(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2008 (18.09.2008)

PCT

(10) International Publication Number
WO 2008/111779 A1

(51) International Patent Classification:
C12N 15/29 (2006.01)

(21) International Application Number:
PCT/KR2008/001343

(22) International Filing Date: 10 March 2008 (10.03.2008)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
10-2007-0024730 13 March 2007 (13.03.2007) KR

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) Title: ANTISENSE DNA OF SWEETPOPATO EXPANSIN CDNA AND METHOD FOR INCREASING STORAGE ROOT YIELD USING THE SAME

Figure 16

	Storage root		Shoot	
	Number	Total weight (g)	Number	Total weight (g)
Wild-type	4.75±0.96	163.71±24.62	2.5±0.58	118.13±19.02
antisense1	5.33±0.58	248.20±33.93	3.3±0.58	118.48±11.66
antisense4	8.25±1.50	224.45±30.55	2.8±0.75	118.48±11.02

(57) Abstract: Disclosed herein are antisense DNA of a sweetpotato expansin (*IbExpansin*) cDNA, a plant transformation vector carrying the same, and a method for increasing storage root production using the same. The transgenic sweetpotatoes prepared using the antisense DNA of *IbExpansin* cDNA have storage root production increased by up to one and half times. Thus, the gene is useful in the generation of highly productive transgenic storage roots for the increase of bioethanol production.

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【DESCRIPTION】

【Invention Title】

ANTISENSE DNA OF SWEETPOTATO EXPANSIN CDNA AND METHOD FOR INCREASING STORAGE ROOT YIELD USING THE SAME

【Technical Field】

<1> The present invention relates to an antisense DNA of sweetpotato expansin cDNA useful in the generation of highly productive storage roots. More particularly, the present invention relates to an antisense DNA of sweetpotato expansin cDNA, a transformation vector carrying the same, and a method for increasing storage root production using the same.

【Background Art】

<2> Since the discovery of expansin by Cosgrove and his colleagues (McQueen-Mason et al., 1992, Plant Cell 4, 1425-1433), intensive studies have been conducted thereon. In early studies, expansins were known as cell-wall-loosening enzymes that mediate, at least in part, pH-dependent extension of the plant cell wall and the growth of the cell (Cosgrove, 2000, Nature 407, 321-326). Since then, expansins were found to be in either α- or β-form (Shcherban et al., 1995, PNAS 92, 9245-9249).

<3> More recently, expansins have been found to be involved in regulating, besides cell expansion, a variety of other plant processes, including morphogenesis (Ruan et al., 2001, Plant Cell 13, 47-60), softening of fruits (Rose et al., 2000, Plant Physiology 123, 1583-1592; Civello et al., 1999, Plant Physiology 121, 1273-1280), growth of the pollen tube (Cosgrove et al., 1997, PNAS 94, 6559-6564), elongation of graviresponding roots (Zhang and Hasenstein, 2000, Plant Cell Physiology 41, 1305-1312), and elongation of root cells (Lee et al., 2003, Plant Physiology 131, 985-997) (for review, Lee et al., 2001, Cur. Opin. Plant Biol. 4, 527-532).

<4> Further, the expression pattern of expansins in flooded rice and tomatoes has been well studied. It has been found that expansins are expressed in the shoot apical meristem of tomato for incipient leaf primordium initiation (Reinhardt et al., 1998, Plant Cell 10, 1427-1437). An

expansin gene (*Exp1*) was cloned and found through transformants therewith to play an important role in the growth and ripening of tomato fruits in (Brummell et al., 1999, Plant Cell, 11: 2203-2216). Expansin mRNA was accumulated just before the rate of growth or the loosening degree of the cell wall started to increase, suggesting that the expression of expansin genes is correlated with cell elongation (Cho and Kende, 1997a, Plant Cell 9, 1661-1671; 1997b, Plant Physiology 113, 1137-1143; 1998, PlantJournal 15, 805-812). Transgenic rice plants in which expansins are overexpressed were observed to further increase the length of cotyledons by 31-97% compared with the wild type (Choi et al., 2003 Plant Cell, 15: 1386-1398). However, the transgenic rice plants are unable to bear seeds due to male sterility.

<5> On the other hand, sweetpotato storage roots are a good energy source for people because they contain a lot of starch and various kinds of inorganic nutrients, and are high value-added crops having beneficial health effects owing to their high content of fiber, which is a material useful in the body.

<6> Recently, as bio-ethanol obtained upon the fermentation of plants comes into the spotlight as an alternative energy source, sweetpotato storage roots are also being considered as a useful alternative energy crop.

<7> It is very important to increase the productivity per unit area under cultivation of alternative energy crops in view of enhancing the price competitiveness of alternative energy.

<8> Further, there has been little research on the molecular mechanisms of storage root production because the material of the storage root is not suitable for molecular study, for the following reasons : (1) it is not easy to extract DNA and RNA because of the large amounts of polysaccharide; and (2) it is not easy to monitor storage root growth because the storage root grows in the ground, and thus studies into molecular breeding to regulate the development of sweetpotato storage root have been limited.

<9> Therefore, there has been a need for molecular breeding to regulate the development of sweetpotato storage root and transgenic sweetpotato that can

remarkably increase production using the molecular breeding.

【Disclosure】

【Technical Problem】

<10> Accordingly, the present invention has been made keeping in mind the above problems occurring in the prior art, and an object of the present invention is to provide an antisense DNA of sweetpotato expansin (*IbExpansin*) cDNA with which highly productive transgenic sweetpotato can be prepared.

<11> It is another object of the present invention to provide a method for increasing storage root production by suppressing the expression of expansin using the antisense DNA of *IbExpansin* cDNA.

<12> It is a further object of the present invention to provide a highly productive transgenic sweetpotato which comprises a vector carrying the antisense DNA of *IbExpansin* cDNA.

