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(54) Title: METHOD FOR MODIFYING LIGNIN BIOSYNTHESIS IN PLANTS

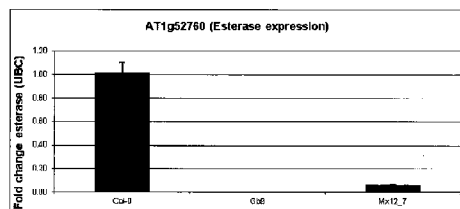


Figure 1A

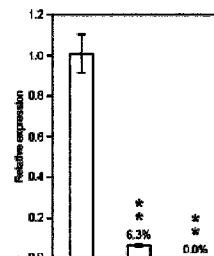


Figure 1B

(57) Abstract: The invention is based on the discovery of genes which influence lignin biosynthesis. In particular, the inventors have observed that if the expression, function and/or activity of these gene(s) (or any protein products thereof) is/are modulated, the lignin content of plants can be altered. As such, this invention provides plants which exhibit modulated expression of one or more lipase/esterase/thioesterase family gene(s) and which may find application in methods for producing biofuels.

METHOD FOR MODIFYING LIGNIN BIOSYNTHESIS IN PLANTS

FIELD OF THE INVENTION

The present invention provides modified plants having altered lignin and the use of such plants in processes which require carbohydrate extraction from plants, including, for example, methods for the production of biofuels.

BACKGROUND OF THE INVENTION

Lignin is a phenolic polymer made from monolignol units that strengthens and waterproofs plant cell walls and influences the downstream processing of plant biomass for agricultural and industrial processes. For example, the presence of lignin in biomass makes it harder for enzymes to gain access to cell wall polysaccharides (cellulose and hemicellulose) in order to release the component sugars for useful purposes such as biofuel, bioplastic or chemical production. Much research effort has focused on manipulating the lignin pathway to make it easier to process biomass for these kinds of applications [1]. Although the monolignol biosynthesis pathway is well-characterized, there are still novel genes involved in lignification that remain to be discovered. For example, two laccases have recently been identified as being involved in lignin synthesis [2]. Other genes that are directly or indirectly involved in lignification that could be targets for useful manipulation, remain to be identified.

SUMMARY OF THE INVENTION

The present invention is based on the discovery of genes which influence lignin biosynthesis. In particular, the inventors have observed that if the expression, function and/or activity of these gene(s) (or any protein products thereof) is/are modulated, the lignin content of plants can be altered.

As such, a first aspect of this invention provides a plant exhibiting modulated expression of one or more lipase/esterase/thioesterase family gene(s), wherein said plant comprises a modified lignin.

For convenience, the plants provided by this invention shall be referred to hereinafter as “modified” plants.

The phrase “modulated expression of a lipase/esterase/thioesterase family gene(s)” should be understood as encompassing any increase or decrease in the expression of one or more gene(s) belonging to the lipase/esterase/thioesterase family. One of skill will appreciate that levels of gene expression in modified plants of this invention may be assessed relative to the expression of a corresponding gene in a control plant of the same species. A control plant may, for example, be a wild type plant

exhibiting a wild-type level of expression of a/the corresponding lipase/esterase/thioesterase family gene(s). It should be understood that modulated gene expression may be detected by quantitative and/or qualitative comparison of gene expression levels between modified plants of this invention and control plants.

5 The methods by which levels of gene expression can be assessed are well known to one of skill and include, for example, PCR based techniques including real-time PCR and the like. Northern Blotting techniques may also be used. Further information on such techniques may be found in *Molecular Cloning: A Laboratory Manual (Third Edition)* By Sambrook, MacCallum & Russell, Pub. CSHL; ISBN 978-087969577-4 – incorporated
10 herein by reference.

 As stated, the modified plants provided by this invention comprise an altered or modified lignin - that is to say, when compared to a control plant (or population of control plants) having a known, standard or wild-type lignin, the modified plants of this invention comprise either more or less lignin and/or lignin having an altered or variant
15 structure/composition. As such, references to “modified plants” or “modified lignin” or “altered lignin” should be taken to encompass plants which, when compared to un-modified plants of the same type (i.e. plants which exhibit wild-type or normal levels of lipase/esterase/thioesterase family gene expression), comprise more or less lignin (i.e. plants which exhibit a modified or altered lignin content) or lignin having an altered or
20 modified composition/structure.

 Regarding lignin structure and/or composition, it should be understood that lignin is largely comprised of hydroxycinnamyl alcohols – more commonly referred to as the monolignols coniferyl alcohol (the G-lignin unit), sinapyl alcohol (the S-lignin unit) and *p*-coumaryl alcohol (the H-lignin unit). The precise lignin structure/composition varies
25 from plant to plant; for example grasses may comprise lignin which comprises an elevated amount of H-unit lignin whereas lignins from gymnosperms may be composed of G-units only. As such, references to altered or modified lignin composition and/or structure may encompass lignin which in comparison to the lignin of an un-modified form of a particular plant, exhibits an altered H, G and/or S unit composition. By way of
30 example, lignins from the modified plants described herein may comprise different proportions of H, G and S-units as compared to the lignins of un-modified forms of the same plants. A modified plant of this invention may comprise an altered S-unit composition. A modified plant of this invention may comprise an altered H, G and/or S unit composition and the amount or proportion of H, G and/or S units might increase or

decrease. For example the amount or proportion of H units might increase and the amount and/or proportion of S and G units might decrease.

In addition to the various structural and compositional modifications described above, it should be understood that the term “modified lignin” may further include
5 modified lignin which, relative to a comparable wild type plant, comprises one or more unusual monomers and/or increased amounts thereof [3-5].

This invention provides plants exhibiting reduced expression of one or more lipase/esterase/thioesterase family gene(s), wherein said plants comprise a reduced or modified lignin; the reduced expression of the lipase/esterase/thioesterase family gene(s)
10 and associated modified lignin, being assessed relative to the lipase/esterase/thioesterase family gene expression and lignin of a control plant having a known or quantified level of lipase/esterase/thioesterase family gene expression and/or lignin.

Lignin is predominantly deposited in the cell wall making them rigid and impermeable and protecting the cell wall polysaccharides from microbial degradation.

15 In wild type plants, the presence of lignin in plant cell walls and other structures protects plant carbohydrates rendering them inaccessible to hydrolysing enzymes etc., this makes methods which require the release of sugars from lignin-containing plant matter (for example methods of biofuel production), inefficient and costly.

The cell walls and vascular structures of the modified plants described herein,
20 may comprise less lignin and/or a modified lignin and one advantage associated with such plants is that material or biomass derived therefrom may be more easily deconstructed to access carbohydrate polymers and enable the release of sugars.

As such, modified plants according to the first aspect of this invention may find application in methods for accessing and/or processing carbohydrate polymers from plant
25 matter and, for example, biofuel production.

In view of the above, biomass derived from plants modified in accordance with this invention may be used as feedstock for processes which require or exploit plant cell wall carbohydrates. By way of example, biomass derived from the modified plants of this invention may be used in biofuel production methods.

30 In one embodiment, and through the teachings of this invention, biomass for use in methods involving plant carbohydrate deconstruction (for example biofuel production) may comprise, for example parts of crops, waste crop material and trees - all of which may be regarded as typically high in lignin.

The term “plant” as used herein may comprise a crop or grass species, hybrids and varieties including, for example, those belonging to the *Saccharum*, *Zea*, *Triticum*, *Secale*, *Hordeum*, *Glycine*, *Oryza*, *Sorghum*, *Lolium*, *Vitis* and *Medicago* genera. In addition, the term “plant” may encompass species, hybrids and varieties of the
5 *Miscanthus*, *Panicum* (switchgrass), *Phalaris* (reed canary grass), *Cannabis* (hemp) genera – plants of this type may be grown as crops for use in bioenergy production (i.e. as dedicated bioenergy crops). In other embodiments, the term “plant” encompass species, hybrids and varieties of trees such as *Salix*, *Populus*, and *Eucalyptus* genera.

In view of the above, it should be understood that the “plant biomass” for use in
10 methods requiring or exploiting plant cell wall carbohydrates, for example biofuel production, may comprise material or matter derived from modified forms (i.e. forms exhibiting modulated expression of one or more lipase/esterase/thioesterase family gene(s)) of any of the plants described herein.

