on a 90×20 mm of Petri dish (Iwaki Co.) containing 20~30 ml of media at the rate of 9 seeds per dish and subjected to growth culture under light for 24 h at 30° C. We used a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. On the 10th day of inductive culture, the calli derived from induced seeds were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20~30 ml of medium and subjected to growth culture under light for 24 h at 30° C. We used a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium.

DNA was extracted from calluses derived from seeds in growth culture for two weeks. To amplify the DNA region containing the transposon DNA by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 8 and 9 as PCR primers as described in Example 2. Each reaction mixture (100 µl) contained 200 ng of DNA, 2.5 units of GOLD AMPLITAQ GOLD® (ABI Co.), 10 µl of GENEAMP10®×PCR buffer (contains 15 mM MgCl₂), 10 µl of GENE AMP MIXTURE® (2 mM each dNTP) and 200 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 1 min. This cycle was repeated 35 times. After the reaction, DNA was separated on 2% LO3 AGAROSE® (Takara Co.). FIG. 3 shows the result of the agarose gel electrophoresis. Only a single DNA band of approximately 850 bp was found. The DNA band of approximately 850 bp indicates the DNA fragment, including the transposon gene. The expected DNA band of 420 bp, wherein transposon gene was deleted, could not be found. The probability that the band of size of approximately 420 bp is found was 0 callus per 64 calli (0%). Therefore, it was confirmed that transposon genes were not mobile in seed (scutellum)-derived calli.

EXAMPLE 3

Spikes of Nihonbare were harvested at pre-emergence, kept in cold treatment for 10 days at 10° C., sterilized in 1% sodium hypochlorite solution for 1 min and washed with sterilized water. Then, anthers were picked out from the floret, seeded in a 35×10 mm Petri dish (CORNING Co.) containing 3 ml of liquid medium at the rate of 50 anthers per dish and subjected to induction culture under light for 24 h at 30° C. Used was a liquid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 30 g of sucrose (Wako) in 1 L of medium. After 3~4 weeks of inductive culture, the calli derived from induced anthers

were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20~30 ml of medium and subjected to growth culture under light for 24 h at 30° C. We used a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 ml of α-naphthalene acetic acid solution (Sigma Co.), 2 ml of kinetin solution (Sigma Co.), 3 g of casamino acids (DIFCO), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium.

DNA was extracted from calli derived from anthers in growth culture for 2 weeks. To amplify the DNA region containing the transposon DNA by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 8 and 9 as PCR primers as described in example 2. Each reaction mixture (100 µl) contained 200 ng of DNA, 2.5 units of AMPLITAQ GOLD® (ABI Co.), 10 µl of GENEAMP10®×PCR buffer (contains 15 mM MgCl₂), 10 µl of GENE AMP MIXTURE® (2 mM each dNTP) and 200 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 1 min. This cycle was repeated 35 times. After the reaction, DNA was separated using 2% LO3 AGAROSE® (Takara Co.). FIG. 4 shows the result of the agarose gel electrophoresis. As shown in FIG. 4, two DNA bands of approximately 850 bp and 420 bp were obtained. The DNA band of approximately 850 bp indicates the DNA band including a transposon gene. While, the band of approximately 420 bp indicates that transposon genes were deleted. The probability that the DNA band of approximately 420 bp is observed was 11 calli per 64 calli (17.2%).

Since the DNA bands of approximately 420 bp, indicating the deletion of the transposon gene, was the PCR products from anther derived calli in this example, the amplified DNA fragments (approximately 420 bp, N=5) were recovered from the gel and subcloned into plasmid PCR II-TOPO USING TA CLONING KIT® (In Vitrogen). The nucleotide sequence of 5 clones thus obtained was determined by 310 DNA SEQUENCER® (ABI Co.). The alignment of these nucleotide sequences clarified the absence of the transposon gene sequence in these 5 clones (data not shown). Therefore, the inventors confirmed the deletion of the transposon gene on the basis of nucleotide sequences.

In comparative example 1, the transposon gene in scutellum (seed) derived cell cultures was not mobile, however, in this example, the transposon gene in anther derived cell cultures was mobile highly frequently.

EXAMPLE 4

Spikes of Nihonbare were harvested at pre-emergence, kept in cold treatment for 10 days at 10° C., sterilized in 1% sodium hypochlorite solution for 1 min and washed with sterilized water. Then, anthers were picked out from the floret, seeded in a 35×10 mm Petri dish (CORNING Co.) containing 3 ml of liquid medium at the rate of 50 anthers per dish and subjected to induction culture under light for 24 h at 30° C. We used a liquid medium consisting of 4 g of CHU(N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 30 g of sucrose (Wako) in 1 L of medium. After 3~4 weeks of inductive culture, the calli derived from induced anthers were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20~30 ml of medium and subjected to growth culture under light for 24 h at 30° C. We used a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma

13

Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 ml of α -naphthalene acetic acid solution (Sigma Co.), 2 ml of kinetin solution (Sigma Co.), 3 g of casamino acids (DIFCO), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. The anther derive calli in growth culture for two weeks were transplanted in a 90×20 mm Petri dish (Iwaki Co.) with 20–30 ml of medium and were cultured for regeneration under light for 24 h, at 30° C. We used a solid medium consisting of 4.3 g of MS BASAL SALT MIXTURE® (Gibcobl Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 10 ml of 6-benzylamino-purine solution (Sigma Co.), 2 ml of α -naphthalene acetic acid solution (Sigma Co.), 2 g of casamino acids (DIFCO), 30 g of sorbitol (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. The regenerated plant, in regeneration culture for 3–4 weeks, was transplanted in a growth culture and, when it was grown up, transferred to the soil. As a growth medium, we used a solid medium consisting of 4.3 g of MS BASAL SALT MIXTURE® (Gibcobl Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 30 g of sucrose (Wako) and 2 g of GELRITE® (Wako) in 1 L of medium.

DNA was extracted from 9 young seedlings, regenerated from anther-derived calli by CTBA method. The extracted DNA was digested with a restriction enzyme HindIII, separated on 0.8% LO3 AGAROSE® (Takara Co.) gel electrophoresis and transferred to a Nylon membrane (HybondN+) (Amersham Co.) by alkaline blotting. The DIG LUMINESCENCE DNA DETECTION KIT® (Roche Co.) was used for Southern hybridization. The PCR DIG PROBE SYNTHESIS KIT® (Roche Co.) was used for preparation of probes. To amplify the internal DNA region containing the transposon DNA by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 10 and 11 (both sequences are located inside the SEQ. ID NO.: 1 (transposon gene)) as PCR primers as described in example 2. FIG. 5 shows the result of the agarose gel electrophoresis. As shown in FIG. 5, two new bands (indicated by arrows), which were not observed in control Nihonbare, were found in Lanes 2 and 6. These two bands show that the transposon gene was inserted in these seedlings and then disrupted.

On the basis of the present example, it was clarified that the transposon gene was inserted to new genetic loci. Furthermore, an example of morphological mutation (the relevant gene is not determined) was found in regenerated rice as shown in FIG. 6 and the example may suggest that genes related to morphology was disrupted by the insertion of the transposon gene.

EXAMPLE 5

Seeds of Nihonbare were sterilized in 3% sodium hypochlorite solution for 15–30 min, washed with sterilized water, inoculated on a 90×20 mm of Petri dish (Iwaki Co.) containing 20–30 ml of media at the rate of 9 seeds per dish and subjected to induction culture under light for 24 h at 30° C. We used a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium.

After several days, calli were started forming from scutellum inside rice seeds and changed to cream-colored ones with 5 mm length on the 10th day of culture. The creamy-colored calli with 5 mm length were transplanted to growth

14

media supplemented with 5-azacytidine (Sigma Co.) at either 0 mM, 0.01 mM, 0.03 mM, 0.05 mM, 0.1 mM or 0.3 mM, respectively, and subjected to growth culture under light for 24 h at 30° C. We used a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium.

DNA was extracted by DNEASY PLANT MINI KIT® (QIAGEN) from calli derived from seeds in growth culture for 2 weeks. To amplify the DNA region containing the transposon DNA by PCR, and oligonucleotides comprising the sequences of SEQ. ID NOS.: 12 and 13 as PCR primers were used.

As PCR reaction mixture, HOTSTARTAQ MASTER MIX KIT® (QIAGEN) was used.