【Technical Solution】

<13> In order to accomplish the objects, therefore, the present invention provides a method for increasing storage root production, comprising suppressing the expression of an expansin gene in cells of a plant.

<14> According to the preferred embodiment of the present invention, the method further comprises introducing antisense DNA of a cDNA from a sweetpotato expansin gene (*IbExpansin*) into the plant.

<15> According to the preferred embodiment of the present invention, the method further comprises inserting an antisense DNA of the expansin gene into a binary vector, and introducing the binary vector into the plant.

<16> The antisense DNA comprises a nucleotide sequence of SEQ ID NO.: 9.

<17> The plant is sweetpotato.

<18> According to another aspect of the present invention, the present invention provides a method for preparing highly productive transgenic sweetpotato, comprising introducing antisense DNA of a sweetpotato expansin cDNA into sweetpotato.

<19> According to another aspect of the present invention, the present invention provides a PCR primer suitable for amplifying a DNA fragment

comprising the nucleotide sequence of SEQ ID NO.: 9, said primer being represented by one of the nucleotide sequences as shown in SEQ ID NO. 10 or 11.

【Advantageous Effects】

<21> The present invention provides an antisense DNA of *IbExpansin* cDNA (expansin cDNA derived from *Ipomoea batatas*) that is useful for transforming plants, and the resulting transgenic plants are capable of accelerating the growth of storage root by suppressing the elongation growth of roots. Therefore, the present invention is useful in the generation of highly productive transgenic storage root.

【Description of Drawings】

<22> The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

<23> FIG. 1 is a view showing the results of primary PCR for cloning a full-length sweetpotato expansin cDNA;

<24> FIG. 2 is a view showing the results of secondary PCR for cloning a full-length sweetpotato expansin cDNA, with the primary PCR product serving as a template;

<25> FIG. 3 is a view showing the full-length sweetpotato expansin cDNA cloned by PCR in accordance with the present invention;

<26> FIG. 4 shows a comparison of the amino acid sequence of the sweetpotato expansin cDNA with amino acid sequences of other plant expansin cDNA;

<27> FIG. 5 shows amino acid sequence homologies between the sweetpotato expansin cDNA and other plant expansin cDNA;

<28> FIG. 6 shows sweetpotato tissues used in examining expression patterns of sweetpotato expansin gene;

<29> FIG. 7 presents the results of Northern Blot Analysis showing the expression patterns of sweetpotato expansin gene in sweetpotato;

<30> FIG. 8 shows results obtained at various process stages of constructing a binary vector for carrying the antisense DNA of sweetpotato expansin cDNA of the present invention into sweetpotatoes;

<31> FIG. 9 is a schematic diagram showing the structure of a p*IbExpansin*-anti binary vector for carrying the antisense DNA of sweetpotato expansin

cDNA of the present invention into sweetpotatoes;

<32> FIG. 10 shows embryogenic callus induced in sweetpotato for transferring the antisense DNA of sweetpotato expansin cDNA of the present invention into sweetpotatoes;

<33> FIG. 11 is a view showing the result of electrophoresis to examine the insertion of antisense DNA of *IbExpansin* cDNA in sweetpotato transformant expressing the antisense DNA of sweetpotato expansin cDNA of the present invention;

<34> FIG. 12 is a view showing the result of electrophoresis to examine the expression of *IbExpansin* in sweetpotato transformant expressing the antisense DNA of sweetpotato expansin cDNA of the present invention;

<35> FIG. 13 shows the comparison of root growth between sweetpotato transformant expressing the antisense DNA of sweetpotato expansin cDNA of the present invention and wild-type at 10 days after cutting in vitro;

<36> FIG. 14 shows the comparison of root growth on a cellular level between sweetpotato transformant expressing the antisense DNA of sweetpotato expansin cDNA of the present invention and wild-type at 10 days after cutting in vitro;

<37> FIG.15 shows the comparison of growth between seetpotato transformant expressing the antisense DNA of sweetpotato expansin cDNA of the present invention and wild-type; and

<38> FIG. 16 shows the comparison of storage root production between sweetpotato transformant expressing the antisense DNA of sweetpotato expansin cDNA of the present invention and wild-type.

【Best Mode】

<39> Hereinafter, the preferred embodiment of the present invention will be described with reference to the accompanying drawings.

<40> First, the present inventors succeeded in cloning a sweetpotato expansin (*IbExpansin*) cDNA, constructing a binary vector suitable for plant transformation using the antisense DNA of the cDNA, and transforming the vector into sweetpotato. The transgenic sweetpotato was found to

significantly increase storage root production.

<41> Therefore, the present invention provides an antisense DNA of sweetpotato expansin cDNA, comprising a nucleotide sequence of SEQ ID NO.: 9.

<42> The antisense DNA of the cDNA has a nucleotide sequence 1,213 bp long.

<43> Further, the present invention provides a binary vector (p*IbExpansin*-anti) for transforming plants, carrying the antisense DNA of sweetpotato (*Ipomoea batatas*) expansin cDNA (*IbExpansin*).

<44> The plant transformation vector is a binary vector capable of stably expressing an exogenous gene of interest in plants.

<45> In a pMBP1 vector, the antisense DNA of sweetpotato expansin cDNA (*IbExpansin*) according to the present invention is located between a CaMV35S promoter and an NOS terminator. It should be understood by those skilled in the art that any other plant transformation vector can be used instead of the pMBP1 vector.

<46> Further, the present invention provides a transgenic sweetpotato carrying the antisense DNA of sweetpotato expansin cDNA (*IbExpansin*) according to the present invention on a binary vector.

<47> The binary vector may be introduced into plants using Agrobacterium or a gene gun. In an embodiment of the present invention, a gene gun method (Sanford etc, 1993) was used for transforming sweetpotato.