One of skill will appreciate that the term “biomass” may comprise any part of a
15 plant, including for example, the stem, flower (including seed heads etc), root and leaves. Where a modified plant provided by this invention exhibits modified lignin content throughout its cells and tissues, any part of that plant may yield biomass which is useful as feedstock for methods requiring plant carbohydrate extraction or methods of producing biofuel – of particular use are the stems, leaves and roots.

In other embodiments, the modulated expression of one or more
20 lipase/esterase/thioesterase family gene(s) and/or the modified lignin may be confined to one or more specific parts or tissues of a plant. For example, the modulated expression of a lipase/esterase/thioesterase family gene and/or modified lignin content may be apparent in one or more of the cells or tissues – including, for example the meristem, stem, root,
25 pistil, anther, flower, leaf, seed, embryo, stalk and/or petiole. In such cases, the parts comprising modified lignin will be most useful as feedstock for methods requiring or exploiting plant carbohydrates or, for example, biofuel production processes.

In one embodiment, a lipase/esterase/thioesterase family gene may encode a lysophospholipase and/or esterase enzyme. Thus, this invention may provide a plant
30 exhibiting modulated expression of one or more lysophospholipase and/or esterase gene(s) and/or one or more lysophospholipase and/or esterase enzymes, wherein said plant comprises a modified or altered lignin.

One of skill will appreciate that modulation of lysophospholipase and/or esterase gene expression may result in a corresponding increase or decrease in lysophospholipase

and/or esterase enzyme expression. A modulated level of lysophospholipase and/or esterase enzyme expression may be determined relative to a level of lysophospholipase and/or esterase enzyme expression in a control plant having a known or quantified level of lysophospholipase and/or esterase enzyme expression. As stated above, a control plant may take the form of a wild-type plant of the same species, the wild type plant exhibiting a wild-type level of lipase/esterase gene expression.

In some embodiments, the invention provides a plant exhibiting reduced expression of one or more lysophospholipase and/or esterase gene(s), wherein said plant comprises a modified lignin.

In a further embodiment, the invention provides a plant exhibiting reduced expression of one or more lysophospholipase and/or esterase protein/enzymes, wherein said plant comprises a modified lignin. One of skill will appreciate that any reduction in the function, activity and/or expression of one or more lipase/esterase/thioesterase family gene(s), may result in an associated (or corresponding) reduction in the function, activity and/or expression of the encoded lysophospholipase and/or esterase. This in turn may result in a plant comprising a modified or altered lignin.

The plant may be *Arabidopsis thaliana* and the lysophospholipase and/or esterase gene is the lysophospholipase 2 gene designated *LysoPL2* and encoding lysophospholipase 2. An exemplary *A. thaliana LysoPL2* gene has been deposited under the accession number AT1G52760 and has the sequence designated SEQ ID NO: 1 below:

