POLYNUCLEOTIDE SEQUENCE OF FRUIT SOFTENING ASSOCIATED *B*-D-*N*-ACETYLHEXOSAMINIDASE AND ITS USES FOR ENHANCING FRUIT SHELF LIFE

FIELD OF INVENTION

The present invention relates to isolated polynucleotides coding for fruit softening associated enzyme, β-D-*N*-acetylhexosaminidase from plants.

BACKGROUND OF THE INVENTION

The post-harvest losses of fruits and vegetables, which are quickly perishable, are a major cause for concern in developing countries because of the fact that a large amount of what is produced never reaches to the consumer. This loss in India, the second largest producer of fruits and vegetables, account for 35-40% of total produce; a loss estimated at 40,000 crores per year, directly affecting economy. The main factor in determining the post-harvest deterioration of fruit and vegetable crops is the rate of softening, which influences shelf life, wastage, infection by post-harvest pathogens, frequency of harvest, and limits transportation and storage, all of which directly affect costs. Attempts to understand molecular basis softening is focused on cell wall metabolism and tomato has provided the principal model system for these studies. However, decreased expression of several proteins acting on cellulose, hemicelluloses and pectin polysaccharides in transgenic tomato fruit has proven to be insufficient to prevent fruit softening [Brummell DA, Harpster MH (2001) Plant Mol Biol. 47:311–340 and Giovannoni J (2001) Annual Review of Plant Physiology and Plant Molecular Biology 52: 725–749], suggesting that modifications of these cell wall components are not the sole factors to determine fruit firmness.

N-Glycoproteins are one of the constituents of plant cell wall and free *N*-glycans are also reported to occur in tomato fruit. Moreover free *N*-glycans are known to induce tomato fruit ripening and blocking of *N*-glycosylation with tunicamycin delayed fruit

ripening, which suggests that *N*-glycoprotein processing is important in ripening process [Priem B, Gitti R, Bush CA, and Gross KC (1993) Plant Physiol. 102: 445-458]. Most glycoprotein of plant source contain substantial amount of paucimannosidic *N*-glycns, which is produced by the removal of terminal GlcNAc residues in the secretary pathway as a consequence of β-D-*N*-acetylhexosaminidase activity (Strasser R, Bondili JS, Schoberer J, Svoboda B, Liebminger E, Glo¨ssl J, Altmann F, Steinkellner H and Lukas Mach (2007) Plant Physiology 145: 5–16.). Moreover, β-D-*N*-acetylhexosaminidase shows consistent increase in activity during ripening of tomato and capsicum and is present in banana, mango, papaya, etc. (Jagadeesh BH and Prabha TN (2002) Phytochemistry, 61(3): 295-300; and Jagadeesh BH, Prabha TN and Srinivasan K (2004) Plant Science 166(6): 1451-1459).

SUMMARY OF THE INVENTION

The present invention relates to purification and characterization of fruit softening associated enzyme, β-D-*N*-acetylhexosaminidase of tomato (*Solanum lycopersicum*) and capsicum (*Capsicum annuum*). The present invention also relates to identification, isolation and cloning of tomato and capsicum β-D-*N*-acetylhexosaminidase genes. The present invention further relates to transgenic plants, with reduced β-D-*N*-acetylhexosaminidase protein accumulation, having enhanced fruit shelf life.

One aspect of the present invention provides an isolated polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

Another aspect of the present invention provides a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

Yet another aspect of the present invention provides an RNAi construct for suppressing expression of hexosaminidase in a transgenic plant, the construct comprising a sense polynucleotide strand comprising at least 20 contiguous nucleotides from the sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, and an antisense polynucleotide strand that hybridizes to said sense polynucleotide strand, wherein the antisense polynucleotide strand and the sense polynucleotide strand form a duplex.

Yet another aspect of the present invention provides a process for delaying fruit softening in plant, the process comprising transforming a plant cell, tissue or any part thereof with the recombinant vector comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is in anti-sense orientation.

Yet another aspect of the present invention provides a process for delaying fruit softening in plant, the process comprising decreasing the level of hexosaminidase in transgenic plant compared to its level in non-transgenic plant by expression of an RNA interference (RNAi) construct comprising at least a fragment of at least 20 contiguous nucleotides of the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5; and screening the resulting plants for reduced level of hexosaminidase relative to the non-transgenic plant.

Yet another aspect of the present invention provides a process for delaying fruit softening in plant, the process comprising decreasing the level of hexosaminidase in transgenic plant compared to its level in non-transgenic plant by expression of an

RNA interference (RNAi) construct comprising a sense polynucleotide strand comprising at least 20 contiguous nucleotides from the sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, and an antisense polynucleotide strand that hybridizes to the sense polynucleotide strand, wherein the antisense polynucleotide strand and the sense polynucleotide strand form a duplex; and screening the resulting plants for reduced level of hexosaminidase relative to the non-transgenic plant.

Still yet another aspect of the present invention there is provided a transgenic plant, seed or progeny thereof, wherein expression of the hexosaminidase in the plant is controlled to delay fruit softening.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS

The above and other features, aspects, and advantages of the subject matter will become better understood with regard to the following description, appended claims, and accompanying drawings.

Figure 1 shows

A) β-D-*N*-acetylhexosaminidase activity during tomato fruit ripening. Data are mean ±s.e.m., n=4. MG-matured green, B-breaker, P-pink, RR-red ripe.

B) 6% non-denaturing PAGE analysis of purified β-D-*N*-acetylhexosaminidase protein from tomato.

C) 6% non-denaturing PAGE analysis of purified β-D-*N*-acetylhexosaminidase protein from capsicum.

D) tomato β-D-*N*-acetylhexosaminidase activity on *N*-glycan substrates. *N*-glycan1 and *N*-glycan2 are biantennary *N*-linked core pentasaccharide and asialo, agalacto, biantennary, respectively.

E) β-D-*N*-acetylhexosaminidase activity during capsicum fruit development and ripening stages. Activity checked in eight stages (S1-S8).

F) 2DE analysis of tomato purified protein. Spots 1, 2 and 3 were identified as SlHEX1.

G) 2DE analysis of capsicum purified protein. Spots 1, 2 and 3 were identified as CaHEXO1.

Figure 2 shows

A) *SlHEX1* gene expression during tomato fruit ripening as determined by quantitative Real Time reverse transcriptase PCR (qRT PCR) analysis. Data are mean ±s.e.m., n=3

B) the protein level expression of SlHEX1, as determined by western blotting using peptide based antibody.

Figure 3 shows

A) effect of transient silencing and over-expression of *SlHEX1* in tomato fruit, by agroinjection, on β-D-*N*-acetylhexosaminidase activity. 5RH1 and 3RH1 are RNAi construct designed from 5′ and 3′ sequence of *SlHEX1*, respectively. 7FH1 represent overexpression of *SlHEX1*.

B) confirmation of gene silencing by northern blotting. C is control. (Empty vector injected)

C) confirmation of gene silencing by western blotting. '+Ve' denotes purified protein.

D) quantitative measurement of firmness of RNAi agro injected fruits 15 days after agroinjection. Data are mean ±s.e.m., n=30, $P<0.0001$.

Figure 4 shows

A) subcellular localization of SlHEX1. C-terminal GFP fusion protein was transiently expressed in tomato fruit by agroinjection. GFP signal was observed in the cell wall.

B) relative expression of *SlHEX1* in ripening impaired mutants *rin, nor* and *Nr* compared to wild type (Ailsa craig) as determined by qRT PCR analysis. Data are mean ±s.e.m., n=3.

C) ACC inducibility of the gene as determined by qRT PCR. Data are mean ±s.e.m., n=3.

Figure 5 shows

A) *SlHEX1* transcript level and *β*-D-*N*-acetylhexosaminidase activity in T_0 fruits of *SlHEX1* overexpression lines as determined by qRT PCR analysis and enzyme assay, respectively. C is control (wild type). Data are mean ±s.e.m., n=3

B) *SlHEX1* transcript level and enzyme activity in *SlHEX1* RNAi/Antisense lines as determined by qRT PCR analysis and enzyme assay, respectively. Data are mean ±s.e.m., n=3.

C) 5 mm compression analysis of fruits from *SlHEX1* suppressed and overexpressed T_0 lines at different stages of ripening. Data are mean +s.e.m., n=15. OR- over ripe (Pink + 10 days).

D) progression of post harvest fruit deterioration recorded by time lapse photography. Fruits from transgenic (T_0) and wild type plants were harvested at pink stage and stored at room temperature (22-24°C in 55-60% relative humidity). Time after harvest is specified by days.

E) the section of pink and red ripe stage fruits stained with toluidine blue dye.

Figure 6 shows diagram of recombinant vectors (p5RH1 and p3RH1) used to generate RNAi lines.

Figure 7 shows diagram of recombinant vector (pOVH1) used to generate overexpression lines.

Figure 8 shows diagram of recombinant vector (pAH1) used to generate antisense lines.