<48> In addition to sweetpotato, the antisense DNA of sweetpotato expansin cDNA (*IbExpansin*) according to the present invention may be introduced into any plant storage root which is adapted to have increased storage root production.

<49> Further, the present invention provides a pair of primers for the PCR amplification of the antisense DNA of sweetpotato expansin cDNA (*IbExpansin*) according to the present invention, which is represented by SEQ ID NO.: 10 and SEQ ID NO.: 11.

<50> Further, the present invention provides a method for increasing storage root production by suppressing the expression of an expansin gene using the antisense DNA of the expansin cDNA.

<51> Further, the present invention provides a method for increasing storage root production by inserting an antisense DNA of the expansin cDNA into a binary vector, and introducing the binary vector into the plant.

<52> As mentioned above, some of the expansin family genes are disclosed, but nowhere has the application of expansin genes for storage root production increase been mentioned in reports predating the present invention. In accordance with the present invention, antisense DNAs of various expansin cDNAs can be introduced into plants in order to increase their storage root production.

<53>

<54> EXAMPLE 1: Cloning of Sweetpotato Expansin cDNA

<55> Total RNA was isolated from a fresh tuber of sweetpotato and was used to construct an EST (Expressed Sequence Tag) library. Using this library, 2,859 ESTs were cloned and deposited in the National Center for Biotechnology Information (NCBI) with NCBI Accession Nos.: BU690119 - BU692977 (You et al., 2003, FEBS Letters 536, 101-105). Of them, *IbExpansin* (NCBI Accession No. BU691452) was found to be about 1 kb long, and was identified as a partial cDNA devoid of the start codon ATG. To obtain full length *IbExpansin*, PCR was performed in the presence of an *IbExpansin*-specific primer (SEQ ID NO.: 3) and a T3 vector primer, with the preexisting EST library of early sweetpotato storage root development serving as a template. However, no bands were visible at the position of the expected 5' full length size (FIG. 1).

<56> DNA fragments were eluted from a gel piece excised from the agarose gel at the expected full length position and used as a template for PCR, with a set of a gene-specific nested primer (SEQ ID NO.: 4) and a T3 primer. As a result, a PCR product having a length of about 350 bp was obtained (FIG. 2).

<57> The PCR product was inserted into a pGEM-T Easy vector for sequencing analysis and identified 5' sequence of *IbExpansin*.

<58> On the basis of the nucleotide sequence, 5' and 3' primers were synthesized with BamHI and KpnI restriction sites provided respectively to

their termini. RT-PCR was performed with the primers to obtain a full-length *IbExpansin* (FIG. 3).

<59>

<60> EXAMPLE 2: Sequencing and Analysis of Nucleotide Sequence of Full-Length *IbExpansin*

<61> A 1.2 kb full-length cDNA was cloned by PCR and inserted into a pGEM-T Easy vector, which was then amplified. Sequencing analysis revealed that *IbExpansin* is 1,213 bp long and consists of a 33 bp 5' -UTR, a 717 bp ORF, and a 463 bp 3' -UTR. This full-length *IbExpansin* cDNA was registered in NCBI, with Accession No.: DQ515800. The *IbExpansin* amino acid sequence, consisting of 238 amino acid residues, is highly conserved, with the exception of the N-terminal region (FIG. 4), and shares homology as high as 78% with expansin amino acid sequences of tomato and pepper (FIG. 5).

<62>

<63> EXAMPLE 3: Northern Blot Analysis of Tissues

<64> 1. Northern blotting

<65> The expression pattern of *IbExpansin* was examined with various tissues at various developmental stages through Northern blotting.

<66> For the isolation of total RNA, roots, stems, leaves and petioles of sweetpotato at various developmental stages were used as RNA sources. That is, total RNA was isolated from tissues in a non-storage root stage, such as roots (FRN: fibrous root in non-storage root stage), stems (stem-FRN), leaves (Leaf-FRN) and petioles (petiole-FRN); tissues in an early storage root stage, such as roots (fibrous root in early storage root stage, FRES); tissues in a storage root stage, such as roots (SR), stems (Stem-SR), leaves (Leaf-SR) and petioles (Petiole-SR); and tissues in a late storage root stage, such as roots (fibrous root in late storage root stage, FRLS) (FIG. 6). Total RNA extraction was performed using a 4.4 M guanidinium-SDS lysis buffer (Chirgwin et al., 1979)/5.7 M CsCl gradient method (Glisin et al., 1974). About 20 μg of the extracted total RNA was electrophoresed on 1% agarose-formaldehyde gel and transferred onto a Tropilon-plusTM nylon

membrane (Tropix, USA).

<67> A probe was obtained by amplification from 2.5 ng of a plasmid carrying a 1 kb Expansin EST clone through PCR, which was performed in a PCR mixture containing 100 μM of dNTP mix exclusive of dCTP, 100 μM of dCTP-biotin, 10 μM of vector (pBluescript II) primers T3 (5' -AATTAACCCTCACTAAAGGG-3' ; SEQ ID NO.: 7) and T7 (3' -CGGGATATCACTCAGCATAATG-5' ; SEQ ID NO.: 8) each, 1x PCR buffer, and 1 unit of Taq polymerase to a final volume of 10 μl, starting with pre-denaturation at 95° C for 5 min before 35 cycles of denaturation at 95° C for 10 sec, annealing at 65° C for 30 sec and extension at 72° C for 30 sec.