SEQ ID NO: 1

```

1  CTTTATCACC ACCAAAAACC AAAATTCAC T GCCAAAAAAA ACACATCAAA
51  ACGATGCCGT CGGAAGCGGA GAGCTCAGCG AATTCAGCTC CGGCAACTCC
25 101 GCCACCACCA CCGAATTTCT GGGGAACCAT GCCGGAGGAA GAGTACTACA
151 CTTCACAAGG AGTACGTAAC AGCAAATCAT ACTTCGAAAC ACCAAACGGC
201 AAGCTCTTCA CTCAGAGCTT CTTACCATTA GATGGTGAAA TCAAAGGCAC
251 TGTGTACATG TCTCATGGAT ACGGATCCGA TACAAGCTGG ATGTTTCAGA
301 AGATCTGTAT GAGTTTCTCT AGTTGGGGTT ACGCTGTTTT CGCCGCCGAT
30 351 CTTCTCGGTC ACGGCCGTTT CGATGGTATC CGCTGCTACA TGGGTTTCGT
401 TACTTCGTTT CTCTGTTTTG ATAAGATAAA TTTTCCATCT TTGTGTAATT
451 GATAAGATAA TTTACGATCT TTAGGTGATT AAAGATTGGA TTTTATGGT
501 TATTAGGTGA TATGGAGAAA GTTGACAGCA CATCATTTGG TTTCTTCAAG
551 CATGTTCGTT GTAGTGATCC ATATAAGGAT CTTCCGGCTT TTCTGTTTGG
35 601 TGAATCGATG GGAGGTCTTG TGACGCTTTT GATGTATTTT CAATCGGAAC
651 CTGAGACTTG GACCGGTTTG ATGTTTTTCG CTCTCTCTT TGTATCCCT

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701 GAGGATATGA AACCAAGCAA GGCTCATCTT TTTGCTTATG GTCTCCTCTT
 751 TGGTTTGGCT GATACGTGGG CTGCAATGCC GGATAATAAG ATGGTTGGGA
 801 AGGCTATCAA GGACCCTGAA AAGCTTAAGA TCATCGCTTC TAACCCGCAA
 851 AGGTACTATT AAACCTTCTTG GAAGCAAACA TAGTATAAAG CTTGAGACTT
 5 901 TACTTTGGAA GCTATAAAAAG TTTGGATTTT GCATTGTAGA TATACAGGGA
 951 AGCCTAGAGT GGAACAATG AGAGAGTTAC TGAGGAAGAC TCAATACGTT
 1001 CAGGAGAATT TCGGGAAAGT TACTATTCCG GTGTTTACGG CGCACGGGAC
 1051 AGCGGATGGA GTAACATGTC CTACATCTTC GAAGCTACTA TACGAAAAAG
 1101 CGTCAAGCGC TGATAAAACG TTGAAGATCT ATGAAGGGAT GTATCACTCG
 10 1151 CTGATTCAAG GAGAGCCTGA CGAGAACGCT GAGATAGTCT TGAAGGATAT
 1201 GAGAGAGTGG ATCGATGAGA AGGTTAAGAA GTATGGATCT AAAACCGCTT
 1251 GAACAAAGCT ACATTTGTGT TACAAGAACT TGAAGAGAAA TGTATATTGA
 1301 TGTATGATC CGTATCGTCG ATTTGACTTG TTTTGTGTC TGTGTAAATC
 1351 CAAGAACATG AATTTTCTGA TGTAAGAACT TATAATATCA TGGATTACAG
 15 1401 AAATCCTTTT ATCATTCT

The protein encoded by this sequence is provided below as SEQ ID NO: 2.

SEQ ID NO: 2

20 1 MPSEAESSAN SAPATPPPPP NFWGTMPEEE YYTSQGVNRNS KSYFETPNGK
 51 LFTQSFLPLD GEIKGTVMYS HGYGSDTSWM FQKICMSFSS WGYAVFAADL
 101 LGHGRSDGIR CYMGDMKVA ATSLAFFKHV RCSDPYKDLP AFLFGESMGG
 151 LVTLLMYFQS EPETWTGLMF SAPLFVIPED MKPSKAHLFA YGLLFGLADT
 201 WAAMPDNKMV GKAIKDPEKL KIIASNPQRY TKGPRVGTMR ELLRKTQYVQ
 25 251 ENFGKVTIPV FTAHGTADGV TCPTSSKLLY EKASSADKTL KIYEGMYHSL
 301 IQGEPDENAE IVLKDMREWI DEKVKKYGSK TA

One of skill will appreciate that functionally equivalent sequences and/or
 sequences identical or similar to, or homologous or orthologous with, the
 30 lysophospholipase and/or esterase sequences described herein, in particular the sequences
 given as SEQ ID NO: 1 or 2 above (or a fragment thereof), may be present in other plant
 species. Examples of such sequences are given below for *Populus trichocarpa*, *Vitis*
vinifera, *Glycine max*, *Medicago truncatula*, *Oryza sativa*, *grandis* and *Panicum*
virgatum. These exemplary protein sequences have been deposited under the accession
 35 numbers XP_002303266.1, CAN62561.1, XP_002298118.1, XP_003542674.1,
 XP_003610038.1, EAY84954.1, Eucgr.F02557, Pavirv0007801m.1 and have the
 sequence designated SEQ ID NO: 3-18 below:

SEQ ID NO: 3

Populus trichocarpa predicted protein, mRNA. ACCESSION XM_002303230

1 t c t c t c c c t c c c g c a a c c a g t t t t a a a a a a a g t t g a a a c a c c a t t a t c c a a c t c c g a a a c g
 61 c c a c c c a c c t a c t c c c t g t a a a a a c c c c t a c c g t t t t c t c t g t t t a a a a g t c a a c c a t c
 5 121 c a a g c c t t a c g a t a a c c g t a a c g a g a c g t g a c c a t g c c a t c c g a a g c g c a g c a g c c c g a a
 181 g c g c c a c c c a a c t t c t g g g g c g a c a t g c c g g a g g a g a g t a c t a t g c a t c g c a a g g a g t g
 241 a c c a a t a c c c a g t c a c a c t t t g a a a c g c c g a a t g g g a a g g t c t t c a c g c a g g g t t t t c t c
 301 c g t t t g g a t a a a a g g t c a a a g c c a c g g t g t a t a t g a c c c a c g g c t a c g g a t c t g a t a c t
 361 g g c t g g c t g t t t c a g a a g a t t t g c a t c a a c t t t g c t a c c t g g g g t t a t g c t g t t t t t g c t
 10 421 g c t g a t c t t c t t g g g c a t g g c a g a t c a g a c g g t t t a c g c t g c t a c a t g g g c g a c a t g g a g
 481 a a a a t t g c t g c a g c g t c c g t a t c g t t c t t c a a g c a t g t g c g c t a c a g c g a g c c a t a c a a g
 541 a a c t t g c c c g c c t t c t t a t t t g g c g a g t c a a t g g g c g g a c t a g c a a c g a t g c t g a t g t a t
 601 t t c c a a t c a g a a c c t g a c a c g t g g a c g g g c g t g a t t t t c t c g g c c c a c t t t t c g t c a t a
 661 c c g g a a c c a a t g a a a c c t a g t a a g g c a c a c c t a t t c a t g t a t g g c c t g c t c t t t g g a t t t
 15 721 g c t g a c a c g t g g g c g g c c a t g c c a g a c a a c a a a a t g g t a g g t a a a g c g a t a a a g g a c c c a
 781 g a g a a a c t c a a g a t c a t a g c a t c c a a c c c a g a a g a t a c a c a g g c a a g c t a g g g t g g g t
 841 a c c a t g a g a g a a a t t g c c a g a g t c t g c c a a t a c a t a c a g g a c a a t t t t c t c a a g g t t a c g
 901 g t g c c g t t t t t g a c t g t c c a c g g g a c c g c c g a t g g g g t g a c a t g c c c a a c a t c a t c a c a g
 961 t t g t t g t a t g a g a a g c c t c g a g t g a g g a t a a g a g c t t g a a g a t g a c g a g g c a t g t a c
 20 1021 c a t t c t t t g a t a c a a g g c g a g c c t g a c g a a a a t g c a a g t c t t g t c t t g a a g g a t a t g a g a
 1081 g a g t g g a t c g a t g a g a g g t t g a g a g g t a t g g g t c t a c a a a g a g t g a t g a t t g a a a t c a t
 1141 a t a t g a a g a a a a a t g g t g g t t t t t t t t c t g g a a a g t g a a g c t t g g t c c a t a g t c t c t t
 1201 g a t g g g a t t a g g g c a a a a c g a a t g c c a a t g t a a t t g a a t a t t t t g a a c t a a c g a a g t c a
 1261 g c t a t t g c t t c t c t g a t t t a a t t t a t a a a a a a t g t t t g a a a c t t t t a a t t t t c

25 The protein encoded by this sequence is provided below as SEQ ID NO: 4.

SEQ ID NO: 4

Predicted protein [Populus trichocarpa] ACCESSION XP_002303266

1 m p s e a q q p e a p p n f w g d m p e e e y y a s q g v t n t q s h f e t p n g k v f t q g f l p l d k k v k a t v y
 30 61 m t h g y g s d t g w l f q k i c i n f a t w g y a v f a a d l l g h g r s d g l r c y m g d m e k i a a a s v s f f k
 121 h v r y s e p y k n l p a f l f g e s m g g l a t m l m y f q s e p d t w t g v i f s a p l f v i p e p m k p s k a h l
