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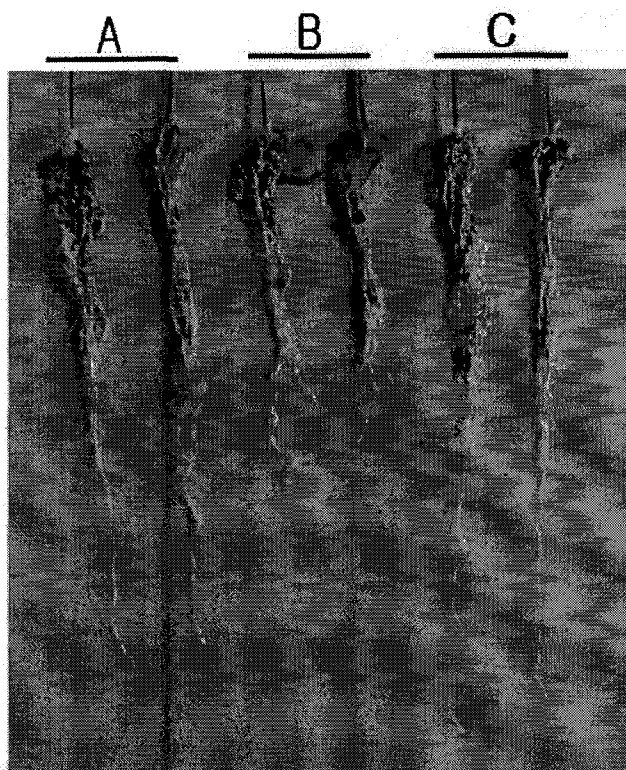
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(54) Title: A NEW GENE CONTROLLING THE DEVELOPMENT AND GROWTH OF ROOT AND ROOT HAIRS, AND A TRANSFORMED PLANT BY THE GENE



(57) Abstract: Disclosed herein are a gene inducing the development of root hairs and a method of using said gene to promote the growth of roots and root hairs in a plant and to increase the nutrient absorption capability of the plant. A protein encoded by the gene is expressed only in the plant roots, a function of inducing the elongation of the plant roots and root hairs, and has an amino acid sequence showing a homology of at least 90% to SEQ ID NO: 1. According to the disclosure herein, the development and growth of the plant roots and root hairs in a plant can be promoted to significantly increase the nutrient absorption capability of the plant.

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【DESCRIPTION】**【Invention Title】**

A NEW GENE CONTROLLING THE DEVELOPMENT AND GROWTH OF ROOT AND ROOT HAIRS, AND A TRANSFORMED PLANT BY THE GENE

【Technical Field】

The present invention relates to a gene inducing the development of root hairs, and a method of using said gene to promote the elongation of roots and root hairs in a plant and to increase the capability to absorb nutrients and water in the plant, as well as a plant produced thereby.

【Background Art】

The primary structure of roots consists of the epidermis, cortex and vascular tissues from the outermost to the innermost. The epidermis consists generally of a single layer of cells. The absorption of water and mineral salts occurs through the epidermis, and most of plants living on the earth develop root hairs to more effectively perform the absorption of water and nutrient elements (e.g., mineral salts) dissolved in water. Such root hairs develop only in a mature portion where the epidermal cell wall is shaped like an elongated cylinder. The absorption of water in roots occurs mainly by osmotic phenomena, which occur because: (1) the activity of water in the soil is higher than that in the epidermal cells, containing various salts, sugars and other organic substances dissolved in water; and (2) the membrane of the epidermal cells is permeable to water, but impermeable to most of substances dissolved in cells. Water and mineral salts dissolved therein is moved across roots from the epidermis to the vascular bundle, and transported to other regions of the plants, so that they are used for the physiological activity of the plants.

Meanwhile, a rice plant is one of the most important crops worldwide, and the entire genomic DNA has been sequenced, and thus many efforts to secure the useful genes thereof in large amounts and to use these genes are

ongoing. Studies associated with the absorption of mineral nutrients in plants have been conducted for a long time in terms of plant cultivation and physiology. However, recently, studies on the use of genes involved in the absorption of mineral nutrients to increase the growth and quantity of crops and to breed crops cultivable even in extremely limited nutrient conditions have been actively conducted worldwide.

It is reported that 37 cellulose synthase-like genes are present in a rice plant (Hazen et al., Plant physiology, 2002, 128:336-340, Cellulose Synthase-Like Genes of Rice). These genes are named cellulose synthase-like genes of rice (Hazen et al. *supra*) on the basis of similarity in the amino acid analysis of the cellulose synthase-like genes. It was reported that *AtCSLD(KOJAK)*, a member of the cellulose synthase-like subfamily D group, is involved in the development of root hairs in *Arabidopsis thaliana*, a dicotyledonous plant, but there is still no report of a gene inhibiting the development of root hairs in dicotyledonous plants. Furthermore, because the root of dicotyledonous plants and the root of monocotyledonous plants are significantly different from each other with respect to the anatomical and morphological structure thereof, the elucidation of the function of specific genes associated with roots in the monocotyledonous plants will provide very important information for the development of roots in monocotyledonous plants.

The present inventors have discovered a mutant showing inhibited development of root hairs during studies on genetic mutants of plants, conducted studies to investigate a gene involved in root hair development in said discovered mutant and isolated said gene. As a result, the present inventors have found that the isolated gene is an *OsSCLD1* (*Oryza sativa* Cellulose Synthase-Like D1) gene, one of four cellulose synthase-like genes belonging to the cellulose synthase-like subfamily D group (Hazen et al, *supra*; classified according to <http://cellwall.stanford.edu/>), and suggested the utility thereof by elucidating the structure and action thereof and the function thereof in a plant, thereby completing the present invention.

【Disclosure】**【Technical Problem】**

Accordingly, it is an object of the present invention to provide a gene promoting the development of root hairs in a plant.

Another object of the present invention is to provide a plant transformed with said gene, which extends the length of hair roots.

Still another object of the present invention is to provide a method for producing a plant transformed with said gene.

【Technical Solution】

To achieve the above objects, the present invention provides a protein, which is expressed only in the root of a plant, has a function of inducing the development of root hairs, and has an amino acid sequence showing a homology of at least 90% to SEQ ID NO: 1, as well as a gene encoding said protein. Said protein and gene according to the present invention may be an *OsCSLD* protein derived from a rice plant, and an *OsCSLD* gene of SEQ ID NO: 2 encoding said protein, respectively.

In another aspect, the present invention provides a vector for the overexpression of root hairs, which contains said gene, and a plant transformed with said vector, which shows the promoted elongation of roots and the overexpression of root hairs.

