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(21) International Application Number: PCT/GB94/02467 (22) International Filing Date: 10 November 1994 (10.11.94) (30) Priority Data: 9323225.4 10 November 1993 (10.11.93) GB (71) Applicant (for AU BB CA GB IE LK MN MW NZ SD only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB). (71) Applicant (for all designated States except US): UNILEVER N.V. [NL/NL]; Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): DE SILVA, Jacqueline [GB/GB]; Pear Tree Cottage, High Street, Pavenham, Bedfordshire MK43 7NJ (GB). WHITEMAN, Sally-Anne [GB/GB]; 14 Hindburn Close, Brickhill, Bedfordshire MK41 7YJ (GB). HELLYER, Susan, Amanda [GB/GB]; The Old Maltings, Church Lane, Upper Dean, Huntingdon, Cambridgeshire PE18 0NE (GB). ARROWSMITH, David, Andrew [GB/GB]; 59 Queen's Street, Rushden, Northamptonshire NN10 0AY (GB).		(74) Agent: KEITH W. NASH & CO.; Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP (GB). (81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). Published <i>With international search report.</i>
(54) Title: TOMATO XYLOGLUCAN ENDO-TRANSGLYCOSYLASE		
(57) Abstract Disclosed is a polypeptide having xyloglucan endo-transglycosylase (XET) activity, comprising substantially residues 21-289 of the amino acid sequence shown in Figure 1 (Seq ID No. 2) or functional equivalents thereof, together with nucleotide sequences encoding the same and various uses thereof.		

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TOMATO XYLOGLUCAN ENDO-TRANSGLYCOSYLASE

Field of the Invention

This invention relates to nucleotide sequences encoding a plant enzyme, vectors containing said nucleotide sequences, hosts containing said nucleotide sequences, amino acid sequences encoded by said nucleotide sequences, recombinant DNA methods of producing the enzyme, and a method of altering the properties of a plant.

Background of the Invention

Fruit and vegetable cell walls are largely polysaccharide, the major components being pectin, cellulose and xyloglucan (Selvendran & Robertson, IFR Report 1989). Numerous cell wall models have been proposed which attempt to incorporate the essential properties of strength and flexibility (e.g. Albersheim, 1975 Sci. Am. 232, 81-95; Albersheim, 1976 "The primary cell wall", Plant Biochemistry, 3rd Edition, Academic Press; and Hayashi, 1989, Ann. Rev. Plant Physiol. & Plant Mol. Biol. 40, 139-168).

Xyloglucans are 1,4- β -glucans that are extensively substituted with α -1,6-xylosyl side chains, some of which are 1,2 β -galactosylated. They are found in large amounts in the primary cell walls of dicots but also in certain seeds, where they serve different roles.

Primary cell wall xyloglucan is tightly hydrogen bonded to cellulose microfibrils and requires concentrated alkali or strong swelling agents to release it. Xyloglucan is thought to form cross-bridges between cellulose microfibrils, the cellulose/xyloglucan network forming the major load-bearing/elastic network of the wall. DCB mutated suspension culture cells (cell walls lacking cellulose) release xyloglucan into their media, suggesting that xyloglucan is normally tightly bound to cellulose.

Hydrolysis of primary cell wall xyloglucan has been demonstrated in segments of dark grown squash hypocotyls, during IAA induced growth (Wakabayashi et al., 1991 Plant

Physiol. 95, 1070-1076). Endohydrolysis of wall xyloglucan is thought to contribute to the wall loosening which accompanies cell expansion (Hayashi, cited previously). The average molecular weight of xyloglucan has also been shown to decrease during tomato fruit ripening and this may contribute to the tissue softening which accompanies the ripening process (Huber, 1983 J. Amer. Soc. Hort. Sci. 108, 405-409).

Certain seeds, e.g. nasturtium, contain up to 30% by weight of xyloglucan, stored in thickened cotyledonary cell walls, which serves as a reserve polysaccharide and is rapidly depolymerised during germination.

An endo 1,4 β -D glucanase which specifically acts on xyloglucan (i.e. a xyloglucanase) has been isolated and purified to apparent homogeneity from germinating nasturtium (*Tropaeolum majus* L.) seeds (Edwards et al., 1986 J. Biol. Chem. 261, 9494).

The purified xyloglucanase gives a single polypeptide band on SDS polyacrylamide gel electrophoresis, (apparent molecular weight, 29-31kDa) and isoelectric focusing (isoelectric point, 5.0). The enzyme displays an absolute specificity for xyloglucan and an endo mode of action, as determined by end product analysis following hydrolysis of tamarind seed xyloglucan (Wakabayashi et al., cited above). Although the natural substrate of the enzyme is nasturtium cotyledonary reserve xyloglucan, it has also been shown to hydrolyse primary cell wall xyloglucans *in vitro* (Edwards et al., cited previously). At high substrate concentrations, xyloglucan endo-transglycosylase (XET) activity has been demonstrated (Fanutti et al., 1993 The Plant Journal 3, 691-700).

The nucleotide sequence of this nasturtium xyloglucanase/XET enzyme has been determined and is disclosed in International Patent Application No. WO 93/17101 and by de Silva et al., (1993 The Plant Journal 3, 701-711).

Similar enzyme activity has been detected in other plant tissue and shown to be positively correlated with growth rate in different zones of the pea stem (Fry et al., 1992 Biochem. J. 282, 821-829). It has been proposed that XET is responsible for cutting and rejoining intermicrofibrillar xyloglucan chains and that this causes the wall-loosening required for

plant cell expansion. XET activity has also been demonstrated in tomato fruit (xyloglucanase apparently activatable by xyloglucan oligosaccharides) where it is reportedly highest at the 2 days post-"breaker" stage of ripening (Machlachlan & Brady, 1992 Aust. J. Plant Physiol. 19, 137-146) and may be involved in the softening process.

This application describes the isolation from tomato plants of a nucleotide sequence which encodes an XET activity.

Summary of the Invention

In a first aspect the invention provides a polypeptide having xyloglucan endo-transglycosylase (XET) activity, comprising substantially residues 21-289 of the amino acid sequence of Figure 1 (Seq ID No. 2), or functional equivalents thereof.

The term "functional equivalent" as used herein and applied to an amino acid sequence is intended to refer to those amino acid sequences having the same functional characteristics as the sequence of the invention but which have slightly different sequences. Generally, for example, it is well known that one may make a "conservative" substitution, exchanging one amino acid residue in a sequence for another residue with very similar properties, without having any significant effect on the function of the polypeptide.

An example of a functionally equivalent polypeptide sequence is shown in Figure 5 (Seq ID No. 8). The amino acid sequences shown in Figures 1 and 5 are directly compared in Figure 6.

Further, one or more amino acid residues may be deleted or added without significant deleterious effect. It will be apparent to those skilled in the art that such changes may particularly be made at points in the sequence which are not evolutionarily conserved. Enzyme precursors are also included within the meaning of "functional equivalents" and also mature, processed enzymes lacking signal sequences. In the present case amino acid residues 1-20 of Figure 1 are believed to constitute such a signal sequence and are therefore not essential for enzyme activity. Likewise, amino acid residues 1-18 of the

sequence shown in Figure 5 are thought to comprise a non-essential signal sequence.

In specific embodiments therefore the invention may provide a polypeptide having XET activity and comprising residues 1-289 of the sequence shown in Figure 1; or residues 19-287 of the amino acid sequences shown in Figure 5; or residues 1-287 of the amino acid sequence shown in Figure 5.

Preferably such functional equivalents will exhibit at least 80% homology, and more preferably at least 85% homology, and most preferably at least 90% homology, with the amino acid sequence of the invention.

The present inventors have, by analysis of the amino acid sequence data, identified a number of peptide sequences which are thought to be relatively conserved within functional equivalents of the sequence of the invention.

Preferably, functional equivalents will comprise substantially one or more of the following peptide sequences (which will generally be perfectly conserved but may contain up to one or, at most, two amino acid differences):

DEIDFEFLGN; SLWNADDWAT; FYSKNEYLFG; and GTVTTFYLSS; (Seq ID Nos. 3-6 respectively).

Desirably the polypeptide is substantially in isolation from other plant derived substances.

In a second aspect the invention provides a nucleotide sequence encoding the amino acid sequence of Figure 1 (Seq ID No. 1), or functional equivalents thereof.

Preferably the nucleotide sequence comprises the nucleotide sequence of Figure 1 or other functionally equivalent sequences obtainable from tomato plants. The term "functional equivalent" as used herein and applied to nucleotide sequences is intended to refer to nucleotide sequences encoding a polypeptide having the same functional characteristics as the amino acid sequences of the first aspect and includes nucleotide sequences which will hybridise under standard conditions (described by Sambrook et al. 1989, Molecular

Cloning: A Laboratory Manual) to the complement of the nucleotide sequence of Figure 1. Preferably the functionally equivalent nucleotide sequence will exhibit at least 70% homology, more preferably 80% homology and most preferably at least 85% homology with the nucleotide sequence of Figure 1. A specific example of a functionally equivalent nucleotide sequence is shown in Figure 5 (Seq ID No. 7).

For the purposes of the present specification, the term "functional equivalent" also refers to "anti-sense" sequences which hybridise under standard conditions to the nucleotide sequence of Figure 1. Such sequences have utility in down-regulating the expression of the nucleotide sequence of the invention. Preferably such anti-sense functional equivalents will exhibit at least 70% homology, more preferably at least 80% homology, and most preferably at least 90% homology with the complement of the nucleotide sequence of Figure 1. In general, anti-sense functional equivalents will preferably exhibit higher percentage homology than sense equivalents. This is because sense constructs will be translated into polypeptides which will be functional and which may contain conservative amino acid substitutions. In contrast, anti-sense constructs will, in the vast majority of instances, function at the level of nucleic acid rather than polypeptide, and thus any base sequence differences will tend to reduce the usefulness of the construct.

Those skilled in the art will understand that, with the benefit of the disclosure herein relating to the nucleotide and amino acid sequences of the invention, it will be simple to detect, isolate and clone (e.g. by PCR) other nucleotide sequences which are functionally equivalent to the nucleotide sequence of the invention. Suitable methods, for example, are taught by Sambrook et al. Of particular usefulness in such routine work may be the peptide sequences disclosed above. Also, the polypeptide of the invention (or peptides derived therefrom) may be used to generate antibodies for use in immunological screening of putative functionally equivalent sequences.

It is preferred that the sequence of the second aspect of the invention further comprises a 5' untranslated region including a suitable promoter, to enable expression in host cells.

The invention also provides a vector comprising the nucleotide sequence of the second

aspect of the invention, and a host cell comprising the nucleotide sequence of the invention.

In one embodiment, the vector is capable of expressing the nucleotide sequence, so as to produce the polypeptide of the invention. (Other vectors may comprise an antisense functionally equivalent nucleotide sequence).

Thus, in a further aspect, the invention provides a method of producing a polypeptide having XET activity, comprising the steps of: introducing into a suitable host cell a vector capable of directing the expression of a polypeptide as defined above, growing the host cell and/or the progeny thereof in suitable culture conditions so that the polypeptide is expressed, followed by obtaining the polypeptide from either the culture medium and/or the host cells.

It is preferable, but by no means essential, that the host cell is eukaryotic, as a eukaryotic host is more likely to express the enzyme in a fully functional conformation.

Suitable vectors are known which can be used to introduce and express the sequence of the invention in plants.