 181 f m y g l l f g f a d t w a a m p d n k m v g k a i k d p e k l k i i a s n p r r y t g k p r v g t m r e i a r v c q y
 241 i q d n f s k v t v p f l t v h g t a d g v t c p t s s q l l y e k a s s e d k s l k m y e g m y h s l i q g e p d e n
 301 a s l v l k d m r e w i d e r v e r y g s t k s d d

35 SEQ ID NO: 5

Populus trichocarpa predicted protein, mRNA. ACCESSION XM_002298082

1 a t g t c a t c c g a a a c g c a c c c g a a a c g c c t c c c a a c t t c t g g g g c g a c a t g c c g g a g
 61 g a g g a g t a c t a t g c g t c a c a a g g a g t g a c c a c t a c c c a a t c a t a c t t c g a g a c g c c a a a t
 121 g g g a a g c t c t t c a c g c a a g g t t t t c t c c c g t t g g a t a a a a a g t c a a a g c c a c g g t a t a t
 40 181 a t g a c c c a c g g c t a t g g a t c t g a t a c t g g c t g g t t g t t c c a g a a g a t t t g c a t c a g c t t t
 241 g c t a a c t g g g g t t a t g c t g t t t t t g c c g c t g a t c t t c t t g g a c a t g g c a g a t c a g a c g g t
 301 a t a c g t t g c t a c a t g g g t g a c a t g g a c a a g a t t g c t g c c a c t t c c c t g t c a t t c t t c a a g
 361 c a c g a g c g c t t c a g c g a a c c a t a c a a g g g c t t a c c a g c c t t c t t a t t t g g t g a a t c a a t g

421 ggtggactca caacaatgct aatgtacttc caatcagaac ctaacatgtg gacgggcttg
 481 attttctcgg cgccactttt tgtcatacca gaagcgatga aaccaagcaa ggtacaccta
 541 ttcattgtatg gcctgctctt tggattggct gatacgtggg cagccatgcc agacaacaaa
 601 atggtaggca aagcgatcaa ggacccagag aagctcaaga tcatagcatc caaccctagg
 5 661 agatacacag gcaagcctag ggtgggaacc atgagggaaa ttgctaggat gtgccaatac
 721 atacaggaca atttctccaa ggttacagcg ccgttcttga cagtccacgg cacggctgat
 781 ggggtgacat gccctacatc atcacagttg ttgtttgaga aagcctctag tgaggacaag
 841 agcttgaaga tgtacgaggg catgtaccat tctttgatac aaggtgagcc cgatgagaat
 901 gctaattctt ttttgaagga tatgagaggg tggattgacg agagggttga gaggtatggg
 10 961 tccaaaaaaa gcgatgactg a

The protein encoded by this sequence is provided below as SEQ ID NO: 6.

SEQ ID NO: 6

Predicted protein [*Populus trichocarpa*] **ACCESSION** XP_002298118

15 1 mssetqqpet ppnfwgdmpe eeyyasqgvt ttqsyfetpn gklftqgflp ldkkvkatvy
 61 mthgygsdtg wlfqkicisf anwgyavfaa dllghgrsdg ircymgdmk iaatslsffk
 121 herfsepykg lpaflfgesm gglttmlmyf qsepnmwtgl ifsaplflvip eamkpskvhl
 181 fmygllf gla dtwaampdnk mvgkaikdpe klkiasnpr rytgkprvgt mreiarncqy
 241 iqdnfsvta pfltvhgtad gvtcptssql lfekassedk slkmyegmyh sliqgepden
 20 301 anlvlkdmrg widerveryg skksdd

SEQ ID NO: 7

EMBL-CDS: CAN62561.1 : *Vitis vinifera* hypothetical protein

1 atgtcgtcgg aatccgaaat ttcggccaac ttctggggcg atatgccgga ggaggagtac
 25 61 tatgcctccc aaggggtgcg caacacccaaa tcatayttcg acacccccaa cggcaagctc
 121 ttcaaccaga gtttctacc cttggatctc cctgtcaagg cttccgtcta catgaccac
 181 ggctacggct ccgacaccgg ctggctcttc cagaagattt gcattaacta cgccacctgg
 241 ggctacgcag tcttcgccgc cgacatcctc ggccacggcc gctccgacgg yatccgctgc
 301 tacctcggcg acatggagaa ggtcgccgcc acctcccttt cyttcttcaa gacgtycgc
 30 361 accagcgaat cctaccgyga cctccctgct ttctctctcg gcgagtccat ggggtggggct
 421 accaccatgc tcgtgtactt ccaatcggag ccggagctgt ggacaggcct gatcttctca
 481 gcccacttt tcgtgatgcc ggagaacatg aagccgtcga aggtgaggct attcctgtac
 541 ggacttctgt ttgggatggc tgacacgtgg gcgacgatgc cggacaacaa gatggtgggg
 601 aaggcgatca aggatccgga gaagctgaag gtcatagcgt cgaatccacg gcggtacacg
 35 661 ggtccgccga ggggtggggac gatgaggggag ctggctaggg tgtgccagta catacaggat
 721 aatttctcga argtgackgc gccgttcttg acgggtgcacg ggacggcrga tggggtgacg
 781 tgtccgacgt cgtcgaagct gctgtacgag aaggtctcga gtgaggacaa agcattgaag
 841 ttgtatgagg ggatgtacca ttctttgata caggagagac ctgatgagaa tkccaatctg
 901 gtgttgaagg atatgaggga atggattgat gagaggggtt agagatacgg accctccaaa
 40 961 tcctag

The protein encoded by this sequence is provided below as SEQ ID NO: 8.

SEQ ID NO: 8

Hypothetical protein VITISV_001366 [*Vitis vinifera*]. **ACCESSION** CAN62561

1 msseseisan fwgdmpeeey yasqgvnrnk syfdtpngkl ftqsflpldl pvkasvymth
 61 gygsdtgwlf qkicinyatw gyavfaadil ghgrsdgirc ylgdmekvaa tslsffksvr
 121 tsesyrdlpa flfgesmgga ttmlvyfqse pelwtglifs aplfvmpenm kpskvrflfly
 5 181 gllfgmadtw atmpdnkmvg kaikdpekkl viasnprryt gprrvgtmre larvcqyiqd
 241 nfskvtapfl tvhgtadgvt cptsskllie kassedkalk lyegmyhsli qgependxn1
 301 vlkdmrewid erverygpsk s

SEQ ID NO: 9

10 Monoglyceride lipase [*Medicago truncatula*] (MTR_4g127220) mRNA. ACCESSION
 XM_003609990

1 aatctctaata tatccatcct caccggtttc catcgctgaa acaacaacgc caatggcaac
 61 gcagcaggaa tcagagattc ccccaaattt ctgggggtcac acccccgaag aagaatacta
 15 121 cacctcccaa ggagttcgca ataccaaatc acacttcgaa acaccaacgc gcaaaatcctt
 181 cacacagtcc tttctccac tcaacgctga aatcaaagct accgtttaca tgactcacgg
 241 ttacggctcc gacaccggct ggctcttcca aaaaatctgc atcacctacg ccacctgggg
 301 ttacgccgtc ttcaccgctg atctcttagg tcacggccgt tccgatggcc tccgttgcta
 361 cctcggggac atggacaaaa tcgccgccac ctcactttca ttttctctcc acgtccgccg
 20 421 ttctctctccc tacaaccacc tcccagcgtt tctcttcggt gagtcaatgg gtgggttagc
 481 tacattgctg atgtatttcc aatcagaacc cgacacgtgg acgggtttta tattctcagc
 541 gccgcttttc gtaatccccg aggatatgaa accgagtaag attcatttgt ttgtgtacgg
 601 tcttttggtt ggtttggctg acacgtgggc agcgatgcct gataacaaaa tggtcggaaa
 661 agcaattagg gatccaaata agttgaagat tattgcttct aatccaagga ggtatacggg
 25 721 cccacctaga gtagggacca tgagggaact tcttagagtc actcaatatg tgcaagataa
 781 tttctgcaat gtaacgggtc cgtttcttac ggcacatggt actgctgatg gtgtcacgtg
 841 cccttcttct tctaagctgt tgtatgagaa agctgaatct aaggataaga ctttgaagct
 901 ttatgagggg atgtatcatt ctttgattca aggggagcct gatgagtctg ctaatcttgt
 961 gttaagggat atgaggaggt ggattgatga gaggggtcgt aggtatggac ctaataatga
 30 1021 taattctcaa tgaaaaacaa ggggtggtgt tgtgtttttt tttcatacaa ttttagttt
 1081 ggaattacct ggtctcgata atcaagattt gattgaggac tattgttatg actatattga
 1141 aatttttatg actatatgaa cgaactgtga tgttggtata tgggtgtgctt cgttttagatc
 1201 cttctataca taacaatatg atcttacggt tc

35 The protein encoded by this sequence is provided below as SEQ ID NO: 10.

SEQ ID NO:10

ACCESSION XP_003610038.1

1 matqqeseip pnfwghtpee eyytsqgvnr tkshfetpng kiftqsflpl naeikatvym
 61 thgygsdtgw lfqkicitya twgyavftad llghgrsdgl rcylgdmcki aatslsfflh
 40 121 vrrsppynhl pafllgesmg glatllmyfq sepdtwtgli fsaplfvipe dmkpskihl
 181 vyglflglad twaampdnkm vgkairdpnk lkiiasnprr ytgpprvgtm rellrvtqyv
 241 qdnfcnvtp fltahgtadg vtcpssskll yekaesdkdt lklyegmyhs liqgepedesa
 301 nlvlrdmrew idervrrygp nndnsq

SEQ ID NO: 11

PREDICTED: Glycine max monoglyceride lipase-like (LOC100785661), mRNA.

ACCESSION XM_003542626