【Advantageous Effects】

According to the present invention, the function of the *OsCSLD* gene capable of controlling the growth and development of roots and root hairs, which are important in experimental and agricultural terms, was elucidated, and thus a plant having an increased capability to absorb water and nutrients was created.

【Description of Drawings】

FIG. 1 is a conceptual diagram showing the chromosomal structure of a mutant *OsCSLD1::Ds* allele according to the present invention.

FIG. 2 is a photograph of the results of Northern hybridization, which shows the expression pattern of the inventive gene in each tissue of a plant.

FIG. 3 is a photograph of the results of GUS staining, which indirectly shows that the inventive gene is expressed in root hairs.

FIG. 4 is a microscopic photograph showing the expression pattern of the inventive gene at the cell level.

FIG. 5 is a photograph showing the comparison of root development between a wild-type plant and a mutant according to the present invention.

FIG. 6 is a photograph showing the comparison of root development between a wild-type plant and a mutant according to the present invention.

FIG. 7 is a scanning electron microscope photograph showing the comparison of root development between a wide-type plant and a mutant according to the present invention.

FIG. 8 is a conceptual diagram of an overexpression vector according to the present invention.

FIG. 9 is a photograph showing that the root hair length of an *OsCSLD1*-overexpressed transgenic plant is much longer than that of a wild-type plant.

FIG. 10 is a graphic diagram showing that an *OsCSLD1*-overexpressed transgenic plant has an increased capability to absorb nutrients, particularly microelements.

【Best Mode】

Hereinafter, the present invention will be described in detail.

The present invention relates to a protein, which is expressed only in the root of plants, has a function of inducing the development of root hairs, and has an amino acid sequence showing a homology of at least 90% to SEQ ID NO: 1, as well as a gene encoding said protein. Said protein and gene according to the present invention may be an *OsCSLD* protein derived from a rice plant, and an *OsCSLD* gene of SEQ ID NO: 2 encoding said protein,

respectively.

Also, the present invention provides a vector for the overexpression of root hairs, which contains said gene, and a plant transformed with said vector, which shows the promoted elongation of roots and the overexpression of root hairs. Although a transformed plant was obtained from a rice plant in Example below, the promotion of root elongation and the length growth of root hairs can also be induced in other monocotyledonous plants having genetic and physiological characteristics similar to those of the rice plant.

The root hair-overexpressed plant according to the present invention shows an excellent nutrient absorption capability compared to that of a wild-type plant.

The present invention has been completed by selecting a gene involved in the production and growth of root hairs in a rice plant, through an *Ac/Ds* tagging system, an insertional mutation system, and elucidating the root hair-specific expression characteristics of the gene through molecular studies on the genes.

As described above, the present invention relates to a protein involved in the production and growth in root hairs in a rice plant, and an *OsCSLD* gene. Specifically, the present inventors obtained a plant mutant through the *Ac/Ds* tagging system (Chin HG et al., 1999 Plant J 19, 615-623; Kim et al., 2004 Plant J 39: 252-263), which is an insertional mutation system in rice, developed by the present inventors. Also, the present inventors analyzed the function of the obtained plant mutant. The *Ac/Ds* tagging system was constructed by genetically manipulating *As* and *Ds*, a transposon family of maize, and then inserting the genetically manipulated factors into the genome of rice by transformation. This system was constructed, considering the fact that *Ds* undergoes transposition when *Ac* exists in the same genome. Also, said system was so designed that a reporter gene is placed in *Ds*, such that, when *Ds* is inserted around or into a gene on a chromosome, the reporter gene is expressed by a host gene. The tagging system is a highly potent research method, which can identify the function of genes through mutation and, at the

same time, observe gene expression through a reporter gene. Although GUS was used as the reporter gene in said paper and the present invention, any gene can be used as the reporter gene, as long as it is a gene having a suitable marker function.

The inventive mutant (*OsCSLD1::Ds*) obtained using the *Ac/Ds* tagging system comprise *Ds* inserted into the *OsCSLD1* gene. Through studies on said mutant, the present inventors have found that the *OsCSLD1* gene is a gene involved in the development and growth of root hairs in plants, thereby completing the present invention.

Hereinafter, each step of the invention will be described in detail.

(1) Selection of mutant showing defective root hair development, and analysis of mutated gene

Large amounts of rice mutants were constructed using the *Ac/Ds* tagging system, an insertional mutation system developed by the present inventors, and genes mutated from mutants showing a specific phenotype were analyzed. A GUS coding region is present in *Ds* of the *Ac/Ds* tagging system. Thus, in the mutants obtained using said system, GUS can be expressed in a specific region, unlike a wild-type plant, and a region having GUS expression can be determined through GUS staining. Also, because the use of said system enables *Ds* flanking DNA to be obtained through TAIL-PCR, a *Ds*-inserted region (gene) on a chromosome can be easily isolated.

In the present invention, among various rice mutants having a transposable element *Ds* inserted therein, a mutant showing the inhibited development of root hairs and the expression of GUS at the region where root hairs were to be developed was selected, and DNA of the selected mutant was extracted to analyze a gene involved in mutation.

After germination of the mutant (*Ds* tagging line) plant seed, DNA was obtained from the leaf, and in order to information on the chromosomal region (gene) inserted with *Ds*, the extracted DNA as a template was subjected to TAIL-PCR three times using three primers (three TAIL3 primers) complementary to the terminus of *Ds*, and one primer (W4 primer). Then, *Ds* flanking DNA

(rice genome DNA that flanks Ds), a PCR product 50 bp shorter in the third-step PCR than in the second-step PCR, was collected. The base sequence of the obtained Ds flanking DNA was analyzed using another primer (Ds3-4) to obtain the flanking sequence of the mutant.

Through database on the already known entire base sequence of rice, the Ds flanking DNA thus sequenced was found to be a gene belonging to the cellulose synthase subfamily D, which is expected to be involved in the cellulose synthesis of a cell wall, and the gene was named *OsCSLD1*. Also, an allele resulting from the insertion of Ds was named *OsCSLD1::Ds*. It could be found that the *OsCSLD1* gene (SEQ ID NO: 2) consists of a 3471-bp base sequence, has two exons and one intron, and encodes a protein (SEQ ID NO: 1) consisting of 1127 amino acids.

It was found through flanking sequence analysis and GUS expression that, in the mutant (*OsCSLD1::Ds*) according to the present invention, Ds is located downstream of the first exon in the direction of expression of the reporter gene.

Example 1 below is technology relating to a process for selecting a Ds mutant (SEQ ID NO: 1) of the *OsCSLD1* gene), the base sequence of *OsCSLD1*, and the *OsCSLD1::Ds* gene.