In a further aspect the invention provides a method of transforming a host cell, comprising the introduction into the host cell of the nucleotide sequence of the second aspect of the invention. Preferably the host cell is a plant cell. Preferably the transformed plant cell comprises part of a plant.

As will be apparent to those skilled in the art, the introduction of the nucleotide sequence of the invention into a plant or part thereof would be expected to alter the characteristics of the plant or part thereof.

In a still further aspect, the invention provides a method of altering the characteristics of a plant or part thereof, comprising introducing into the plant or part thereof an effective portion of the nucleotide sequence of the invention or a functional equivalent thereof, so

as to alter the level of XET activity in the plant or part thereof. Preferably the method is such as to reduce the level of XET activity in the plant or part thereof, typically by introducing an antisense sequence. Preferably the effective portion will comprise at least 50%, more preferably at least 70%, and most preferably at least 90% of the nucleotide sequence of the invention?

In another aspect, the invention provides a genetically engineered plant having characteristics altered by the method defined above, or the progeny of such a plant.

The altered plant is preferably any commercially important plant (including fruit or vegetable plants) where there is sufficient knowledge to perform the method of the invention and in which xyloglucan has a structural function (i.e. dicots and non-Graminaceous monocots).

Such plants include: alfalfa, apple, broccoli, cabbage, carrot, cauliflower, celery, cotton, cranberry, cucumber, eggplant, flax, grape, horseradish, kiwi, lettuce, mangoes, melon, oilseed rape, papaya, pea, peaches, pears, peppers, plum, poplar, potato, raspberry, soybean, spruce, strawberry, sugarbeet, sweet potato, tobacco, tomato and walnut.

It will be appreciated that the characteristics of the whole plant need not be altered. It may be desirable to alter the properties of parts of the plant (e.g. seeds, fruit). This could be achieved, for example, by the use of tissue-specific promoters to regulate transcription of the introduced sequence.

The method of the invention would be expected to alter levels of XET activity and thus the frequency of breaking and re-forming of cell wall cross-links (consisting of xyloglucan molecules) resulting in wall loosening which may permit cell expansion. Moreover, in view of the important structural role of xyloglucan, one might expect the characteristics which might be altered to include: size, rate of growth, and texture, especially during ripening.

The invention is further described by reference to the following illustrative example and

drawings, in which:

Figure 1 shows an amino acid and nucleotide sequence (clone B1) in accordance with the invention;

Figure 2 shows partial amino acid sequences of a polypeptide in accordance with the invention and a functional equivalent thereof, compared with two prior art amino acid sequences;

Figure 3 shows the sequencing strategy used to determine the sequence shown in Figure 1, (E = EcoRI, P = Pst, H = HindIII);

Figure 4 shows the sequencing strategy used to determine the sequence of a nucleotide (clone B2) in accordance with the invention (E = EcoRI, P = Pst);

Figure 5 shows a nucleotide sequence (clone B2) and amino acid sequence in accordance with the invention;

Figure 6 shows a comparison of two functionally equivalent amino acid sequences (dot = identical amino acid; triangle = predicted cleavage site); and

Figures 7 and 8 are schematic illustrations of various plasmid constructs.

Examples

Example 1

Following complete sequencing of a cDNA encoding nasturtium seed XET (described in pending International Patent Application No. WO 93/17101), primers were designed to attempt PCR amplification of similar gene fragments from tomato. Two of the primers (NXG2H and NXG2B, shown below) overlapped with a region homologous to a *Bacillus macerans* β -1,3-1,4-glucanase (the region having the amino acid sequence DEIDIEFLG).

The other primers corresponded to regions adjacent to or surrounding histidine residues; (these are often present in active sites of hydrolytic enzymes, and active sites of enzymes of similar function often having primary sequence homology). These corresponded to amino acid sequences PNHNRVD (primer NXG1H), HDEIDIE (primers NXGH2H and NXG2B), HLWFDPT (primers NXG3H and NXG3B) and TRDHTLT (primer NXG4B).

NXG1H	5' AAGCTTCCTCAACATCAAAGGGTAGA 3'	(sense)
NXG2H	AAGCTTCATGATGAAATCGATATTGA	(sense)
NXG2B	GGATCCTCAATATCGATTTTCATCATG	(antisense)
NXG3H	AAGCTTCATTTATGGTTTGATCCAAC	(sense)
NXG3B	GGATCCGTTGGATCAAACCATAAATG	(antisense)
NXG4B	GGATCCGTTAACGTGTGGTCTCGTGT	(antisense)

(Seq ID Nos. 9-14, respectively)

These primers were completely conserved with respect to the nasturtium sequence and incorporated restriction enzyme recognition sites at their 5' ends to facilitate cloning of PCR products. However all attempts to amplify tomato DNA by PCR, using any combination of the above primers, proved unsuccessful.

The deduced amino acid sequence of nasturtium seed XET shares some regions of homology (42% identity in a 151aa overlap) with a sequence deduced from a genomic clone and cDNA from *Arabidopsis thaliana* (Medford et al., 1991 The Plant Cell 3, 359-370). The polypeptide encoded by the genomic clone is termed meri-5 and its function is currently unknown.

It was decided to attempt PCR amplification of tomato DNA using a mixture of degenerate oligonucleotides (XG 117 and XG 162, shown below) corresponding to two of these regions conserved between nasturtium XET and meri-5, although there was little expectation of success in view of the previous failures and the lack of knowledge concerning the function (if any) of meri-5.

Surprisingly, PCR succeeded in amplifying a 122bp tomato genomic DNA fragment

having significant sequence homology to both meri-5 and nasturtium XET.

The experiment was performed as described below.

Genomic DNA was isolated from young tomato leaf tissue (cultivar VF 143B-7879, available from the Tomato Genetics Stock Centre, Department of Vegetable Crops, University of California, Davis, CA95616), as described by Dellaporta et al. (1983, Plant Mol. Biol. 1, 19-21).

The degenerate oligonucleotide mixtures were designed corresponding to the conserved regions DEID(I/F)EFLG (XG 117) and HLWFDPT (XG 162), as shown below:

XG 117 5' GA(C/T) GA(A/G) ATI GA(C/T) (A/T)T(A/C/T) GA(A/G) TT 3' (sense)

XG 162 3' GT(G/A) (G/A)A(G/A/T/C) ACC AA(G/A) CT(G/A) GGI TG 5' (antisense)
(Seq ID Nos. 15 and 16 respectively)

The degenerate oligonucleotides were then used as primers in the following reactions (each reaction having a total reaction volume of 100 μ l): 1-2 μ g genomic DNA; 100 picomoles each of XG 117 and XG 162; dGTP, dATP, dTTP and dCTP all at 0.2mM; 10mM Tris-HCl pH 8.3; 50mM KCl; 1.5mM MgCl₂; 0.01% (W/V) gelatin; and 2.5 units Taq DNA polymerase (Stratagene).

Thermal cycling was carried out in an MJ Research programmable thermal controller with an initial denaturing step of 94°C for 2 minutes, followed by 35 cycles of 94°C for 1 minute, 40°C for 2 minutes and 72°C for 2 minutes. Amplification products were fractionated on a 2% agarose/TBE gel containing 0.5 μ g/ml ethidium bromide and viewed on a UV transilluminator. DNA fragments were purified from gels using DEAE paper, as described by Sambrook et al. (Molecular Cloning: a laboratory Manual).

The main PCR product was found to be 122bp in size. This was ligated into pT7Blue (obtained from AMS Biotechnology) and subjected to DNA sequence analysis. Of the four

clones sequenced, three had identical DNA sequences (PX3) and one differed in both DNA and deduced amino acid sequence (PX1), as shown in Figure 2. In Figure 2, Tom 3 and Tom 1 represent the deduced amino acid sequence for PX3 and PX1 respectively, as compared with Meri-5 and nasturtium XET.

In order to obtain more extensive sequence information, a tomato cDNA 5' stretch library was obtained from Clontech Laboratories Inc. (prepared using mRNA from the "breaker" stage of ripening fruit from tomato, "Ailsa craig" cultivar VFN8); this was primed with oligo-(dT) and random primers to synthesise cDNA, which was packaged in phage lambda gt11.

Approximately 200,000 cDNA clones from this library were screened using the 122bp PCR fragment as a radiolabelled probe. Briefly, 25-30ng of gel-purified 122bp amplification product were denatured in 9 μ l H₂O by heating at 100°C for 5 minutes and then cooling on ice. The fragments were radio-labelled with ³²P-dCTP (Amersham International plc) using a kit supplied by United States Biochemicals Inc. (Sequenase v2.0). Unincorporated nucleotides were removed by chromatography through a Sephadex G-50 column.

Using this probe, a number of positively-reacting clones were detected in the cDNA library. These were eluted from top layer agarose into 0.1-ml SM (50mM Tris-HCl pH 7.5, 10mM MgSO₄, 100mM NaCl, 0.01% w/v gelatin) and purified by further rounds of screening. The insert was isolated from the bacteriophage either by direct PCR of the stock suspension or by digestion of isolated DNA.

For PCR of the stock suspension, purified bacteriophage stocks (5 μ l, 10⁵-10⁶pfu) were heated at 100°C for 5 minutes and added to a solution identical to that described previously except that 20pmols each of gt11 5' and 3' oligonucleotides (Clontech) were used instead of the degenerate ones. Thermal cycling was as follows: 2 minutes at 94°C, 25 cycles of 1 minute at 94°C, 1 minute at 55°C and 2 minutes at 72°C, followed by a final step of 5 minutes at 72° C. Amplification products were gel-purified on 1% agarose gels as above.

Gel-purified PCR-amplified products were ligated into pT7 Blue PCR cloning vector (AMS Biotechnology Limited) and transformed into competent *E. coli* according to the manufacturer's instructions.

Colonies containing recombinant plasmids were used to inoculate 30ml Lennox broth containing 50 μ g/ml ampicillin and 12.5 μ g/ml tetracycline; after overnight growth at 37°C, purified plasmid DNA was obtained from the culture using a "Plasmid midi" kit supplied by Qiagen Inc. Restriction analysis of the cloned products was carried out using enzymes and buffers supplied by Amersham International plc, followed by agarose gel electrophoresis.

DNA was sequenced using kits and protocols supplied by United States Biochemicals Inc. (Sequenase v2.0). Recombinant plasmids (Qiagen-prepared) were either sequenced directly or restriction fragments were subcloned into M13 to produce single-stranded templates. Reaction products were fractionated on 30cm x 40cm x 0.4mm polyacrylamide gels using BRL 6% acrylamide solutions. An IBM computer with DNASTar software was used to analyse resultant sequences.

Clones were found to vary in size between 500 and 1400 bp. One clone, termed clone tXET-B1 (or "B1" for simplicity), was found to possess internal HindIII and SacI recognition sites which facilitated subcloning into M13. Internal primers were also used in order to sequence the clone fully and in both directions (Fig. 3). The sequences of these internal primers (8-TH, 8-HS sense primers and 8-TB, 8-HT antisense primers) are as follows:

8-TH: 5' CGATGCAGCTGGAATTTCA 3'

8-TB: 5' AGTTTGAGCTGCATATCGAA 3'

8-HS: 5' TTTGATCCACCTCGCCGTT 3'

8-HT: 5' CAGCTGACCAAACACAAGCA 3'

(Seq ID Nos. 17-20 respectively)

The sequences of the other primers were:-

T3: 5' ATTAACCCTCACTAAAGGGA 3'

T7: 5' TAATACGACTCACTATAGGG 3'

U19: 5' GTTTTCCCAGTCACGACGT 3'

(Seq ID Nos. 21-23 respectively)

U represents the M13 (-40) primer. Initially, two sequences were found, each from a different PCR amplification from the lambda stock. They differed in 2 base pairs within the putative coding region. Since these differences were probably due to the inaccuracy of the thermostable DNA polymerase used in the PCRs, lambda DNA was prepared and the cDNA ligated into pBluescript SK+, in order to obtain definitive sequence data (Fig. 1). The definitive nucleotide sequence of clone B1 is shown in Figure 1, together with the deduced amino acid sequence.