```

5      1  acccaatcgc aatggcaccg gaatcagagg ctccccctaa cttctggggc cacaccccg
      61  aagaagaata ctacacctcc caaggcggtc gcaacaccaa gtcccacttc gaaaccccca
     121  acggcaaaat cttcaccagc tccttcctcc ctctcaacct ccaacccac caagtcaaag
     181  ccaccgtctt tatgaccac ggctacggct cgcacaccgg ctggctcttc cagaaaatct
     241  gcatcaactt cgccacctgg ggctacggcg tcttcgcccgc cgacctctc ggccacggcc
10    301  gctccgacgg tctccagtgc tacctcggcg acatggacaa aatcgccgcc acctccctct
     361  ccttcttctt ccacgtccgc aatagccacc cctacaaaaa cctcccggca ttctcttctg
     421  gcgagtccat gggaggactc gccacgctcc tcatgtactt caaatcgga cgggacacgt
     481  ggacgggcct gatgttctcc ggcacctct tctgtattcc cgaggacatg aaaccagca
     541  gggtagattt gttcatgtac ggtctcttgt tccgtctcgc cgacacgtgg gcggccatgc
15    601  cggataacaa aatggtcgga aaggccatca gggatcccga gaagttgaag gtcatacgct
     661  cgaacccgag gcgctacacg ggcccaccca ggggtggggc catgcgggag ctgcttaggg
     721  tgacacagta tgtacaggat aatttctcca aggtaacgac gccgtttttc actgctcacg
     781  gaacttctga cggcgttacc tgcccttctt cgtccaagct gctgtatgag aagggttcca
     841  gtgaggataa gacgttgaag ctctacgatg gaatgtatca ctctttgatt caggagagagc
20    901  ccgatgagtc tgcaaatctc gtgttggggg acatgagaga gtggattgat gagagggttc
     961  gacggtatgg acctaacaaa aattcccagt gaaacaaacc attactaaat tcctattttg
    1021  gttccacatt gcatattttg tgtctatcaa aactttatta aagttgttat gtgaagacgg
    1081  aagagtatcc ttcttctatc atatttggat ttcaatcaaa aatgacattt aatcaatcca
    1141  gttatcggtt tcgatgcatg attaacttta gtcctaactc ctcaggatat agtagtaata
25    1201  aattcctcat agtcagggtt tcaaagttta tattagtoga aaaattatgt gaaacctaag
     1261  gaagtttaca aaaatcagat agagagagat atttc

```

The protein encoded by this sequence is provided below as SEQ ID NO: 12.

SEQ ID NO: 12.

30 PREDICTED: monoglyceride lipase-like [Glycine max]ACCESSION XP_003542674