(2) Analysis of expression of *OsCSLD1* gene

The expression pattern of the *OsCSLD1* gene in a wild-type rice plant was analyzed.

It was found through RNA Northern hybridization that the *OsCSLD1* gene was expressed specifically in roots, and it was also observed through GUS staining that the *OsCSLD1* gene was expressed through a GUS coding region present in Ds, further demonstrating that the gene was expressed specifically in roots.

Furthermore, it could be found that GUS was strongly expressed only in the epidermal cells where root hairs develop, suggesting that the *OsCSLD1* gene was expressed only in cells showing the development of root hairs. In-situ hybridization enabling the direct observation of *OsCSLD1* mRNA in cells

revealed that the gene was expressed in the epidermal cells showing the development of root hairs.

Example 2 below relates to test results for the expression pattern of the *OsCSLD1* gene as described above.

(3) Verification of function of gene through *OsCSLD1* mutant (*OsCSLD1::Ds*)

Whether the *OsCSLD1* gene mutant (*OsCSLD1::Ds*) shows any morphological difference from a wide-type plant was examined.

The *OsCSLD1* gene was observed in cytological terms and, as a result, it was found that the growth of root hairs in the mutant was inhibited. The results of observation by a scanning electron microscope showed that the development of root hairs in the mutant was inhibited compared to that in the wide-type plant.

Example 3 below relates to test results showing the function of the *OsCSLD1* gene involved in root growth and root hair elongation.

(4) Construction of overexpression vector using *OsCSLD1* gene, and construction of overexpressed transgenic plant using said vector

To overexpress the *OsCSLD1* gene, a vector capable of overexpressing *OsCSLD1* cDNA was constructed. The constructed vector was used to obtain an *OsCSLD1*-overexpressed transgenic plant.

Example 4 below relates to a process of constructing the *OsCSLD1*-overexpressed transgenic plant.

(5) Examination of root elongation and root hair characteristics of *OsCSLD1*-overexpressed transgenic plant

The root and root hair lengths of the *OsCSLD1*-overexpressed transgenic plant were compared to those of the wide-type plant and, as a result, the root and root hairs of the transgenic plant were significantly longer than those of the wide-type plants.

Examples 5 and 6 show test results for the extension of root and root hair lengths of the *OsCSLD1*-overexpressed transgenic plant.

(6) Examination of increase in capability to absorb nutrients in

OsCSLD1-overexpressed transgenic plant

The absorption of microelements in the overexpressed transgenic plant was tested in water culture using nutrient solution and, as a result, the overexpressed plant could absorb the nutrients in amounts much more than those of the wide-type plant and the mutant plant.

Example 7 relates to test results for nutrient absorption.

Hereinafter, the present invention will be described in further detail with reference to examples. It is to be understood, however, that these examples are presented for illustrative purposes only and are not to be construed to limit the scope of the present invention.

Example 1: Discovery and base sequencing data of *OsCSLD1* gene

From a mutant population created by the *Ac/Ds* tagging system previously constructed by the present inventors, an *OsCSLD1* mutant was selected, and analyzed to identify the function thereof.

Mutant plant DNA was extracted and cloned through PCR. The base sequence of the cloned *Ds* flanking DNA was analyzed to select an *OsCSLD1* mutant gene. The method of identifying the *OsCSLD1* gene through PCR will now be schematically described.

The seeds of a mutant plant (*Ds* tagging plant) showing the immature development of root hairs was surface-sterilized, germinated and grown in a pot. Then, the leaf in the 3-leaf stage was collected, and DNA was extracted from the collected leaf using a DNA extraction solution according to a conventional method. 50 ng/ μ l of the extracted DNA was subjected to TAIL PCR sequentially using TAIL3-1 (SEQ ID NO: 4), TAIL3-2 (SEQ ID NO: 5), TAIL3-3 (SEQ ID NO: 5) and W4 (SEQ ID NO: 7) primers. The second-step and third-step PCR products were electrophoresed on 2% agarose gel and then stained with ethidium bromide, thus eluting bands showing that the third-step PCR product was 50 bp (base pair) than the second-step PCR product. The eluted DNA was directly sequenced using a *Ds*3-4 primer (SEQ ID NO: 8) to obtain the flanking sequence (SEQ ID NO: 3) of the mutant. The base sequence of each of the primers is shown in Table 1 below.

[Table 1]

Primers	SEQ ID NO	Base sequences
TAIL3-1	4	5'-ttcgactttaacccgaccggatcgtatcggttttcg-3'
TAIL3-2	5	5'-cccgttcgttttcgttaccggtatatcccggttttcg-3'
TAIL3-3	6	5'-cccgcaagttaaataatgaaaatgaaaacggtagagg-3'
W4	7	5'-AGW GNA GWA NCA NAG A-3'
Ds3-4	8	5'-gttaccgaccggtttcatcc-3'

The already found entire base sequence of rice (Science, vol 296, 79-92; <http://btn.genomics.org.cn/rice>) was searched for an about 10-kb DNA sequence containing the above-analyzed *Ds* flanking DNA sequence. Through SoftBerry (<http://www.softberry.com/berry.phtml>) and NCBI, the coding region of the gene, inserted with *Ds*, was predicted and the region inserted with *Ds* was found to be an *OsCSLD1* gene. Through the comparison of the entire base sequence of DNA, the insertion location and direction of *Ds* was examined.

Through base sequence analysis, it was found that the *OsCSLD1* gene consists of a 3471-bp base sequence (SEQ ID NO: 2), has two exons (total length of 3384 bp) and one intron (87 bp), and encodes a protein having a sequence of 1127 amino acids (SEQ ID NO: 1).

Also, it was found that flanking sequence analysis and GUS expression that, in the *OsCSLD1* mutant according to the present invention, a reporter gene was located at the terminus of the first exon of the *OsCSLD1* gene in the direction of expression (FIG. 1; 3'-*Ds*-5' was inserted between 2360 bp and 2361 bp of SEQ ID NO: 2; not shown). In FIG. 1, two squares represent exons, the empty space between the two squares represents an intron, and an inverted triangle represents inserted *Ds*.

Example 2: Analysis of expression pattern of *OsCSLD1*

The expression pattern of the *OsCSLD1* gene in rice was examined.

It was observed that the *OsCSLD1* gene was expressed specifically only in root tissue.

The expression level of *OsCSLD1* mRNA in each tissue of rice was

observed through Northern hybridization, and the observation results were the same as those of an observation process in which GUS bonded with a reporter gene in inserted Ds was expressed using X-Gluc. The Northern hybridization and GUS staining processes will now be described.