Figure 9 shows diagram of recombinant vector (p7FH1) used for transient overexpression and subcellular localization experiments.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to tomato and capsicum fruit softening associated β-D-*N*-acetylhexosaminidases. The instant invention particularly relates to polynucleotide sequence coding for β-D-*N*-acetylhexosaminidase, wherein RNAi and antisense mediated suppression of β-D-*N*-acetylhexosaminidase expression in plants delays fruit softening and overexpression enhances fruit softening.

The inventors of the present invention focused on the beneficial physiological functions of β-D-*N*-acetylhexosaminidases such as regulatory role in fruit softening. The present invention was conducted by taking such requirement into account, and its objective is to provide a method for delaying the fruit softning for enhancing the shelf life of fruit by reducing the levels of hexosaminidase in transgenic plant.

The instant invention provides the DNA constructs, recombinant vectors and recombinant host cells comprising the polynucleotide coding for the polypeptide having the β-D-*N*-acetylhexosaminidase activity. The instant invention further provide a method for delaying fruit softening in plants using the polynucleotide sequence of β-D-*N*-acetylhexosaminidase disclosed in the present invention. In addition the instant invention provides the transgenic plants, progeny and seed thereof, wherein suppression of β-D-*N*-acetylhexosaminidase in the plant is resulted in enhanced fruit shelf life.

The resultant transgenic plant obtained by the suppressing the expression β-D-*N*-acetylhexosaminidase gene using the antisense and RNAi technology produced fruits which were about 1.5 to 2 times firmer than the non transgenic fruits. Moreover the transgenic fruits produced by RNAi technology showed no signs of softening up to 45 days of harvest which is almost 30 days more than the non-transgenic fruits. The

transgenic fruits produced by the antisense technology showed no signs of softening up to 35 days of harvest which is almost 20 days more than the non-transgenic fruits. These results are surprising and unexpected. In addition, suppression of the gene in transgenic plants had no negative effect on vegetative growth, flowering and fruit development, days to maturity and yield.

In one embodiment, the instant invention provides purification and biochemical characterization of β-D-*N*-acetylhexosaminidase enzyme from tomato and capsicum fruit pericarp and cloning of cDNA and genomic DNA of the corresponding genes from tomato and capsicum.

In another embodiment, the instant invention relates to studying the expression of the gene coding for the polypeptide having the β-D-*N*-acetylhexosaminidase activity in wild type and different tomato ripening impaired mutants. Furthermore, ethylene inducibility and cell wall localization of β-D-*N*-acetylhexosaminidase protein was determined. In addition, the functional characterization of the gene was carried out by *Agrobacterium* mediated transient and stable transformation experiments.

The enzyme exhibited ~3.5 fold enhancement of its specific activity during ripening of tomato fruit (Figure 1A). Similarly, in capsicum the enzyme showed highest activity during fruit ripening (Figure 1E). β-D-*N*-acetylhexosaminidase was purified from capsicum and tomato fruits to electrophoretic homogeneity from total protein extracted from fruit pericarp by subjecting ammonium sulphate precipitation, followed by ion exchange chromatography on DEAE Sepharose, affinity chromatography on ConA Sepharose and gel filtration chromatography on Sephadex G-100 column. The purified enzyme was a single polypeptide of 80 kDa on SDS-PAGE. However, the native size of the protein was 300 kDa on non-denaturing PAGE (Figure 1B, C). β-D-*N*-acetylhexosaminidase is a glycoprotein as it was able to bind ConA Sepharose column and was positive with PAS staining. Biochemical

characterization of tomato and capsicum hexosaminidase was carried out using *p*NP-N-acetyl-β-D glucosaminide (*p*NP-GlcNAc) substrate. The optimum pH and temperature for *p*NP-GlcNAc hydrolysis were pH6.0 and 45°C, respectively for both tomato and capsicum hexosaminidase and this enzyme was stable between wide ranges of pH (pH4.0-pH8.0 for tomato and pH3.0-pH9.0 for capsicum) without significant loss of activity. The tomato hexosaminidase could retain 90% of its activity at 37°C for 60 min but was 50% inactive at 42°C after incubation for 60 min. Capsicum hexosaminidase is stable at 42°C for 60 min. Km was 0.225 mM for tomato hexosaminidase and 0.141 mM for capsicum hexosaminidase. *N*-glycans processing function of β-D-*N*-acetylhexosaminidase was determined by incubating 10µg of *N*-glycan substrates at 37 °C for 12 hr with 0.5µg of purified protein in a 100µl reaction mixture. Then the sample was filtered through PVDF membrane to remove the protein and 10 µl of the filtered sample was loaded on to Carbopac PA-1 (4 x 250 mm) column and the release of GlcNAc was determined through high performance anion exchange chromatography (Figure 1D). Two dimensional gel electrophoresis (2DE) of purified tomato protein revealed the presence of four polypeptides with different isoelectric points. Mass spectrometry (LC-MS/MS) analysis reveled that spot numbers 1, 2 and 3 are identical and it is named as *Solanum lycopersicum* β-D-*N*-acetylhexosaminidase1 (SlHEX1) (Figure 1F). Further, 2DE of purified capsicum protein revealed similar pattern of polypeptide separation (Figure 1G). Spots 1, 2 and 3 are depicted similar polypeptide and named as *Capsicum annum* β-D-*N*-acetylhexosaminidase1 (CaHEXO1).

On the basis of peptide sequence obtained from mass spectrometry analysis and conserved motifs identified from multiple sequence alignment of *Arabidopsis* and rice β-D-*N*-acetylhexosaminidases, we designed degenerate gene specific oligonucleotides that were used to clone *SlHEX1* from tomato and *CaHEXO1* from capsicum through rapid amplification of cDNA ends. Total RNA was isolated from

fruit pericarp of tomato and reverse transcribed to generate cDNA using polyA tail specific oligonucleotide (RACE adapter primer-5' GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT 3' -SEQ ID NO: 6). A gene specific degenerate primer corresponding to the peptide KLNVLHWH (5' AARYTIAATGTTYTICAYTGGCA 3' -SEQ ID NO: 7) and a nested primer derived from RACE adapter primer (5' GGCCACGCGTCGACTAGTAC 3' -SEQ ID NO: 8) were able to amplify DNA fragments which were cloned into pGEM-T Easy vector and sequenced. Analysis of the derived sequences by BlastX (www.ncbi.nlm.nih.gov) identified as partial sequences of *SlHEX1*. The gene belongs to glycosyl hydrolase family 20 (CaZy family). Further, RACE PCR (Invitrogen) was performed to determine the 5' end sequence of the gene. Using similar approach *CaHEXO1* gene of capsicum was cloned.

Quantitative Real Time PCR (qRT PCR) analysis revealed the appearance of ~6 fold more transcripts for tomato *SlHEX1* in pink and red ripe fruits compared to mature green fruits (Figure 2A). Protein accumulation pattern as determined by western blotting analysis using peptide-based antibody raised against SlHEX1 protein was similar to mRNA expression (Figure 2B). The nature *SlHEX1* gene expression pattern, during fruit ripening, correlates with β-D-*N*-acetylhexosaminidase activity.

To develop RNAi constructs for *SlHEX1*, 600 bp 5' or 3' coding region of the gene including the respective UTRs was cloned into pHANNIBAL (Wesley SV, Helliwell CA, Smith NA, Wang MB, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP, Gleave AP, Green AG and Waterhouse PM (2001) Plant J 27: 581-590) in sense and antisense orientation separated by an intron. Finally, the *Not*I fragment of the construct was cloned into a binary vector, pART27 and named as p5RH1 (vector designed from 5' end sequence of *SlHEX1*) and p3RH1 (vector designed from 3' end sequence of *SlHEX1*) (Figure 6). Over-expression

constructs were developed either in gateway vector PK7FWG2, 0 or pBI121 vector (named as p7FH1 and pOVH1, Figures 7 and 9). Further, these binary vectors were mobilized to *Agrobacterium tumefaciens* strain EHA 105 by electroporation and grown up to mid log phase. Other *Agrobacterium* strains which can be used are LBA 4404, GV3101 etc. The grown *Agrobacterium* cells were collected and resuspended in infiltration media and agroinjected into the pericarp of the matured green tomato fruits. Injected fruits were harvested 3-5 days after injection at pink stage and analyzed for the reduction in transcript and protein level in RNAi injected fruits compared to empty vector injected fruits. Real Time PCR and western blot analysis showed reduction in mRNA and protein level, which was resulted in the loss of β-D-*N*-acetylhexosaminidase activity in the suppressed fruits (Figure 3A, C). To determine whether this reduction of enzyme activity in RNAi injected fruits was due to the silencing of *SlHEX1* gene expression, we checked the generation of gene specific small interfering (siRNA) in silenced fruits by northern hybridization. We could able to detect 21nt small RNAs specific to *SlHEX1* in RNAi injected fruits (Figure 3B). These observations again confirmed the correlation between *SlHEX1* gene expression and enzyme activity. This gene was also transiently overexpressed in tomato fruit. For overexpression fruits were agroinjected at matured green stage and harvested at pink stage, where overexpression of *SlHEX1* gene resulted in enhanced β-D-*N*-acetylhexosaminidase activity (Figure 3A). To obtain a better understanding of *SlHEX1* in fruit ripening its transcript accumulation was determined in tomato ripening impaired mutants *rin, nor* and *Nr* fruits. Since, mutant fruits do not undergo normal ripening process; comparison to wild type was made at the same chronological age rather than similar stages of ripening. qRT PCR analysis revealed reduction in *SlHEX1* transcript level upto 78% in *rin* and 89% in *nor* at both pink and red ripe stages. However in case of *Nr* fruits transcript level was reduced upto 63% in pink stage and 88% in red ripe stage (Figure 4B). All these mutations