Each cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 1 min. This cycle was repeated 45 times. After the reaction, DNA was separated on 2% LO3 AGAROSE® (Takara Co.).

Two DNA bands of approximately 730 bp and 300 bp were observed for calli cultured supplemented with 0.03 mM–0.3 mM of 5-azacytidine (FIG. 7). The DNA band of approximately 730 bp indicates the DNA band including a transposon gene. While, the DNA band of approximately 300 bp indicates the DNA bands not including the transposon gene (430 bp).

These DNA fragments (300 bp) were recovered from the gel and subcloned into plasmid PCRII-TOPO USING TA CLONING KIT® (Invitrogen) The nucleotide sequence of 4 clones thus obtained was determined by 310 DNA SEQUENCER® (ABI Co.). The nucleotide sequences of 4 clones were subjected to a multiple alignment as shown in FIG. 8. No transposon gene sequence was found in these 4 clones.

This example showed that the transposition of the transposon gene of this invention could be induced even for seed-derived calli by means of 5-azacytidine, a demethylating agent. Based on this result, it can be expected that several hundreds of clones transposed by this transposon gene could be obtained from a single seed.

EXAMPLE 6

Spikes of Nihonbare were harvested at pre-emergence, kept in cold treatment for 10 days at 10° C., sterilized in 1% sodium hypochlorite solution for 1 min and washed with sterilized water. Then, anthers were picked out from the floret, seeded in a 35×10 mm Petri dish (CORNING Co.) containing 3 ml of liquid medium at the rate of 50 anthers per dish and subjected to induction culture under light for 24 h at 30° C. A liquid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 30 g of sucrose (Wako) in 1 L of medium was used. After 3–4 weeks of inductive culture, the calli derived from induced anthers were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20–30 ml of medium and subjected to growth culture under light for 24 h at 30° C. We used a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 ml of α -naphthalene acetic acid solution (Sigma

Co.), 2 ml of kinetin solution (Sigma Co.), 3 g of casamino acids (DIFCO), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium.

DNA was extracted from calli derived from anthers in growth culture for 2 weeks (Kikuchi et al. (1998) Plant Biotechnology 15: 45-48). To amplify the DNA region containing the transposon DNA by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NO.: 14 (88933rd-88962nd bases of AP004236) and SEQ. ID NO.: 15 (95545th-95574th bases of AP004236) as PCR primers. Each reaction mixture (100 µl) contained 200 ng of DNA, 2.5 units of TAKARA LA TAQ® (Takara Co.), 10 µl of 10×LA PCR BUFFER II®, 6 µl of 25 mM MgCl₂, 8 µl of dNTP mixture (2.5 mM each dNTP) and 100 pmol of primers.

Each cycle of the polymerase reaction consisted of a denaturation step at 94° C. for 30 sec and an extension step at 68° C. for 12 min. This cycle was repeated 35 times. After the reaction, DNA was separated on 0.8% LO3 AGAROSE® (Takara Co.) gel electrophoresis. The DNA band of approximately 6.6 kbp indicates the DNA band including the transposon gene (5341 bp) of this invention. Since the size of the transposon of this invention is approximately 5.4 kbp, the DNA band of approximately 1.2 kbp could be expected when this transposon gene is deleted. A DNA band of approximately 1.2 kbp was obtained in this example (FIG. 13). These results show that the transposon gene is mobile in anther-derived calli. The probability, that the DNA band of approximately 1.2 kbp was observed in calli, was three calli per 64 calli (4.7%). This example proved that the MITE of rice with the nucleotide sequence of SEQ. ID NO.: 2 was mobile in anther-derived calli.

COMPARATIVE EXAMPLE 2

Seeds of Nihonbare, a rice cultivar, were sterilized in 3% sodium hypochlorite solution for 15-30 min, washed with sterilized water, inoculated on a 90×20 mm of Petri dish (Iwaki Co.) containing 20-30 ml of media at the rate of 9 seeds per dish and subjected to induction culture under light for 24 h at 30° C. Used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. On the 10th day of inductive culture, the calli derived from induced seeds were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20-30 ml of medium and subjected to growth culture under light for 24 h at 30° C. Used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. DNA was extracted from calli derived from seeds in growth culture for two weeks (Kikuchi et al. (1998) Plant Biotechnology 15: 45-48). To amplify the DNA region containing the transposon DNA by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 14 and 15 as PCR primers. Each reaction mixture (100 µl) contained 200 ng of DNA, 2.5 units of TAKARA LA TAQ® (Takara Co.), 10 µl of 10×LA PCR BUFFER II®, 6 µl of 25 mM MgCl₂, 8 µl of dNTP mixture (2.5 mM each dNTP) and 100 pmol of primers. Each cycle of the poly-

merase reaction consisted of a denaturation step at 94° C. for 30 sec and an extension step at 68° C. for 12 min. This cycle was repeated 35 times. After the reaction, DNA was separated on 0.8% LO3 AGAROSE® (Takara Co.) gel electrophoresis. A single DNA band of approximately 6.6 kbp, but not a band of approximately 1.2 kbp, was observed for all calli samples from seeds (FIG. 13). The result implied that transposon genes were not mobile in seed derived-calli. The probability that the DNA band of approximately 1.2 kbp was observed in seed-derived callus is 0 callus per 64 calli (0%).

EXAMPLE 7

In this example, to look for a cultivar, wherein the autonomous transposon gene (SEQ. ID NO.: 2) is not included and to clarify whether there is a difference in the efficiency of the transposition of a nonautonomous transposon gene between calli with the autonomous transposon gene (SEQ. ID NO.: 2) and those without the gene, we induced anther-derived callus from cultivars not including autonomous transposon gene (SEQ. ID NO.: 2) and checked the efficiency of the transposition of a nonautonomous transposon gene.

DNA was isolated by CTAB method from leaves of 4 kinds of rice cultivars, Nihonbare, Koshihikari, Taichung No. 65 and Kasarasu. The isolated DNA was digested by a restriction enzyme, HindIII, separated by 1.0% LO3 AGAROSE® gel electrophoresis, transferred to a Nylon membrane (HybondN+, Amersham Co.) by an alkaline blotting and detected by DIG LUMINESCENCE DNA DETECTION KIT® (Roche Co.) using Southern hybridization. The PCR DIG PROBE SYNTHESIS KIT® (Roche Co.) was used for preparation of probes. To amplify the DNA region specific to the autonomous transposon gene by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 16 and 17 as PCR primers. The result of Southern hybridization shows that one copy of autonomous transposon gene exists in both Nihonbare and Koshihikari, but not in Taichung No. 65 and Kasarasu (FIG. 14).

Then, we induced callus derived from anthers of Taichung No. 65 and examined whether nonautonomous transposon gene (SEQ. ID NO.: 1) was transposed.

Spikes of Taichung No. 65 were harvested from the head spout before forming the spikes, kept in cold treatment for 10 days at 10° C., sterilized in 1% sodium hypochlorite solution for 1 min and washed with sterilized water. Then, anthers were picked out from the caryopsis, seeded in a 35×10 mm Petri dish (CORNING Co.) containing 3 ml of liquid medium at the rate of 50 anthers per dish and subjected to induction culture under light for 24 h at 30° C. Used was a liquid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 30 g of sucrose (Wako) in 1 L of medium. After 3-4 weeks of inductive culture, the calli derived from induced anthers were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20-30 ml of medium and subjected to growth culture under light for 24 h at 30° C. Used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURES (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 ml of α-naphthalene acetic acid solution (Sigma Co.), 2 ml of kinetin solution (Sigma Co.), 3 g of casamino acids (DIFCO), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium.

DNA was extracted from calli derived from anthers in growth culture for 2 weeks according to the method

described (Kikuchi et al. (1998) Plant Biotechnology 15: 45–48). To amplify the DNA region specific to the autonomous transposon gene by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 18 and 19 (L02), SEQ. ID NOS.: 20 and 21 (L06) and SEQ. ID NOS.: 22 and 23 (L07) as PCR primers. Each reaction mixture (100 μ l) contained 200 ng of DNA, 2.5 units of AMPLITAQ GOLD® (ABI Co.), 10 μ l of GeneAmp10 \times PCR buffer (contains 15 mM MgCl₂), 10 μ l of GENE AMP MIXTURE® (2 mM each dNTP) and 200 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 1 min. This cycle was repeated 35 times. After the reaction, DNA was separated on 2% LO3 AGAROSE® (Takara Co.). The results show that there are no transpositions of the nonautonomous transposon gene, located in L02, L06 and L07 loci, in Taichung N. 65, not carrying the autonomous transposon gene (SEQ. ID NO.: 1) (Table 2, the first line). However the nonautonomous transposon gene transposed in high frequency at 10.9–31.3% in anther-derived callus of Nihonbare, which carries the autonomous transposon gene (SEQ. ID NO.: 2), as shown in the following comparative Example 3.