<68> The PCR-amplified biotinylated probe was purified using a QIAquickTM PCR purification kit (QIAGEN, Germany) and was added in an amount of about 100 ng onto the membrane, followed by hybridization at 65° C for 18 hrs. The membrane was washed twice with 2× SSC/1% SDS at room temperature for 5 min, then twice with 0.1× SSC/1% SDS at room temperature for 15 min, and finally twice with 1× SSC at room temperature for 5 min. Probe detection was performed using a Southern-starTM kit (Tropix, USA). The blots were treated with a blocking buffer (1× PBS, 0.2% I-BlockTM Reagent and 0.5% SDS) and labeled with alkaline phosphatase-conjugated streptavidin, followed by treatment with CDP-StarTM (Ready-to-Use). The membrane was exposed to an X-ray film (Fujifilm, Japan) for a period ranging from 10 min to 1.5 hrs.

<69>

<70> 2. Expression pattern of *IbExpansin*

<71> An about 1 kb *IbExpansin* EST clone of sweetpotato was labeled with biotin in PCR and was used in Northern blotting as probe.

<72> Expression of *IbExpansin* was detected in the tissues in a non-storage root stage, including FRN, Stem-FRN, Leaf-FRN and Petiole-FRN, with the highest level in FRN and Petiole-FRN. However, a remarkably decreased level of expression of *IbExpansin* was detected in the tuberous tissue in a late storage root stage, along with significantly low levels in stems and leaves at storage root-stage (FIG. 7). These expression patterns strongly imply that

IbExpansin is related to the elongation growth of roots in the early stage of storage root development, and that its activity does not promote the development of storage root.

<73>

<74> EXAMPLE 4: Construction of Binary Vector

<75> A 5' primer (SEQ ID NO.: 10) was synthesized with KpnI restriction site and a 3' primer was synthesized with BamHI restriction site to construct a knock-out binary vector. PCR was performed with the primers to obtain a full-length *IbExpansin* (NCBI accession number: DQ515800) cDNA. The PCR product was inserted into a pGEM-T Easy vector, and digested the pGEM-T Easy vector with BamHI and KpnI to excise the cDNA therefrom. The cDNA digest was inserted in the antisense direction between a CaMV35S promoter and an NOS terminator in a pMBP1 vector to construct the binary vector p*IbExpansin*-anti (FIG. 9). The insertion was confirmed by colony PCR and restriction enzyme digestion (FIG. 8)

<76>

<77> EXAMPLE 5: Induction of Embryogenic Callus in Sweetpotato

<78> The stem of the sweetpotato, named 'Youlmi' was cut to be with an axillary bud and cultured on MS basal medium (Murashige and Skoog, 1962) under a cool-white fluorescent lamp with 1,000 lux and a photoperiod condition of 16 hours for the in vitro culturing of sweetpotato. An apical meristem having a height of about 150um and a diameter of about 350um was removed from the cultured stem after removing young leaves under a dissecting microscope, and the medium was contacted with the cut surface of the apical meristem (Cantliffe et al., 1987; Liu et al., 1989). For induction of the embryogenic callus, MS basal medium was prepared by adding 100 mg/L myo-inositol, 0.4 mg/L thiamine-HCl, 30 g/L sucrose, and 4 g/L Gelrite to MS inorganic salt, adjusted to pH 5.8, added 1 mg/L 2,4-D, and used in the Petri dish (Resulting medium was referred as MS1D).

<79>

<80> This callus was subcultured on the same medium a one month intervals

(FIG. 10).

<81>

<82> EXAMPLE 6: Transformation of Sweetpotato using the gene gun and Screening of the Sweetpotato Transformant

<83> The embryogenic callus was cut into cell clusters 1-2mm in diameter. About 50-60 callus clusters were put in the center part of a Petri dish containing MS1D medium, cultured for one day, and then bombarded. Particle bombardment was carried out under vacuum condition with 1,100 PSi using helium gas. The bombardment was carried out according to the method of Sanford et al., which involves bombardment with gold particles coated with DNA at intervals of 6cm from the callus clusters, using 1.0 μg DNA and helium gas with 1,100 PSi pressure. After culturing in the dark at a temperature of 25℃ for 2 days, the bombardment was carried out once more under the same conditions. After culturing in the dark at a temperature of 25 ℃ for one week, the callus clusters were subcultured on MS1D solidified medium containing 100 mg/L kanamycin (selection medium) at a temperature of 25℃ and about 2,000 lux for 2 months. To facilitate the recovery of the callus stressed on the selection medium and the differentiation of shoots, the selected callus clusters were transferred to MS1D solidified medium containing 10 mg/L 2iP and 100 mg/L kanamycin. One month after transfer to the selection medium, the callus with a light green color was preferentially transferred to MS basal medium to induce a young bud, and transferred to the medium containing 20mg/L NAA to induce root development. Subculture was carried out approximately once every two weeks.

<84> The callus, beginning to develop buds and roots, was regenerated into a plant on the MS basal medium. DNA was isolated from the leaves of the transformed plant with the aid of QIAquickTM plant DNA miniprep kit (QIAGEN, Germany).

<85> PCR was performed using the isolated DNA, using primer NPT II 5' (GAGGCTATTCGGCTATGACTG- SEQ ID NO.: 12), and primer NPT II 3' (ATCGGGAGCGGCGATACCGTA-SEQ ID NO.: 13) together, starting from pre-

denaturation at 95° C for 5 min, with 30 cycles of denaturation at 95° C for 30 sec, annealing at 65° C for 30 sec and extension at 72° C for 1 min. The PCR products thus produced were separated by 1% agarose gel electrophoresis to detect a 700 bp band (FIG. 11).