The putative open reading frame of clone B1 encodes a 289 amino acid polypeptide of predicted molecular weight 32.7kDa. This has 39% identity in 270 overlapping amino acids with nasturtium XET and 59% identity in an overlap of 186 amino acids with meri-5.

A signal peptide is predicted on the basis of hydrophobicity of amino acids in positions 1-25, and statistical analysis of the amino acid sequence predicted that the precursor is cleaved between alanine and aspartate residues at positions 20 and 21 (Von Heijne, 1983 *Eur. J. Biochem.* 113, 17-21).

The region of clone B1 corresponding to the genomic PCR fragments (aa 103-129) shows differences in the deduced amino acid sequence compared to both of the genomic fragment sequences. This suggests that clone B1 is part of a multi-gene family in tomato. A putative N-glycosylation site (Asn-X-Ser/Thr ; aa 105-107) was identified, which is also present in meri-5 and the genomic fragments, but not nasturtium XET.

The second clone obtained, termed clone tXET-B2 (abbreviated to "B2"), was larger than clone B1 but had no convenient restriction sites for subcloning, so several internal primers were used to complete DNA sequencing. The strategy used to determine the sequence of

clone B2 is shown schematically in Figure 4. In figure 4, E = EcoRI site, P = PstI site; (a) shows sequencing using vector primers, (b) and (c) shows sequencing with internal primers. A number of primers were employed, including:

16-1: 5' AATATCAGCGGAAACAGGG 3'
16-1R: 5' CCCTGTTTCCGCTGATAATT 3'.
16-2: 5' CTATTAGCATCTGCACAGTA 3'
16-2R: 5' TACTGTGCAGATGCTAATAG 3'
16-3R: 5' GGAGGACGGGTGAAGACAGA 3'
16-4: 5' GTGTAAGGTAGTCCTGATGA 3'
16-4R: 5' TCATCAGGACTACCTTACAC 3'
(Seq ID Nos. 24-30 respectively)

A deduced open reading frame of 287 amino acids was identified. The determined nucleotide sequence and deduced amino acid sequence of clone B2 are shown in Figure 5. Figure 6 shows the cDNA sequence of clone B1 and deduced amino acid sequence thereof, in direct comparison with the deduced amino acid sequence for clone B2. (In Figure 6, dots indicate identical amino acids, and the arrow indicates the predicted signal peptide cleavage site.) The predicted signal peptide for clone B2 was 2 amino acids shorter than that of clone B1, leaving a mature polypeptide of 269 amino acids. A putative N-glycosylation site was found in the same position as for clone B1. Sixteen amino acid differences between the predicted mature polypeptides of clones 8 and 16 were identified, 14 of which were conservative.

Hydrophobic Cluster Analysis (HCA, as described by Gaboriaud et al., 1987 FEBS Letters 224, 149-155 and Henrissat et al., 1988 Biochem. J. 255, 901-905) was performed to investigate possible conformational homology of the polypeptide encoded by clone B1 with other known proteins.

HCA plots were produced from the deduced amino acid sequences of nasturtium XET, meri-5 and tomato clone B1 using WordPerfect™ 5.1 macros, using a modified 2D representation of an α -helix (Henrissat et al, cited above). Hydrophobic clusters were

encircled by hand, aligned manually and HCA homology scores calculated.

Similar folding was predicted for all three proteins on the basis of (a) "continuous presence of conserved clusters" throughout most of the length of the sequence and (b) overall HCA homology scores for all three comparisons being more than 75% (meri-5 v. tomato XET clone B1, 86%; nasturtium XET v. tomato XET clone B1, 80%; meri-5 v. nasturtium XET, 78%).

This supports data from primary sequence homology and secondary structure predictions (Kyte & Doolittle 1982, J Mol Biol 157, 105-132) which suggest that these three proteins are likely to have broadly similar folding and therefore similar function, whilst differing in detail.

It was decided to attempt to express clone B2 in *E. coli*. This is described below in Example 2.

Example 2

Manipulation of Clone B2 for expression in *E. coli*

A BamHI recognition sequence was introduced close to the N-terminal coding sequence of the putative mature protein by PCR, using a mutagenic primer (16-1 B: CCCTTGGATCCGCTATAATT Seq ID No. 31) and a pBluescript primer (T3; Stratagene). Reaction conditions were: 1ng XET clone B2 in pBluescript SK+, 0.4mM each of dATP, dCTP, dGTP and dTTP, 25 pmols of each primer, 1 x VENT polymerase buffer and 0.25 units VENT DNA polymerase (New England Biolabs Inc.). Thermal cycling was 2 minutes at 93°C, 25 cycles of 1 minute at 93°C, 30 seconds at 55°C and 1 minute at 72°C.

Over-expression of recombinant tomato XET in *E. coli*

Expression of tomato XET in *E. coli* was achieved using the QIAGEN QIAexpress™

system. A BamHI-HindIII fragment of the mutagenised PCR product was ligated into the expression vector pQE30 and transformed into *E. coli* M15 cells containing the pREP4 repression plasmid. The expression vector allows incorporation of a 6 x histidine tagged N-terminal which has high affinity for Ni-NTA (Ni-NTA is nickel-nitrilo tri-acetic acid) resin provided by QIAGEN. Cells were grown in Lennox broth containing appropriate antibiotics and induced with 2mM IPTG when the optical density of the culture at 600nm was 0.7-0.8. Growth was then allowed to continue for 3-5 hours. For analysis, cells were pelleted from 0.5ml of the culture, boiled in sample buffer (Laemmli 1970 Nature 227, 680-685) and run on SDS-polyacrylamide gels (10%); separated proteins were stained in Coomassie blue. Localisation of tagged proteins was established as in protocol 4 of "The QIAexpressionist" 2nd edition (1992; QIAGEN Inc.).

Recombinant tomato XET protein purification and refolding

Recombinant tomato XET was purified under denaturing conditions on Ni-NTA resin as described in "The QIAexpressionist" protocol 7, except that after the final wash, the column with bound protein was equilibrated in 6M urea, 0.5M NaCl, 20% glycerol, 40mM Tris-HCl pH7.4. An FPLC-mediated 6M-1M urea gradient (24ml at a rate of 0.25ml/min) in the above solution was then used to refold the bound protein, which was eluted in 10ml 1M urea, 0.5M NaCl, 20% glycerol, 40mM Tris-HCl pH7.4, 250mM imidazole.

Assays for XET activity

³H-labelled XG oligosaccharides were obtained from S C Fry, (University of Edinburgh) and their incorporation into polymeric XG assayed as described by Fry et al. (1992 Biochem. J. 261, 9489-9494). Protein concentration was estimated using a kit supplied by BioRad.

N-terminal amino acid sequencing

Purified recombinant proteins were subjected to electrophoresis as above, using high

quality reagents. They were then electroblotted (0.5A, 2 hours) onto Problott™ membrane (a derivatised PVDF material, obtained from ABI) and stained in 1% Coomassie Brilliant Blue, 50% methanol. The blotted protein was subjected to sequential Edman degradation on an ABI gas phase sequencer model 740, with pulse liquid update, as described by Cottingham et al. (1988 Biochim. Biophys. Acta 956, 201-207).

Expression and characterisation of recombinant tomato XET

Tomato XET was expressed in *E. coli* with an N-terminal 6 x His tag to facilitate purification. A tagged protein of the predicted molecular weight (32kDa) was found in the insoluble fraction; phase contrast microscopy of the expressing culture showed strongly refracting bodies in each cell, suggesting the presence of insoluble inclusion bodies (data not shown). Following large-scale purification under denaturing conditions, the tagged protein was subjected to N-terminal amino acid sequencing. The resulting sequence (MRGSHHHHHHGSADNFYQDA Seq ID No. 32) confirmed both the identity of the protein as recombinant tomato XET, and the presence of the N-terminal 6 x His tag.

Recombinant tomato XET was refolded whilst bound to the Ni-NTA agarose resin, by a reverse urea gradient. Following elution from the column, 15µl was assayed for incorporation of ³H-XG oligosaccharides (XGOs) into polymeric XG: approximately 400 Bq of ³H-XGOs were incorporated per kBq provided, per hour, per µg protein. Following dialysis against 0.5M NaCl, 50mM Tris-HCl pH7.2, 20% glycerol, specific activity increased to approximately 700 Bq/kBq hour µg.

Clone B2 was thus over-expressed in *E. coli* with the apparent N-terminal signal peptide replaced with a 6 x histidine tag which facilitated purification. Following purification and refolding, the recombinant protein was able to catalyse incorporation of radiolabelled XG oligosaccharides into polymeric XG, confirming it as an XET. The specific activity of the refolded XET is relatively low; a crude stem extract from a growing tomato plant has an XET specific activity of about 100 Bq/kBq hour µg, compared to 700 Bq/kBq µg for refolded XET. Since the recombinant protein is greater than 90% pure, this is presumably due to most of the protein not being correctly refolded.

Unlike nasturtium seed XET, a putative N-glycosylation site is present in both clones' amino acid sequences. It is interesting to note that, recombinant expression in *E. coli*, which does not glycosylate proteins, resulted in active XET. This shows that the glycosylation is not necessary for XET activity *in vitro*, raising questions about its role *in vivo*. The lack of glycosylation in nasturtium seed XET may reflect its specialist role in storage reserve mobilisation.

Evidence for the function of XET in plants is rapidly accumulating. The hypothesis that XET plays a role in plant growth is supported by its correlation with growth along the length of pea stems (Fry et al, 1992 Biochem. J. 282, 821-828) and in maize roots (Pritchard et al, 1993 J. Exp. Bot. 44, 1281-1289). It is thought to act by loosening the cell wall to permit cell expansion.

XET is also likely to be involved in fruit ripening, as suggested by its changing levels in fruits as diverse as tomato, pear (unpublished observations of the inventors) and kiwi (Fry 1993 Current Biology, 3 (no. 6), 355-357). Here XET may improve access of other enzymes to the tightly packed networks of the cell wall. This would be supported by the small, compact nature of XET (Edwards et al., 1986 J. Biol. Chem. 261, 9489-9494). Alternatively, XET may be involved (possibly in conjunction with other cell wall hydrolases) in the depolymerisation of xyloglucan which occurs during fruit ripening.

Example 3

In addition to the recombinant DNA work described above, attempts were made to isolate and purify a polypeptide having XET activity from tomato plants.

Step 1: Assay for activity of XET

XET activity in various crude tomato fruit extracts was measured by incorporation of ³H-labelled xyloglucan oligosaccharides into xyloglucan polymer. This assay measures transglycosylase activity. Each assay contained the following components:

10 μ l 0.2M Na-Mes, pH 6.0 (Na-Mes is Na 2-[N-Morpholino] ethanesulphonic acid), 10 μ l

8 mg/ml xyloglucan, and 5 μ l [3 H] XGO (0.7 KBq).