TABLE 2

	L02	L06	L07
Anther-derived callus	0/64	0/64	0/64
Gene-transduced anther-derived callus	2/38	1/38	0/38
	5.3%	2.6%	0%

COMPARATIVE EXAMPLE 3

Spikes of Nihonbare were harvested at pre-emergence, kept in cold treatment for 10 days at 10° C., sterilized in 1% sodium hypochlorite solution for 1 min and washed with sterilized water. Then, anthers were picked out from the floret, seeded in a 35 \times 10 mm Petri dish (CORNING Co.) containing 3 ml of liquid medium at the rate of 50 anthers per dish and subjected to induction culture under light for 24 h at 30° C. A liquid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 30 g of sucrose (Wako) in 1 L of medium was used. After 3–4 weeks of inductive culture, the calli derived from induced anthers were transplanted to a 90 \times 20 mm of Petri dish (Iwaki Co.) containing 20–30 ml of medium and subjected to growth culture under light for 24 h at 30° C. Used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 ml of α -naphthalene acetic acid solution (Sigma Co.), 2 ml of kinetin solution (Sigma Co.), 3 g of casamino acids (DIFCO), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. DNA was extracted from calli derived from anthers in growth culture for 2 weeks. To amplify the DNA region containing the transposon DNA by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 5 and 1 as PCR primers as described in example 7. Each reaction mixture (100 μ l) contained 200 ng of DNA, 2.5 units of AMPLITAQ GOLD® (ABI Co.), 10 μ l of GeneAmp10 \times PCR buffer (contains 15 mM MgCl₂), 10 μ l of GENE AMP MIXTURE® (2 mM each dNTP) and 200 pmol of primers. Each

cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 1 min. This cycle was repeated 35 times. After the reaction, DNA was separated using 2% LO3 AGAROSE® (Takara Co.). We obtained the result that there are two DNA bands of approximately 850 bp and 420 bp. The DNA band of approximately 850 bp indicates the DNA band including a transposon gene. While, the band of approximately 420 bp indicates that transposon genes were deleted. The probability, that the DNA band of approximately 420 bp is observed, was 11 calli per 64 calli (17.2%).

EXAMPLE 8

In this example, the DNA region including the autonomous transposon gene (SEQ. ID NO.: 2) was isolated, transduced to a cultivar (Taichung No. 65), which does not include the autonomous transposon gene (SEQ. ID NO.: 2), and examined the possibility of transposition of nonautonomous transposon gene (SEQ. ID NO.: 1) in this cultivar.

To amplify the DNA region including the autonomous transposon gene (SEQ. ID NO.: 2) from Nihonbare, a cultivar of rice, we designed two primer nucleotide sequences, SEQ. ID NOS.: 24 and 25, whose sequences are located at the adjacent upstream and downstream, respectively, of the target DNA region (SEQ. ID NO.: 2) for the PCR reaction. We synthesized the oligonucleotides of the sequences of SEQ. ID NOS.: 24 and 25 and used them as primers. DNA was isolated from leaves of Nihonbare by CTAB method. Each reaction mixture (100 μ l) contained 200 ng of DNA, 2.5 units of TAKARA LA TAQ® (Takara Co.), 10 μ l of 10 \times LA PCR BUFFER II® (Takara Co.), 6 μ l of 25 mM MgCl₂, 8 μ l of dNTP mixture (2.5 mM each dNTP) and 100 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 94° C. for 30 sec and an extension step at 68° C. for 12 min. This cycle was repeated 35 times. After the reaction, DNA was separated on 0.8% LO3 AGAROSE® (Takara Co.) gel electrophoresis. We obtained the DNA band of approximately 6.6 kbp, including the autonomous transposon gene. The DNA fragment (approximately 6.6 kbp) was recovered from gel slices, subcloned into plasmid PCR II-TOPO USING TA CLONING KIT® (In Vitrogen), cut out using the multicloning sites (ApaI and KpnI) in pCRII-TOPO using TA CLONING KIT®, subcloned to a binary vector, which contains a selectable marker gene, hygromycin resistant gene, and could be used for plant infection, and transduced to Agrobacteria EHA101 by electroporation. On three days before the infection of the Agrobacteria to anther-derived callus, the Agrobacteria were streaked onto AB medium containing kanamycin (Wako) and hygromycin (Wako).

Then, mediated by the *Agrobacterium*, the DNA fragments (approximately 6.6 kbp), wherein the autonomous transposon gene (SEQ. ID NO.: 2) was included, were transduced to the anther-derived calli of Taichung No. 65, a rice cultivar.

Spikes of Taichung No. 65 were harvested at pre-emergence, kept in cold treatment for 10 days at 10° C., sterilized in 1% sodium hypochlorite solution for 1 min and washed with sterilized water. Then, anthers were picked out from the floret, seeded in a 35 \times 10 mm Petri dish (CORNING Co.) containing 3 ml of liquid medium at the rate of 50 anthers per dish and subjected to induction culture under light for 24 h at 30° C. A liquid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-

dichloro-phenoxyacetic acid (Sigma Co.), 30 g of sucrose (Wako) in 1 L of medium was used. After 3~4 weeks of inductive culture, the calli derived from induced anthers were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20~30 ml of medium and subjected to growth culture under light for 24 h at 30° C. A solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 ml of α -naphthalene acetic acid solution (Sigma Co.), 2 ml of kinetin solution (Sigma Co.), 3 g of casamino acids (DIFCO), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium was used.

The *Agrobacteria* were infected to anther-derived calli originated from Taichung No. 65, at 2 weeks of growth culture in growth medium. The *Agrobacteria*, streaked onto the surface of AB medium and kept for three days, were scraped by a spatula, mixed with AAM medium (25 ml) supplemented with 10 mg/L acetosyringone. The mixed medium with the *Agrobacterium* for infection was kept in a Petri dish (IWAKI). The calli, in wire cage, derived from anther in growth culture for 2 weeks was immersed into the mixed medium for infection for 2 min. After the immersion, the mesh cage was put on a sterilized paper towel and removed the excess medium. The callus was put on a filter paper on a symbiotic medium by a forceps and cultured at 28° C. for 3 days under dark, sealed by a surgical tape. The symbiotic medium used was a solid medium containing 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 30 g of Sucrose (Wako), 10 g of Glucose (Wako), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 2 g of GELRITE® (Wako) and 10 mg of acetosyringone in 1 L of medium. After symbiotic culture for 3 days, the callus was added to an Erlenmeyer flask with 100 ml of sterilized water, shaken well and the water was discarded. After washing with sterilized water several times and with washing solution supplemented with 500 mg/ml of carbenicillin, the callus was transplanted on Petri dish at the rate of 9 calli/dish, sealed by a surgical tape and was cultured under light at 25° C. for a month. As a selection medium, used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS Vitamine Solution (Sigma Co.), 30 g of sucrose (Wako), 0.3 g of casamino acids (DIFCO), 2.878 g of proline (ICN), 0.1 g of mioinositol (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 500 mg of hygromycin (Wako), 50 mg of carbenicillin (Wako), 2 g of GELRITE® (Wako) in 1 L of medium.

Then, we examined the possible transduction of the autonomous transposon gene of Nihonbare and the possible transposition of the nonautonomous transposon gene (SEQ. ID NO.: 1) in hygromycin-resistant calli, grown in a selection medium for 3~4 weeks after seeded on the medium.

DNA was isolated from the resistant calli according to the method described (Kikuchi et al. (1998) Plant Biotechnology 15: 45~48). To amplify the DNA region including the autonomous transposon gene (SEQ. ID NO.: 2), we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 24 and 25, located at the adjacent upstream and downstream, respectively, of the target DNA region (SEQ. ID NO.: 2), as primers.