<86>

<87> EXAMPLE 7: Expression Analysis of Transgenic Sweetpotato

<88> RNA was isolated from leaves of the transformed sweetpotato with the aid of 4.4M guanidinium-SDS lysis buffer (chirgwin et al., 1979), and 5.7M CsCl gradient method (Glisin et al., 1974). The total amount of sample containing total RNA 6㎍, and dNTP Oligo dT primer 10 pmole was adjusted to 13ul, denatured at 68° C for 5 min, and then immediately transferred to ice. After adding 5X RT-buffer 4㎕, RNase inhibitor(RNasin) 1㎕, 0.1M DTT 1㎕, and 200 U/㎕ of reverse transcriptase (Superscript III) to each sample, reverse-transcription was performed at 50℃ for 80 min, and was then denatured immediately at 70℃ for 15 min. 30 ㎕ of distilled water was added to each sample. RT-PCR was performed using the obtained cDNA as a template. PCR was performed using *IbExpansin* gene specific primers (5' TTC CAGATA AGG TGT GTG AAC 3'; SEQ ID NO.: 14, 5' ACT GTC TCC ACA CTC AGC 3'; SEQ ID NO.: 15), starting from pre-denaturation at 95° C for 5 min, with 30 cycles of denaturation at 95° C for 30 sec, annealing at 58° C for 30 sec and extension at 72° C for 1 min. To construct an internal equal loading control, PCR was performed using primer tublin-1(5' CAA CTA CCA GCC ACC AAC TGT 3'; SEQ ID NO.: 16) and primer tublin-2(5' CAA GAT CCT CAC GAG CTT CAC 3'; SEQ ID NO.: 17) under the same conditions as the *IbExpansin*. The PCR products thus produced were separated by 1% agarose gel electrophoresis. It was found that antisense line No.1 and No.4 were perfect knock-outs and that antisense line No.3 was a knock-down, decreasing the expression level (FIG. 12).

<89>

<90> EXAMPLE 8: Analysis of Transgenic Sweetpotato root Development

<91> Cuttings of the *IbExpansin*-antisense No.1 sweetpotato and wild-type were conducted in MS basal medium on the same date. 10 days after the

cutting, development patterns of roots were compared between transgenic sweetpotato No.1 and wild-type (FIGS. 13 and 14). The fibrous roots were observed with the naked eye. It was found that the fibrous roots of the transgenic sweetpotato No.1 was shorter (2-3 cm) in length and 2-3 times bigger in diameter than those of the wild-type with thin diameter and 5-6cm length (FIG. 13).

<92> The maturation zone of the root was cut, fixed at 4 ℃ for 10 days in 25mM potassium phosphate buffer(pH 7.0) including 2% Paraformaldehyde and 2.5% Glutaraldehyde, washed with buffer twice, 50, 60, 70, 80, 90, 95, and 100 % ethanol were changed serially to remove moisture, and then put in the capsule holding resin which had solidified one day earlier, after serially replacing the mixtures having ratios of resin to ethanol of 1:3, 1:1, 3:1 and 1:0, and solidified at 58℃ after adding the undiluted resin on the solidified resin in the capsule once more.

<93> After the resin hardened, the sample was cut widthwise and lengthwise respectively, and the shape of root cells was observed under a microscope. It was found that cells of the wild type were thin and long compared with the transformant, and on the contrary, cells of the transformant were short and thick compared with the wild-type (FIG. 14). In addition, in the case of transformant, it was observed that cell differentiation occurred actively within the primary cambium, so that cell differentiation was begun within the second cambium to develop storage root. Therefore, expression of the antisense DNA of *IbExpansin* cDNA according to the present invention can advance the onset of the storage root development.

<94>

<95> EXAMPLE 9: Analysis of Transgenic Sweetpotato storage root Development

<96> Cuttings of the transgenic sweetpotato No.1, No.4, and wild-type were conducted on the same day. 5 months after the cutting, development patterns of their aerial parts and storage roots and production were compared with one another (FIGS. 15 and 16). As for the growth of the aerial part, the wild type had a fewer number of branches and its first branch was very long. On

the other hand, it was found that the transgenic sweetpotato had more branches but that the length of its branches was about the same, and that the first branch were much shorter than the first branch of the wild type. Total aerial part biomass showed no difference between the transgenic sweetpotato and the wild type. There was a great difference between the transformant and the wild type in the development of storage root. In the wild type a part of its primary roots developed into storage roots, but in the transformant most of its primary roots developed into storage roots, so that transformant was superior to the wild-type in total storage root number by up to two times, and in total biomass by up to one and half times. There was a difference in the position from which storage root develop, namely, the primary roots of the wide type grew to about 5-10cm in length and then developed into storage roots, but the primary roots of the transformant developed into storage roots at the beginning point of the primary roots' development.

<97> Therefore, the antisense DNA of *IbExpansin* cDNA according to the present invention can be applied to the generation of highly productive transgenic sweetpotato to increase the storage root production usefully.

<98> While this invention has been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not limited to the disclosed embodiment and the drawings, but, on the contrary, it is intended to cover various modifications and variations within the spirit and scope of the appended claims.

【CLAIMS】

【Claim 1】

<100> A method for increasing storage root production, comprising:

<101> suppressing the expression of an expansin gene in cells of a plant.

【Claim 2】

<102> The method of claim 1, further comprising introducing antisense DNA of a cDNA from a sweetpotato expansin gene (*IbExpansin*) into the plant.

【Claim 3】

<103> The method of claim 1, further comprising inserting an antisense DNA of a cDNA of the expansin gene into a binary vector, and introducing the binary vector into the plant.

【Claim 4】

<104> The method of claim 2 or claim 3, wherein the antisense DNA comprises a nucleotide sequence of SEQ ID NO.: 9.

【Claim 5】

<105> The method of any one of claims 1 to 3, wherein the plant is sweetpotato.

【Claim 6】

<106> A method for preparing high productive transgenic sweetpotato, comprising:

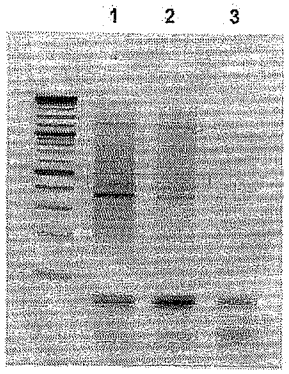
<107> introducing antisense DNA of the cDNA of a sweetpotato expansin gene into sweetpotato.