affect either ripening associated ethylene biosynthesis or ethylene perception and ultimately had reduced fruit softening. The lack of expression of *SlHEX1* in these mutants strongly suggests its role in ripening related softening and its regulation by ethylene. The expression of *SlHEX1* gene was ~4 fold upregulated in 0.1mM ACC treated tomato seedlings which confirmed its ethylene regulation (Figure 4C). Furthermore, the contribution of β-D-*N*-acetylhexosaminidase activity towards the ripening related loss of fruit firmness was determined by analyzing the rate of softening of *SlHEX1* RNAi fruits by quantitative measurement of fruit firmness using Texture Analyzer (TA-XT plus, Stable Microsystem, UK). Agroinjected fruits were harvested at pink stage and stored at room temperature (22-24°C) in 60-65% relative humidity. 5mm compression analysis of intact RNAi and empty vector injected fruits was done 10 days after harvested which showed statistically significant difference in fruit firmness. RNAi injected fruits were ~1.6 times firmer than control fruits (Figure 3D). To gain a detailed knowledge about its function and the site of action the subcellular location of SlHEX1 protein was determined. For this, GFP was fused to the C- terminal end of the protein using gateway vector pK7FWG2,0 and the fusion protein was transiently expressed in tomato fruit by agroinjection. Injection was done at mature green stage and harvested at pink stage. Injected fruits pericarp was sectioned in a cryostat and viewed under a fluorescence microscope with appropriate filter. GFP signal was detected in the cell wall region of the cell, confirming its localization in cell wall where it can act to cell wall glycoproteins (Figure 4A).

We used *SlHEX1* gene to develop stably transformed suppression and overexpression lines in tomato. Two RNAi constructs, p5RH1 and p3RH1 were developed in pHANNIBAL using 600 bp 5′ and 3′ region of the gene, respectively (Figure 6). Antisense and over-expression construct was generated in pBI121 after removing GUS coding sequence (named as pAH1 and pOVH1, Figure7 and 8).

These binary vectors were mobilized to *Agrobacterium tumefaciens* strain EHA 105 through electroporation. Transformed *Agrobacterium* was then grown up to mid log phase and used for plant transformation. Cotyledons of 14 days old tomato seedlings were co-cultivated with *Agrobacterium* and regenerated to through tissue culture techniques. Expression of *SlHEX1* gene and β-D-*N*-acetylhexosaminidase activity was increased up to 32 fold in overexpression lines (Figure 5A). The gene expression and enzyme activity was suppressed up to 99% in RNAi lines, whereas in case of antisense lines the suppression was in the range of 55%-83% (Figure 5B). *SlHEX1* transgenic fruits undergo essentially normal climacteric ripening, with all the organoleptic characteristics and quality properties that are essential for commercialization. Firmness of the transgenic tomato fruits were measured at different stages of ripening and compared to control fruits (Figure 5C). Fruit firmness of RNAi and antisense lines was similar to wild type at matured green and breaker stages but it increased from ~1.4 times at pink stage to ~2 times at over ripe stage in RNAi lines and ~1.2 to 1.5 times in antisense lines. Transgenic *SlHEX1* constitutive overexpression resulted in enhancement of fruit softening even at matured green stage. The fruits were ~10% and ~30% softer than wild type at matured green and pink stage, respectively. Overexpression and suppression of *SlHEX1* in transgenic plants had no effect on vegetative growth, flowering and fruit development, days to maturity and yield.

In addition to compression analysis, the loss of fruit firmness during ripening and consequent spoilage of fruit could be determined qualitatively by seeing the degree of wrinkling of fruit skin. For this, wild type and transgenic fruits, stored at 22-24°C in 55-60% relative humidity, were examined to see visible signs of fruit softening and photographed at 10-15 days intervals. Although ripening was normal in both wild type and RNAi fruits under these post-harvest storage conditions, the wild type fruits started deteriorating fast after 13-15 days of harvest in comparison to RNAi

fruits which showed no signs of softening upto 45 days of harvest (Figure 5D). We further investigated cell wall changes during ripening of transgenic fruits which revealed reduced cell separation and compact cell wall in RNAi fruits as compared to wild type (Figure 5E).

In capsicum using similar approach we have transiently silenced the expression of *CaHEXO1* which resulted delayed fruit softening compared to control.

In addition to tomato and capsicum, the present invention encompasses producing transgenic plant having delayed fruit softening wherein the plant is selected from a group consisting of tomato, capsicum, mango, banana, papaya, citrus, pineapple, guava, avocado, strawberry, apple, pomegranate and other plants.

One embodiment of the present invention is to provide an isolated polynucleotide coding for a polypeptide having β-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of said polynucleotide is selected from the group consisting of

a. a nucleotide sequence coding for a polypeptide having 90% identity with an amino acid sequence as set forth in SEQ ID NO: 3;
b. a nucleotide sequence coding for a polypeptide having 90% identity with an amino acid sequence as set forth in SEQ ID NO: 5;
c. a nucleotide sequence coding for a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3;
d. a nucleotide sequence coding for a polypeptide having amino acid sequence as set forth in SEQ ID NO: 5;
e. a nucleotide sequence complementary to the nucleotide sequence of (a),(b), (c) or (d);
f. a nucleotide sequence as set forth in SEQ ID NO: 1;
g. a nucleotide sequence as set forth in SEQ ID NO: 2;
h. a nucleotide sequence as set forth in SEQ ID NO: 4; and

i. a nucleotide sequence complementary to the nucleotide sequence of (f), (g) or (h).

Yet another embodiment of the present invention provides the polynucleotide sequence from tomato, wherein the polynucleotide codes for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity.

Yet another embodiment of the present invention provides the polynucleotide sequence from capsicum, wherein the polynucleotide codes for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity.

Still yet another embodiment of the instant invention provides a DNA construct comprising the polynucleotide sequence coding for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity, wherein the polynucleotide sequence is operably linked to a promoter sequence.

Yet another embodiment of the instant invention provides a DNA construct comprising the part of polynucleotide sequence coding for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of the polynucleotide sequence is as set forth in SEQ ID NO: 1, wherein the polynucleotide sequence is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense and antisense orientation separated by an intron.

Still yet another embodiment of the instant invention provides a DNA construct comprising the part of polynucleotide sequence coding for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of the polynucleotide sequence is as set forth in SEQ ID NO: 2, wherein the polynucleotide sequence is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense and antisense orientation separated by an intron.

Still yet another embodiment of the instant invention provides a DNA construct comprising the part of polynucleotide sequence coding for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of the polynucleotide sequence is as set forth in SEQ ID NO 4, wherein the polynucleotide sequence is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense and antisense orientation separated by an intron.

İn further embodiment, the instant invention provides a recombinant vector comprising the DNA construct disclosed in the invention.

Still yet another embodiment of the instant invention provides a recombinant vector comprising the part of polynucleotide sequence coding for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of the polynucleotide sequence is as set forth in SEQ ID NO: 1, wherein the polynucleotide sequence is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense and antisense orientation separated by an intron.

Still yet another embodiment of the instant invention provides a recombinant vector comprising the part of the polynucleotide sequence coding for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of the polynucleotide sequence is as set forth in SEQ ID NO: 2, wherein the polynucleotide sequence is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense and antisense orientation separated by an intron.

Still yet another embodiment of the instant invention provides a recombinant vector comprising part of the polynucleotide sequence coding for the polypeptide having the *β*-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of the polynucleotide sequence is as set forth in SEQ ID NO: 4, wherein the polynucleotide sequence is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense and antisense orientation separated by an intron.

Yet another embodiment of the present invention relates to a recombinant host cell comprising the recombinant vector disclosed in the invention.

Yet another embodiment of the present invention relates to a recombinant host cell comprising the polynucleotide sequence coding for the polypeptide having the β-D-*N*-acetylhexosaminidase activity, wherein the nucleotide sequence of the polynucleotide sequence is selected from a group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

One embodiment of the present invention relates to the host cell of the invention, wherein the host cell is selected from the group consisting of *Agrobacterium*, *E. coil* and yeast.

In one embodiment, the instant invention provides a process for delaying fruit softening in plant; said method comprising transforming a plant cell, tissue or any part thereof with the recombinant vector of the present invention, wherein said vector comprises the part of the polynucleotide sequence of *SlHEX1 or CaHEXO1* is in sense and antisense direction separated by an intron.

In one embodiment, the instant invention provides a process for delaying fruit softening in plant; said method comprising transforming a plant cell, tissue or any part thereof with the recombinant vector of the present invention, wherein said vector comprises the polynucleotide sequence *SlHEX1 or CaHEXO1* is in antisense direction.