(1) Northern hybridization analysis of expression pattern in each tissue (FIG. 2)

Using Easy-BLUE (intron cat. No. 17061), RNA was extracted from each tissue of seedling, root, booting and heading tissues. The extracted RNA was electrophoresed on 1.3% formaldehyde gel and stained with ethidium bromide to confirm equal RNA loading. Then, the RNA was transferred to a hybond N⁺ membrane (Amersham Pharmacia Biotech) in 10XSSC and subjected to Northern blot analysis using a ³²P-labeled *OsCSLD1* probe (DNA cloned by PCR using forward 5'-TGG AGT CTT CAG CTT CTG-3' and reverse CAG GTT CCA AGT TCC GAG C-3 primers) in Church buffer (1% BSA, 200 μM EDTA, 0.5M sodium phosphate, 7% SDS) at 65 °C (see FIG. 2). As a result, as can be seen in FIG. 2, the *OsCSLD1* gene was expressed specifically only in roots. The bottom photograph (EtBr) in FIG. 2 is a control used to whether RNA was loaded in a relatively equal amount between the samples.

(2) GUS staining analysis of expression of root hairs

The mutant was observed for the expression of GUS bonded with a reporter gene in an *Ac/Ds* system.

The root tissue of the mutant was immersed in X-Gluc solution (50 mM NaH₂PO₄, 10 mM EDTA, 0.1% Triton, 2 mM potassium ferrocyanide, 0.204 mg/ml chloroampenicol, 10% X-Gluc) and then stored in dark conditions at 37 °C for 48 hours. When the expression of blue GUS was observed, the tissue solution was treated sequentially with 30% ethanol, 50% ethanol and 70% ethanol, and then stored in 70% ethanol while it was observed under a 40x microscope (Leica L2) (FIG. 3). As a result, it was observed that GUS was strongly expressed in a root region showing the development of root hairs.

(3) Observation of gene expression at gene level using GUS (FIG. 4A)

To anatomically observe cells showing the expression of GUS, the root tissue of the mutant was dehydrated with each of 50%, 70%, 85%, 95% and 100% ethanol for 90 minutes. Then, the analyte was stained with eosin for 2 hours and treated with each of mixed solvents ethanol and tert-butanol (7:3, 5:5 and 3:7) for 30 minutes. Then, the analyte was treated with 100% tert-butanol for 1 hour, and was incubated four times in melt paraffin for 12 hours for each time. The treated samples in dissolved paraffin were placed in boats and hardened at room temperature. The paraffin sample thus prepared was sectioned with a microtome, placed on a microscope slide, treated with xylene to remove paraffin, and observed with a microscope (FIG. 4A). The observation results showed that GUS was expressed only in the root epidermal cells showing the production of root hairs.

(4) Observation of gene expression at gene level through *in situ* mRNA hybridization (FIG. 4B)

While GUS expression can be observed in the *OsCSLD1::Ds* mutant, and *in situ* mRNA hybridization allows direct observation of *OsCSLD1* mRNA expressed in a wild-type plant. Thus, to complement the results obtained by the above GUS staining, the expression of *OsCSLD1* mRNA in a wild-type plant was directly observed by an *in situ* mRNA hybridization assay.

The plant root tissue was treated with a fixing solution (0.25% glutaldehyde, 4% paraformaldehyde, 100 mM Na-phosphate, pH 7.5) reported by Kouchi and Hata (Mol Gen Genet. 1993 238: 106-119) for 12 hours and was prepared into a tissue analyte for microscope observation according to the same method as described in section (3) above. Separately constructed *OsCSLD1* antisense mRNA was hybridized to said tissue, and the hybridized tissue was observed for cells showing the expression of mRNA, using anti-DIG-AP and BCIP/NBT (see FIG. 4B). As a result, it could be observed that *OsCSLD1* mRNA appeared only in the root epidermal cells producing root hairs in the same manner as in the expression of GUS.

Example 3: Analysis of function of *OsCSLD1* gene according to the present invention

(1) Morphological characteristics of roots

The root of the mutant plant (*OsCSLD1::Ds*) was morphologically observed to identify the function of the *OsCSLD1* gene in the root.

The seeds of a wild-type plant and the seed of the mutant plant (*OsCSLD1::Ds*) were surface-sterilized with prochloraz emulsion and grown in pot soil for 10 days, and then the phenotypes of the plants were observed. As a result, it was found that the root growth of the mutant plant was at least 20% lower than that of the wild-type plant. Thus, it could be found that the *OsCSLD1* gene expressed in the root hairs also had an effect on the root elongation (see FIG. 5 and Table 2).

[Table 2]Length growth of roots in wild-type plant, mutant plant and overexpressed plant^a (unit: mm)

	Wild-type	<i>OsCSLD1::Ds</i> (mutant)	<i>35S::CSLD1</i> (overexpressed)
Root length	102 ± 12.9 ^b	83 ± 15	126.4 ± 7.6

^a: measurement of seminal roots grown in soil for 10 days

^b: average value (± standard deviation) for 20 root hairs

(2) Morphological characteristics of root hairs

The effect of the *OsCSLD1* gene on the root cells was analyzed at the cytological aspect.

The wild-type plant and the mutant plant (*OsCSLD1::Ds*) were grown in a medium comprising MS solution containing 2.5% phytigel and were stained with a 0.05% toluidine blue reagent, followed by microscopic observation (see FIGS. 6A and 6B). The observation results showed that the development of root hairs in the wild-type plant was normal, whereas the development of root hairs in the mutant plant was inhibited. FIG. 6A shows the root tissue of the wild-type plant, and FIG. 6B shows the root tissue of the mutant plant (*OsCSLD1::Ds*).

The fine configuration of the root hairs was observed with an electron microscope.

The root tissue of the plant obtained in Example 2 was fixed with a fixation solution (2.5% glutaraldehyde, 0.1% Na-cacodylate, pH 7.5) for 12 hours and placed in a 1% OsO₄ reagent at 4 °C for 2 hours. Then, the tissue was treated with each of 20%, 50%, 70% and 90% ethanol for 15 minutes and then treated three times with 100% ethanol for 30 minutes for each time. Then, the treated tissue was hardened using a critical point dryer (Tosimis SAMDRI-795), and the tissue surface was subjected to ion spotting using a sputter coater (JFC-1100E) at 100 mM for 20 seconds. The prepared sample was observed through SEM (see FIG. 7), and the observation results showed that the development of root hairs in the mutant was inhibited compared to that in the wild-type plant. FIG. 7A shows the root hairs of the wild-type plant, and FIG. 7B shows the root hairs of the mutant plant (*OsCSLD1::Ds*).