【Claim 7】

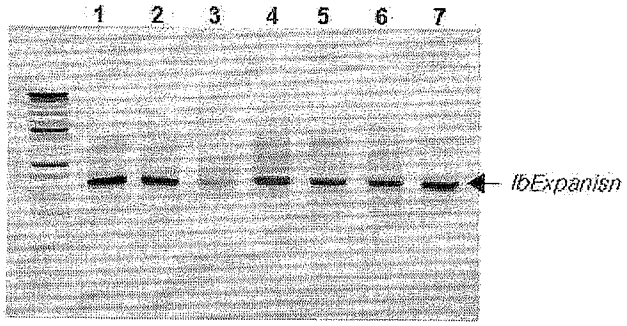
<108> A PCR primer suitable for amplifying a DNA fragment comprising the nucleotide sequence of SEQ ID NO.: 9, said primer being represented by one of the nucleotide sequences as shown in SEQ ID NO.: 10 or 11.

【DRAWINGS】

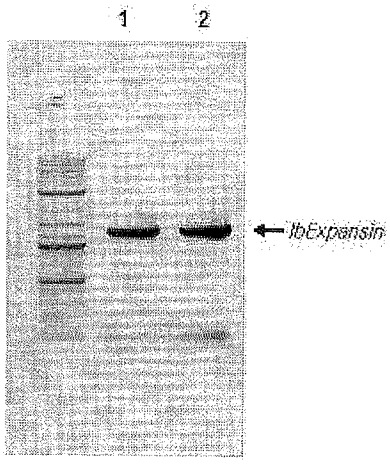
【Figure 1】



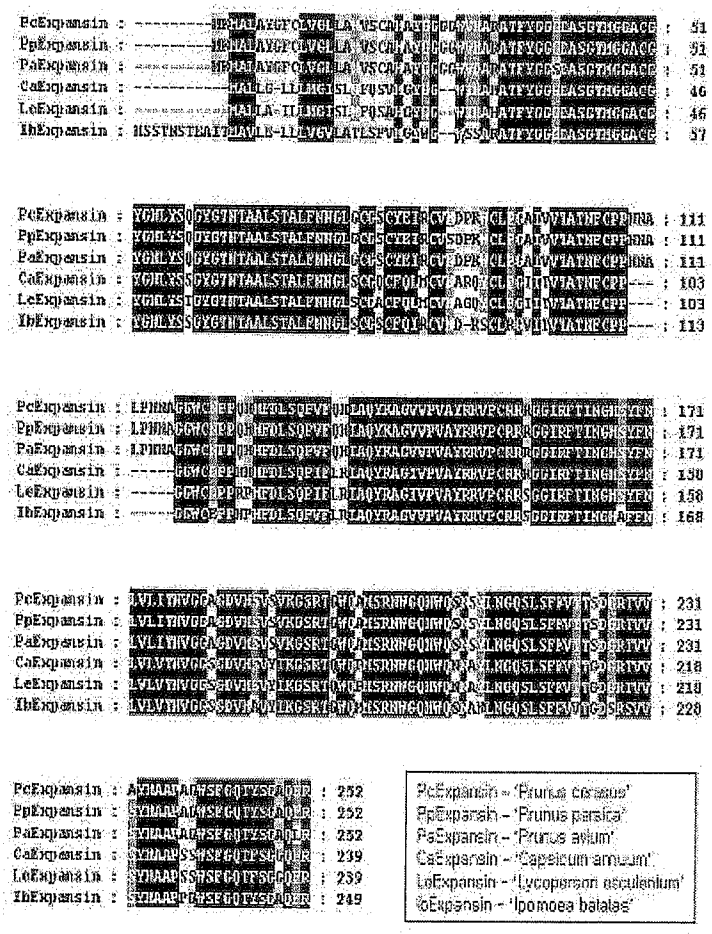
【Figure 2】



【Figure 3】



【Figure 4】

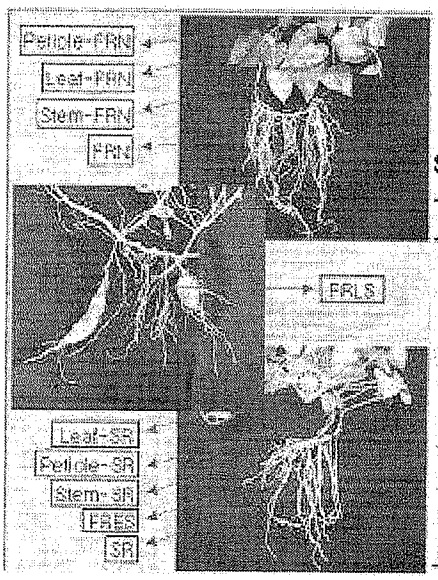


【Figure 5】

Homology among expansin amino acid sequences

Sequence comparison		Identity (%)
		overall
IbExpansin	*LeExpansin*	78
	CaExpansin	79
	PcExpansin	73
	PaExpansin	73
	PpExpansin	73