In another embodiment, the present invention provides a process for early fruit softening in plant; said method comprising transforming a plant cell, tissue or any part thereof with the recombinant vector of the present invention, wherein said vector comprises the polynucleotide sequence *SlHEX1 or CaHEXO1* is in sense direction that codes for a polypeptide having β-D-*N*-acetylhexosaminidase activity.

One embodiment of the present invention relates to a transgenic plant produced by the process disclosed in the instant invention, wherein expression of β-D-*N*-acetylhexosaminidase in said plant is modulated to effect fruit softening, wherein the plant is selected from the group consisting of tomato, capsicum and papaya etc.

One embodiment of the present invention relates to a transgenic plant produced by the method disclosed in the instant invention, wherein expression of β-D-*N*-acetylhexosaminidase in said plant is modulated to effect fruit softening, wherein the plant is tomato.

One embodiment of the present invention relates to a transiently transformed plant produced by the method disclosed in the instant invention, wherein expression of β-D-*N*-acetylhexosaminidase in said plant is modulated to effect fruit softening, wherein the plant is capsicum.

One embodiment of the present invention relates to seed or progeny of the transgenic plant produced by the method disclosed in the instant invention, wherein expression of β-D-*N*-acetylhexosaminidase in said plant is modulated to effect fruit softening.

In one embodiment of the present invention there is provided an isolated polynucleotide coding for a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 88% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

In another embodiment of the present invention there is provided an isolated polynucleotide coding for a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 90 % identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

Another embodiment of the present invention provides an RNAi constructs comprising at least 20 contiguous nucleotides from the polynucleotide encoding a polypeptide having hexosaminidase activity are selected in such a way that these nucleotides form only hairpin structure and do not form the secondary loop within its length, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

Another embodiment of the present invention provides an RNAi constructs comprising at least 20 contiguous nucleotides from the polynucleotide encoding a polypeptide having hexosaminidase activity are selected in such a way that these nucleotides form only hairpin structure and do not form the secondary loop within its length, wherein the nucleotide sequence of said polynucleotide is as set forth in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 4.

The RNAi constructs disclosed in the present invention form hairpin structure after transcription in the plant.

The RNAi constructs disclosed in the present invention is a hairpin nucleic acid.

The RNAi construct disclosed in the present invention form hairpin structure after transcription in the plant, and does not form the secondary loop structure.

Another embodiment provides an RNAi constructs comprising the polynucleotide sequence as disclosed in the present invention form hairpin structure after transcription in the plant.

In one embodiment of the present invention there is provided an isolated polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

In another embodiment of the present invention there is provided a polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the polynucleotide encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

In another embodiment of the present invention there is provided a polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide is as set forth in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 4.

In another embodiment of the present invention there is provided a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

In another embodiment of the present invention there is provided a DNA construct comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is operably linked to a promoter sequence.

In another embodiment of the present invention there is provided a DNA construct comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the polynucleotide encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is operably linked to a promoter sequence.

In another embodiment of the present invention there is provided a DNA construct comprising a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide is as set forth in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 4, wherein the polynucleotide is operably linked to a promoter sequence.

In another embodiment of the present invention there is provided a DNA construct comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense orientation.

In another embodiment of the present invention there is provided a DNA construct comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the polynucleotide encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense orientation.

In another embodiment of the present invention there is provided a DNA construct comprising a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide is as set forth in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 4, wherein the polynucleotide is operably linked to a promoter sequence, wherein the polynucleotide sequence is in sense orientation.

In another embodiment of the present invention there is provided a DNA construct comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is operably linked to a promoter sequence, wherein the polynucleotide sequence is in anti-sense orientation.

In another embodiment of the present invention there is provided a DNA construct comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the polynucleotide encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is operably linked to a promoter sequence, wherein the polynucleotide sequence is in anti-sense orientation.

In another embodiment of the present invention there is provided a DNA construct comprising a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide is as set forth in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 4, wherein the polynucleotide is operably linked to a promoter sequence, wherein the polynucleotide sequence is in anti-sense orientation.

In another embodiment of the present invention there is provided an RNAi construct for suppressing expression of hexosaminidase in a transgenic plant, the construct comprising a sense polynucleotide strand comprising at least 20 contiguous nucleotides from the sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, and an antisense polynucleotide strand that hybridizes to said sense polynucleotide strand, wherein the antisense polynucleotide strand and the sense polynucleotide strand form a duplex .

In another embodiment of the present invention there is provided an RNAi construct for suppressing expression of hexosaminidase in a transgenic plant, wherein the RNAi construct is a hairpin nucleic acid.

In another embodiment of the present invention there is provided an RNAi construct for suppressing expression of hexosaminidase in a transgenic plant, the construct comprising a sense polynucleotide strand comprising at least 20 contiguous nucleotides from the sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, and an antisense polynucleotide strand that hybridizes to said sense polynucleotide

strand, wherein the antisense polynucleotide strand and the sense polynucleotide strand form a duplex , wherein said sense strand comprises 100 to 600 nucleotides.

In another embodiment of the present invention there is provided a recombinant vector comprising the DNA construct as disclosed in the present invention.

In another embodiment of the present invention there is provided a recombinant host cell comprising the recombinant vector as disclosed in the present invention.

In another embodiment of the present invention there is provided host cell selected from the group consisting of *Agrobacterium*, *E. coil* and yeast.

In another embodiment of the present invention there is provided a process for delaying fruit softening in plant, the process comprising transforming a plant cell, tissue or any part thereof with the recombinant vector comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein the polynucleotide is in anti-sense orientation.

In another embodiment of the present invention there is provided a process for delaying fruit softening in plant, the process comprising transforming a plant cell, tissue or any part thereof with the recombinant vector comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

In another embodiment of the present invention there is provided a process for delaying fruit softening in plant, the process comprising decreasing the level of hexosaminidase in transgenic plant compared to its level in non-transgenic plant by expression of an RNA interference (RNAi) construct comprising at least a fragment

of at least 20 contiguous nucleotides of the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5; and screening the resulting plants for reduced level of hexosaminidase relative to said non-transgenic plant.

In another embodiment of the present invention there is provided a process for delaying fruit softening in plant, the process comprising decreasing the level of hexosaminidase in transgenic plant compared to its level in non-transgenic plant by expression of an RNA interference (RNAi) construct comprising at least a fragment of at least 20 contiguous nucleotides of the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of the polynucleotide is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

In another embodiment of the present invention there is provided a process for delaying fruit softening in plant, the process comprising decreasing the level of hexosaminidase in transgenic plant compared to its level in non-transgenic plant by expression of an RNA interference (RNAi) construct comprising a sense polynucleotide strand comprising at least 20 contiguous nucleotides from the sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, and an antisense polynucleotide strand that hybridizes to the sense polynucleotide strand, wherein the antisense polynucleotide strand and the sense polynucleotide strand form a duplex; and screening the resulting plants for reduced level of hexosaminidase relative to said non-transgenic plant.

In another embodiment of the present invention there is provided a process for delaying fruit softening in plant using the RNAI constructs, wherein the RNAi construct is a hairpin nucleic acid.

In another embodiment of the present invention there is provided a transgenic plant produced by the process as disclose in the present invnetion, wherein expression of the hexosaminidase in the plant is controlled to delay fruit softening.

The transgenic plant disclosed in the present invention encompasses tomato, capsicum, papaya, mango, banana, peach, pear, citrus, pineapple, guava, avocado, strawberry, apple and pomegranate.

The transgenic plant as disclosed in the present invention is tomato or capsicum.

In another embodiment of the present invention there is provided a transgenic seed or progeny of the transgenic plant as disclosed in the present invention.

EXAMPLE

It should be understood that the following examples described herein are for illustrative purposes only and that various modifications or changes in light will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

Example 1

Enzyme assay and purification of β-D-N-acetylhexosaminidase from tomato and capsicum

Tomato and capsicum seeds were germinated in pre-sterilized soil and 15 days old seedlings were transplanted to the greenhouse with 25/23°C temperature, 70% humidity and 14/10 hr light/dark regime. Flowers were tagged at anthesis and fruits were harvested at different stages of ripening.

Enzyme reaction was done using *p*NP-GlcNAc (0.4 mM) in 10 mM Tris-Cl, pH 6.0 at 37°C for 15 min and reaction was stopped by adding 20 mM Na_2CO_3. The colour thus developed was recorded at 405 nm and the amount of *p*NP released was determined using a standard curve.

The frozen fruit pericarp was powdered in liquid nitrogen and suspended in one fourth volume of extraction buffer (100 mM Tris-Cl, pH 7.0 with 0.25 M NaCl and 1 mM PMSF) and extracted overnight at 4^0C. After passing through four layers of cheesecloth, the extract was centrifuged at 10,000g to settle down all the debris. The supernatant thus obtained was subjected to 30-80% ammonium sulphate precipitation. The pellet obtained after the precipitation was reconstituted and dialyzed overnight against 25 mM Tris-Cl, pH 7.0 with one change. The sample was then chromatographed on DEAE Sepharose pH 7.0 and eluted with increasing gradient of NaCl. The enzyme eluted at 120 mM salt concentration. The samples containing the activity were pooled and subjected to affinity chromatography on ConA sepharose column and eluted with increasing gradient of α-D-methylmannopyranoside. The enzyme eluted at 100 mM concentration, pooled and concentrated by 0-90% ammonium sulphate saturation. The pellet thus obtained was dissolved in 25 mM Tris-Cl, pH 7.0 and directly loaded on the gel filtration column of Sephadex G100. Fractions were collected after the void volume, assayed for enzyme activity and resolved on 12.5% SDS-PAGE. The enzyme rich fractions were concentrated using CentriconYM30 (Millipore) and stored at 4^0C.