```

      1  mapeseappn fwghtpeeey ytsqgvntk shfetpngki ftqsflplnl qphqvkatvf
     61  mthgygsdtg wlfqkicinf atwgyavfaa dllghgrsdg lqcyldgmdk iaatslsffl
    121  hvrnshpykn lpaflfgesm gglatlmyf ksepdtwtgl mfsaplfvip edmkpsrvhl
    181  fmygllfgla dtwaampdnk mvgkairdpe klkviasnpr rytgpprvgt mrellrvtqy
35    241  vqdnfskvtt pfftahgtsd gvtcpssskl lyekgssedk tlklydgmymh sliqgepdes
     301  anlvlgdmre widervrryg pnknsq

```

SEQ ID NO: 13

EMBL-CDS: EAY84954.1 : Oryza sativa Indica Group hypothetical protein

```

40    1  atggcgccgccaccgcccaccgacggcgacgaagtacttctggggcgactccccggag
     61  cccgacgagtactacgcctcgctgggtctccgccacgcgagggcctacttccagtcctcc
    121  tgccggcgcctcttcacgcactcgttccaccgcctctccgccgcgacggcgacgtc

```

181 aagggcgctcgtcttcatgagccacggctacggctccgactcctcgtggatgttccagaac
 241 atcgccatcagctacgcgcggtgggggtacccgtcttctgcgccgacctgctcggacac
 301 ggccgctccgacggcgtccgcggtacctcggcgacacggaggccgtcgcgagggcggcg
 361 ctctccttcttctctcctcctgctgcggcgagcggcgccctacgcctccctcccgcgcttctc
 5 421 ttccggcgagtcacatgggcggcgccaccacctgctcgcctacctccgctccccgcccgc
 481 gccgggtggcggggatcatcctgtcggcgccgctgctcgtcttccccgacgacatgtac
 541 ccgtcccgctgcggctcttctgtacggcctcctcttcgggtctagccgacacatgggcg
 601 gtgatgccgacaagaggatggtggggagatcgatccgcgacccggcgaagctgagggtg
 661 atcgcgctccaaccgcggctgtaccgcggctcgcgcgggtggggacgatgcgggagctc
 10 721 gcacgcgtgacggcgctgctgcgggagagcttcggggaggtggcgcgccggttctctggtg
 781 gtgcacggcaccgacgacggggtgacctcgcggaggggtccaggatgctgtacgagcgc
 841 gcggcgagcgaggacaagagcctcatcctctacgacgggatgtaccactcgctcatccag
 901 ggggagtcgacgagaaccgcgaccgcgtgctcgcgacatgcgcgcctggatcgacgag
 961 cgcgtccgcgctacggcgccggcgccggcgccgcggcg

The protein encoded by this sequence is provided below as SEQ ID NO: 14.

SEQ ID NO: 14

Putative uncharacterized protein A2X294 (A2X294_ORYSI)

1 MAPPPPPPTATKYFWGDSPEPDEYYASLGLRHAEAYFQSPCGRFLTSHFHLPLSAASDGDV
 20 61 KGVVFM SHGYGSDSSWMFQNI AISYARWGYAVFCADLLGHGRSDGVRGYLGDEAVARAA
 121 LSFFLSVRRSGAYASLPAFLFGESMGGATTLLAYLRSPPDAGWAGIILSAPLLVFPDDMY
 181 PSRVRLFLYGLLFLGLADTWAVMPDKRMVGRSIRDPAKLRVIASNPRLYRGSPRVGTMREL
 241 ARVTALLRESFGGEVAAPFLVHGTDDGVTSPEGSRMLYERAASEDKSLILYDGMHYHSLIQ
 301 GESDENRDRVLADMRAWIDERVRRYGAGAGAAAADGHAEAPAA

SEQ ID NO: 15

Eucalyptus grandis predicted protein mRNA .Eucgr.F02557.1

1 ttctggggggc acatgcccga ggtatgagtag tacgcgtcgc aaggggtgcg
 30 51 caactcccag tctacttctg agacccccaaa cggcaagctc ttcacgcaga
 101 gcttctcttc cttggatcag gaagtcaagg cctcgggtcta catgacccac
 151 ggctacggat ccgacacccg ctggctcttc cagaagatct gcatcaactt
 201 cgccacctgg ggctacgcg tcttcgcgcg cgatctcttc ggccacggcc
 251 gctccgacgg cctccgttgc tacatgggtg acatggagaa gatcgtgcc
 35 301 acctccgtat cgttcttcac ccacgtccgc aagagcggag cctacaagga
 351 cctgccggcc tctctgttct gcgagtcctt gggcggggcg acgacaatgc
 401 tgatgtactt ccaatccgag ccgacgcctt ggacgggatt gatcttctctg
 451 gcgcgcgtct tcgtgatccc ggagaacatg aagcccagca aggtacggct
 501 gttctctctac ggcatgctct tcgggggtgc cgacacgttg gcgagcatgc
 40 551 cggacaacaa gatggtgggg aaggccatca aggaccccca gaagctcaag
 601 atcatcgctg cgaacccgcg gaggtagacg ggcaagccga gggtcggcac
 651 gatgagggag atcgcccggt tctgcccagta catacaggac aacttcgcca
 701 gggtagagcgc ccggttctct acggtccacg ggacgtcggc cggggtcacg

751 tgcacacacct cgtcgcagct cctgtacgag aaggcgtcca gctcggacaa
 801 gaccctgaag ctgtacgacg ggatgtacca ctgcctgato caggggggagc
 851 ccgacgagaa cgcgcacccg gtgttgggag acatgagggg gtggatcgac
 901 gagcgggtcg cgaggtacgg gccgaagato gcc

5

The protein encoded by this sequence is provided below as SEQ ID NO: 16.

SEQ ID NO: 16

Eucalyptus grandis predicted protein .Eucgr.F02557.1

10 1 FWGHMPEDEY YASQGVNRSQ SYFETPNGKL FTQSFLPLDQ EVKASVYMTN
 51 GYGSDTGWLF QKICINFATW GYAVFAADLL GHGRSDGLRC YMGDMKIAA
 101 TSVSFFTHVR KSEPYKDLPA FLFGESMGGG TMLMYFQSE PDAWTGLIFS
 151 APLFVIPENM KPSKVRLFLY GMLFGVADTW ASMPDNKMVG KAIKDPEKLE
 201 IIASNPRRYT GKPRVGTMR E IARVCQYIQD NFARVSAPFL TVHGTSDGVT
 15 251 CPTSSQLLYE KASSSDKTLK LYDGMYSLSI QGEPDENADR VLGDMREWID
 301 ERVARYGPKI A

SEQ ID NO: 17

Panicum virgatum predicted protein mRNA. Pavirv0007801m.1

20

1 accaagtact tctggggcga caccoccgag ccgacgagt actaogccgc
 51 gcagggggtc cggcacgcgc agtcctactt ccagtcocct cagggccgc
 101 tcttcaccca cgccttcac cgcctcgcgc gcgacgtcaa gggcgtcgtc
 151 ttcattgacc aaggtctacg ttcgcactcc tegtgggtct tccagacgc
 201 cgcctcgcgc tacgcgcgcg gggggtaagc cgtctctctg gccgacctcc
 251 tcggccacgc cgcctcgcgc ggcctcgcgc ggtacgtcgc cgcctcgcgc
 301 gcgcgcgcgc cggcgtccct cgtctctctc ctctcgcgc gcgcgcgcgc
 351 gggtacgcgc gcgcctcgcgc cgtctctctg cggcgagtc atgggggggc
 401 ccgcacgcgc gtcctctgac ctccgcctcc cgcgcgcgc gcgcgtggac
 30 451 gggctcgtgc tctcgggcgc gtcctcgcgc atccccgac gcatgtaccc
 501 gtccgcgcgc cgcctctctc tgtacggcct cctctctcgc ctgcgcgcgc
 551 cctgggcgcgc gtcctcgcgc aagaggatgg tggggaaggc gatcaaggac
 601 ccgcacaagc tgcggcttat cgcgtccaac cgcctcgcgc accgcgcgcgc
 651 gcgcggggtg ggcacgatgc gggagctggc cgcgcgtgac gatctgctgc
 35 701 gggagagcct cggggaggtg gcggcgccgc tctcgcgcgc gcacgggacg
 751 gacgacgggc tgacctcgc ggaggggtcc aggatgctgt acgagcgcgc
 801 gacgacgcgc gacaaggagc tcatcctgta cgggggatg taccactcgc
 851 tcatccaggg ggagccgcgc gagaaccgcgc accgcgtgct cgcgcacatg
 901 cgcaggtgga tcgacgagcg cgtcgcgcgc tac

40

The protein encoded by this sequence is provided below as SEQ ID NO: 18.

SEQ ID NO: 18

Panicum virgatum predicted protein. Pavirv0007801m.1

1 TRYFWGDTPE PDEYYAAQGL RHAESYFQSP HGRLETHAFH PLAGDVKGVV
 51 FMTHGYGSDS SWLFQTAAIS YARWGYAVFC ADLLGHGRSD GLRGYVGDME
 5 101 AAAAASLAFF LSVRASAAYA ALPAFLFGES MGAATLLMY LRSPPSARWT
 151 GLVLSAPLLV IPDGMYP SRL RFLYGLLFG LADTWAVLPD KRMVGKAIKD
 201 PDKLRLIASN PLGYRGAPRV GTMRELVRVT DLLRESLGEV AAPFLAVHGT
 251 DDGVTSPEGS RMLYERASSE DKELILYEGM YHSLIQGEPD ENRDRVLADM
 301 RRWIDERVRR Y

10 As such, it should be understood that this invention encompasses modified plants
 other than modified *Arabidopsis* species, exhibiting modulated function, activity and/or
 expression of a gene comprising a sequence being functionally similar to or having a
 degree of homology or identity with, SEQ ID NO: 1 or a fragment thereof and/or
 modulated expression, function and/or activity of a protein or peptide comprising a
 15 sequence having a degree of homology or identity with SEQ ID NO: 2 or a fragment
 thereof. For example, the invention provides modified plants exhibiting modulated
 expression, function and/or activity of a gene comprising a sequence selected from the
 group consisting of the sequences provided by SEQ ID NOS: 3; 5; 7; 9; 11, 13, 15 and 17.
 Additionally or alternatively, the invention may relate to modified plants exhibiting
 20 modulated function, activity and/or expression of a protein or peptide comprising a
 sequence selected from the group consisting of the sequences provided by SEQ ID NOS:
 4, 6, 8, 10, 12, 14, 16 and 18.

25 It should be understood that the terms “functionally similar” or “functional
 equivalent” means a protein which exhibits esterase and/or lysophospholipase 2 like
 activity or activity which is characteristic of an esterase or lysophospholipase 2. As such,
 a functionally similar or functionally equivalent esterase and/or lysophospholipase 2 gene
 may encode a protein exhibiting esterase and/or lysophospholipase 2 like activity or
 activity which is characteristic of an esterase or a lysophospholipase 2.

30 The invention may relate to modified *Populus trichocarpa*, *Vitis vinifera*, *Glycine*
max, *Medicago truncatula*, *Oryza sativa*, *Eucalyptus grandis* and/or *Panicum virgatum*
 each exhibiting modulated expression of gene exhibiting a degree of homology/identity to
 or with the *Arabidopsis thaliana* lysophospholipase 2 gene (designated *LysoPL2*) as
 described above.

A modified *Populus trichocarpa* may exhibit modulated expression, function and/or activity of a gene encoded by SEQ ID NOS: 3 or 5 (encoding the proteins of SEQ ID NOS: 4 and 6 respectively).

5 A modified *Vitis vinifera* may exhibit modulated expression, function and/or activity of a gene encoded by SEQ ID NO: 7 (encoding the protein of SEQ ID NO: 8).

A modified *Glycine max* may exhibit modulated expression, function and/or activity of a gene encoded by SEQ ID NO: 11 (encoding the protein of SEQ ID NO: 12).

10 A modified *Medicago truncatula* may exhibit modulated expression, function and/or activity of a gene encoded by SEQ ID NO: 9 (encoding the protein of SEQ ID NO: 10).

A modified *Oryza sativa* may exhibit modulated expression, function and/or activity of a gene encoded by SEQ ID NO: 13 (encoding the protein of SEQ ID NO: 14).

15 A modified *Eucalyptus grandis* may exhibit modulated expression, function and/or activity of a gene encoded by SEQ ID NO: 15 (encoding the protein of SEQ ID NO: 16).

A modified *Panicum virgatum* may exhibit modulated expression, function and/or activity of a gene encoded by SEQ ID NO: 17 (encoding the protein of SEQ ID NO: 18).

20 The term “degree of homology/identity” may encompass nucleic acid and/or amino acid sequences which exhibit at least about 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology or identity with SEQ ID NO: 1 or 2 (or any of the sequences given as SEQ ID NOS: 3-18 herein), or fragments thereof.

25 The degree of (or percentage) “homology” between two or more (amino acid or nucleic acid) sequences may be determined by aligning the sequences and determining the number of aligned residues which are identical and adding this to the number of residues which are not identical but which differ by redundant nucleotide substitutions - the redundant nucleotide substitution having no effect upon the amino acid encoded by a particular codon, or conservative amino acid substitutions. The combined total is then divided by the total number of residues compared and the resulting figure is multiplied by
30 100 – this yields the percentage homology between aligned sequences.

A degree of (or percentage) “identity” between two or more (amino acid or nucleic acid) sequences may also be determined by aligning the sequences and ascertaining the number of exact residue matches between the aligned sequences and

dividing this number by the number of total residues compared – multiplying the resultant figure by 100 would yield the percentage identity between the sequences.

Proteins and/or peptides exhibiting homology or identity to/with a lysophospholipase protein or to/with a protein/peptide encoded by SEQ ID NO: 2 or a