Each reaction mixture (100 μ l) contained 200 ng of DNA, 2.5 units of TAKARA LA TAQ® (Takara Co.), 10 μ l of 10×LA PCR BUFFER II®, 6 μ l of 25 mM MgCl₂, 8 μ l of dNTP mixture (2.5 mM each dNTP) and 100 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 94° C. for 30 sec and an extension step

at 68° C. for 12 min. This cycle was repeated 35 times. After the reaction the PCR products were separated on 0.8% LO3 AGAROSE® (Takara Co.) gel electrophoresis. As shown the results in FIG. 15, we confirmed the transduction of the autonomous transposon gene of Nihonbare in hygromycin-resistant calli from Taichung No. 65.

Then to amplify the DNA region including the nonautonomous transposon gene by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 18 and 19 (L02), SEQ. ID NO.: 20 and 21 (L06) and SEQ. ID NOS.: 22 and 23 (L07) as primers. As PCR reaction mixture, we used HOTSTARTAQ MASTER MIX KIT® (QIAGEN). Each cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 55° C. for 1 min and an extension step at 72° C. for 2 min. This cycle was repeated 45 times. After the reaction, DNA was separated on 2% LO3 AGAROSE® (Takara Co.). We examined the possibility of deletion of the nonautonomous transposon gene for 38 calli, wherein the autonomous transposon gene was transduced, and obtained a DNA band (shown by an arrow), suggestive of deletion of the nonautonomous transposon gene in L06 gene locus (FIG. 16). The frequency of deletion of the nonautonomous transposon gene in L02, L06 and L07 gene loci was around 0~5.3% (Table 2, the 2nd line).

The DNA fragments suggestive of the deletion of nonautonomous transposon gene in L02 and L06 gene loci were recovered from the gel and subcloned into plasmids pCRII-TOPO using TA CLONING KIT® using a TA cloning kit (In Vitrogen). The nucleotide sequences of the clones obtained were determined by 310 DNA SEQUENCER® (ABI Co.). There was no nonautonomous transposon gene in these clones (FIGS. 17 and 18).

These result show that transposition of the nonautonomous transposon gene was induced in anther-derived calli originated from Taichung No. 65, wherein the autonomous transposon gene of Nihonbare was transduced.

On the basis of these results, we can conclude that the transposon gene expressed by SEQ. ID NO.: 2 not only transposes autonomously in anther-derived calli but also regulates the transposition of the nonautonomous transposon gene.

EXAMPLE 9

In this example, we examined the transposition activity of the nucleotide sequence of SEQ. ID NO.: 3 in anther-derived calli and in scutellum-derived calli treated with 5-azacytidine.

Spikes of Nihonbare were harvested at pre-emergence, kept in cold treatment for 10 days at 10° C., sterilized in 1% sodium hypochlorite solution for 1 min and washed with sterilized water. Then, anthers were picked out from the floret, seeded in a 35×10 mm Petri dish (CORNING Co.) containing 3 ml of liquid medium at the rate of 50 anthers per dish and subjected to induction culture under light for 24 h at 30° C. Used was a liquid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 30 g of sucrose (Wako) in 1 L of medium. After 3~4 weeks of inductive culture, the calli derived from induced anthers were transplanted to a 90×20 mm of Petri dish (Iwaki Co.) containing 20~30 ml of medium and subjected to growth culture under light for 24 h at 30° C. Used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLU-

21

TION® (Sigma Co.), 2 ml of α -naphthalene acetic acid solution (Sigma Co.), 2 ml of kinetin solution (Sigma Co.), 3 g of casamino acids (DIFCO), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. DNA was extracted from calli derived from anthers in growth culture for 2 weeks according to the method described (Kikuchi et al. (1998) Plant Biotechnology 15: 45–48).

Seeds of Nihonbare, a rice cultivar, were sterilized in 3% sodium hypochlorite solution for 15–30 min, washed with sterilized water, inoculated on a 90×20 mm of Petri dish (Iwaki Co.) with 20–30 ml of media at the rate of 9 seeds per dish and subjected to induction culture under light for 24 h at 30° C. Used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. On the 10th day of inductive culture, the calli derived from induced seeds were transferred to a growth medium supplemented with 5-azacytidine (Sigma) at 0 mM, 0.1 mM or 0.5 mM and were subjected to growth culture under light for 24 h at 30° C. Used was a solid medium consisting of 4 g of CHU (N6) BASAL SALT MIXTURE® (Sigma Co.), 1 ml of MS VITAMIN SOLUTION® (Sigma Co.), 2 mg of 2,4-dichloro-phenoxyacetic acid (Sigma Co.), 0.3 g of casamino acids (DIFCO), 0.1 g of myo-inositol (Sigma Co.), 2.878 g proline (Wako), 30 g of sucrose (Wako), 2 g of GELRITE® (Wako) in 1 L of medium. DNA was extracted from calli derived from seeds in growth culture for two weeks by DNEASY PLANT MINI KIT® (Qiagen).

To amplify the DNA region containing the transposon DNA by PCR, we used the oligonucleotides comprising the sequences of SEQ. ID NOS.: 26 and 27 as PCR primers. Each reaction mixture (100 μ l) contained 200 ng of DNA, 2.5 units of TAKARA LA TAQ® (Takara Co.), 10 μ l of 10×LA PCR bufferII, 6 μ l of 25 mM MgCl₂, 8 μ l of dNTP Mixture (2.5 mM each dNTP) and 100 pmol of primers. Each cycle of the polymerase reaction consisted of a denaturation step at 94° C. for 30 sec and an extension step at 68° C. for 12 min. This cycle was repeated 35 times. After the reaction, PCR products were separated on 0.8% LO3 AGAROSE® (Takara Co.) gel electrophoresis.

It was found that the transposon gene with the nucleotide sequence of SEQ. ID NO.: 3 was not transposed in anther-

22

derived calli and in scutellum-derived calli, but high frequency of transposition was taken place in scutellum-derived calli treated with 5-azacytidine (Table 3).

TABLE 3

	The frequency of deletion
Anther-derived callus (Nihonbare)	0/64 (0%)
Scutellum-derived callus (Nihonbare)	0/64 (0%)
0 mM 5-azacytidine	0/8 (0%)
0.1 mM 5-azacytidine	2/8 (25%)
0.5 mM 5-azacytidine	7/8 (87.5%)

The transposon gene with the nucleotide sequence of SEQ. ID NO.: 3 has the structure of an autonomous transposon gene with a coding sequence of a transposase, can be activated by the treatment with 5-azacytidine and may control the transposition of nonautonomous transposon gene (SEQ. ID NO.: 1).

EXAMPLE 10

DNA was extracted from mature leaves of Kasarasu, a cultivar of rice, by DNEASY PLANT MINI KIT® (QIAGEN). To amplify the DNA region adjacent to the inserted transposon gene by PCR, we used an inverse PCR method. We used the oligonucleotide comprising the sequence (5'-CCATTGTGACTGGCC-3') (SEQ. ID NO.: 29) of 15 bases from the 5'-end of SEQ. ID NO.: 1 as a primer for inverse PCR. GENEAMP9600 SYSTEM® (ABI Co.) was used for PCR. HOTSTARTAQ MASTER MIX KIT® (QIAGEN) was used for PCR reaction solution. Each cycle of the polymerase reaction consisted of a denaturation step at 96° C. for 30 sec, an annealing step at 44–58° C. for 1 min and an extension step at 72° C. for 1 min. This cycle was repeated 45 times. After the reaction, PCR products were separated on 2% LO3 AGAROSE® (Takara Co.) gel electrophoresis. The amplified DNA fragments were subcloned into a plasmid PCRII-TOPO USING TA CLONING KIT® (In Vitrogen). The nucleotide sequence of the obtained clone was determined by 310 DNA SEQUENCER® (ABI Co.).