Example 2

2DE, SDS-PAGE, LC-MS/MS and western blotting

Purified protein was diluted in dilution buffer (200 mM Tris-Cl pH 8.5, 40% glycerol, 8% SDS, 0.4 mM PMSF and 50 mM DTT), boiled for 5 min and precipitated with 9 volumes of 100% chilled acetone. Electrofocusing was performed

using IPGphor system (Amersham biosciences) at 20°C for 40,000 Vhr after passive rehydration of 13 cm long immobiline dry strip (pH 4.0-7.0) for 12 hr with 20 μg of purified protein sample in 250 μl 2DE rehydration buffer (9 M urea, 4% chaps, 2% thiourea, 20 mM DTT, 0.8% IPG buffer and trace amount of bromophenol blue). The focused strips were subjected to reduction with 1% (w/v) DTT in 10 ml of equilibration buffer (50 mM Tris-Cl pH 8.8, 6 M urea, 30% glycerol and 2% SDS) followed by alkylation with 2.5% (w/v) iodoacetamide in the same buffer. The strips were then loaded on top of 12.5% polyacrylamide gels for second dimensional separation on SDS-PAGE. The electrophoresed proteins were stained with coomassie blue stain and gel images were digitized with a FluorS imaging system (Bio-Rad, CA). Experimental molecular mass and pI were calculated from digitized 2DE images using standard molecular mass marker proteins. The spots were cut from the gel and analyzed by electrospray ion trap time-of-flight mass spectrometry (LC-MS/MS) (Q-Star Pulsar *i*, Applied Biosystems). The spectra were analyzed by Mascot sequence matching software (www.matrixscience.com) against the Viridiplantae (green plants) database.

For western blot analysis 50 μg of the protein, quantified by Bio-Rad protein assay kit and resolved on 12.5% SDS-PAGE was electrotransferred to Hybond-C (Amersham biosciences) membrane at constant current of 150 mA for 2-3 hours. Non specific sites on the membrane were blocked by Blotto in TBS for 1 hr and incubated with the primary antibody (1:2500) overnight at 4°C. Immunodetection was carried out with horse raddish peroxidase conjugated anti-rabbit antiserum as secondary antibody for 1 hr and exposing the blot to chemiluminescence substrate (Pierce Biotechnology).

SDA-PAGE analysis of the purified protein reveled a single protein band of 80 kD and native molecular weight of the protein was found to be 300kD. On 2DE gel

purified protein sepearted into different spots of 80 kD which were found to be representative of β-D-N-acetylhexosaminidase as confirmed by LC-MS/Ms analysis.

Example 3

High performance anion exchange chromatography

The standard sugar (GlcNAc, Sigma) was prepared in deionized water and injected at a concentration of 100 nmoles before and after the analysis of sample. An HPAE-PAD system (Dionex DX 500 BioLC) equipped with a gradient pump (GP 40), an anion exchange column (Carbopac PA-1, 4 x 250 mm), an eluant degas module (EDM-2) for pressurizing the eluants with argon was used for the analysis of monosaccharide. The separated monosaccharide was detected by an ED 40 detector equipped with a gold electrode and an Ag/AgCl reference electrode. The resulting chromatographic data was integrated and plotted using a PC based oracle 2 data acquisition system (Indtech Analytical, Bombay). 10 μg of *N*-glycan substrates were incubated at 37°C for 12 hr with 0.5 μg of purified protein in a 100 μl reaction mixture. Then the sample was filtered through PVDF membrane to remove the protein and 10 μl of the filtered sample was loaded on to the column.

The tomato enzyme was able to cleave terminal GlcNAc residue from different N-glycans, thus confirming its N-glycan processing ability.

Example 4

Cloning of tomato and capsicum β-D-N-acetylhexosaminidases and sequence analysis

Degenerate gene specific oligonucleotides were designed based on the peptide tags obtained from mass spectrometry (LC-MS/MS) analysis and motifs identified from multiple sequence alignment and were used for rapid amplification of cDNA ends (RACE). Total RNA was isolated from pericarp of pink stage tomato fruit and reverse transcribed to generate cDNA using polyA tail specific oligonucleotide

(RACE adapter primer). A gene specific degenerate primer corresponding to the peptide KLNVLHWH (5′ AARYTIAATGTTYTICAYTGGCA 3′ -SEQ ID NO: 7, R is A/G, Y is C/T and I is inosine) and a nested primer derived from RACE adapter primer were able to amplify DNA fragment which was cloned into pGEM-T Easy vector (Promega) and sequenced. Analysis of the derived sequences by blastx (www.ncbi.nlm.nih.gov) identified as partial sequence of *SlHEX1* of glycosyl hydrolase family 20 (CaZy family). Further, RACE PCR (Invitrogen) was performed to determine the 5′ end sequence of the gene. Using similar approached, capsicum CaHEXO1 was cloned. Protein sequences were deduced by back translation. Related protein sequences from other species were taken and ClustalW and phylogenic analysis (MEGA4) were performed.

Tomato SlHEX1 (1876 bp) was claoned into pGEM-T Easy vector and the recombinant vector was named as pGHT. Similarly capsicum CaHEXO1 (1725 bp) was cloned into pGEM-T Easy vector and the recombinant vector was named as pGHC.

Example 5

Subcellular localization

To generate C-terminal GFP fusion protein, coding sequence lacking the stop codon were PCR amplified using attB appended primers (5′ GGGGACAAGTTTGTACAAAAAAGCAGGCTATGAGAGGAGAGAAAACAT TCTCC 3′ -SEQ ID NO: 9 and 5′ GGGACCACTTTGTACAAGAAAGCTGGGTGCTAGTAAATGAATGACCTGT GTTAC 3′ -SEQ ID NO: 10). attB flanked PCR product was then cloned into entry vector pDONR221 (Invitrogen) by performing a BP recombination reaction. Further LR reacombination rection was carried out between entry clone and Gateway expression vector pK7FWG2, 0. This recombinant binary vector was used for

Agrobacterium mediated transient expression of fusion protein in tomato fruit. Agroinjected fruit pericarp was mounted on to the cryostat (Lieca CM1510S) and frozen. Then 10-15 μm slices were cut and taken on the slides to view under fluorescence microscope.

This confirms that β-D-N-acetylhexosaminidase is localized in cell wall.

Example 6

***Agrobacterium* based transient transformation**

Agroinjection was done as described previously (Orzaez DF, Mirabel S, Wieland WH and Granell A (2006) Plant Physiol 140: 3–11) with few modifications. For agroinjection *Agrobacterium* pre-cultures (3mL) were grown for 48 hrs form individual colonies at 28°C in YEP (Yeast extract 1%, peptone 1% and NaCl 0.5%) media with antibiotics. This was transferred to 50mL induction medium (0.5% beef extract, 0.1% yeast extract, 0.5% Peptone, 0.5% Sucrose, 2mM $MgSO_4$, 20 mM acetosyringone, 10mM MES , pH 5.6) with antibiotics and was grown overnight. Next day the culture recovered by centrifugation when the OD reached 1 and resuspended in infiltration medium (10mM $MgCl_2$, 10mM MES, 200 mM acetosyringone, pH 5.6) and incubated at room temperature with gentle agitation for 4 hr. 1mL syringe with 0.5-mm needle was used for agroinjection. Needle was introduced 1 to 2mm in depth into the fruit pericarp near the stylar apex, and infilteration solution was gently injected in the fruit. Maximum of 500mL solution was able to be injected. Beyond breaker stage fruits were unfit for injection.

Example 7

RNA isolation and quantitative real time reverse transcriptase PCR (qRT PCR)

RNA was isolated according to LiCl method and quantified using nanodrop (ND 100). Total RNA (5 μg) was reverse transcribed using superscript II (Invitrogen) and diluted five times before using in the qRT PCR reaction. qRT PCR was performed

using One Step Real Time RT PCR (Applied Biosystems) with cyber green. The oligonucleotide primers used in the amplification are RTH1F-5′ TATGTTCTGG TGGCCCG 3′ (SEQ ID NO: 11) and RTH1R- 5′ TCTGCTCCTCCGTGAAAG 3′ (SEQ ID NO: 12) for *SlHEX1* target amplification and RTTAL-5′ TTATCACCATT GGTGCTGAG 3′ (SEQ ID NO: 13) and RTTAR- 5′ CGATGTTTCCATAC AGATCCTT 3′ (SEQ ID NO: 14) for endogenous control actin. Relative gene expression was analyzed using $2^{-\Delta\Delta Ct}$ method.