Assays were incubated at 25°C for 1 hour, and stopped by addition of 100 μ l 20% formic acid. The assay mixture was then spotted onto a 5 x 5 cm disc of cellulose filter paper (Whatman 3MM Chromatography paper). It has been established that xyloglucan polymer (whether labelled or unlabelled with [3 H]XGO) will bind to the cellulose filter, whereas [3 H]XGO oligosaccharides will not. The filters were allowed to dry and unincorporated [3 H]XGO was removed by washing under running tap water for 30 min. The filters were dried again at 60°C, then rolled, loaded face outwards, into a cylinder and placed in a 22ml scintillation vial. Scintillant (2ml of Betamax ES) was added and the filters counted for 3 H. This assay is essentially as described by Fry et al. (1992), cited above.

Step 2: Extraction of XET from tomato pericarp

A variety of extraction buffers were used and their effectiveness assessed. It was found that XET was efficiently extracted by 0.1 M sodium phosphate buffer, pH 7.2. It was also found that retention of XET activity was highly dependent on ionic strength, and activity was lost if the ionic strength of chromatography buffer etc. was less than 0.2M.

Step 3: Choice of starting material for purification

Crude extracts were made (in 0.1 M sodium phosphate buffer, pH 7.2) from tomato pericarp (*Lycopersicum esculentum*, var. Moneymaker) tissues taken from all stages of growth and ripening. It was consistently found (from several experiments) that small green fruit (i.e. less than 35mm in diameter) had the highest XET activity, whether expressed as total or specific activity. Therefore the pericarp of small green fruit was used as the starting material for purification.

Step 4: Ammonium sulphate precipitation

Pericarp tissue (100g) from small green fruit of less than 35mm in diameter was harvested, frozen in liquid nitrogen, and stored at -20°C or -70°C. The tissue was

homogenised in 1 g:1.5 vol 0.1 M sodium phosphate, pH 7.2 with 1.0% (w/v) insoluble PVP (polyvinyl pyrrolidone) in a blender. The homogenate was stirred for 1 hour at 4°C to allow diffusion of cell wall enzymes. Insoluble material was removed by centrifugation at 20,000g, 30 minutes, 4°C in the SS34 rotor of the Sorvall RC5B centrifuge. The supernatant was passed through glasswool, and brought to 90% ammonium sulphate saturation by addition of solid ammonium sulphate (60.3 g/100ml). The mixture was stirred for 40 min at 4°C, and the precipitated proteins collected by centrifugation (38,000g, 30 minutes, 4°C). The ammonium sulphate precipitated proteins were stored at -20°C without resuspension.

Step 5: S-sepharose cation exchange chromatography

0-90% ammonium sulphate precipitated proteins were resuspended in 10ml 50 mM sodium acetate buffer, pH 5.3 with 0.2 M NaCl (Buffer A) containing 1 mM EDTA, 20 µg/ml chymostatin, 1 mM PMSF. The resuspended pellet was dialysed for 2 hours against 2.5 l of Buffer A using 3,500 M^r-cut off dialysis tubing. The sample was diluted with 50 mM sodium acetate buffer pH 5.3, 1 mM EDTA to bring the conductivity down to a suitable value for loading onto the S-Sepharose column. The sample was centrifuged at 38,000g for 15 minutes, 4°C, then loaded onto a 7ml S-Sepharose Fast Flow column (Pharmacia) equilibrated in Buffer A. The column was washed with Buffer A (flow rate 1ml/minute) until all unbound proteins were removed. Bound proteins were eluted with a 0-100% gradient of Buffer B (as Buffer A, but with 1 M NaCl) over 10 column volumes. Fractions (2 ml) were collected and assayed for XET activity.

Step 6: Concanavalin A affinity chromatography

Fractions from S-sepharose ion exchange chromatography with high XET activity were pooled and stored at -20°C. Prior to concanavalin chromatography, the fractions were brought to 1 mM MgCl₂ and MnCl₂. The S-Sepharose pool was centrifuged and the supernatant loaded onto a Concanavalin-A column equilibrated in 50 mM sodium acetate buffer pH 5.7 containing 0.2 M NaCl, 1 mM MgCl₂, 1 mM CaCl₂ and 1 mM MnCl₂ (Buffer C). Bound proteins were eluted from the column with a single step elution with

Buffer D (as Buffer C plus 0.5 methyl α -D-mannopyranoside). Fractions collected were assayed for XET activity.

Step 7: N-terminal amino acid sequencing

Samples from the Concanavalin A column with high XET activity were separated according to apparent molecular weight on a 15% SDS polyacrylamide gel. After electrophoresis, proteins were transferred onto the Problott™ membrane (transfer buffer: 10 mM CAPS pH 11 in 10% methanol). The Problott™ membrane was stained in 0.1% Coomassie Brilliant Blue R250 in 1% acetic acid, 40% methanol and destained in 50% methanol. A single band (i.e. the protein had been successfully purified to homogeneity) of approximate Mr36,000 was visible; this was excised and subjected to N-terminal amino acid sequencing on an ABI model 475 protein sequencer. The sequence determined (Seq ID No. 33) was: GYPRRPVDVPFWKNY. The level of the sequence was consistent with the intensity of the stained protein band.

In another aspect therefore, the invention provides a substantially pure polypeptide with xyloglucan endotransglycosylase (XET) activity with an apparent molecular weight of 36kDa, as judged by SDS-PAGE.

Preferably the enzyme is obtainable from tomato plants, most preferably from the pericarp of green tomato fruit, and preferably has substantially the N-terminal amino acid sequence GYPRRPVDVPFWKNY.

The enzyme is preferably purified by one or more of ammonium sulphate precipitation, ion exchange chromatography and/or concanavalin A affinity chromatography.

Example 4

Expression of XET in tomato

The XET assay described above has been used to monitor XET activity throughout the tomato plant (var. Moneymaker). Extracts (0.2M phosphate) were prepared from root, leaf and petiole segments isolated from different parts of the growing plant and fruit at four stages of development and three stages of ripening. Highest XET activity was recorded in extracts prepared from petioles isolated from the top of the growing plant and developing (small green) fruit, consistent with XETs involvement in plant growth.

Total RNA was extracted from different parts of the tomato plant using a Qiagen extraction procedure. tXET-B1 (tomato XET clone B1) transcript levels were determined by RNase protection analysis. An antisense tXET-B1 specific probe was synthesised by incubating HindIII linearised ptXET-B1 plasmid with 1 μ l T3 RNA polymerase in the presence of 0.5mM rATP/rGTP/rCTP, 5nM UTP, 50 μ Ci³²P-UTP (800Ci/mmol), 1 μ l RNase inhibitor and 2 μ l reaction buffer (Ambion probe synthesis kit) in a final reaction volume of 20 μ l for 1 hour at 25°C. Following DNase digestion and phenol/chloroform extraction, probe was recovered by ethanol precipitation. 10⁵cpm labelled probe was incubated with 5 μ g total RNA and RNase protection analysis carried out using an RPAII kit supplied by Ambion. Protected probe was quantified by denaturing gel electrophoresis followed by analysis on a Molecular Dynamics PhosphorImager. Highest levels of tXET-B1 transcripts were detected in fruit pericarp, with levels increasing through ripening and peaking in pink fruit. Maximum levels in stem (equivalent to levels seen in mature green fruit) were found approximately 10cm from the top of a 30cm plant. Lower levels of tXET-B1 transcript were detected in leaf with negligible levels in root. The differences in the patterns of activity and transcript level suggest either that total XET activity is encoded by a set of differentially expressed genes or that selective isolation of isoforms is occurring at 0.2M phosphate. The pattern of expression of tXET-B1 suggests that this isoform may be involved in fruit ripening.

Example 5

Reduction of XET enzyme levels in transgenic tomato plants

A constitutive 2' mannopine synthase promoter was recovered on a BglII fragment from

the plasmid pSLJ2'Lnos, and ligated into the plasmid ptXET-B1. (pSLJ2'Lnos was derived from pSLJ1006 [Jones *et al.*, 1992 transgenic Research 1, 285-297] by replacing the DNA sequence encoding a β -glucuronidase enzyme with a polylinker sequence). This placed the tXET-B1 coding sequence in the antisense orientation with respect to, and downstream of, the 2' promoter (Figure 7). An Xba - Sst fragment containing the promoter and 798bp (nucleotides 187 to 984) of tXET-B1 sequence was then cloned into the plant transformation vector, pGPTV-kan (Becker D. *et al.*, 1992, Plant Molecular Biology 20, 1195-1197 and Figure 8). The resultant plasmid, pGPTV-kan-2'tXET-B1 was used to transform *Agrobacterium* LBA4404 and the recombinant *agrobacterium* strain used to infect cotyledonary segments of tomato (variety Moneymaker). Transformed plants were selectively regenerated in the presence of kanamycin. The presence of the transferred DNA was confirmed by PCR amplification of a DNA segment between the primers NPTIIb (5' ATCGGGAGCGGCGATACCGTA 3', Seq ID No. 34) and 8-HS (see above). XET activity was monitored in extracts prepared from pink fruit (15 plants) by the method described above. Levels were reduced by up to approximately 80% in plants transformed with an antisense tXET construct.

24

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Unilever PLC
(B) STREET: Unilever House, Blackfriars
(C) CITY: London
(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): EC4P 4BQ
(G) TELEPHONE: (0234) 222268
(H) TELEFAX: (0234) 222633

(A) NAME: Unilever NV
(B) STREET: Weena 455
(C) CITY: Rotterdam
(E) COUNTRY: Netherlands
(F) POSTAL CODE (ZIP): 3013 AL

(ii) TITLE OF INVENTION: Novel Plant Enzyme

(iii) NUMBER OF SEQUENCES: 34

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 994 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 79..945