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 27

<210> SEQ ID NO 1

<211> LENGTH: 430

<212> TYPE: DNA

<213> ORGANISM: *Oryza sativa*

<400> SEQUENCE: 1

```

ggccagtcac aatgggggtt tcactggtgt gtcatgcaca tttaataggg gtaagactga      60
ataaaaaatg attatttgca tgaaatgggg atgagagaga aggaaagagt ttcacacctg      120
tgaaaactcgt cagcgtcggt tccaagtcct cggtaacaga gtgaaacccc cgttgaggcc      180
gattcgtttc attcaccgga tctcttgcgt ccgcctccgc cgtgcgacct ccgcattctc      240

```

-continued

ccgcgcccgcg ccggatcttg ggtacaaatg atcccagcaa cttgtatcaa ttaaatgctt	300
tgcttagtct tggaaacgtc aaagtgaac cctccactg tggggattgt ttcataaaag	360
atctcatttg agagaagatg gtataatatt ttgggtagcc gtgcaatgac actagccatt	420
gtgactggcc	430

<210> SEQ ID NO 2
 <211> LENGTH: 5341
 <212> TYPE: DNA
 <213> ORGANISM: Oryza sativa

<400> SEQUENCE: 2

ggccagtcaac aatggagggt tcaactggtg gtcactgcaca tttaataggg gtaagactga	60
ataaaaaatg attatctgca tgaatgggg atgagagaga aggaaagagt ttcactctgg	120
tgaaactcgt cagcgtcgtt tccaagtcct cggtaacaga gtgaaacccc cgttgaggcc	180
gattcgttcc attcaccgga tctcttgctg ccgcctcccg cgtgcgacct ccgcattctc	240
ccgcgcccgcg ccgcgccacg cctccttccc gcgtgaacat tcctccttcc cgcgcgagcg	300
attccacat ctccccgctc cggcgcctac ggagtacacc gcaaccggtc gcccaatcc	360
ggcgcctaga ccgtgaccca cccgccatct tccgcaagac cgaatcccca acccaccac	420
catcttccgc cgcctccgct cccgtccccg gccatggatc cgtcgcgggc cgtggatccg	480
tcgcccggccg tggatccgct gccggctgct gaaacccggc ggctgcaac cgggaaagga	540
ggcaaacagc gcgggggcaa gcaactagga ttgaagaggc cgcgcgggat ttctgtcccg	600
gccaccccgct ctcctgctgc gacgtcttca tcccctgctg cgcgcgagcc catcccacca	660
cgaccaccgc aatcttcgct gattttctgc cccgattcgc cgaatccgct accggctgcg	720
ccgacctcct ctcttgcttc ggggacatcg acggcaagc caccgcaacc acaaggagga	780
ggatggggac caacatcgac catttcccca aactttgcat ctttctttgg aaaccaacaa	840
gacccaaatt catggtacat gtatcttctt ctttttctgt tactttcaac ctacggtaac	900
tctaattcat ggatgagact actgccattg tgcagttcaa tgcttttctt tcatgttata	960
tttctgcag ctgtgagtta tggtttgaag attgctgtgg ttgtttcatt gctgagtatg	1020
tgaaagatag atggatgaaa gagagaatta tatttttagt tgtaactctg ctcatccagt	1080
tgctcatgta tgacctgggt tctagaatgt tgccctgact gtatgcttaa tgttcagaga	1140
agtgatgcct aaagcagtgat gatcagtggt atcagattag ctatcgacat ataatttag	1200
ctatctcagt tgtgaaagag agatgggtga aaaggcacc cttggattaa ttctgtagta	1260
tcaaatctg cacctgtct gtcctatgt tctgcttgggt tgggtgggtgc agtgcatttg	1320
taaaaaatag tttgctctg atcottaata tatgtaacag ggaatgaatt ttcaccatc	1380
tcagttgtaa aggtactgct ttgctatgca atatgtgtaa attgacaaac ctgaaaatag	1440
tctgtttgga atttgcaaaa gcaattcgat agtttggaa ttccaaacct cagtcagcag	1500
taggcaatcc attttagttc ttgctatgca caaaaacagt acacctgata tgctcatttt	1560
aatacaactt tttgtctct gttacagttt ggtcaggggt tatcctccag gagggtttgt	1620
caatcttatt caaaaaatt gtcgcccga gccacaacg caaggtgaaa attttcattt	1680
cgttggtcac aatatgggat tcaacccaat atctccacag ccaccaagtg cctacggaac	1740
accaacaccc caagctacga accaaggcac ttcaacaac attatgattg atgaagagga	1800
caacaatgat gacagtaggg cagcaagaa aagatggact catgaagag aagagagact	1860
ggtattcatc ggatactttt acatttccat atgtctttgt tttgactaat acttgacagg	1920

-continued

tcattaactg attctttag gccagtgcct ggttgaatgc ttctaaagac tcaattcatg	1980
ggaatgataa gaaaggtgat acattttgga aggaagtcac tgatgaattt aacaagaaag	2040
ggaatggaaa acgtaggagg gaaattaacc aactgaaggt tcaactggta aggttgaagt	2100
cagcgatctc tgagtccaat gactattgga gtacgggtac tcaaatgcac acaagcggat	2160
actccgacga catgcttgag aaagaggcac agaggctgta tgcaaacagg tttggaaaac	2220
cttttgcggtt ggtccattgg tggaagatac tcaaagatga gcccaaatgg tgtgctcagt	2280
ttgaatcaga gaaagacaag agcgaaatgg atgctgttcc agaacagcag tcacgtccta	2340
ttggtagaga agcagcaaaag tctgagcgca atggaaagcg caagaaagaa aatggtatgg	2400
aaggcattgt cctcctaggg gacaatgtcc agaaaattat aaaggtccac gaagaccgga	2460
gggtggatcg tgaaaaggcc accgaagcac agattcagat atcaaatgca acattgttgg	2520
ccgctaagga gcagaaggaa gcaaagatgt tcgatgtgta caaactccta ttaagtaagg	2580
atacaagcaa catgtctgaa gatcaaatgg ctagccacca gagggcaata cggaaattag	2640
aggagaagct atttgcggtt taaggtgagt ttataaaact gaccactatt ttctgaaatg	2700
tatgaattct gaaatttata tacaattgtg taaacatgga aaattagata atgtatgcat	2760
gatgcacaac atgtgctgac agcactatct aatggcagtt tcacaagtgt gaaaactgac	2820
cactatagta ctattgtggt gtgaaaactg accactacta ttgtggtgtg aatgctactg	2880
tggtgtgaaa actgaccact atagtttcac attcctggat gcagccctcc tctatatata	2940
tagatacagt cctcatctct tcctggcata cacacagccc tcttctctaa ttcttgagc	3000
cagtcctcat ctcttctctg catagacgca gcccttctct cttcctgttt agttcaaaa	3060
cattgaggtg atctgccttt ctttgaagtt tctatctttt ttcactgctg tgaatgatta	3120
tttctctgct gtgaatgatt atttctccaa tcttctcttg ttcaccttct ctctttctct	3180
gctgtgaaga tgtctggaaa tgaaaatcag attcctgtgt ccttgttggc cgagtttctc	3240
gctgaggatg agatcatgga tgagataatg gatgatgttc tccatgaaat gatggtgta	3300
ttgcagtcct ccatcggaga tcttgaaga gaggctgctg accatcgttt gcatccaagg	3360
aagcacatca agaggccacg agaggaagca catcaaaatt tggatgaatga ttatttctct	3420
gaaaatcctc tataatcttc caatattttt cgccgaagat ttcgatgta caggccgctg	3480
tttttacgta ttgtggagcg attaggccag tggtcagatt actttactca gagggtagat	3540
gcoctggtga ggcaagggtc tagtccatta caaaagtgtc ctgcagcaat tcgccaattg	3600
gctactggtg gtggtgctga tgaactagat gagtatttga agattggaga gactactgct	3660
atggatgcta tgaaaaattt tgtgaaagga attagagaag tatttgggtga aagatatctc	3720
aggcgtccca ctgtagaaga tactgaacga ctactcagac ttggtgagag acgcggtttt	3780
cctggtatgt tcggtagcat tgactgtatg cattggcaat gggaaaggtg cccaactgcy	3840
tggaagggtc agttcactcg tggatgataa aaagtgccaa cgctgattct tgaggcagtg	3900
gcatcacatg atctttggat ttggcatgcy tcttttgag tagcaggttc taacaatgat	3960
atcaatgttt tgagccgacg tactgtgttt atcaatgagc tgaaaggaca agctcctaga	4020
gtgcagtaca tggtaaatgg gaatcaatac aacgaaggtt attttcttgc tgatggaatt	4080
taccctgaat ggaaggtatt tgctaagtca tatcgactcc ctatcactga gaaggagaag	4140
ttgtatgcac aacatcaaga aggggcaaga aaggatctcg agagagcatt tgggtgttcta	4200
caacgtcgat tctgcatctt aaaacgacca gcccgctctat atgaccgaggtgtactcct	4260
gatgttgcct taggttgcac catacttcac aatgatgatg ttgaagatga gaaggaagcg	4320