Example 8

Small RNA isolation and northern blotting

Total RNA was enriched for small molecular weight RNA. The RNA after the precipitation with LiCl, was washed with 70% ethanol and the pellet was air dried. The pellet was then dissolved in 1ml of DEPC water and heated for 65°C for 5 min, and then placed on ice for 2 min. To precipitate the high molecular weight RNA, polyethylene glycol (molecular weight 8000) and NaCl was added to a final concentration of 5% and 0.5 M, respectively. After 30 minutes of incubation on ice, the RNA was centrifuged at 13,000 rpm for 30 min. The supernatant was precipitated with 3 volumes of ethanol and 1/10 volume of 3M Sodium acetate (pH 5.2) and the tube was placed in -20°C overnight. Next day the low molecular weight RNA was recovered by centrifugation at 13,000 rpm for 10 min. The pellet was dried and dissolved in 50 µl of DEPC water. Small molecular weight RNA was resolved on 15% urea PAGE containing 0.5X TBE for 4-5 hr at 70 V. Then the gel was electrotransferred to nylon membrane using 0.5X TBE for 1 hr at 100 V constant. The nylon membrane was UV cross-linked and pre-hybridized in 50% formamide, 7% SDS and 50 mM Na_2HPO_4 and NaH_2PO_4, pH 7.2 at 35°C. After 4 hours, the denatured probe was added and hybridization was done for 14-16 hours at 35°C. The blot was washed using 2X SSC and 1% SDS for 2 min at RT, then washed with 0.5X SSC and 0.1% SDS and exposed to the film.

The analysis confirmed the generation of gene specific siRNA in transgenic fruit produced with the RNAi technology.

Example 9

Vectors and tomato transformation

To develop RNAi constructs, 500-600 bp 5′ or 3′ coding region of the gene including UTR was cloned into pHANNIBAL in sense and antisence orientation separated by an intron. To develop RNAi construct from 5′ end sequence of the *SlHEX1*, PCR amplification was done using primers 5′ CCGCTCGAGAAGCAGTGGTATCAACGCAGAGTACGC 3′ (SEQ ID NO- 15) or 5'CCGCTCGAGGAGAAAAAAATGAGAGGAGAGAAAAC 3' (SEQ ID NO- 16) and 5′ GGAATTCCAGCCTTAACAAGTGATCGACTCCG 3′ (SEQ ID NO: 17) for cloning in sense direction. Similarly, PCR was done for cloning in antisense direction using primers 5′ GCTCTAGAAAGCAGTGGTATCAACGCAGAGTACGC 3′ (SEQ ID NO: 18) or 5' GCTCTAGAGAGAAAAAAATGAGAGGAGAGAAAAC 3' (SEQ ID NO: 19) and 5′ CCCAAGCTTCAGCCTTAACAAGTGATCGACTCCG 3′ (SEQ ID NO: 20). To develop RNAi construct from 5′ end sequence of the *SlHEX1*, PCR amplification was done using primers 5′ CCGCTCGAGCCGTGTGATTGTGTCATCTGC 3′ (SEQ ID NO: 21) and 5′ GGAATTCGTAAGAAATTCCCAGATTCATTTGC 3′ (SEQ ID NO: 22). Similarly, PCR was done for cloning in antisense direction using primers 5′ GCTCTAGACCGTGTGATTGTGTCATCTGC 3′ (SEQ ID NO: 23) and 5′ CGGGATCCGTAAGAAATTCCCAGATTCATTTGC 3′ (SEQ ID NO: 24). Finally, the NotI fragment of the construct was cloned into a binary vector, pART27. Antisense and overexpression constructs were prepared in pBI121 vector after removing GUS coding sequence. By PCR amplification, XbaI restriction site was incorporated in the cDNA sequence before start and after stop codon for preparation

of overexpression and antisense vectors, respectively. For amplification of sense strand PCR was done using primers 5' GCTCTAGAATGAGAGGAGAGAAAACATTCTCC 3' (SEQ ID NO: 25) and 5' TCAGCTAGTAAATGAATGAACTG 3' (SEQ ID NO: 26). For cloning in antisense direction PCR was done using primers 5' ATGAGAGGAGAGAAAACATTCTCC 3' (SEQ ID NO: 27) and 5' GCTCTAGAGTAAGAAATTCCCAGATTCATTTGC 3' (SEQ ID NO: 28). PCR product was then ligated into XbaI / Ecl136II site of pBI121.

Transgenic tomato plants were generated by *Agrobacterium* mediated transformation of tomato cotyledon leaves. After cutting, explants were placed on MS media (Sigma) containing zeatin (10 µg/ml) as callusing and shooting hormone (shooting media) and kept for one day under tissue culture condition (24°C, 65% relative humidity, 14/10 hr light-dark cycle). After one day these explants were used for transformation. *Agrobacterium* were grown in YEP media for transformation. *Agrobacterium* cells were pellet suspended in liquid MS containing Acetosyringone. The cell suspension were added to explants and co-cultivated. After co-cultivation explants were transferred to MS (shooting media) and kept at 28°C. After two days, explants were transferred to shooting media containing Cefotaxime and maintained at tissue culture condition. Then explants were further transferred to shooting media containing Cefotaxime and Kanamycin. Explants were maintained in this media for two to three and half months with regular change. Shoots (3-4 cm in length) were then transferred to rooting media with hormone IAA (10 µg/ml) and antibiotics (Cefotaxime and Kanamycin) and maintained for 1-2 months. Leaves were collected from rooted plants for DNA extraction (CTAB method) and Polymerase Chain Reaction (PCR) was performed to determine putative transformants. 7-10% frequency of regeneration and transformation was achieved through this

Agrobacterium mediated gene transfer method. PCR positive plants were transferred to green house after hardening

Example 10

Textural analysis

Fruit firmness was determined using TA-XT Plus (Stable Microsystems, UK). Each fruit was analyzed with 75 mm wide P75 compression plate loading at 1 mm s^{-1}. The fruits were compressed to a vertical displacement of 5 mm. Firmness was defined as the response force to a 5 g applied force. The values were subjected to Student's t-test to find the significant difference.

The textural analysis reveled 2 fold increase in the fruit firmness of the transgenic fruit as compared to the non transgenic fruit.

Example 11

ACC treatment to the seedlings

Fifteen days old tomato seedlings (Pusa ruby), germinated and grown in MS medium were used for ACC treatment. Seedlings from MS media were transferred to liquid MS containing 0.1 mM ACC and harvested after 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 16 hr of treatment. Seedlings were frozen immediately in liquid nitrogen and RNA was isolated as mentioned above. Further RNA was reverse transcribed to cDNA and used for qRT-PCR. Expression was calculated relative to the control seedlings (0 hr).

This experiment confirms the up-regulation of SlHEX1 gene by ethylene.

Example 12

Staining and microscopy

Sections were cut in a cryostat microtome (Leica 1050) and were allowed to dry on the poly-l-lysine coated slides. The slides were dipped in aqueous solution of 0.05% toluidine blue (Sigma) in 0.1 M phosphate buffer at pH 6.8 for 2 min. and washed in

milliQ water for 2 minutes. The stained sections mounted in milliQ water under a cover slip and photographed at 40X magnification using Nikon 80i epiflouresent/ phage contrast/ Bright field microscope.

Staining of tomato fruit cell wall followed by microscopy revealed reduced cell separation and compact cell wall in transgenic fruit as compared to non transgenic fruit.

SEQ ID NO: 1 Complete genomic sequence of *Solanum lycopersicum* β-D-*N*-acetylhexosaminidase1 (2510 nts)