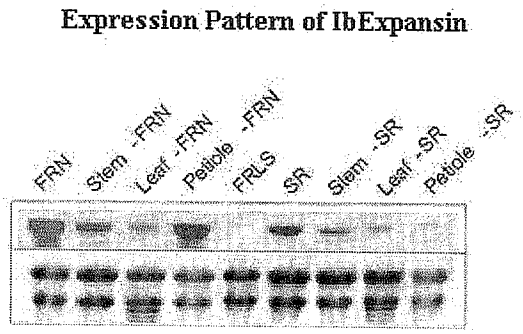
【Figure 6】



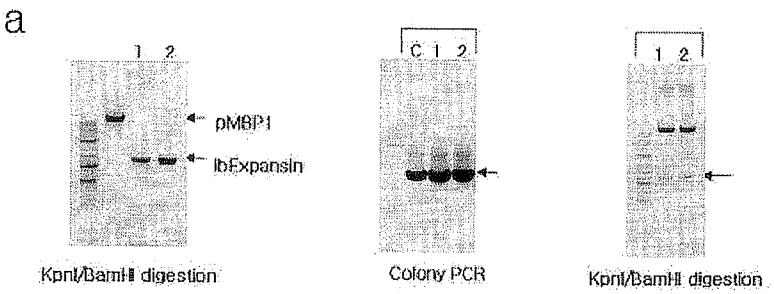
Sweetpotato tissues used in Nothern blot analysis

Sample	Description
FRN	Fibrous root of non-storage root stage
Stem-FRN	Stem tissue of non-storage root stage
Leaf-FRN	Leaf tissue of non-storage root stage
Petiole-FRN	Petiole tissue of non-storage root stage
FRES	Fibrous root of early storage root stage
FRLS	Fibrous root of late storage root stage
SR	Storage root (< 1.5 cm in diameter)
Stem-SR	Stem tissue of storage root stage
Leaf-SR	Leaf tissue of storage root stage
Petiole-SR	Petiole tissue of storage root stage