-continued

cgacttattg aagaaaatct agatttaa	at agcctgcta gttcatcaac ggttcaggca	4380
ccagaattct ctcctgacca gcatgttcca	ttagaaagaa ttttagaaaa ggatactagt	4440
atgagagatc gtttggctca tcgccgactc	aagaatgatt tggatggaaca tatatggaat	4500
aagtttggtg gtggtgcaca ttcattctgt	aattatgttt ttatttttga ttattagtta	4560
tctatggtac taagatatgt acaagtttct	ctaaattgca ctaaactctgt ggttcatatt	4620
ggatatgtgt aaactatgaa tgtagcctga	ctaaaacat cattcatgct gaactggttt	4680
ttgttttga tatgcaggat gaaacaagga	actaggttcc tgaacgcatt acggactgaa	4740
ggttgagggg cagaatgac caccagttg	cttctatcag atcactaaag ttccatttca	4800
ctgttttatt ttggacactt gatgcttgtg	tgcacccgat gaatgtttaa ttgggtcacc	4860
tgatgcttgt gtgcatccga tgaatgttta	atttggtcac ctgatgcttg tatgcagtta	4920
tctatcttat ttgttaatgt tgctgttact	gaggattttt agaagtgaaa tgcacaagtt	4980
gctgtgtttt ttgactgatc cttgtgtgca	cttgacgttg tatgtgacaa atgatggttc	5040
ccagttgtgc acctgattca tgattcagtt	atccagttta aattgacgtt gtttgtgtgc	5100
accttttgc agttagccag ttacggctgg	aagttgtgta agtttgtgtg acgcctggct	5160
acaggatttt gggtaacaat gatcccagca	acttgtatca attaaatgct ttgcttagtc	5220
ttggaacgtc caaagtgaaa cccctccact	gtggggattg ttccataaaa gatttcattt	5280
gagagaagat ggtataatat tttgggtagc	cgtgcaatga cactagccat tgtgactggc	5340
c		5341

<210> SEQ ID NO 3

<211> LENGTH: 5166

<212> TYPE: DNA

<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 3

ggccagtcac aatgggtggt tcatttgagt	gtcatgcgca ttttaatacag tgacaagtca	60
gcaaaagagc aatatttga tgaatgggt	aggagagaga gtaaactcgt ttccatagc	120
tgacaagaga tagcgcggtt tcccaggctc	ctgaaacggg gtgaaacagc attgagagtt	180
catcgtttca cctccgggat cccgtgcgag	cgctgctctt cgccatcttc gcgcgcatcg	240
ccggattctt cccgcgcgag tccccatct	tcccgcgcag cacctccatg ttcccgccc	300
caaagcactg gctcgaagct tttttcccca	atctcacctg caaccctagc gccagactca	360
gtccccatcg ccccgctcgt ccataaccct	agcgcaagaa ccacgagcgg agattgcgga	420
gctggatcca caagtaggtg gtgaatcctg	tccatctgcc gccgtccgcc gtccagcagc	480
catggatcca caaggaggtg gtggatcccg	tctgagcgcc gccggcagag gagggaataa	540
gcgtgggggc aagcagctgg gcctgaagag	gtcgtcggcg cctgctccat caccggcaac	600
agctcagcca ccgctgcctg caagttcccc	tectgaagct ccatgcgagg caacagttca	660
gcgcctact ccatogtcaa gtcctgctgt	tgetgcccc agttcatccc ctgctgtacc	720
gatgtcaacc atgccccat ggccaccgca	aggagcagga tggggctctg taccocccaa	780
ttttgctttt ctgcaaggaa accaacaagg	cccaagtcca tggatatttc tccttgtcac	840
agattattca ctgtacacta tgatacatga	tatgaactct ttcttcatgc attagtaatt	900
agttcctggt tatgctcaat gaaattgtt	agaatcagta tgtcagtaca ttggtaattt	960
gatatatgcc tgagtaatga atagaaaaa	tgtagtattc agtatggatt gcagtaatac	1020
ttgttagtg aaaattcagt atccagtatg	cagtatggat tgcggcttgt ataacagaaa	1080

-continued

ttgaaagcaa aagattcagt ttgcaatctg gacagtgtag tgtacaacat gtaattcaca	1140
tacgtaaagc ttgttaaata tctccttgtc agtacattgg taacaaatgc tttgagtgtg	1200
aatgccaagg gtatcatcct aacattggta tatatTTTTA gccttctgta tggaatgcag	1260
acatggctct ctttgcaacc acagcaacag cttgccctac actctgtgct gtcgtcatag	1320
ctaaccAAAT aacctgtag tactgatata tatggctctc tttgcaacca cagcaacagc	1380
ttgccctaca tggctctctg tatgcttgac taaacttggt acttgacata tatgcttgac	1440
tgaacttggt gcttgactga attattcctt acacatactg tagtacttgc ttgactgaac	1500
tatgtcagga tcttattaaa aaaaatctat gtcagcactg ctactatgct aggatcatca	1560
gtatgatgct taagtaacct gttagtagt cagtacttac tatgtcagga tcatcttctg	1620
gaacttacta tgtttgatTT tcttatgctg ccatacggtt caattggatt tgcttcttat	1680
gttttcaggt tgtatcctac agaaggcttc gtaaattttc tccaacagaa ctgtctgccg	1740
cagccacaag aaggTgaaaa ttttcacctt gttggtcaga ctaccaacac aatgtctact	1800
ccaccaccaa caccCCAAGC tgcagctaac aatacagtcc aaattgatat tcatgaagat	1860
gcaatcaatg atgcaagtgc taaaagaga agtttgagat attggactca tgatgaggaa	1920
gagagattgg ctagtgtctg gttgaatgct tctaagatc ccattcatgg gaatgaaaag	1980
aaagtgata cgTTTTGgaa agaggttact gatgagttca acagaaaagg gaatgggaa	2040
cgtaCaaggg aaataaatca attgaagggt cattggtcac gcctcaaatc atcgattgga	2100
gaattcaatg attactggac taaggtaact caaatgaata caagcggata tgacgatgac	2160
atgctggaga aggaggcaca acagatgtat gcaatacat ttggaaagcc ttttgcactt	2220
gtgcattggt ggaagatact gagaaaagag cccaagtggg gtgcaatgat tgagaaggac	2280
aaaaacaagg ctgaagtggg tgatattcca gatgaacaaa agcgtcccat tggtagagaa	2340
gcagcacaag ccgagcgcaa tggaaaacgc aagaaggaca gtatgtcaga aggaattgtc	2400
atcctagggg acaatattga aaaaattatc aaagtgacgc aagatcggaa gctggagcgt	2460
gagaaggTca ctgaagcaca gattcacatt tcaaacgtaa atttgaaggc agcagaacag	2520
caaaaagaag caaagatggt tgaggTatac aattcctctg tcaactcaaga tacaagtaac	2580
atgtctgaag aacagaaggc tcgccgagac aaggcattac aaaagctgga ggaagttta	2640
tttgctgact aaggttagat atctaattca atctgagctg cactattatt tataataatt	2700
aaagaatgct gcaatattta gttatattgt ctgtatatct gtgctgact atgcagtca	2760
ctgcatatca cgaatttgct aaatctgagc tgcatatctg tgaatggTgc aatatttagt	2820
tatattaatt acccagtgTg aatgatgtat tgctgtcagt ttcacatata gtatgaatgc	2880
tgcactatgc agtcagtttc acatgcagtg tgaatgctgc actaggcagt cagtTtoca	2940
tgcaGtgggc gcctatttat gcagagttta gccatctctc tactcctctc agaaactcat	3000
tccctctttt ctcatagcaa gacctcctcc cttttatctt tactgtttct ctcttcttca	3060
aagatgtctg agcaaaatc tgatggagT caagttccag tgaacttggt ggatgagttc	3120
ctggctgagg atgagatcat agatgatctt ctcaactgaag ccacggTggg agtacagtcc	3180
actatagaag gtcttcaaaa cgaggcttct gaccatcgac atcatccgag gaagcacatc	3240
aagaggccac gagaggaagc acatcagcaa ctagtgaatg attacttttc agaaaatcct	3300
ctttaccctt ccaaaatttt tcgtcgaaga ttctgtatgt ctaggccact ttttcttcgc	3360
atcgTtgagg cattaggcca gtggctcagTg tatttcaacac aaagggtgga tgctgttaat	3420
cggaaaggac tcagTccact gcaaaagTgt actgcagcta ttcgccagTt ggctactggt	3480