ATGAGAGGAGAGAAAACATTCTCCTTCTTTCTTCTATTATTCTTTATCTTAATTTCACAAA
CAACAGCCACAAATTACCCAATCAATGTCTGGCCCAAGCCCACAACATTCCTTTGGCCCA
ACCCAAAATCCATCTTCCTCTCCACAAACTTCACCATCTCCCACCCGTACCACCGGTACCT
CACTCCCGCCGTCGACCGTTACCGCCACCTCATCCTCTCCGAACACCACCGTCCCATCAT
AACTCCCGCTATCAACCTCACTTCATCAATTCCGTTACAAAGCCTTGTCATCTCCGTCTCC
GATGTCACTTCACCACTCGCTCACGGAGTCAACGAATCCTACTCTCTCTCCACACCTTCCG
ACGGCTCCGCCTCCGCCTACATATCTGCGGCGACTGTATGGGGAGCCATGCGAGGGCTG
GAGACATTCTCACAACTCGTGTACGGAAACCCTACCAGAGTTTCCGCCGGCGTGTATATA
CACGATCTACCGATTTTTACTCACAGAGGTGTGATGTTGGATACTTCGAGAAATTTCTAC
GGAGTCGATCACTTGTTAAGGCTGATTAAAGCTATGAGTATGAACAAGTTGAATGTTTTT
CACTGGCATATAACTGATTCACATTCATTTCCGCTTGTGATCCCGTCGGAGCCTGAGCTC
GCCGGAAAAGGAGCGTACAGCAACGAGATGATGTATTCGCCGGCGGACGTGCAGAAGA
TCGTGGAATATGGAATGGAACACGGAGTTAGGGTTTTACCTGAAATTGACATGCCTGGTA
AGTTATGGAATCACGATAAAGTATAATAGAATAATGTATGGATAATTTTCGTTTACAATT
TCTAGGTAATCCCGAAAAGGAACAAGATTGGACTTTTGGAAGTATTATTAATTTATCAGG
ATATGGGGATCATTAATTTATTAATATTAACTTAATGGGACCTTGGTGGATTTTTTGGAAC
TTTTTCTTCTGGCAAATGAAAATGTATATATCTTTTTATACAGGAAACCAAAACCTTTAAG
CCTGGGTTTGGGAAGGTAGTGTGGGTTTGGGAAGGTAGTGTGTATGAACCGCTGTTCTTT
TGAGTACTCTTCTCGATACGAATAAGAGATTATGTTTGATACTTTCAGTGAGATGTGAAT
GAGAGTTTAGATGGTTGACGATCTCAATCTTGATATTCGGCTCCATTTTTTTTATGGTAGT
TCCGTAGGTAGGATTTTGACAATTTTGTTTACATGGTGTGCAAAGAACTATGAATTTTAG
ATTATCTTGTAAACCAGCTTGATAAAGGGTAATGGAAGGGACAGTCTTGTCTTTGGTACC
ACTGAAAGTGCGATAGTCCTTATTACTGCAATTGATATTTTGTTAAAATCCAAATGAGAG
GCAAATTGCTATATCCTCCACAGAAGAAGGTTATATCATGTTGTAAAGCACGTCAAAATT
TCTTTTGTTAAACATAGAATCACATATGGATATCTTTGTGGACATAGGAACAAAGGTAAG
TGTATTGGTAACACTATTAAATGTCTGAAGTTTGTCCCATTTTGCAAATATTGCAGCACAT
ACAGGATCCTGGGCTGAAGCTTACCCTGAGATAGTCACTTGTGCAAATATGTTCTGGTGG
CCCGCTGGAAGTAGTCCAGCTCTTGCAGCTGAACCAGGCACTGGCCAACTGAACCCATC
GATTCCCAAGACCTATGAAGTAGTCAAGAATGTCATCCAGGGCACTATCGCCATGTTTCC
GGATTCACTCTTTCACGGAGGAGCAGATGAGATCAATTCAGACTGTTGGAATACTGATCT
ATCAGTCCAAAAGTTTGTTGCTAGCAATGGAACTCTCAGTCAGCTACTAGAGAAGTTTAT
CAATAATACCTTACCTGAAATCCTCTCACTCAATCGTACGGTGGTCTACTGGGAGGATGT

TATATTGAGTGGTAATGTGAAAGTGAATCCATCTCTGCTTCCTCCACAGAATGTTATTAT
GCAAACTTGGAATAATGGACCAAACAATACAAAGCAGCTTGTCACTTCTGGCTACCGTGT
GATTGTGTCATCTGCAGATTACTATTACTTGGATTGTGGCCATGGAAGCTTCGTTGGGAA
TGACAGCCGCTATGATCAGCCACCAGGTACTGACCAAGGCAATGGCGGATCATGGTGTG
GGCCTTTCAAGACGTGGGAAACCATTTACAACTATGATATAACCTACGGCCTAACTGATG
AGGAGGCTCCATTGGTAATTGGAGGGGAAGTAGCATTATGGTCCGAACAAGCTGATTCA
ACTGTTATGGACTCAAGGATTTGGCCAAGAGCATCAGCAATGGCAGAAGCATTGTGGTC
AGGAAATCGTGATGAAACAGGAATGAAGAGATATGCAGAGGCTACTGATCGACTGAATG
AATGGAGGTACAGAATGGTTTCTAGGGGAATAGGTGCTGAATCGATTCAACCACTTTGGT
GTCTCAAAAACCCAGGCATGTGTAACACAGTTCATTCATTTACTAGCTGA

SEQ ID NO: 2 Complete cDNA sequence of *Solanum lycopersicum* β-D-*N*-acetylhexosaminidase1 (1876 nts)

GAGAAAAAAATGAGAGGAGAGAAAACATTCTCCTTCTTTCTTCTATTATTCTTTATCTTA
ATTTCACAAACAACAGCCACAAATTACCCAATCAATGTCTGGCCCAAGCCCACAACATTC
CTTTGGCCCAACCCAAAATCCATCTTCCTCTCCACAAACTTCACCATCTCCCACCCGTACC
ACCGGTACCTCACTCCCGCCGTCGACCGTTACCGCCACCTCATCCTCTCCGAACACCACC
GTCCCATCATAACTCCCGCTATCAACCTCACTTCATCAATTCCGTTACAAAGCCTTGTCAT
CTCCGTCTCCGATGTCACTTCACCACTCGCTCACGGAGTCAACGAATCCTACTCTCTCTCC
ACACCTTCCGACGGCTCCGCCTCCGCCTACATATCTGCGGCGACTGTATGGGGAGCCATG
CGAGGGCTGGAGACATTCTCACAACTCGTGTACGGAAACCCTACCAGAGTTTCCGCCGG
CGTGTATATACACGATCTACCGATTTTTACTCACAGAGGTGTGATGTTGGATACTTCGAG
AAATTTCTACGGAGTCGATCACTTGTTAAGGCTGATTAAAGCTATGAGTATGAACAAGTT
GAATGTTTTTCACTGGCATATAACTGATTCACATTCATTTCCGCTTGTGATCCCGTCGGAG
CCTGAGCTCGCCGGAAAAGGAGCGTACAGCAACGAGATGATGTATTCGCCGGCGGACGT
GCAGAAGATCGTGGAATATGGAATGGAACACGGAGTTAGGGTTTTACCTGAAATTGACA
TGCCTGCACATACAGGATCCTGGGCTGAAGCTTACCCTGAGATAGTCACTTGTGCAAATA
TGTTCTGGTGGCCCGCTGGAAGTAGTCCAGCTCTTGCAGCTGAACCAGGCACTGGCCAAC
TGAACCCATCGATTCCCAAGACCTATGAAGTAGTCAAGAATGTCATCCAGGGCACTATCG
CCATGTTTCCGGATTCACTCTTTCACGGAGGAGCAGATGAGATCAATTCAGACTGTTGGA
ATACTGATCTATCAGTCCAAAAGTTTGTTGCTAGCAATGGAACTCTCAGTCAGCTACTAG
AGAAGTTTATCAATAATACCTTACCTGAAATCCTCTCACTCAATCGTACGGTGGTCTACT
GGGAGGATGTTATATTGAGTGGTAATGTGAAAGTGAATCCATCTCTGCTTCCTCCACAGA
ATGTTATTATGCAAACTTGGAATAATGGACCAAACAATACAAAGCAGCTTGTCACTTCTG
GCTACCGTGTGATTGTGTCATCTGCAGATTACTATTACTTGGATTGTGGCCATGGAAGCTT
CGTTGGGAATGACAGCCGCTATGATCAGCCACCAGGTACTGACCAAGGCAATGGCGGAT
CATGGTGTGGGCCTTTCAAGACGTGGGAAACCATTTACAACTATGATATAACCTACGGCC
TAACTGATGAGGAGGCTCCATTGGTAATTGGAGGGGAAGTAGCATTATGGTCCGAACAA
GCTGATTCAACTGTTATGGACTCAAGGATTTGGCCAAGAGCATCAGCAATGGCAGAAGC
ATTGTGGTCAGGAAATCGTGATGAAACAGGAATGAAGAGATATGCAGAGGCTACTGATC
GACTGAATGAATGGAGGTACAGAATGGTTTCTAGGGGAATAGGTGCTGAATCGATTCAA
CCACTTTGGTGTCTCAAAAACCCAGGCATGTGTAACACAGTTCATTCATTTACTAGCTGA
TCCTTCATCAGTGTCTGTACCAGTCATTTATCTGTATTATCTTCTATATTCAGATCCAGTTT
ATTATTGTAATGTTACCTTCTATTGTCACCACATTACCATGATTTTACTAGGCAAATGAAT
CTGGGAATTTCTTACA

SEQ ID NO: 3 Amino acids sequence of *Solanum lycopersicum* β-D-*N*-acetylhexosaminidase1 (575 aa)

MRGEKTFSFFLLLFFILISQTTATNYPINVWPKPTTFLWPNPKSIFLSTNFTISHPYHRYLTPAVD
RYRHLILSEHHRPIITPAINLTSSIPLQSLVISVSDVTSPLAHGVNESYSLSTPSDGSASAYISAAT
VWGAMRGLETFSQLVYGNPTRVSAGVYIHDLPIFTHRGVMLDTSRNFYGVDHLLRLIKAMS
MNKLNVFHWHITDSHSFPLVIPSEPELAGKGAYSNEMMYSPADVQKIVEYGMEHGVRVLPEI
DMPAHTGSWAEAYPEIVTCANMFWWPAGSSPALAAEPGTGQLNPSIPKTYEVVKNVIQGTIA
MFPDSLFHGGADEINSDCWNTDLSVQKFVASNGTLSQLLEKFINNTLPEILSLNRTVVYWED
VILSGNVKVNPSLLPPQNVIMQTWNNGPNNTKQLVTSGYRVIVSSADYYYLDCGHGSFVGN
DSRYDQPPGTDQGNGGSWCGPFKTWETIYNYDITYGLTDEEAPLVIGGEVALWSEQADSTV
MDSRIWPRASAMAEALWSGNRDETGMKRYAEATDRLNEWRYRMVSRGIGAESIQPLWCLK
NPGMCNTVHSFTS