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAAGTAGACG ATGCAGCTGG AATTTCAAAC ATACATTCCC AAAAACAAC TACTACATAT

60

25

TCCTTAAGAT	TTCCTATA	ATG Met 1	TTG Leu	CTG Leu	CAG Gln	CAG Gln 5	CTA Leu	TCT Ser	GTT Val	CTT Leu	GCT Ala 10	CTA Leu	111			
CTT Leu	CTC Leu	TTG Leu	CTA Leu 15	TGT Cys	CCT Pro	GTT Val	TGG Trp	GCT Ala 20	GAC Asp	AAT Asn	TTC Phe	TAC Tyr	CAA Gln 25	GAT Asp	GCA Ala	159
ACG Thr	GTT Val	ACC Thr 30	TTT Phe	GGT Gly	GAT Asp	CAG Gln	CGA Arg 35	GCT Ala	CAG Gln	ATA Ile	CAA Gln	GAT Asp 40	GGT Gly	GGG Gly	CGC Arg	207
CTT Leu	CTC Leu 45	GCC Ala	TTG Leu	TCC Ser	CTT Leu	GAC Asp 50	AAA Lys	ATT Ile	TCA Ser	GGT Gly	TCA Ser 55	GGA Gly	TTT Phe	CAG Gln	TCT Ser	255
AAG Lys 60	AAT Asn	GAA Glu	TAT Tyr	TTA Leu	TTT Phe 65	GGA Gly	AGG Arg	TTC Phe	GAT Asp	ATG Met 70	CAG Gln	CTC Leu	AAA Lys	CTA Leu	GTA Val 75	303
CCT Pro	GGA Gly	AAT Asn	TCT Ser	GCT Ala 80	GGC Gly	ACT Thr	GTC Val	ACT Thr	ACC Thr 85	TTC Phe	TAT Tyr	TTG Leu	TCT Ser	TCT Ser 90	CAA Gln	351
GGA Gly	GCA Ala	GGG Gly	CAC His 95	GAC Asp	GAA Glu	ATT Ile	GAT Asp	TTT Phe 100	GAG Glu	TTT Phe	CTG Leu	GGA Gly	AAT Asn 105	TCA Ser	TCA Ser	399
GGC Gly	CAA Gln	CCG Pro 110	TAC Tyr	ACG Thr	GTT Val	CAT His	ACT Thr 115	AAT Asn	GTC Val	TAC Tyr	TCT Ser	CAA Gln 120	GGA Gly	AAA Lys	GGC Gly	447
AAC Asn	AAA Lys 125	GAA Glu	CAA Gln	CAG Gln	TTT Phe	CGC Arg 130	CTA Leu	TGG Trp	TTT Phe	GAT Asp	CCC Pro 135	ACC Thr	TCG Ser	CCG Pro	TTC Phe	495
CAC His 140	ACC Thr	TAC Tyr	TCT Ser	ATT Ile	GTT Val 145	TGG Trp	AAC Asn	TCT Ser	CAA Gln	CGC Arg 150	ATC Ile	ATA Ile	TTT Phe	TTG Leu	GTG Val 155	543
GAT Asp	AAT Asn	ATC Ile	CCA Pro	ATA Ile 160	AGA Arg	GTA Val	TTC Phe	AAC Asn	AAC Asn 165	CAC His	GAA Glu	AAG Lys	CTT Leu	GGT Gly 170	GTT Val	591
GCA Ala	TTC Phe	CCA Pro	AAG Lys 175	AAC Asn	CAA Gln	GCA Ala	ATG Met	AGA Arg 180	GTT Val	TAT Tyr	GCC Ala	AGT Ser	TTA Leu 185	TGG Trp	AAT Asn	639
GCT Ala	GAT Asp	GAC Asp 190	TGG Trp	GCA Ala	ACA Thr	CAA Gln	GGA Gly 195	GGG Gly	CGA Arg	GTG Val	AAG Lys	ACG Thr 200	GAT Asp	TGG Trp	TCA Ser	687
ATG Met	GCT Ala 205	CCG Pro	TTT Phe	ACA Thr	GCT Ala	TCT Ser 210	TAC Tyr	AGG Arg	AAT Asn	TTC Phe	AAC Asn 215	ACA Thr	AAT Asn	GCT Ala	TGT Cys	735

26

GTT TGG TCA GCT GCA TCG TCT ACT TCG TCC TGT GGA GGC TCT AAG ACT	783
Val Trp Ser Ala Ala Ser Ser Thr Ser Ser Cys Gly Gly Ser Lys Thr	
220 225 230 235	
GAT TCA GTA AAC AAT GAT CAG GCA TGG CAA ACT CAA GAA CTG AAC GGT	831
Asp Ser Val Asn Asn Asp Gln Ala Trp Gln Thr Gln Glu Leu Asn Gly	
240 245 250	
AAT GAC AGA AAT AGG CTT CGA TGG GTT CAG CAG AAA TAC ATG ATC TAC	879
Asn Asp Arg Asn Arg Leu Arg Trp Val Gln Gln Lys Tyr Met Ile Tyr	
255 260 265	
AAT TAC TGT GCA GAT GCT AAA AGG TTC TCT CAA GGC CTT TCT CCT GAA	927
Asn Tyr Cys Ala Asp Ala Lys Arg Phe Ser Gln Gly Leu Ser Pro Glu	
270 275 280	
TGC AAA CGT TCA AGG TTC TAAAGGATCA AATCTACGAA TGTTGTCTGT	975
Cys Lys Arg Ser Arg Phe	
285	
AATATTATCC CGGAATTCC	994

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 289 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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

27

Val His Thr Asn Val Tyr Ser Gln Gly Lys Gly Asn Lys Glu Gln Gln
 115 120 125
 Phe Arg Leu Trp Phe Asp Pro Thr Ser Pro Phe His Thr Tyr Ser Ile
 130 135 140
 Val Trp Asn Ser Gln Arg Ile Ile Phe Leu Val Asp Asn Ile Pro Ile
 145 150 155 160
 Arg Val Phe Asn Asn His Glu Lys Leu Gly Val Ala Phe Pro Lys Asn
 165 170 175
 Gln Ala Met Arg Val Tyr Ala Ser Leu Trp Asn Ala Asp Asp Trp Ala
 180 185 190
 Thr Gln Gly Gly Arg Val Lys Thr Asp Trp Ser Met Ala Pro Phe Thr
 195 200 205
 Ala Ser Tyr Arg Asn Phe Asn Thr Asn Ala Cys Val Trp Ser Ala Ala
 210 215 220
 Ser Ser Thr Ser Ser Cys Gly Gly Ser Lys Thr Asp Ser Val Asn Asn
 225 230 235 240
 Asp Gln Ala Trp Gln Thr Gln Glu Leu Asn Gly Asn Asp Arg Asn Arg
 245 250 255
 Leu Arg Trp Val Gln Gln Lys Tyr Met Ile Tyr Asn Tyr Cys Ala Asp
 260 265 270
 Ala Lys Arg Phe Ser Gln Gly Leu Ser Pro Glu Cys Lys Arg Ser Arg
 275 280 285
 Phe

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asp Glu Ile Asp Phe Glu Phe Leu Gly Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser	Leu	Trp	Asn	Ala	Asp	Asp	Trp	Ala	Thr
1				5					10

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Phe	Tyr	Ser	Lys	Asn	Glu	Tyr	Leu	Phe	Gly
1				5					10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

29

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gly	Thr	Val	Thr	Thr	Phe	Tyr	Leu	Ser	Ser
1				5				10	

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1289 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 232..1092

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GAATTCTGGG TAGGCTTTGG TTGCAATAAG CGTCTGTTCT TCGCTTACTA CTTTGGAAAA	60
ATTGACTTTA GGCCTGCGGT CCCTAGCATT AAATTCATCG ACCGCTGTGT CATATAGACG	120
CCGCTTTGCA AGATCGTTGA CCAAGGTATT GTTACCTCGG ACGGTCTCAA GCAAAGCGGA	180
CGATGTGGCT GTTTGTGTGT ATGCCATTGT AGTTGTAGAA TGTAATAATAT A ATG TTG	237
	Met Leu
	1
CTG CAG CTT TCT CTT CTT ACA CTA GTC TTA CTA TCC CCT GTT TCC GCT	285
Leu Gln Leu Ser Leu Leu Thr Leu Val Leu Leu Ser Pro Val Ser Ala	
5 10 15	
GAT AAT TTC TAC CAA GAC GCG GCG GTC ACG TTT GGT GAC CAG CGC GCT	333
Asp Asn Phe Tyr Gln Asp Ala Ala Val Thr Phe Gly Asp Gln Arg Ala	
20 25 30	
CAG ATA CAA GAT GGA GGG CGC CTT CTC ACA TTG TCA CTT GAT AAA ATT	381
Gln Ile Gln Asp Gly Gly Arg Leu Leu Thr Leu Ser Leu Asp Lys Ile	
35 40 45 50	
TCA GGT TCC GGA TTT CAG TCT AAG AAT GAG TAT TTA TTC GGA AGG TTC	429
Ser Gly Ser Gly Phe Gln Ser Lys Asn Glu Tyr Leu Phe Gly Arg Phe	
55 60 65	

30

GAT Asp	ATG Met	CAG Gln	CTT Leu 70	AAA Lys	CTC Leu	GTA Val	CCT Pro	GGA Gly 75	AAT Asn	TCT Ser	GCT Ala	GGC Gly	ACT Thr 80	GTC Val	ACC Thr	477
ACA Thr	TTC Phe	TAT Tyr 85	TTG Leu	TCT Ser	TCT Ser	CAA Gln	GGA Gly 90	GCA Ala	GGG Gly	CAT His	GAT Asp	GAA Glu 95	ATT Ile	GAT Asp	TTT Phe	525
GAG Glu 100	TTT Phe	CTA Leu	GGA Gly	AAT Asn	TCA Ser	TCA Ser 105	GGA Gly	CTA Leu	CCT Pro	TAC Tyr	ACG Thr 110	GTT Val	CAT His	ACC Thr	AAT Asn	573
GTT Val 115	TAC Tyr	TCT Ser	CAA Gln	GGA Gly 120	AAA Lys	GGC Gly	AAT Asn	AAA Lys	GAA Glu 125	CAA Gln	CAA Gln	TTT Phe	CGT Arg	CTC Leu	TGG Trp 130	621
TTT Phe	GAT Asp	CCA Pro	ACT Thr	TCG Ser 135	TCG Ser	TTC Phe	CAC His	ACT Thr 140	TAC Tyr	TCT Ser	ATT Ile	GTT Val	TGG Trp	AAC Asn 145	TCT Ser	669
CAA Gln	CGG Arg	ATC Ile	ATA Ile 150	TTT Phe	TTG Leu	GTG Val	GAT Asp	AAT Asn 155	ATC Ile	CCA Pro	ATT Ile	AGA Arg	GTG Val 160	TTC Phe	AAC Asn	717
AAC Asn	CAC His	GAA Glu 165	GCA Ala	CTT Leu	GGT Gly	GTT Val	GCA Ala 170	TAC Tyr	CCA Pro	AAG Lys	AAT Asn	CAA Gln 175	GCA Ala	ATG Met	AGA Arg	765
GTT Val 180	TAC Tyr	GCG Ala	AGT Ser	CTA Leu	TGG Trp	AAT Asn 185	GCT Ala	GAT Asp	GAT Asp	TGG Trp	GCT Ala 190	ACA Thr	CAA Gln	GGA Gly	GGA Gly	813
CGG Arg 195	GTG Val	AAG Lys	ACA Thr	GAT Asp	TGG Trp 200	TCT Ser	ATG Met	GCT Ala	CCG Pro	TTT Phe 205	ACA Thr	GCT Ala	TCT Ser	TAC Tyr	AGG Arg 210	861
AAT Asn	TTC Phe	AAT Asn	ACA Thr	AAT Asn 215	GCT Ala	TGT Cys	GTT Val	TGG Trp	TCA Ser 220	GCT Ala	GCT Ala	ACG Thr	TCT Ser	ACT Thr 225	TCG Ser	909
TCT Ser	TGT Cys	GGA Gly	GGT Gly 230	TCT Ser	AAG Lys	ACT Thr	GAG Glu	TCA Ser 235	GTA Val	AAC Asn	AAT Asn	GAT Asp	GAG Glu 240	ACA Thr	TGG Trp	957
CAA Gln	ACG Thr	CAA Gln 245	CAA Gln	CTG Leu	AAC Asn	GCT Ala 250	AAT Asn	GGA Gly	AGA Arg	AAT Asn	AGA Arg	ATA Ile 255	CGA Arg	TGG Trp	GTT Val	1005
CAG Gln 260	CAG Gln	AAG Lys	TAC Tyr	ATG Met	ATC Ile	TAC Tyr 265	AAT Asn	TAC Tyr	TGT Cys	GCA Ala 270	GAT Asp	GCT Ala	AAT Asn	AGG Arg	TTC Phe	1053
TCT Ser 275	CAA Gln	GGC Gly	TTT Phe	TCT Ser	CCT Pro 280	GAA Glu	TGC Cys	AAG Lys	CGT Arg	TCA Ser 285	AGG Arg	TTC Phe	TAAGGCGGAT			1102