5 fragment thereof (or 4, 6, 8, 10, 12, 14, 16 or 18 or a fragment thereof) may comprise one or more conservative amino acid substitutions. One of skill in this field will understand that a conservative substitution, represents one or more residues which are different from the residues present in a reference sequence (for example SEQ ID NOS: 2, 4, 6, 8, 10, 12,
14, 16 or 18 or a wild type esterase and/or lysophospholipase protein sequence), but
10 which do not substantially alter the physico-chemical properties and/or structure or function of the protein.

As is well known in the art, the degeneracy of the genetic code permits substitution of one or more bases in a codon without changing the encoded primary amino acid sequence. Consequently, although the sequences described in this application (for
15 example SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 or 17) are known to encode esterase and/or lysophospholipase enzymes, the degeneracy of the nucleic acid code may be exploited to yield variant nucleic acid sequences which encode the same primary amino acid sequences.

It should be understood that fragments of any of the sequences described herein
20 (for example those designated SEQ ID NOS: 1-18) may comprise any size from about 10 residues to (n-1) residues, where “n” is the total number of residues in the complete or native amino acid/nucleic acid sequence.

By way of example, fragments of SEQ ID NO: 1 may comprise short oligomeric sequences comprising 30-1418 nucleic acids. In one embodiment, the fragments may
25 comprise 60, 90 120, 150 180 210, 300, 390, 480, 570, 690, 780, 810 900, 990, 1080, 1170, 1260, 1350 or 1410 nucleotides or consecutive nucleotides of SEQ ID NO: 1. Similarly, fragments of SEQ ID NO: 2, may comprise about 10 to about 331 amino acids – for example, 10, 20, 30 40, 50, 60, 70, 80, 90 ,100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 300, 310, 320 or 330 amino acids (for
30 example contiguous amino acids) of SEQ ID NO: 2.

In view of the above, one embodiment of this invention provides a plant exhibiting modulated expression, function and/or activity of one or more lipase/esterase/thioesterase family gene(s) - the one or more lipase/esterase/thioesterase family gene(s) being selected from the group consisting of:

- (i) a gene encoded by SEQ ID NO: 1 (or a fragment thereof);
 - (ii) a gene having a degree of identity or homology with SEQ ID NO: 1 (or a fragment thereof);
 - (iii) a gene encoded by any of the sequences designated SEQ ID NOS: 3, 5, 7, 9, 11, 13, 15 or 17 or a fragment thereof;
 - (iv) a gene having a degree of identity or homology with and of SEQ ID NOS: 3, 5, 7, 9, 11, 13, 15 or 17; and
 - (v) a gene encoding a protein functionally similar or equivalent to a protein encoded by any of the sequences designated SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15 or 17;
- wherein said plant comprises modified lignin.

Additionally or alternatively, an embodiment of this invention provides a plant exhibiting modulated expression, function and/or activity of one or more esterase/lysophospholipase enzyme(s), the esterase/lysophospholipase enzyme(s) being selected from the group consisting of:

- (i) an esterase/lipophospholipase enzyme encoded by SEQ ID NO: 2 (or a fragment thereof);
- (ii) an esterase/lipophospholipase enzyme encoded by a protein having a degree of homology/identity with SEQ ID NO: 2 (or a fragment thereof);
- (iii) an esterase/lipophospholipase enzyme encoded by a protein having a sequence corresponding to a sequence designated SEQ ID NO: 4, 6, 8, 10, 12, 14, 16 or 18 or a fragment thereof;
- (iv) an esterase/lipophospholipase enzyme encoded by a sequence having a degree of homology/identity with any of SEQ ID NOS 4, 6, 8, 10, 12, 14, 16 or 18; and
- (v) an esterase/lipophospholipase enzyme functionally similar or equivalent to a lipophospholipase enzyme encoded by any of SEQ IS NOS: 2, 4, 6, 8, 10, 12, 14, 16 or 18;

wherein said plant comprises modified lignin.

The plants provided by this invention may be genetically modified so as to exhibit modulated expression of one or more lipase/esterase/thioesterase family gene(s) and/or a modulated level of esterase/lysophospholipase expression.

As such, this invention encompasses plants which comprise modified lipase/esterase/thioesterase family gene sequence(s). In the context of this invention, a “modified sequence” may comprise one or more mutations such as, for example, one or

more nucleic acid or amino acid additions, deletions, substitutions and/or inversions (collectively referred to as modifications) – which modifications affect the level of expression, function and/or activity of a lipase/esterase/thioesterase family gene or a protein encoded thereby. In one embodiment, the one or more mutations of the modified sequences may ablate or reduce the expression of a lipase/esterase/thioesterase family gene and/or the activity and/or function of any lipase/esterase/thioesterase encoded thereby. Such mutations may be collectively referred to as loss-of-function mutations.

It should be understood that the level of expression of a lipase/esterase/thioesterase family gene or a protein encoded thereby may be assessed relative to the expression of a corresponding lipase/esterase/thioesterase family gene or a protein encoded thereby in a control plant.

One of skill will appreciate that there are many ways of introducing genetic modifications into plant genomes and all of these techniques apply here. For example, it may be possible exploit random mutagenesis methods such as irradiation, random DNA integration and/or chemical mutagen processes in order to modify lipase/esterase/thioesterase family gene(s) so as to provide plants exhibiting a modified lignin content. Additionally or alternatively, lipase/esterase/thioesterase family gene(s) may be modified or mutated by techniques which may include, for example, Agrobacterium-mediated transformation, biolistics, site or oligonucleotide-directed mutagenesis, oligonucleotide-directed repair, zinc finger nuclease technology, TALE-based hybrid nucleases, and site-specific recombination.

In one embodiment, a plant may be modified using any of the techniques described above, such that expression of lipase/esterase/thioesterase family gene/protein(s) is/are partially or completely ablated – such plants may exhibit a modified or altered lignin.

However, one of skill will appreciate that in some cases a degree of lignin production may be desirable and modified plants of this invention may be further modified by the introduction of expression vectors which encode one or more expressible lipase/esterase/thioesterase family gene sequences. In one embodiment, the expression vectors may direct reduced expression of one or more functional lipase/esterase/thioesterase family gene(s) leading to reduced expression of lipase/esterase/thioesterase family protein(s) in transformed plant tissues (again “reduced” expression of a lipase/esterase/thioesterase family gene/protein(s) may be assessed relative to the levels of expressions observed in a control plant). Alternatively, a vector

may encode (or direct the expression of) one or more fully or partially functional lipase/esterase/thioesterase family gene/protein(s) in a wild type plant, or in a plant that does not express the endogenous lipase/esterase/thioesterase gene or protein.

5 In other embodiments, wild-type or unmodified plants may be modified by the introduction of one or more vectors which encode one or more expressible lipase/esterase/thioesterase family gene/protein(s). The introduction of such vectors may trigger co-suppression of endogenous lipase/esterase/thioesterase family gene/protein(s) or may (in some cases) bring about an increase in lignin production.

10 In other embodiments, the modified plants provided by this invention may comprise one or more nucleic acid sequences which are complementary to a sequence provided by this invention - for example, a sequence derived from SEQ ID NO: 1 (or SEQ ID NOS 3, 5, 7, 9, 11, 13, 15 or 17). Such sequences may be known as sense or antisense sequences. Antisense oligonucleotides sequences may comprise DNA that gives rise to a variety of small/short interfering and/or silencing RNAs – such molecules being
15 referred to hereinafter as siRNA.

In one embodiment, the modified plants of this invention may comprise one or more inverted repeat elements designed to silence one or more lipase/esterase/thioesterase family gene sequences. One of skill will appreciate that an inverted repeat element may comprise an antisense sequence and sense sequence separated by a hairpin structure. Such
20 elements may be introduced into plants via vectors which encode one or inverted repeat elements.

Antisense oligonucleotides sequences for use in this invention (such as those designed to modulate the expression, function and/or activity of a sequence of SEQ ID NO:1) may be comprised within a nucleic acid construct operably linked to, for example,
25 a suitable promoter sequence. In one embodiment, a construct of this invention may comprise a constitutive or tissue specific promoter sequence or a tissue, cell, seed or organelle specific promoter.

In view of the above, the invention extends to plants comprising a modified lignin content and one or more antisense sequences or inverted hairpin constructs which affect
30 the expression, function and/or activity of one or more lipase/esterase/thioesterase family gene(s). In one embodiment, the modified plants of this invention may comprise (exogenous) nucleic acid sequences which encode sections or parts of one or more lipase/esterase/thioesterase family gene(s). For example, such sequences may comprise approximately 200bp-1kb of a lipase/esterase/thioesterase family gene sequence and be

introduced as part of an expression cassette or vector – such as, for example, T-DNA (for Agrobacterium-mediated transformation) or by biolistics.

This invention extends to plants generated by new breeding techniques such as Zinc finger nuclease (ZFN) technology (ZFN-1, ZFN-2 and ZFN-3), Oligonucleotide
5 directed mutagenesis (ODM), Cisgenesis and intragenesis, RNA-dependent DNA methylation (RdDM) [3, 12].

As mentioned above, the modified lignin of the plants described herein, ensures sugars can be more efficiently released. As such, this invention further provides a method of increasing the level or availability of one or more carbohydrate(s) in a plant, said