SEQ ID NO: 4 Complete cDNA sequence of *Capsicum annuum* β-D-*N*-acetylhexosaminidase1 (1725 nts)

ATGAGAGGAGACACAACATTCCCATTCATTCTTTCACTATTCGTTATCTTCATTACTCAAA
CAATAGCTACAAATTACCCAATTAATGTCTGGCCCAAGCCCACAACATTCAATTGGCCCA
ACCCAAAAATCCATCTTCCTCTCCCCAACTTCACCATCTCCCACCCGACCCACCGTTACCT
AACTCCCACCGTTTACCGGTACCGCCGTCTCATCCTCTCCGAGCACTACCGCCACATCAT
CACTCCGTCGATCAACCTGACGTCATCAACTCCGTTACAACACCTCATCATCTCCGTCTCC
GACGTCACTTCACCGTTATCTCACGGCGTCAACGAATCCTACTCTCTCTCCACGCCTAAC
GGTTCTTCCGCCGCCTACATAACTGCCGGTACCGTATGGGGAGCCATGAGAGGACTCGA
GACATTCTCACAGCTCGTGTACGGAAACCCTACTCGAGTCGCCGCAGGCGTGTACATATC
CGATCTGCCGATTTTCACTCACCGTGGTGTGATGTTGGACACTTCGAGAAACTTCTACGG
AGTAGATGATTTGTTGAGGCTTATCAAAGCTATGAGTATGAACAAGCTGAATGTTTTTCA
CTGGCACATAACTGATTCACATTCGTTTCCGCTTGTGGTTCCATCGGAGCCGGAGCTTGC
CGGAAAAGGAGCATACGGCAATGAGATGATGTACTCGCCGGCGGATGTGGAGAAGATTG
TGGAATTTGGAATGGAACATGGAGTTAGGGTTTTACCTGAGATTGATATGCCCGCACATA
CGGGATCATGGGCTGAAGCTTACCCTGAGATTATCACTTGTGCAAATATGTTCTGGTGGC
CTGCTGGAAATAGTCCAGCTCTTGCAGCTGAACCAGGCACTGGTCAACTGAACCCTTTGA
TTCCCAAAACCTATGAAGTAGTCAAGAATGTCATCCACGATACCATCGCCATGTTTCCAG
ATTCCCTCTTTCACGGGGGAGCAGATGAGATCAATTCAGCATGTTGGAATACTGATCCAT
CAATCCAAACGTTTGTTGCTAGCAATGGAACTCAGAGTCAGCTACTCGAAATGTTTATCA
ATAATACCTTACCTGAAATCCTCTCACTCAACCGTACTGTGGTCTACTGGGAGGATGTTA
TATTGAGTGCTAATGTGAAGGTGGATCCATCTCTGCTTTCGCCACAACATGTTATCATGC
AAACTTGGAATAATGGACCAAGCAATACAAAGCAGCTCGTCACTTCTGGGTACCGTGTC
ATTGTGTCATCTGCAGATTACTATTACTTGGATTGTGGCCATGGGAGTTTCGTTGGAAAT
GACAGCCGCTATGACCAGCCCCCAGGTACTGACCAAGGCAATGGTGGATCCTGGTGTGG
ACCTTTCAAGACCTGGGAAACCATTTACAACTATGATATAACCTATGGTCTGACTGATAA
GGAGGCTCAATTGGTAATCGGAGGGGAAGTAGCATTATGGTCCGAACAAGCTGATTCAA
CTGTTATGGACTCACGAATTTGGCCTAGAGCATCAGCAATGGCAGAGACATTGTGGTCAG
GGAATTGCGATGAAACAGGAATGAAGAGATATGCAGAGGCTACTGATCGACTGACTGAA
TGGAGGTACAGAATGGTTGCTAGGGGAATAGGTGCTGAACCTATTCAACCACTTTGGTGT
GTCAAAAACTCAGGAATGTGTAACACAGTTCATTCATTTACTAGCTGA

SEQ ID NO: 5 Amino acids sequence of *Capsicum annuum* β-D-*N*-acetylhexosaminidase1 (574 aa)

MRGDTTFPFILSLFVIFITQTIATNYPINVWPKPTTFNWPNPKIHLP
LPNFTISHPTHRYLTPTVYRYRRLILSEHYRHIITPSINLTSSTPLQH
LIISVSDVTSPLSHGVNESYSLSTPNGSSAAYITAGTVWGAMRGLE
TFSQLVYGNPTRVAAGVYISDLPIFTHRGVMLDTSRNFYGVDDLL
RLIKAMSMNKLNVFHWHITDSHSFPLVVPSEPELAGKGAYGNEM
MYSPADVEKIVEFGMEHGVRVLPEIDMPAHTGSWAEAYPEIITCA
NMFWWPAGNSPALAAEPGTGQLNPLIPKTYEVVKNVIHDTIAMFP
DSLFHGGADEINSACWNTDPSIQTFVASNGTQSQLLEMFINNTLPE
ILSLNRTVVYWEDVILSANVKVDPSLLSPQHVIMQTWNNGPSNTK
QLVTSGYRVIVSSADYYYLDCGHGSFVGNDSRYDQPPGTDQGNG
GSWCGPFKTWETIYNYDITYGLTDKEAQLVIGGEVALWSEQADST
VMDSRIWPRASAMAETLWSGNCDETGMKRYAEATDRLTEWRYR
MVARGIGAEPIQPLWCVKNSGMCNTVHSFTS

What is Claimed:

1. An isolated polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.
2. The polynucleotide as claimed in claim 1 encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.
3. The polynucleotide as claimed in claim 1, wherein the nucleotide sequence of the polynucleotide is as set forth in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 4.
4. A polypeptide having amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5.
5. A DNA construct comprising the polynucleotide as claimed in claims 1 to 3, wherein the polynucleotide is operably linked to a promoter sequence.
6. The DNA construct as claimed in claim 5, wherein the polynucleotide sequence is in sense orientation.
7. The DNA construct as claimed in claim 5, wherein the polynucleotide sequence is in anti-sense orientation.
8. An RNAi construct for suppressing expression of hexosaminidase in a transgenic plant, said construct comprising
   - a sense polynucleotide strand comprising at least 20 contiguous nucleotides from the sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, and
   - an antisense polynucleotide strand that hybridizes to said sense polynucleotide strand, wherein the antisense polynucleotide strand and the sense polynucleotide strand form a duplex
9. The RNAi construct as claimed in claim 8, wherein the RNAi construct is a hairpin nucleic acid.

10. The RNAi construct as claimed in claim 8, wherein said sense strand comprises 100 to 600 nucleotides.

11. A recombinant vector comprising the DNA construct as claimed in claim 5 to 9.

12. A recombinant host cell comprising the recombinant vector as claimed in claim 11.

13. The recombinant host cell as claimed in claim 12 is selected from the group consisting of *Agrobacterium*, *E. coil* and yeast.

14. A process for delaying fruit softening in plant, said process comprising transforming a plant cell, tissue or any part thereof with the recombinant vector comprising the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5, wherein said polynucleotide is in anti-sense orientation.

15. The process as claimed in claim 14, wherein the nucleotide sequence of the polynucleotide is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

16. A process for delaying fruit softening in plant, said process comprising decreasing the level of hexosaminidase in transgenic plant compared to its level in non-transgenic plant by expression of an RNA interference (RNAi) construct comprising at least a fragment of at least 20 contiguous nucleotides of the polynucleotide encoding a polypeptide having hexosaminidase activity, wherein the nucleotide sequence of said polynucleotide encodes a polypeptide having at least about 85% identity with an amino acid sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 5; and screening the resulting plants for reduced level of hexosaminidase relative to said non-transgenic plant.

17. The process as claimed in claim 16, wherein the nucleotide sequence of the polynucleotide is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

18. A process for delaying fruit softening in plant, said process comprising decreasing the level of hexosaminidase in transgenic plant compared to its level in non-transgenic plant by expression of an RNA interference (RNAi) construct comprising a sense polynucleotide strand comprising at least 20 contiguous nucleotides from the sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, and an antisense polynucleotide strand that hybridizes to said sense polynucleotide strand, wherein the antisense polynucleotide strand and the sense polynucleotide strand form a duplex; and screening the resulting plants for reduced level of hexosaminidase relative to said non-transgenic plant.

19. The process as claimed in claim 18, wherein the RNAi construct is a hairpin nucleic acid.

20. A transgenic plant produced by the process as claimed in claims 14 to 19, wherein expression of the hexosaminidase in said plant is controlled to delay fruit softening.

21. The transgenic plant as claimed in claim 20 is selected from a group consisting of tomato, capsicum, papaya, mango, banana, peach, pear, citrus, pineapple, guava, avocado, strawberry, apple and pomegranate.

22. The transgenic plant as claimed in claim 20 is tomato or capsicum.

23. A transgenic seed or progeny of the transgenic plant as claimed in claims 18 to 22.