31

ATATATAGTA TATGAATGTA AAATTATGTT TGTTTCACTT TTCTATTCTT TTAATTTTGA 1162
 TCAGGTAAAA AAAAAAAGA ACATAGTGTA ATTATTTGTG TATGCAATAT ATTCTTTATT 1222
 CTTTTTGTA TCATGAAATA GAAATAATA TGAATTGTTT TCCTGAAAAA AAAAAACCG 1282
 GAATTCC 1289

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 287 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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

32

Gly Gly Arg Val Lys Thr Asp Trp Ser Met Ala Pro Phe Thr Ala Ser
 195 200 205
 Tyr Arg Asn Phe Asn Thr Asn Ala Cys Val Trp Ser Ala Ala Thr Ser
 210 215 220
 Thr Ser Ser Cys Gly Gly Ser Lys Thr Glu Ser Val Asn Asn Asp Glu
 225 230 235 240
 Thr Trp Gln Thr Gln Gln Leu Asn Ala Asn Gly Arg Asn Arg Ile Arg
 245 250 255
 Trp Val Gln Gln Lys Tyr Met Ile Tyr Asn Tyr Cys Ala Asp Ala Asn
 260 265 270

 Arg Phe Ser Gln Gly Phe Ser Pro Glu Cys Lys Arg Ser Arg Phe
 275 280 285

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AAGCTTCCTC AACATCAAAG GGTAGA

26

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AAGCTTCATG ATGAAATCGA TATTGA

26

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGATCCTCAA TATCGATTTC ATCATG

26

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AAGCTTCATT TATGGTTTGA TCCAAC

26

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGATCCGTTG GATCAAACCA TAAATG

26

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iii) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGATCCGTTA ACGTGTGGTC TCGTGT

26

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iii) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GAYGARATNG AYWTHGARTT

20

(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iii) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTNGGRTCRA ACCANARTG

20

35

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CGATGCAGCT GGAATTTCA

19

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AGTTTGAGCT GCATATCGAA

20

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TTTGATCCCA CCTGCGCGTT

20

36

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CAGCTGACCA AACACAAGCA

20

(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

ATTAACCCTC ACTAAAGGGA

20

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TAATACGACT CACTATAGGG

20

37

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GTTTTCCCAG TCACGACGT

19

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AATATCAGCG GAAACAGGG

19

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CCCTGTTTCC GCTGATAATT

20

38

(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CTATTAGCAT CTGCACAGTA

20

(2) INFORMATION FOR SEQ ID NO: 27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TACTGTGCAG ATGCTAATAG

20

(2) INFORMATION FOR SEQ ID NO: 28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GGAGGACGGG TGAAGACAGA

20

39

(2) INFORMATION FOR SEQ ID NO: 29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GTGTAAGGTA GTCCTGATGA

20

(2) INFORMATION FOR SEQ ID NO: 30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TCATCAGGAC TACCTTACAC

20

(2) INFORMATION FOR SEQ ID NO: 31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CCCTTGATC CGCTATAATT

20

40

(2) INFORMATION FOR SEQ ID NO: 32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Met	Arg	Gly	Ser	His	His	His	His	His	His	Gly	Ser	Ala	Asp	Asn	Phe
1				5					10					15	
Tyr Gln Asp Ala															
20															

(2) INFORMATION FOR SEQ ID NO: 33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Gly	Tyr	Pro	Arg	Arg	Pro	Val	Asp	Val	Pro	Phe	Trp	Lys	Asn	Tyr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO: 34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

41

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

ATCGGGAGCG GCGATACCGT A

21

Claims

1. A polypeptide having xyloglucan endo-transglycosylase (XET) activity, comprising substantially residues 21-289 of the amino acid sequence shown in Figure 1 (Seq ID No. 2) or functional equivalents thereof.
2. A polypeptide according to claim 1, comprising substantially residues 1-289 of the amino acid sequence shown in Figure 1.
3. A polypeptide according to claim 1, comprising substantially residues 19-287 of the amino acid sequence shown in Figure 5 (Seq ID No. 8).
4. A polypeptide according to claim 1, comprising substantially residues 1-287 of the amino acid sequence shown in Figure 1.
5. A polypeptide according to any one of the preceding claims, having at least 80% homology with the sequence of residues 21-289 of the sequence shown in Figure 1.
6. A polypeptide according to any one of the preceding claims, having at least 85% homology with the sequence of residues 21-289 of the sequence shown in Figure 1.
7. A polypeptide according to any one of the preceding claims, having at least 90% homology with the sequence of residues 21-289 of the sequence shown in Figure 1.
8. A polypeptide according to any one of the preceding claims comprising one or more of the following peptides (possibly with up to one, or at most two, amino acid differences in each peptide): DEIDFEFLGN; SLWNADDWAT; FYSKNEYLFG; and GTVTTFYLSS
9. A polypeptide in accordance with any one of the preceding claims, substantially in isolation from other plant-derived substances.
10. A nucleotide sequence encoding substantially the amino acid sequence of residues 21-

289 of the amino acid sequence shown in Figure 1, or functional equivalents thereof.

11. A nucleotide sequence according to claim 10, comprising substantially the DNA sequence shown in Figure 1 (Seq ID No. 1).

12. A nucleotide sequence according to claim 10, encoding substantially the amino acid sequence of residues 19-287 of the amino acid sequence shown in Figure 5.

13. A nucleotide sequence according to claim 12 comprising substantially the DNA sequence shown in Figure 5 (Seq ID No. 7).

14. A nucleotide sequence according to claim 10 or 12, further comprising a promoter to enable expression in a host cell.

15. A sequence according to claim 14, wherein the promoter is not one with which the sequence is naturally associated in a plant.

16. A nucleotide sequence according to claim 10, having at least 70% homology with the nucleotide sequence of Figure 1.

17. A nucleotide sequence according to claim 16, having at least 80% homology with the nucleotide sequence of Figure 1.

18. A nucleotide sequence according to claim 16, having at least 85% homology with the nucleotide sequence of Figure 1.

19. A nucleotide sequence according to any one of claims 10-18, encoding a polypeptide in accordance with any one of claims 1-9.

20. A nucleotide sequence according to claim 10, being an antisense functional equivalent having at least 70% homology with the complement of the nucleotide sequence of Figure 1.

21. A nucleotide sequence according to claim 20, having at least 80% homology with the complement of the nucleotide sequence of Figure 1.
22. A nucleotide sequence according to claim 20, having at least 90% homology with the complement of the nucleotide sequence of Figure 1.
23. A DNA vector comprising a sequence in accordance with any one of claims 10-22.
24. A vector according to claim 23 capable, when introduced into a suitable host, of directing the expression of a polypeptide according to any one of claims 1-9.
25. A host cell into which has been introduced a nucleotide sequence in accordance with any one of claims 10-22, or the progeny thereof.
26. A host cell according to claim 25, comprising a vector in accordance with claim 23 or 24.
27. A method of making a polypeptide having xyloglucan endo-transglycosylase (XET) activity, comprising the steps of: introducing a vector in accordance with claim 24 into a suitable host cell; growing the host cell and/or the progeny thereof in suitable culture conditions such that the polypeptide is expressed; and obtaining the polypeptide from the culture medium and/or the host cells.
28. A method according to claim 27, wherein the host cell is a plant cell or a micro-organism.
29. A method according to claim 27 or 28, wherein the host cell is a yeast cell.
30. A method of altering the characteristics of a plant or part thereof, comprising introducing into the plant or part thereof an effective portion of a nucleotide sequence in accordance with any one of claims 10-22, so as to alter the level of XET activity in the plant or part thereof.

31. A method according to claim 30, wherein the level of XET activity in the plant or part thereof is reduced.
32. A method according to claim 30 or 31, wherein one or more of the following characteristics are altered: size, rate of growth, texture, ripening.
33. A genetically engineered plant made by the method of claim 30, 31 or 32, or the progeny of such a plant.

GAAGTAGACGATGCAGCTGGAAATTTCAAACATACATTCCCAAAAACAATACTACATAT
 TCCTTAAGATTTCCCTATAATGTTGCTGCAGCAGCTATCTGTTCTTCTCTACTTCTCTTG
 M L L Q Q L S V L A L L L L
 ^5 ^10
 CTATGTCCTGTTTGGGCTGACAAATTTCTACCAAGATGCAACGGTTACCTTTGGTGATCAG
 L C P V W A D N F Y Q D A T V T F G D Q
 ^15 ^20 ^25 ^30
 CGAGCTCAGATACAAGATGGTGGCGCCTTCTCGCCTTGTCCTTGACAAAATTTACGGT
 R A Q I Q D G G R L L A L S L D K I S G
 ^35 ^40 ^45 ^50
 TCAGGATTTTCAGTCTAAGAATGAATATTATTTGGAAGTTCGATATGCAGCTCAAACTA
 S G F Q S K N E Y L F G R F D M Q L K L
 ^55 ^60 ^65 ^70
 GTACCTGGAAATTCGTGCTGGCAGTGTCACTACCTTCTATTTGTCTTCTCAAGGAGCAGGG
 V P G N S A G T V T T F Y L S S Q G A G
 ^75 ^80 ^85 ^90
 CACGACGAAATTGATTTTGAGTTTCTGGGAAATTCATCAGGCCAACCGTACACGGTTCAT
 H D E I D F E F L G N S S G Q P Y T V H
 ^95 ^100 ^105 ^110
 ACTAATGTCTACTCTCAAGGAAAGGCAACAAGAAACAAGTTTCGCCCTATGGTTTGAT
 T N V Y S Q G K G N K E Q Q F R L W F D
 ^115 ^120 ^125 ^130
 CCCACCTCGCCGTTCCACACCTACTCTATTGTTTGGAACTCTCAACGCATCATATTTTG
 P T S P F H T Y S I V W N S Q R I I F L
 ^135 ^140 ^145 ^150

Fig.1 SHEET 1

GTGGATAATATCCCAATAAGAGTATTCAACAACCAACGAAAGCTTGGTGTTCATTCCTCCA
V D N I P I R V F N N H E K L G V A F P
^155 ^160 ^165 ^170

AAGAACCAAGCAATGAGAGTTTATGCCAGTTTATGGAATGCTGATGACTGGGCAACACAA
K N Q A M R V Y A S L W N A D D W A T Q
^175 ^180 ^185 ^190

GGAGGGCGAGTGAAGACGGATTGGTCAATGGCTCCGTTTACAGCTTCTTACAGGAATTTC
G G R V K T D W S M A P F T A S Y R N F
^195 ^200 ^205 ^210

AACACAAATGCTTGTGTTTGGTCAGCTGCATCGTCTACTTCGTCCTGTGGAGGCTCTAAG
N T N A C V W S A A S S T S S C G G S K
^215 ^220 ^225 ^230

ACTGATTCAGTAAACAATGATCAGGCATGGCAAACCTCAAGAACTGAACGGTAATGACAGA
T D S V N N D Q A W Q T Q E L N G N D R
^235 ^240 ^245 ^250

AATAGGCTTCGATGGGTTTCAGCAGAAATACATGATCTACAATTACTGTGCAGATGCTAAA
N R L R W V Q Q K Y M I Y N Y C A D A K
^255 ^260 ^265 ^270