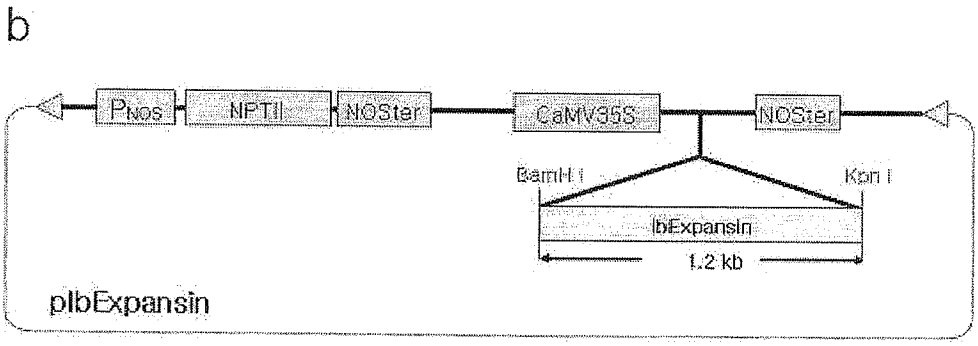
【Figure 7】



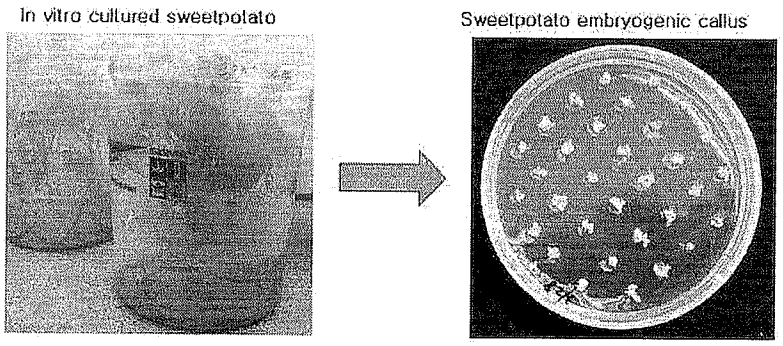
【Figure 8】



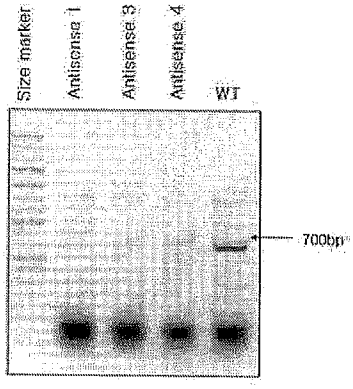
【Figure 9】



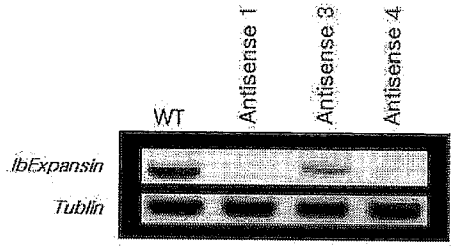
【Figure 10】



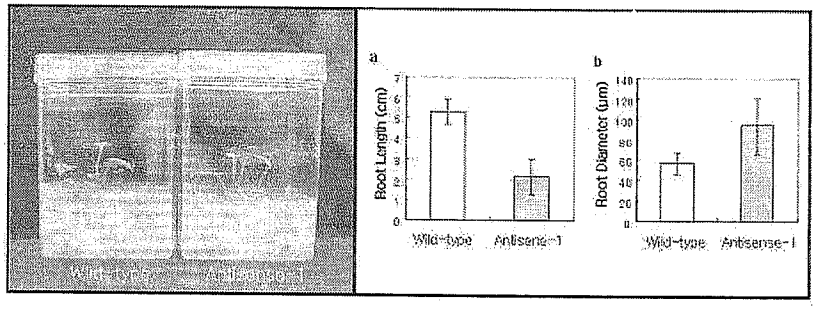
【Figure 11】



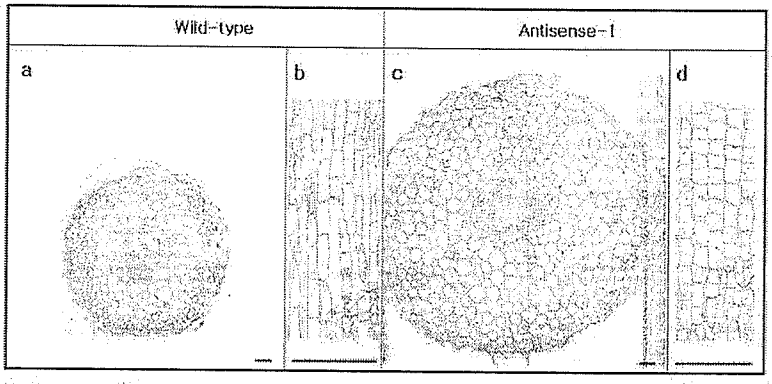
【Figure 12】



【Figure 13】



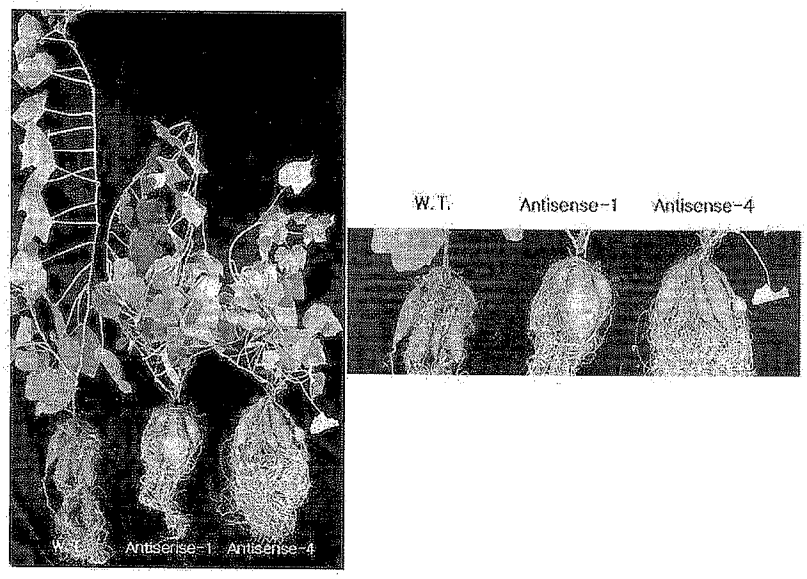
【Figure 14】



a, c: cross section
b, d: longitudinal section

Bars are 50μm.

【Figure 15】



【Figure 16】

	Storage root		Shoot	
	Number	Total weight (g)	Number	Total weight (g)
Wild-type	4.75±0.96	163.71±24.62	2.5±0.58	118.13±19.02
antisense1	5.33±0.58	248.20±33.93	3.3±0.58	118.48±11.66
antisense4	8.25±1.50	224.45±30.55	2.8±0.75	118.48±11.02

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/001343

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)

IPC 8 A01H 1/00, C12N 15/82, C07H 21/04, C12N 9/24, C12N 5/04

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

Korean Utility Models and Applications for Utility Models since 1975

Japanese Utility Models and Applications for Utility Models since 1975

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

eKIPASS, WPI, USPTO, PAJ, NCBI, CAPLUS(STN), INSPECT "expansin, storage root, sweetpotato, antisense, storage root production, IbExpansing, binary vector etc."

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Plant Physiol, Vol.141: 1149-1158 (2006) "Molecular cloning and characterization of b-expansin gene related to root hair formation in barley" - see the whole document	2, 4-7
A	Mol. Gen. Genomics, Vol.274: 548-556 (2005) "Isolation and characterization of 18 genes encoding a- and b-expansins in wheat (Triticum aestivum L.)" - see the whole document	2, 4-7
A	Plant Physiology, Vol.131: 985-997 (2003) "Expression of an expansin gene is correlated with root elongation in soybean" - see the whole document	2, 4-7
A	Genome Biology, Vol.6;242 (2005) "The expansin superfamily" - see the whole document	2, 4-7

☒ 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	Date of mailing of the international search report
25 JULY 2008 (25.07.2008)	**28 JULY 2008 (28.07.2008)**

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

KIM Kee-Yeun

Telephone No. 82-42-481-8387

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/001343

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Plant Physiol. Biochem., Vol.44(5-6): 301-307 (2006) "Expression of expansin genes in strawberry varieties with contrasting fruit firmness" - see the whole document	2, 4-7
A	Plant Physiol., Vol.143(1): 278-290 (Epub 2006) "Association of specific expansins with growth in maize leaves is maintained under environmental, genetic, and developmental sources of variation" - see the whole document	2, 4-7
A	Plant Physiol., Vol,140(2): 603-612 (Epub 2005) "Progressive inhibition by water deficit of cell wall extensibility and growth along the elongation zone of maize roots is related to increased lignin metabolism and progressive stelar accumulationof wall phenolics" - see the whole document	2, 4-7
A	US 2005/0246795 A1, (J.S., Lee, et al., KR) 3 Nov 2005 - see the whole document	2, 4-7
A	US 2003/0054533 A1, (Daniel J.C., et al., US) 20 Mar 2003 - see the whole document	2, 4-7
A	US 2004/0049809 A1 (S.C.M. Fonseca, et al., PT) 11 Mar 2004 - see the whole document	2, 4-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/001343

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

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

   a. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [ ] on paper
   - [x] in electronic form

   c. time of filing/furnishing
   - [ ] contained in the international application as filed
   - [x] 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/KR2008/001343

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1, 3
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 1 and 3 are directed at or refer to a method for increasing storage root production by suppressing the expression of an expansin gene without limiting the method suppressing the expression, an expansin gene and are considered too broad and so inadequately supported by the description that no meaningful search could be made.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2008/001343

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005-246795 A1	03.11.2005	KR 10-2004-0084186	06.10.2004
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		BR 200113366 A	29.07.2003
		EP 1322770 A2	02.07.2003
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