Example 4: Construction of *OsCSLD1*-overexpressed transgenic plant

To examine a phenotype occurring upon the overexpression of the *OsCSLD1* gene, an overexpression vector was constructed using a 35S promoter, a potent promoter, and was used to transform a rice plant.

(1) Construction of transformation vector

OsCSLD1 cDNA was inserted into vector pCAMBIA 1302 (manufactured by CAMBIA, Australia) having a 35S promoter and GFP, thus constructing overexpression vector pCAMBIA-CSLD1. The vector pCAMBIA 1302 is a vector constructed for plant transformation, which consists of a total of 10,549 base pairs, has hygromycin- and kanamycin-resistant genes as plant selection markers, and can be expressed with GFP and a fusion protein.

A process of constructing the overexpression vector pCAMBIA-CSLD1 according to the present invention will now be described in detail. (1) *OsCSLD1* cDNA having *SpeI* restriction enzyme digestion sites at both termini thereof was amplified by PCR and cloned in a pBSK plasmid vector (Stratagene, USA). (2) T-DNA vector pCAMBIA 1302 and a plasmid vector, containing CaMV35S promoter and *CSLD1* cDNA, were digested with *SpeI* enzymes. (3) The 35S promoter and the DNA of *CSLD1* were ligated to pCAMBIA 1302 at 16 °C overnight. (4) The ligated DNA was transformed into *E. coli* competent cells

to obtain a clone, and DNA was extracted from the clone and digested with a *SpeI* enzyme to confirm whether it was ligated. The resulting vector was used as a transformation vector.

The main elements of the constructed overexpression vector according to the present invention are shown in FIG. 8. As shown in FIG. 8, *OsCSLD1* cDNA according to the present invention is inserted between the 35S promoter and the GFP gene.

(2) Construction of overexpressed plant

The transformation of plants was performed using the *Agrobacterium* method, which is generally used in the art.

First, pCAMBIA-CSLD1 containing *OsCSLD1* cDNA was transformed into an *Agrobacterium* LBA4404 strain. The bacterial strain was cultured in an AB plate at 30 °C for 3 days, and then DNA was extracted from the cultured bacterial strain and analyzed for the cointegration thereof. The confirmed bacterial strain was further cultured for 3 days and used in infection. The transformed *Agrobacterium* cells were infected through the calli of rice and subjected to dark room incubation (AAM, 2N6-AS medium) for 3 days, selective incubation (50 mg N6-CH-Hyg) in a dark room for 3-4 weeks, pre-regeneration (N6-7-CH) in a dark room for 10 days, regeneration I (N6S3-CH-I) in a light room for at least 3 weeks, regeneration II (N6S3-CH-II) in a light chamber until shooting, and then incubation in bottle medium (MS) in a light chamber, thus obtaining a transformed plant. Whether the plant was transformed was confirmed by extracting genomic DNA from the plant and subjecting the extracted DNA to PCR Southern blot analysis using a hygromycin probe.

Example 5: Examination of promoted elongation of overexpressed plant root

The seeds of a wild-type plant, a mutant plant (*OsCSLD1::Ds*) and an overexpressed plant (*35S::OsCSLD1*) were surface-sterilized with prochloraz emulsion and grown in pot soil, and then the phenotypes of the plants were observed. The observation results showed that the root growth of the mutant plant was at least 20% lower than that of the wild-type plant, and the root

growth of the overexpressed plant was at least 20% higher than that of the wild-type plant (see FIG. 5 and Table 4). The reason that the elongation of the overexpressed plant root is promoted is believed to be because, as confirmed in Examples 6 and 7 below, the root hairs grow longer, while the absorption of nutrients in the root hairs becomes relatively better. FIG. 5, A, B and C represent the wild-type plant, the mutant plant and the overexpressed plant, respectively.

Example 6: Examination of morphological characteristics of overexpressed plant root hairs

The seeds of a wild-type plant, a mutant plant (*OsCSLD1::Ds*) and an overexpressed plant (35S::*OsCSLD1*) were surface-sterilized and then grown in MS medium, and then the root tissues of the plants were treated according to the treatment method for SEM observation as described in Example 3(2) and were observed with an electron microscope (FIG. 9). As can be seen in FIG. 9, the length of root hairs in the mutant plant (FIG. 9B) was shorter than that in the wild-type plant (FIG. 9A), and the length of root hairs in the overexpressed plant (FIG. 9C) according to the present invention was significantly longer than that in the wild-type plant (see Table 3).

[Table 3] Root hair lengths of wild-type plant, mutant plant and overexpressed plant^a (unit: μm)

	Wild-type	<i>OsCSLD1::Ds</i> (mutant)	35S:: <i>CSLD1</i> (overexpressed)
Root hair length	178.5 $\pm$ 13.3 ^b	77.3 $\pm$ 9.1	328.1 $\pm$ 18.2

^a: Measurement of root hair length of seminal root grown for 5 days in 0.5X MS containing 0.5% phytoigel

^b: average value ($\pm$ standard deviation) for 200 root hairs

However, there was no significant difference in the number of root hairs between the wild-type plant, the mutant plant (*OsCSLD1::Ds*) and the overexpressed plant (35S::*OsCSLD1*) (see Table 4).

[Table 4] Number of root hairs produced per unit area of root

epidermis^a

	Wild-type	<i>OsCSLD1::Ds</i> (mutant)	35S::CSLD1 (overexpressed)
Number of root hairs	15.2 ± 1.2 ^b	14.6 ± 1.5	15.6 ± 1.1

^a: Measurement of root hair number of seminal root grown for 5 days in 0.5X MS containing 0.5% phytoigel

^b: average value (± standard deviation) for 200 root hairs

Example 7: Analysis of nutrient absorption capability of overexpressed plant

Whether the inventive *OsCSLD1*-overexpressed plant showing the promoted stimulation of roots and root hairs has any difference in nutrient absorption capability was analyzed.

The seeds of a wild-type plant, a mutant plant (*OsCSLD1::Ds*) and an overexpressed plant (35S::*OsCSLD1*) were surface-sterilized with 0.05% prochloraz for 24 hours and washed with distilled water. Then, the seeds were placed onto Whatmman No. 2 filter paper on 9-cm diameter Petri dishes and then germinated in dark conditions at 30 °C for 5 days. The germinated seeds were transferred into boxes containing a nutrient solution [Carmak and Marschner (1992); 0.88 mM K₂SO₄, 1 mM Ca(NO₃)₂, 1 mM (NH₄)₂SO₄, 1 mM MgSO₄, 0.25 mM KH₂PO₄, 0.1 mM KCl, 40 mM FeEDTA, 10 mM H₃BO₄, 1 mM MnSO₄, 1 mM ZnSO₄, 0.1 mM CuSO₄ and 0.01 mM (NH₄)₆MoO₂₄] and were growth in growth chambers (16-hr photoperiod at 30/22 °C day/night and 70% RH, 250 Me⁻² s⁻¹) for 15 days while replacing the nutrient solution at 2-day intervals. After 15 days, the roots were cut, dried at 70 °C for 3 days and then powdered. Each of the root powders was added into 1N HCl, stirred for 24 hours and then filtered through Whatmman No. 2 paper to obtain filtrates.