AGGTCTCTCAAGGCCCTTCTCCTGAATGCAAACGTTCAAGTTCTTAAAGGATCAAATCT
R F S Q G L S P E C K R S R F
^275 ^280 ^285 ^290

ACGAATGTTGTCTGTAATATTATCCCGGAATTCC

Fig 1 SHEET 2

2/
11

Tom3
Tom1
Meri5
XET

DEMDLEFF
DEIDFEFF
DEIDFEFF
DEIDIEFF

LG
LG
LG
LG

NLS
NVS
NMS
TIP

GG
GG
GG
GG

GD
GD
GD
GD

PP
PP
PP
PP

Y
Y
Y
Y

TL
TL
TL
TL

LH
LH
LH
LQ

TN
TN
TN
TN

V
V
V
V

FS
FY
FY
FY

QQ
QQ
QQ
QQ

GG
GG
GG
GG

KGN
KGN
KGD
KGD

YN
YN
YN
YN

IG
IG
IG
IG

REL
REL
REL
REL

R
K
K
K

EQ
EQ
EQ
EQ

QQ
QQ
QQ
QQ

FF
FF
FF
FF

HLW
HLW
HLW
HLW

F
F
F
F

FD
FD
FD
FD

P
P
P
P

T
T
T
T

Fig. 2

4 / 11

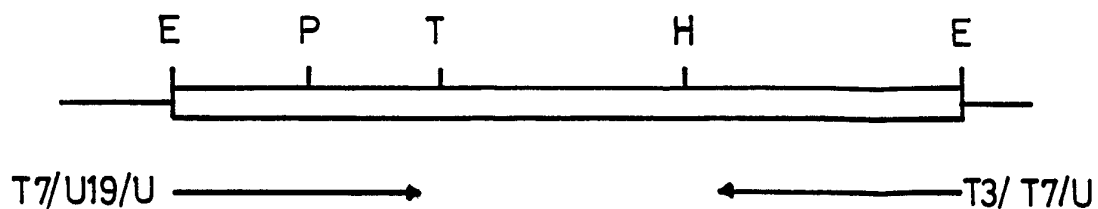


Fig. 3(a)

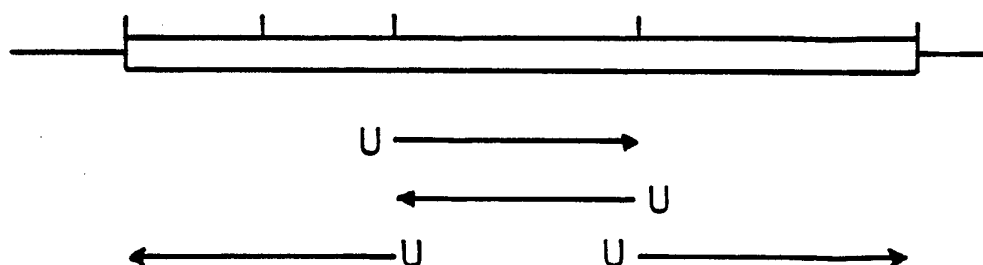


Fig. 3(b)

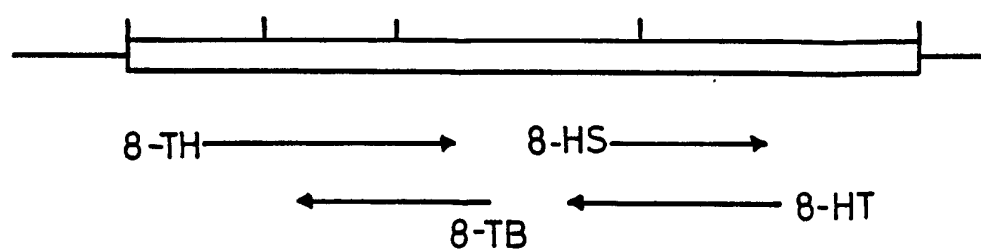


Fig. 3(c)

8-TH:	CGATGCAGCTGGAATTTCA	(SENSE)
8-TB:	AGTTTGAGCTGCATATCGAA	(ANTISENSE)
8-HS:	TTTGATCCACCTCGCCGT	(SENSE)
8-HT:	CAGCTGACCAAACACAAGCA	(ANTISENSE)

Fig. 3(d)

5/11

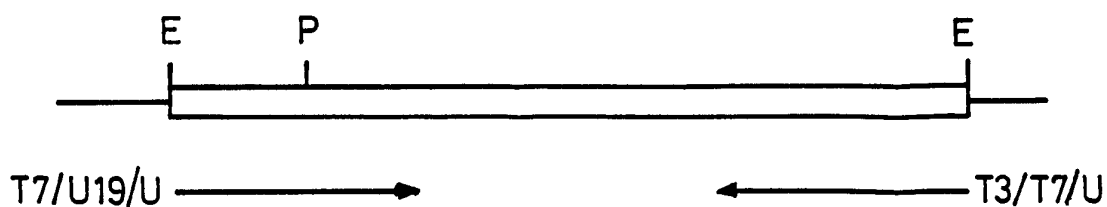


Fig. 4(a)

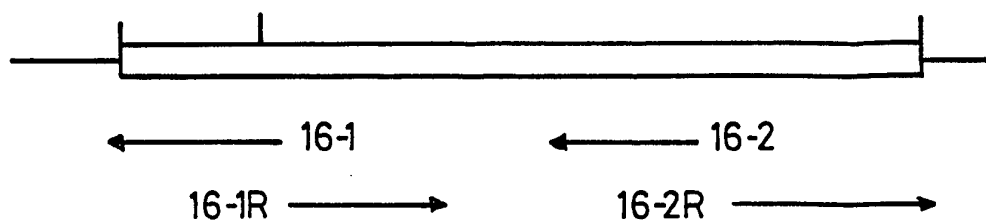


Fig. 4(b)

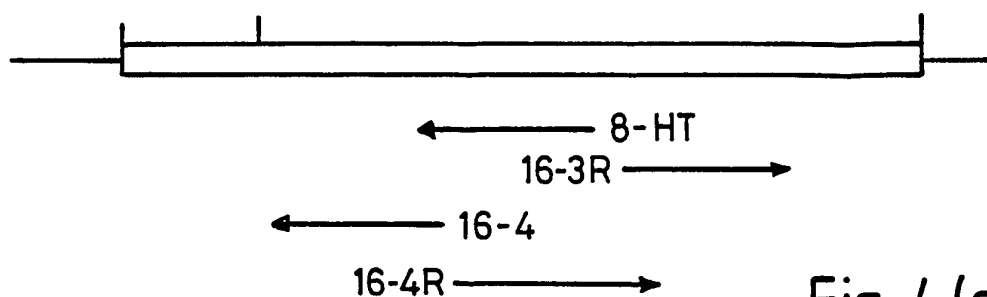


Fig. 4(c)

16-1:	AATTATCAGCGGAAACAGGG
16-1R:	CCCTGTTTCCGCTGATAATT
16-2:	CTATTAGCATCTGCACAGTA
16-2R:	TACTGTGCAGATGCTAATAG
16-3R:	GGAGGACGGGTGAAGACAGA
16-4:	GTGTAAGGTAGTCCTGATGA
16-4R:	TCATCAGGACTACCTTACAC

Fig. 4(d)

GAATCTGGGTAGGCTTTGGTTGCAATAAGCGTCTGTCTTCCGCTTACTAC
TTTGAAAAAATTGACTTTAGCCCTGCGGTCCCTAGCATTTAAATTCAATCGACCGCTGTGTC
ATATAGACCGCGCTTTGCAAGATCGTTGACCAAGTATTGTTACCTCGGACGGTCTCAAG
CAAAGCGGACGATGTGGCTGTTGTGTGTATGCCATTGTAGTGTAGTAATGTAAAAATATA
ATGTTGCTGCAGCTTTCTCTTCTTACACATAGTCTTACTATCCCCTGTTCCTCCGCTGATAAT
M L L Q L S L L T L V L L S P V S A D N
^5 ^10 ^15
TTCTACCAAGACGGCGGTCAAGTTTGGTGACCAAGCGGCTCAGATACAAGATGGAGGG
F Y Q D A A V T F G D Q R A Q I Q D G G
^25 ^30 ^35
CGCCTTCTCACATTGTCACTTGATAAAATTTCAGGTTCCGGATTTCAGTCTAAGAATGAG
R L L T L S L D K I S G S G F Q S K N E
^45 ^50 ^55
TATTATTCGGAAGGTTTCGATATGCAGCTTAAACTCGTACCTGGAAATTCTGCTGGCACT
Y L F G R F D M Q L K L V P G N S A G T
^65 ^70 ^75
GTCACCACATTCTATTGTCTTCTCAAGGACGAGGCGCATGATGAAATTGATTTGAGTTT
V T T F Y L S S Q G A G H D E I D F E F
^85 ^90 ^95
CTAGGAAATTCAATCAGGACTACCTTACACGGTTCAACCAATGTTTACTCTCAAGGAAA
L G N S S G L P Y T V H T N V Y S Q G K
^105 ^110 ^115
GGCAATAAAGAACAAATTCGTCTCTGTTGATCCAACTTCGTCGTTCCACACTTAC
G N K E Q Q F R L W F D P T S S F H T Y
^125 ^130 ^135
TCTATTGTTGGAACTCTCAACGGATCATATTTTGGTGGATAATATCCCAATTAGAGTG
S I V W N S Q R I I F L V D N I P I R V
^145 ^150 ^155

6/11

Fig.5 SHEET 1

Tomato XET cDNA (B1) and encoded
amino acid sequences (B1 & B2)

TCCTTAAGATTTCCTATAATGTTGCTGCAGCAGCTATCTGTTCTTCTCTACTTCTCTTG
 M L L Q Q L S V L A L L L L
 B1
 CTATGTCCTGTTGGCTGACAAATTCTACCAAGATGCAACGGTTACCTTGGTGATCAG
 L C P V A A D N F Y Q D A T V T F G D Q
 B2
 - S . S . A A
 CGAGCTCAGATACAGATGGTGGCGCCTTCTCGCCTTGTCCTTGACAAAATTTCAGGT
 R A Q I Q D G G R L L A L S L D K I S G
 T
 TCAGGATTTCAGTCTAAGAAATGAATATTATTGGAAGGTTTCGATATGCAGCTCAAACTA
 S G F Q S K N E Y L F G R F D M Q L K L

 GTACCTGGAAATTCTGCTGGCACTGTCACTACCTTCTATTGTCTTCTCAAGGAGCAGGG
 V P G N S A G T V T T F Y L S S Q G A G

 CACGACGAAATTGATTTTGAGTTCCTGGGAAATTTCATCAGGCCAACCGTACACGGTTTCAT
 H D E I D F E F L G N S S G Q P Y T V H
 L
 ACTAATGTCTACTCTCAAGGAAAGGCAACAAAGAACACAGTTTCGCCCTATGGTTTGTAT
 T N V Y S Q G K G N K E Q Q F R L W F D