-continued

```

agtggcgag atgaactaga tgaatatctg aagataggag agactacagc aatggaggca 3540
atgaagaatt ttgtcaaagg tcttcaagat gtgtttggtg agaggtatct taggcgcccc 3600
accatggaag ataccgaacg gcttctccaa cttggtgaga aacgtggttt tcctggaatg 3660
ttcggcagca ttgactgcat gcaactggcat tgggaaagat gccagtagc atggaaggg 3720
cagttcactc gtggagatca gaaagtgcc accctgattc ttgaggctgt ggcatcgcat 3780
gatctttgga tttggcatgc attttttggg gcagcgggtt ccaacaatga tatcaatgta 3840
ttgaaccaat ctactgtatt tatcaaggag ctcaaaggac aagctcctag agtccagtac 3900
atggtaaatg ggaatcaata caatactggg tattttcttg ctgatggaat ctaccctgaa 3960
tgggcagtgt ttgttaagtc aatcagctc ccaaacactg aaaaggagaa attgtatgca 4020
gatatgcaag aaggggcaag aaaagatata gagagagcct ttggtgtatt gcagcgaaga 4080
ttttgcatct taaaacgacc agctcgtcta tatgatcgag gtgtactgag agatgttgtt 4140
ctagcttgca tcatacttca caatatgata gttgaagatg agaaggaaac cagaattatt 4200
gaagaagatt tagatctaaa tgtgcctcct agttcatcaa ccgttcagga acctgagttc 4260
tctcctgaac agaacacacc atttgataga gttttagaaa aagatatttc tatccgagat 4320
cgagcggctc ataaccgact taagaaagat ttggtggaac acatttgga taagtttgg 4380
ggtgctgcac atagaactgg aaattgagaa tcagtaaatg taattatttt atttttcttg 4440
taatttatat atctatggtc cacttgtaaa tttctgaatg ctcatcgcca tttttttaa 4500
tctctgcagg ttccaatcta tttacagggt ccctaaaaaa aaatctattt gcaggttcca 4560
gtctgtgtgc ttcacaatgt aagttctgag aatcaaatca ctatgttttt ctcttttttg 4620
gtagctacag ggtgttagaa catgtgttat tttctttact atgcaattgt gatcctccaa 4680
tatttatcta ctgcatgtgt aaacctgttt gtcatgtctg aactactttc atttgtacag 4740
ggtgaaagaa tcaatgaaat ctatgggtgc atcgtcaatt tgcctccagt tacctgcttg 4800
tcatcgtcat ttgtagctta gttctgtcat atttcacctc gagttaacat ctattcagtt 4860
atctaaactt tgctatgtag tgaacttgg  tgaatggca tttaaattta tcaagtgaac 4920
aatcgtacct atctgtgctg aatgcatgta ttttgttttg tgttcaagtg gctacacag 4980
tttgtgttac atacgatccc actatgtggc tggaaataaa tgccttgaat ttgcattgga 5040
aacgctagag tgaacacag cattgagaag gtctgtttca ttgtacgttt caactgttt 5100
catcttcggt tcagctgatg tggcgtctgg gaaacagtgt aatgaaacac tgcattgtga 5160
atggcc 5166

```

```

<210> SEQ ID NO 4
<211> LENGTH: 455
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa

```

```

<400> SEQUENCE: 4

```

```

Met Ser Gly Asn Glu Asn Gln Ile Pro Val Ser Leu Leu Asp Glu Phe
1           5           10          15

Leu Ala Glu Asp Glu Ile Met Asp Glu Ile Met Asp Asp Val Leu His
20          25          30

Glu Met Met Val Leu Leu Gln Ser Ser Ile Gly Asp Leu Glu Arg Glu
35          40          45

Ala Ala Asp His Arg Leu His Pro Arg Lys His Ile Lys Arg Pro Arg
50          55          60

```

-continued

Glu Glu Ala His Gln Asn Leu Val Asn Asp Tyr Phe Ser Glu Asn Pro
 65 70 75 80
 Leu Tyr Pro Ser Asn Ile Phe Arg Arg Arg Phe Arg Met Tyr Arg Pro
 85 90 95
 Leu Phe Leu Arg Ile Val Asp Ala Leu Gly Gln Trp Ser Asp Tyr Phe
 100 105 110
 Thr Gln Arg Val Asp Ala Ala Gly Arg Gln Gly Leu Ser Pro Leu Gln
 115 120 125
 Lys Cys Thr Ala Ala Ile Arg Gln Leu Ala Thr Gly Ser Gly Ala Asp
 130 135 140
 Glu Leu Asp Glu Tyr Leu Lys Ile Gly Glu Thr Thr Ala Met Asp Ala
 145 150 155 160
 Met Lys Asn Phe Val Lys Gly Ile Arg Glu Val Phe Gly Glu Arg Tyr
 165 170 175
 Leu Arg Arg Pro Thr Val Glu Asp Thr Glu Arg Leu Leu Glu Leu Gly
 180 185 190
 Glu Arg Arg Gly Phe Pro Gly Met Phe Gly Ser Ile Asp Cys Met His
 195 200 205
 Trp Gln Trp Glu Arg Cys Pro Thr Ala Trp Lys Gly Gln Phe Thr Arg
 210 215 220
 Gly Asp Gln Lys Val Pro Thr Leu Ile Leu Glu Ala Val Ala Ser His
 225 230 235 240
 Asp Leu Trp Ile Trp His Ala Phe Phe Gly Val Ala Gly Ser Asn Asn
 245 250 255
 Asp Ile Asn Val Leu Ser Arg Ser Thr Val Phe Ile Asn Glu Leu Lys
 260 265 270
 Gly Gln Ala Pro Arg Val Gln Tyr Met Val Asn Gly Asn Gln Tyr Asn
 275 280 285
 Glu Gly Tyr Phe Leu Ala Asp Gly Ile Tyr Pro Glu Trp Lys Val Phe
 290 295 300
 Ala Lys Ser Tyr Arg Leu Pro Ile Thr Glu Lys Glu Lys Leu Tyr Ala
 305 310 315 320
 Gln His Gln Glu Gly Ala Arg Lys Asp Ile Glu Arg Ala Phe Gly Val
 325 330 335
 Leu Gln Arg Arg Phe Cys Ile Leu Lys Arg Pro Ala Arg Leu Tyr Asp
 340 345 350
 Arg Gly Val Leu Arg Asp Val Val Leu Gly Cys Ile Ile Leu His Asn
 355 360 365
 Met Ile Val Glu Asp Glu Lys Glu Ala Arg Leu Ile Glu Glu Asn Leu
 370 375 380
 Asp Leu Asn Glu Pro Ala Ser Ser Ser Thr Val Gln Ala Pro Glu Phe
 385 390 395 400
 Ser Pro Asp Gln His Val Pro Leu Glu Arg Ile Leu Glu Lys Asp Thr
 405 410 415
 Ser Met Arg Asp Arg Leu Ala His Arg Arg Leu Lys Asn Asp Leu Val
 420 425 430
 Glu His Ile Trp Asn Lys Phe Gly Gly Gly Ala His Ser Ser Gly Asn
 435 440 445
 Tyr Val Phe Ile Leu His Tyr
 450 455