The content of microelements in each of the filtrates was measured (see FIG. 10). As can be seen in FIG. 10, the overexpressed plant according to the present invention was significantly superior to the wild-type plant with

respect to the capability to absorb microelements, including Mg, Zn, Fe, Mn and Cu.

The measurement in this Example was repeated three times in order to reduce test errors.

【Industrial Applicability】

According to the present invention, the function of the *OsCSLD* gene capable of controlling the growth and development of roots and root hairs, which are important in experimental and agricultural terms, was elucidated, and thus a plant having an increased capability to absorb water and nutrients was created.

【CLAIMS】**【Claim 1】**

A protein, which is expressed only in the roots of a plant, has a function of inducing the elongation of the plant roots and root hairs, and has an amino acid sequence showing a homology of at least 90% to SEQ ID NO: 1.

【Claim 2】

The protein of Claim 1, which is an *OsCSLD* protein having an amino acid sequence of SEQ ID NO: 1.

【Claim 3】

A gene encoding the protein of Claim 1.

【Claim 4】

The gene of Claim 3, which is an *OsCSLD1* gene having a base sequence of SEQ ID NO: 2.

【Claim 5】

An expression vector containing the gene of Claim 3.

【Claim 6】

An expression vector containing the gene of Claim 4.

【Claim 7】

The expression vector of Claim 6, which is expression vector pCAMBIA-CSLD1.

【Claim 8】

A transgenic plant transformed with the expression vector of Claim 5, which shows induced elongation of roots and root hairs.

【Claim 9】

A transgenic plant transformed with the expression vector of Claim 6, which shows induced elongation of roots and root hairs.

【Claim 10】

A transgenic plant transformed with the expression vector of Claim 7, which shows induced elongation of the roots and root hairs.

【Claim 11】

The transgenic plant of Claim 8, wherein the plant is a monocotyledonous plant.

【Claim 12】

The transgenic plant of Claim 11, wherein the plant is a rice plant.

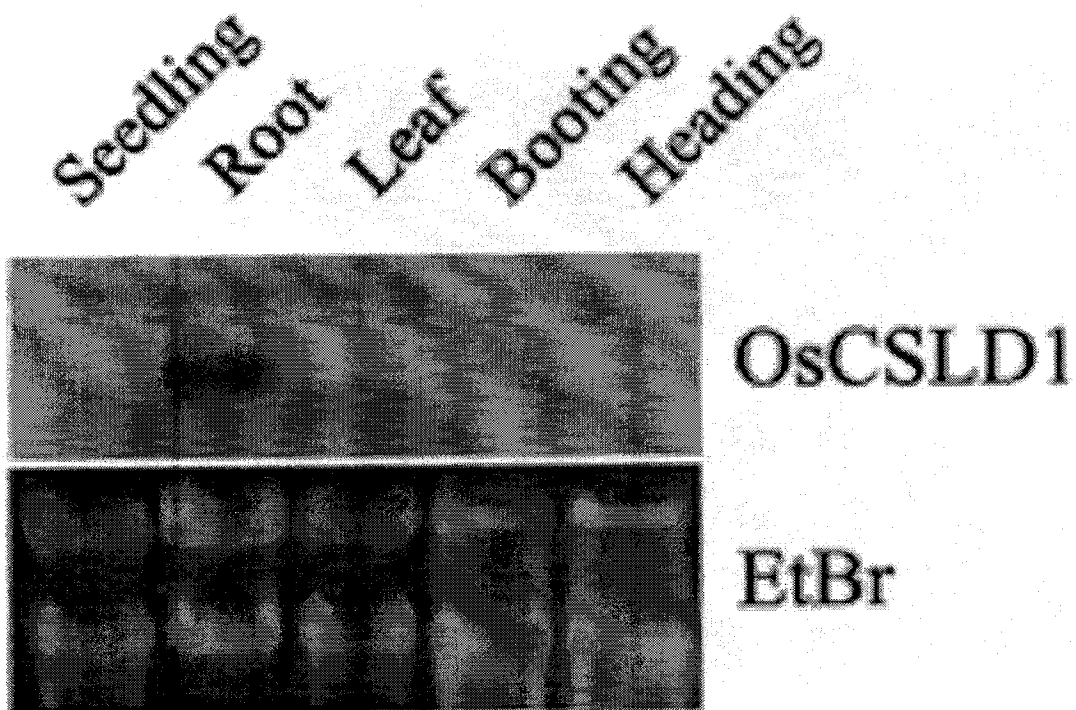
【Figure 1】

Exons: 3471bp (1127aa)

Intron: 87bp



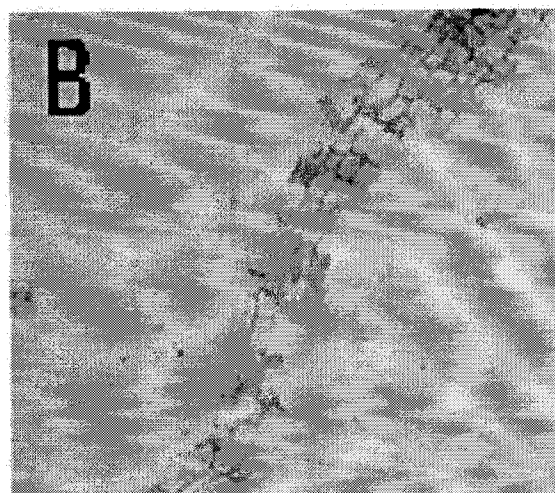
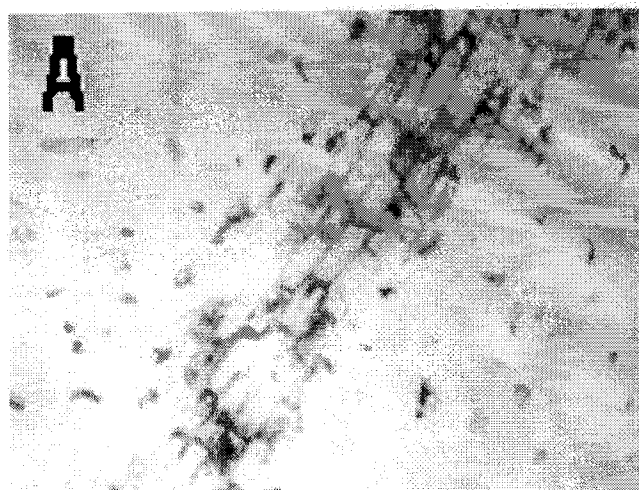
【Figure 2】