 CCCACCTCGCCGTTCCACACCTACTCTATTGTTGGAACTCTCAACGCATCATATTTTG
 P T S P F H T Y S I V W N S Q R I I F L
 . . . S

8/11

Fig. 6 SHEET 1

9/11

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GTGGATAATATCCCAATAAGAGTATTCAACAACCAAGAAAGCTTGGTGTGCTATCCCA
V D N I P I R V F N N H E K L G V A F P
. . . . .
AAGAACCAAGCAATGAGAGTTTATGCCAGTTTATGGAATGCTGATGACTGGGCAACACAA
K N Q A M R V Y A S L W N A D D W A T Q
. . . . .
GGAGGGCGAGTGAAGACGAGTGGTCAATGGCTCCGTTTACAGCTTCTTACAGGAATTTC
G G R V K T D W S M A P F T A S Y R N F
. . . . .
AACACAAATGCTTGTGTTGGTCAAGCTGCATCGTCTACTTCGTCCTGTGGAGGCTCTAAG
N T N A C V W S A A S S T S S C G G S K
. . . . .
ACTGATTCAAGTAAACAATGATCAGGCATGGCAAACTCAAGAACTGAACGGTAATGACAGA
T D S V N N D Q A W Q T Q E L N G N D R
. . . . .
AATAGGCTTCGATGGGTTTCAGCAGCAAAATACATGATCTACAATTACTGTGCAGATGCTAAA
N R L R W V Q Q K Y M I Y N Y C A D A K
. . . . .
AGGTTCTCTCAAGGCCCTTCTCCTGAATGCAAACTCAAGGTTCTAAAGGATCAAAATCT
R F S Q G L S P E C K R S R F X
. . . . .
ACGAATGTTGCTGTAATATATATCCCGGAATTCC

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• = IDENTICAL AMINO ACID
▲ = PREDICTED SIGNAL PEPTIDE CLEAVAGE SITE

Fig. 6 SHEET 2

10/11

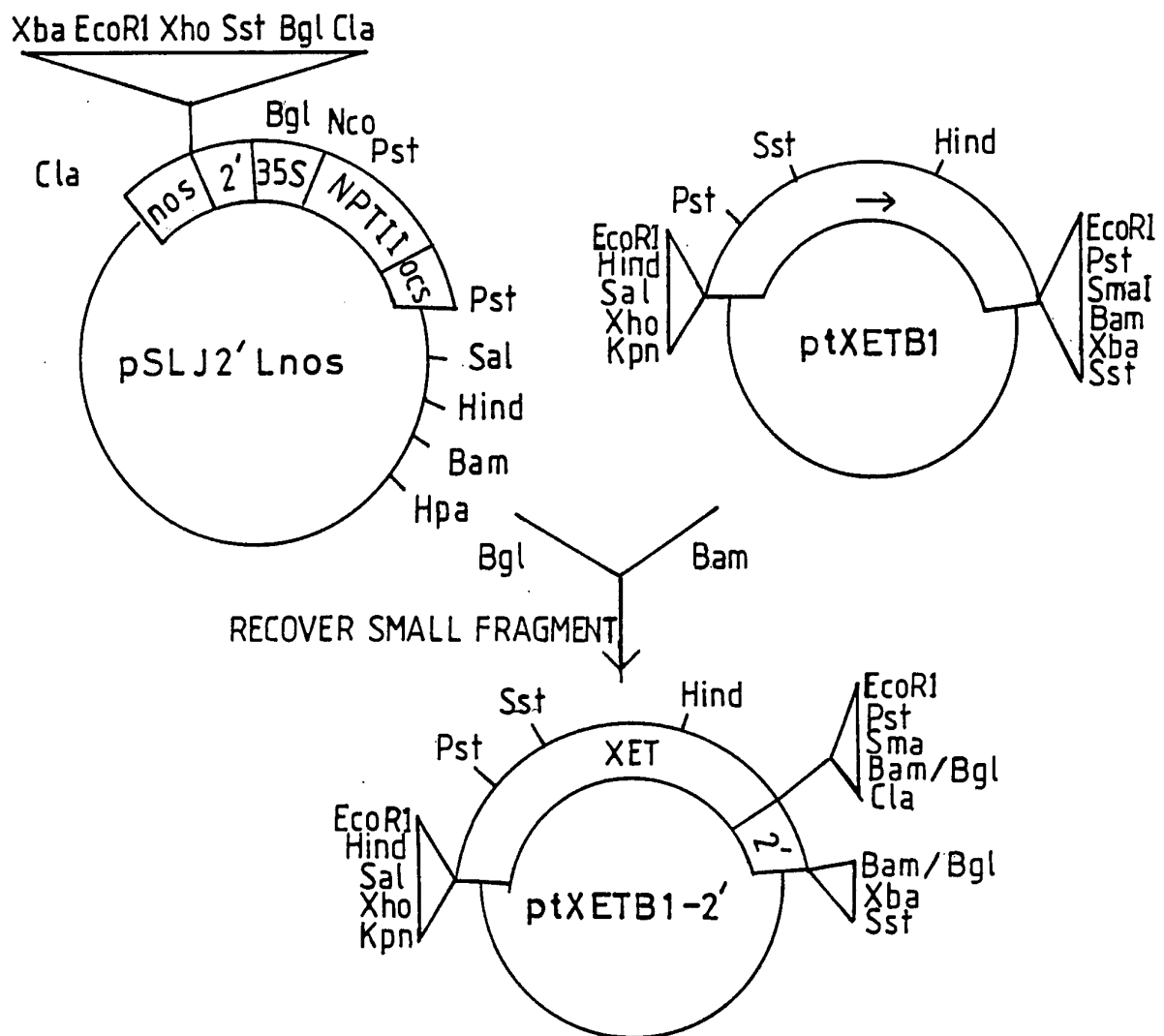
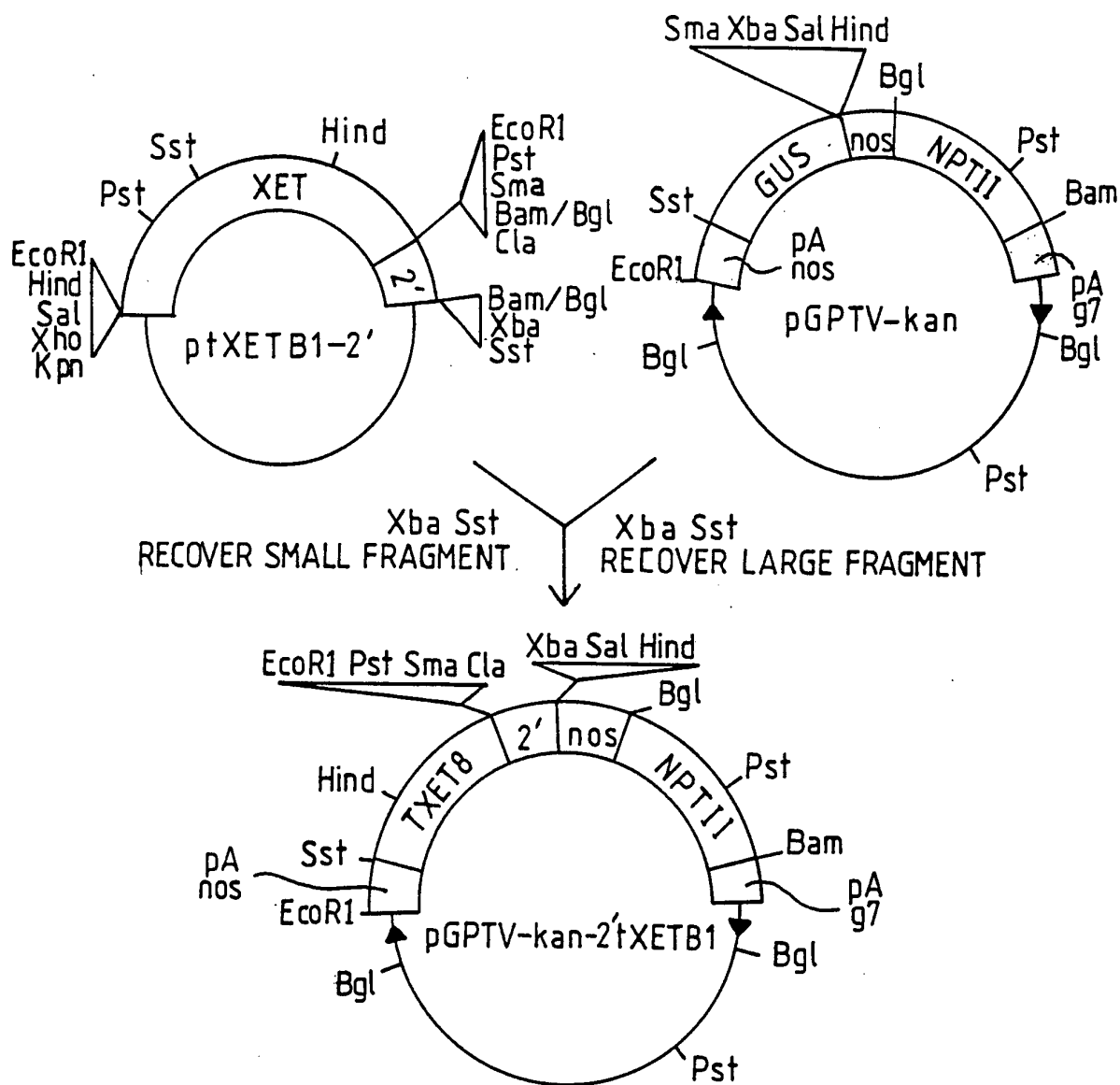
SITES NOT SHOWN: *DraI*, *EcoRV*, *Spe*, *NotI*, *BstXI*

Fig. 7

11/11



SITES NOT SHOWN: DraI, EcoRV, Spe, Not, BstXI

Fig. 8

INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/GB 94/02467

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/56 C12N15/82 C12N15/54 C12N5/10 C12N9/24
C12N9/10 A01H5/00

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 6 C12N A01H

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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 562 836 (TAKARA SHUZO) 29 September 1993 see the whole document ---	1,8,10, 14,15, 19, 23-28, 30-33
X	WO,A,93 17101 (UNILEVER) 2 September 1993 see the whole document --- -/--	1,8,10, 14,15, 19, 23-28, 30-33

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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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

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Date of the actual completion of the international search

23 January 1995

Date of mailing of the international search report

06 -02- 1995

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Maddox, A

INTERNATIONAL SEARCH REPORT

Internat. Application No.

PCT/GB 94/02467

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE PLANT JOURNAL, vol.3, no.5, May 1993 pages 701 - 7111 DE SILVA, J., ET AL. 'Molecular characterization of a xyloglucan-specific endo-(1-4)-beta-D-glucanase (xyloglucan endo-transglycosylase) from nasturtium seeds' see the whole document ---	1,8,10, 14,15, 23-28, 30-33
A	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US DN BA93:138777 MACLACHLAN, G., ET AL. 'Multiple forms of 1,4-beta glucanase in ripening tomato fruits include a xyloglucanase activatable by xyloglucan oligosaccharadies' see abstract & AUST. J. PLANT PHYSIOL., vol.19, no.2, 1992 pages 137 - 146 ---	1-33
A	THE PLANT CELL, vol.3, 1991 pages 359 - 370 MEDFORD, J.I., ET AL. 'Molecular cloning and characterization of genes expressed in shoot apical meristems' see figure 3 ---	16
A	EMBL SEQUENCE DATABASE ENTRY L22162 RELEASE 36, 23-7-93. Glycine max brassinosteroid-regulated protein mRNA, complete cds. -----	16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 94/02467

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0562836	29-09-93	JP-A- 6086670 AU-B- 3540593	29-03-94 30-09-93
WO-A-9317101	02-09-93	NONE	