-continued

```

<210> SEQ ID NO 5
<211> LENGTH: 482
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 5

Met Gln Ser Leu Ala Ile Ser Leu Leu Leu Ser Glu Thr His Ser Leu
1          5          10          15

Phe Ser His Thr Lys Thr Ser Ser Leu Leu Ser Leu Leu Phe Leu Ser
          20          25          30

Ser Ser Lys Met Ser Glu Gln Asn Thr Asp Gly Ser Gln Val Pro Val
          35          40          45

Asn Leu Leu Asp Glu Phe Leu Ala Glu Asp Glu Ile Ile Asp Asp Leu
          50          55          60

Leu Thr Glu Ala Thr Val Val Val Gln Ser Thr Ile Glu Gly Leu Gln
          65          70          75          80

Asn Glu Ala Ser Asp His Arg His His Pro Arg Lys His Ile Lys Arg
          85          90          95

Pro Arg Glu Glu Ala His Gln Gln Leu Val Asn Asp Tyr Phe Ser Glu
          100         105         110

Asn Pro Leu Tyr Pro Ser Lys Ile Phe Arg Arg Arg Phe Arg Met Ser
          115         120         125

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

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

Leu Gln Lys Cys Thr Ala Ala Ile Arg Gln Leu Ala Thr Gly Ser Gly
          165         170         175

Ala Asp Glu Leu Asp Glu Tyr Leu Lys Ile Gly Glu Thr Thr Ala Met
          180         185         190

Glu Ala Met Lys Asn Phe Val Lys Gly Leu Gln Asp Val Phe Gly Glu
          195         200         205

Arg Tyr Leu Arg Arg Pro Thr Met Glu Asp Thr Glu Arg Leu Leu Gln
          210         215         220

Leu Gly Glu Lys Arg Gly Phe Pro Gly Met Phe Gly Ser Ile Asp Cys
          225         230         235         240

Met His Trp His Trp Glu Arg Cys Pro Val Ala Trp Lys Gly Gln Phe
          245         250         255

Thr Arg Gly Asp Gln Lys Val Pro Thr Leu Ile Leu Glu Ala Val Ala
          260         265         270

Ser His Asp Leu Trp Ile Trp His Ala Phe Phe Gly Ala Ala Gly Ser
          275         280         285

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

Leu Lys Gly Gln Ala Pro Arg Val Gln Tyr Met Val Asn Gly Asn Gln
          305         310         315         320

Tyr Asn Thr Gly Tyr Phe Leu Ala Asp Gly Ile Tyr Pro Glu Trp Ala
          325         330         335

Val Phe Val Lys Ser Ile Arg Leu Pro Asn Thr Glu Lys Glu Lys Leu
          340         345         350

Tyr Ala Asp Met Gln Glu Gly Ala Arg Lys Asp Ile Glu Arg Ala Phe
          355         360         365

Gly Val Leu Gln Arg Arg Phe Cys Ile Leu Lys Arg Pro Ala Arg Leu
          370         375         380

```

-continued

Tyr	Asp	Arg	Gly	Val	Leu	Arg	Asp	Val	Val	Leu	Ala	Cys	Ile	Ile	Leu
385					390					395					400
His	Asn	Met	Ile	Val	Glu	Asp	Glu	Lys	Glu	Thr	Arg	Ile	Ile	Glu	Glu
			405						410					415	
Asp	Leu	Asp	Leu	Asn	Val	Pro	Pro	Ser	Ser	Ser	Thr	Val	Gln	Glu	Pro
			420					425					430		
Glu	Phe	Ser	Pro	Glu	Gln	Asn	Thr	Pro	Phe	Asp	Arg	Val	Leu	Glu	Lys
		435					440					445			
Asp	Ile	Ser	Ile	Arg	Asp	Arg	Ala	Ala	His	Asn	Arg	Leu	Lys	Lys	Asp
	450					455					460				
Leu	Val	Glu	His	Ile	Trp	Asn	Lys	Phe	Gly	Gly	Ala	Ala	His	Arg	Thr
465				470						475					480

Gly Asn

<210> SEQ ID NO 6
 <211> LENGTH: 900
 <212> TYPE: DNA
 <213> ORGANISM: Oryza sativa

<400> SEQUENCE: 6

tttcaagtac aatctcaact tagggaaagt tgtgattgag ggaggatggt agataatggt	60
agttagtttg ttatagagat agattagttc tgttaccgca tgtactttct tgtatctatc	120
tctatatcca ggattgtctc aggttgttga gattaatcct atcctttgta cacgccacgg	180
tagaggctct ttctgcctat atcaacaaag gtgcggcccc gtaaaggggt tcaacgcttc	240
tcattccggt ttacaatcct ccttcttctc cctggtgttg gaaattcgtt gatcgagttg	300
aaactctcat ccttcatcat gtgctgcaga aactaacgcg tgcacagatg atggatgggt	360
gtggtgtgac atgaaagtgg atcaatgaca cgcggcacat ttaggggagt gtgtcgtgtc	420
ttgacttctt catgcaaaag tataccaacc ctgtataagg ccagtcacaa tggctagtgt	480
cattgcacgg ctacccaaaa tattatacca tcttctctca aatgaaatct tttatgaaac	540
aatccccaca gtggaggggt ttcactttga cgtttccaag actaagcaaa gcatttaatt	600
gatacaagtt gctgggatca tttgtaccca aaatcggcgg cggcgcggga gaatgcggag	660
gtcgcacggc ggaggcggac gcaagagatc cgggtaatga aacgaatcgg cctcaacggg	720
ggtttcactc tgttaccgag gacttggaaa cgacgctgac gagtttcacc aggatgaaac	780
tctttccttc tctctcatcc ccatttcatg caaataatca tttttattc agtcttacc	840
ctattaatg tgcatgacac accagtgaaa cccccattgt gactggccta agcatctttg	900

<210> SEQ ID NO 7
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 7

ttaggccagt cacaatgg	18
---------------------	----

<210> SEQ ID NO 8
 <211> LENGTH: 24
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

-continued

<400> SEQUENCE: 8

gtgctgtggtt ggtctcggct ttat 24

<210> SEQ ID NO 9

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 9

cctccttctt cctcctgggtg ttgg 24

<210> SEQ ID NO 10

<211> LENGTH: 28

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 10

gtaagactga ataaaaaatg attatttg 28

<210> SEQ ID NO 11

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 11

catcttctct caaatgaaat ctttta 26

<210> SEQ ID NO 12

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 12

atgtagtttg tcgtaagtt tga 23

<210> SEQ ID NO 13

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 13

atgtgttggtg attgatggga taa 23

<210> SEQ ID NO 14

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 14

gggtgagtga agtgagtgag tgagcagcat 30

-continued

<210> SEQ ID NO 15
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 15

agttagggga ggagagttgg gcataggaat 30

<210> SEQ ID NO 16
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 16

gcctcctgct ggcagctctt cat 23

<210> SEQ ID NO 17
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 17

caactggatg agcaagatta cagactaaaa 30

<210> SEQ ID NO 18
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 18

cagtagccca ccaatcacca tcat 24

<210> SEQ ID NO 19
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 19

ctcatctcga acgcaaccta aata 24

<210> SEQ ID NO 20
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 20

tgtgatgaac agaacaccac cgaga 25

<210> SEQ ID NO 21
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

-continued

<400> SEQUENCE: 21
cccaaagata cagagcacct acaca 25

<210> SEQ ID NO 22
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 22
tgatccagat acaacctcca t 21

<210> SEQ ID NO 23
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 23
gaaaagaaaa acaacaaga a 21

<210> SEQ ID NO 24
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 24
gggtgagtga agtgagtgag tgagcagcat 30

<210> SEQ ID NO 25
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 25
agttagggga ggagagttgg gcataggaat 30

<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 26
cctcacaacc aatccctacc a 21

<210> SEQ ID NO 27
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 27
agccaccaca ataaccaaag t 21

45

The invention claimed is:

1. A method for transposing the transposon gene of rice comprising a nucleotide sequence at least 95% identical to SEQ ID NO: 1 comprising,
culturing anthers of rice, or
treating seeds, leaves, roots, or stems of axillary buds of rice, or a callus derived from them of with 5 azacytidine or 5-azadeoxycytidine.
2. A method for determining the integration site of a transposon comprising a nucleotide sequence at least 95% identical to SEQ ID NO:1, said method comprising the steps

46

- of a) amplifying a segment of genomic DNA utilizing a primer comprising at least 15 contiguous nucleotides of SEQ ID NO:1 for inverse PCR, b) isolating the amplified DNA, and c) determining the sequence of the genomic plant DNA flanking said transposon, thereby determining the integration site of said transposon.
3. The method of claim 2, wherein the plant species is selected from the group consisting of rice, barley, wheat and maize.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,132,587 B2
APPLICATION NO. : 10/494944
DATED : November 7, 2006
INVENTOR(S) : Kazuhiro Kikuchi et al.


Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page, item (73) Assignee should read: --Japan Science and Technology Agency--

Signed and Sealed this

Twenty-ninth Day of May, 2007

A handwritten signature in black ink on a light gray dotted background. The signature reads "Jon W. Dudas" in a cursive style.

JON W. DUDAS
Director of the United States Patent and Trademark Office