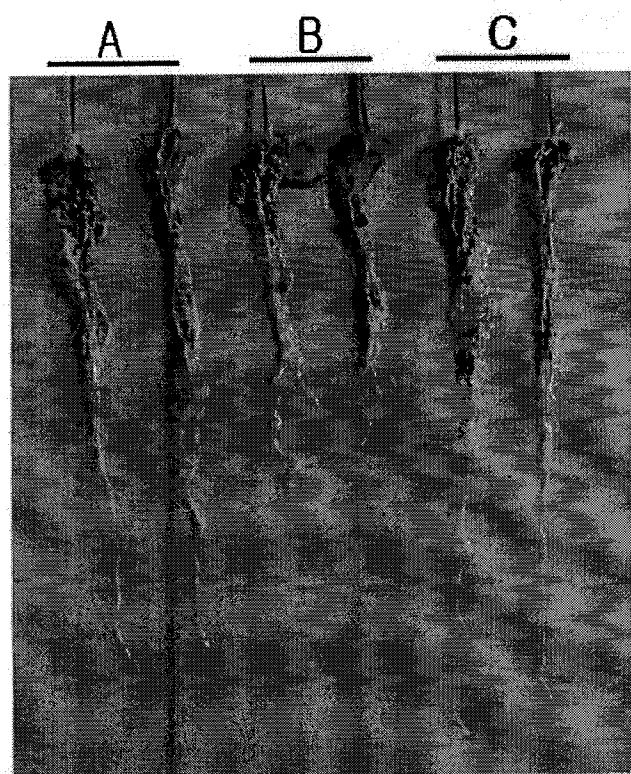
【Figure 3】



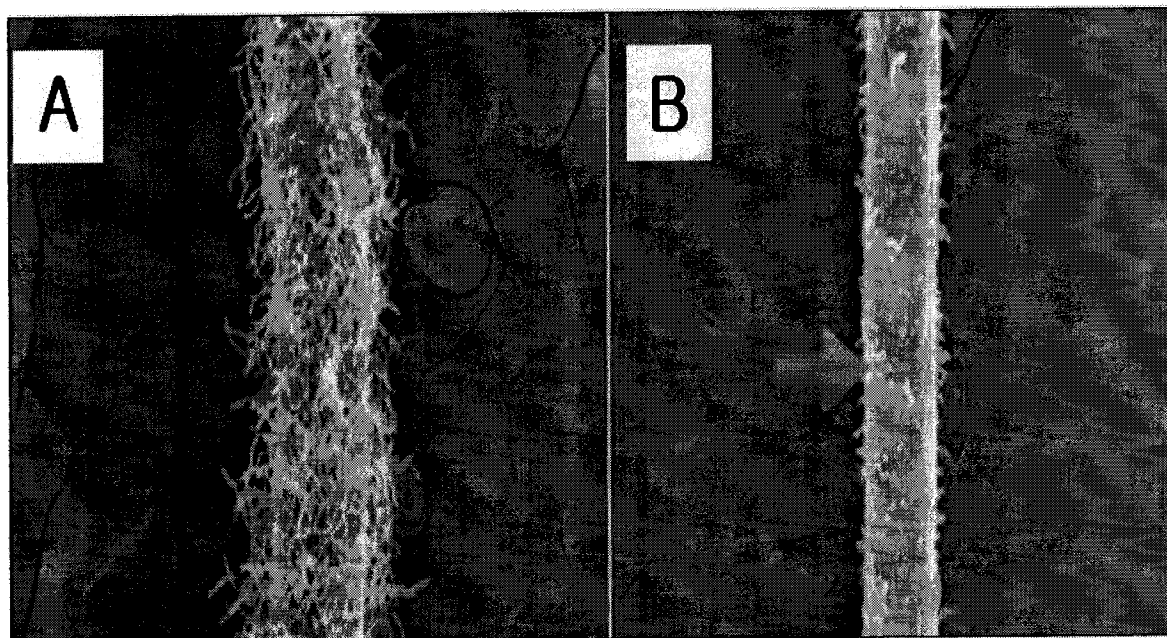
【Figure 4】



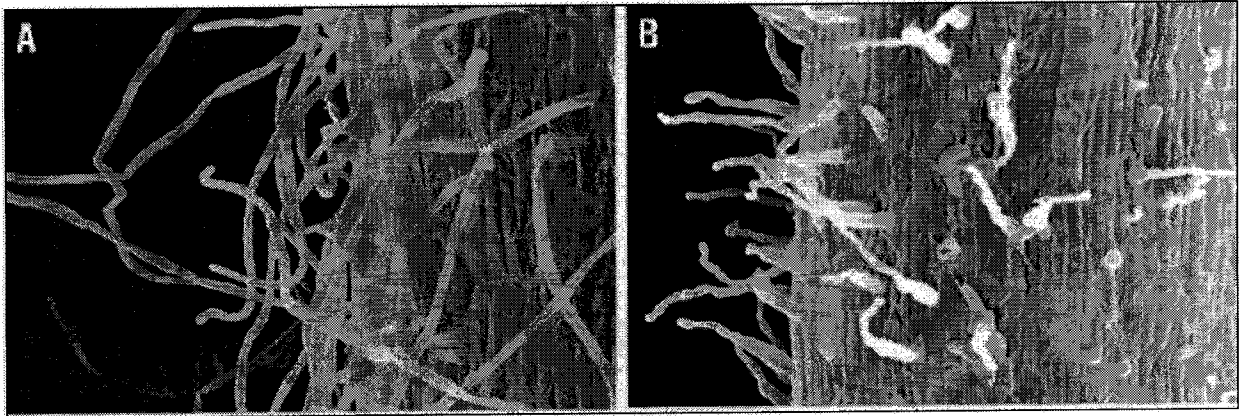
【Figure 5】



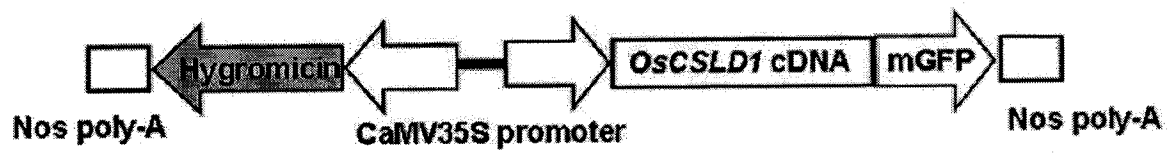
【Figure 6】



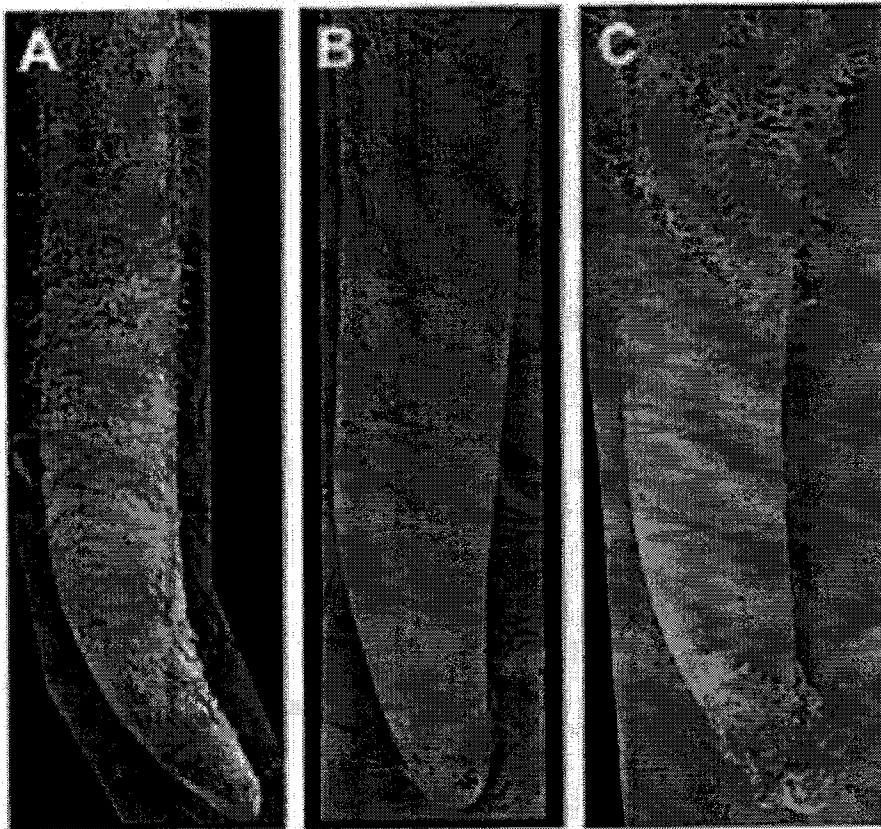
【Figure 7】



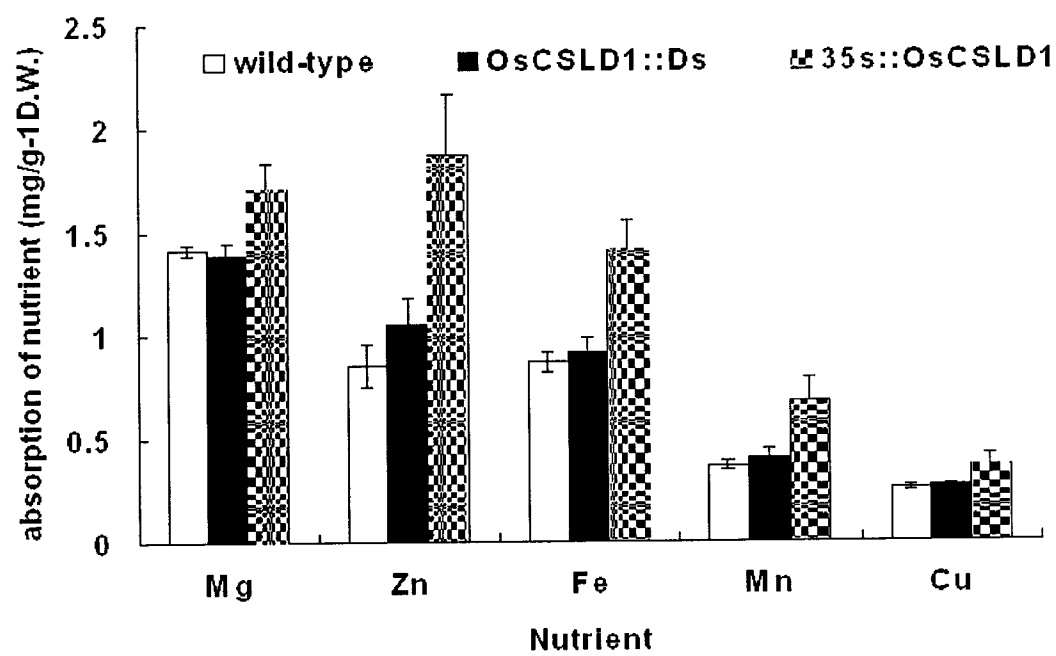
【Figure 8】



【Figure 9】



【Figure 10】



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2006/003049

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material

☒

a sequence listing

☐

table(s) related to the sequence listing

b. format of material

☐

on paper

☒

in electronic form

c. time of filing/furnishing

☐

contained in the international application as filed

☒

filed together with the international application in electronic form

☐

furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/003049**A. CLASSIFICATION OF SUBJECT MATTER***C12N 15/29(2006.01)i*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC8: C12N 15/29

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Pubmed, Esp@snet, Delphion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NCBI Genbank Accession No. DAA01752 (3 September 2003) See the whole document.	1-4
X A	HAZEN, S. P. et al. : 'Cellulose synthase-like genes of rice' Plant Physiology, Vol. 128, pp. 336-340 (February 2002) Cited in the application. See the whole document.	1-4 1--12
A	WANG, X. et al. : 'AtCSLD3, a cellulose synthase-like gene important for root hair growth in arabidopsis' Plant Physiology, Vol. 126, pp. 575-586 (June 2001) See the whole document.	1--12
A	FAVERY, B. et al. : 'KOJAK encodes a cellulose synthase-like protein required for root hair cell morphogenesis in Arabidopsis' Genes & Development, Vol. 15, pp. 79-89 (1 January 2001) Cited in the application. See the whole document.	1--12
A	DOBLIN, M. S. et al. : 'Pollen tubes of Nicotiana glauca express two genes from different beta-glucan synthase families' Plant Physiology, Vol. 125, pp. 2040-2052 (April 2001) See the abstract.	1--12

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 NOVEMBER 2006 (20.11.2006)

Date of mailing of the international search report

20 NOVEMBER 2006 (20.11.2006)

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