10 method comprising the steps of modulating the expression of one or more lipase/esterase/thioesterase family gene(s) and/or the expression, function and/or activity of one or more lipase/esterase/thioesterase family protein(s).

In one embodiment, the one or more carbohydrates are fermentable carbohydrates such as, for example cellulose, hemicelluloses, or glucose. In a further embodiment, the
15 plant may be a plant grown as a biofuel crop.

In a further aspect, there is provided a plant or plant material for use in methods which require release (or exploitation of) carbohydrates from plants, wherein the plant is a plant according to the first aspect of this invention and/or the plant material is derived from a modified plant provided by the first aspect of this invention.

20 In one embodiment, the method is, for example, a biorefinery method or a method of biofuel, animal feed, bioplastic, chemical, pulp or paper production.

In one embodiment, there is provided a modified plant of the first aspect of this invention, or material derived therefrom, for use in methods of producing biofuels.

In a further aspect, the invention provides a method of producing a biofuel, the
25 method comprising the steps of obtaining material from a plant according to the first aspect of this invention and subjecting the material (or carbohydrates thereof) to a fermentation protocol. In one embodiment, the biofuel is a bioethanol.

In another aspect, the invention provides a method of modifying the lignin content of a plant, said method comprising the step of modifying the expression of one or more
30 lipase/esterase/thioesterase family gene(s) and/or the expression, function and/or activity of a lipase/esterase/thioesterase family protein(s). In one embodiment, the modified lignin content comprises a reduced lignin content and/or lignin having an altered composition and/or structure.

In a further aspect, the present invention provides a biofuel, animal feed, bioplastic, chemical, pulp or paper produced by a method exploiting material (biomass) derived from the modified plants described herein.

It should be understood that the modified plants provided by this invention may
 5 comprise one or more other modifications which affect lignin biosynthesis. For example, in addition to exhibiting modulated expression of one or more lipase/esterase/thioesterase family gene, the plants of this invention may exhibit modulated expression of one or more other genes involved in lignin biosynthesis.

DETAILED DESCRIPTION

10 The present invention will now be described in detail with reference to the following figures which show:

Figure 1: (A and B) Arabidopsis mutants in AT1G52760 (encoding a lysophospholipase / thioesterase LysoPL2) have reduced (mutant Mx12_7) or virtually abolished (mutant Gb9) expression of AT1G52760 RNA compared to wild-type Col-0.
 15 The gene AT1G52760 was identified as being tightly co-expressed with lignin biosynthesis genes using methods similar to [6-8]. Levels of AT1G52760 mRNA were estimated by qRT-PCR. ** 0.01>p>0.001.

Figure 2: (A) Stem cross sections stained with Maule reagent reveal that mutant Gb9 with defective AT1G52760 expression (bottom) shows less red staining in fibres and
 20 more irregular shaped xylem vessels compared to wild type plants (top). (B) Stem cross sections autofluorescence (top) and stained with Maule reagent (bottom) reveal that mutant Gb9 (right hand side) with defective AT1G52760 expression shows less lignin autofluorescence, less Maule red staining in fibres, and more irregular shaped xylem vessels compared to wild type plants (left hand side).

25 Figure 3: (A, B and C) Arabidopsis mutant *Gb9* with defective AT1G52760 expression has reduced lignin, with levels significantly lower than wild type plants (WT). Lignin was determined by the acetyl bromide method similar to [9]. (C) Shows the altered monomer structure; lignin was determined by the acetyl bromide method similar to [9] and by thioacidolysis to determine H, G and S units.

30 Figure 4: (A) Arabidopsis mutant *Gb9* with defective AT1G52760 expression has increased sugar yield on cell wall saccharification, with levels comparable to known lignin mutants *ccr1* and *ref3-3*, and significantly higher than Col-0 wild type control plants. Saccharification was determined in a relatively mild assay using methods similar to [10]. Greater improvements in saccharification might be expected under conditions

with higher enzyme loading left for longer time. (B) Figure 4: Arabidopsis mutants *Gb9* and *Mx12_7* with defective AT1G52760 expression have increased cellulose-to-glucose conversion on cell wall saccharification compared to wild type plants. Error bars represent the standard error. * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $0.001 > p$.

Figure 5 (A, B and C): Phenolic profiling reveals that there are differences in metabolite accumulation between wild type (wt) and the lysophospholipase/thioesterase mutants *Mx12_7* and *Gb9*. PCA plots show that the wild type profiles cluster differently from those of the mutants while an S-plot analysis also confirms that some metabolites accumulate differentially between mutants and wild type (revealed by outlying dots in the tails of the S-plot).

Figure 6: Differential accumulation of three compounds that are present in different levels in the lysophospholipase/thioesterase mutant and the wild type is illustrated in the right hand side column of graphs while the mass spectra of these compounds is shown in the left hand side column.

Figure 7: Arabidopsis mutant with defective AT1G52760 expression (green line) has more ferulate esters and glucosides on phenolic profiling than wild type plants (red line). Two peaks that accumulate in the thioesterase mutant correspond to ferulate glucose ester and one to ferulic acid glucoside. The peak that accumulates 70-fold is feruloyl malate.

Figure 8: Arabidopsis mutant with defective AT1G52760 expression (green line) has less lignin oligomers than wild type plants (red line).

Figure 9: Multiple alignment of Arabidopsis AT1G52760 amino acid sequence and similar sequences from other plant species. AT1G52760 has previously been described as a lysophospholipase 2 (LysoPL2) involved in tolerance to cadmium-induced oxidative stress [11]. No basis for a role in lignin biosynthesis has previously been proposed.

Materials & Methods

Co-expression analysis and selection of Arabidopsis mutants

A variety of tools [6-8] including ACT and CressExpress were used to search for genes that have similar expression patterns to individual lignin biosynthesis genes. In total, 255 genes were retrieved, with some of them shared between different analyses; 102 of them were chosen for further investigation. To investigate the potential biological function of these genes, we searched the Nottingham Arabidopsis Stock Centre (NASC) for available T-DNA insertion mutants in these genes and obtained

66 homozygous mutants, including two, renamed Gb9 and Mx12-7, that are mutated in AT1G52760, a gene annotated as encoding a lipase/thioesterase enzyme and later described as a lysophospholipase [11] with no known role in lignin biosynthesis. Figure 1 shows that Mx12-7 retains a very small level of the AT1G52760 lysophospholipase/thioesterase expression while in Gb9, no expression was detected. Expression was quantified by standard QRT-PCR analysis.

Lignin determinations, saccharification analysis, and phenolic profiling

Histochemical staining with Maule reagent (which stains S lignin) of transverse stem sections from the *Gb9* mutant showed reduced lignin staining and collapsed xylem indicative of a cell wall defect (Figure 2). Acetyl bromide lignin determinations [9] carried out on Arabidopsis mutant *Gb9* showed that it has reduced lignin, with levels significantly lower than wild type plants (Figure 3). This suggests that the AT1G52760 lysophospholipase plays some unknown role in determining the amount of lignin deposited in Arabidopsis and possibly other plants. We subsequently demonstrated a significant improvement in the release of sugar from plant cell walls of the *Gb9* mutant (Figure 4) which releases levels comparable to that of known lignin mutants (*ccr1*), and significantly higher than the levels released by Col-0 wild type plants. This saccharification assay is a very mild treatment and does not indicate the maximum possible sugar release from these genotypes but merely reveals differences between them under mild conditions. Saccharification was evaluated by grinding stem material to a fine powder, pretreating it with mild acid (typically 1% H₂SO₄), washing the residue and subjecting it to enzymic hydrolysis with Novozymes 188 plus Celluclast. Levels of simple reducing sugars released was determined by MBTH detection using methods similar to [10]. These data illustrating improved saccharification of *Gb9* suggest that it may be a novel point at which to manipulate lignin biosynthesis to improve sugar release for biofuel production. Phenolic profiling by Ultrahigh Pressure Liquid Chromatography (UPLC) of methanol-soluble phenolic compounds revealed that some metabolites accumulate differentially between the wild type and the lysophospholipase/thioesterase mutant. PCA plots (Figure 5, top) confirmed that the wild type profiles differ from those of the mutant, as did an S-plot analysis (figure 5, bottom). Dots in the tails of the S-plot designate metabolites that accumulate differentially between mutant and wild type. The mass spectra of three compounds that accumulate differentially between the lysophospholipase/thioesterase mutant and the wild type are shown in Figure 6. Two peaks that accumulate in the mutant correspond to ferulate glucose ester and one to ferulic

acid glucoside. A peak that accumulates 70 fold is feruloyl malate (Figure 7). Arabidopsis mutants with defective AT1G52760 lysophospholipase/thioesterase expression (esterase; green line) also have less lignin oligomers than wild type plants (red line) (Figure 8). The figure shows regions in the chromatogram that are rich in small lignin oligomers.

5 Chromatograms of the thioesterase mutant have lower peak heights compared to the wild type. Structures of some oligolignols that are reduced in the thioesterase mutant are shown. These data are being studied further to try to deduce the exact role of AT1G52760 mutants in lignin biosynthesis and new lines of investigation are being pursued to the same end.

10 *Protein complex purification*

In order to determine whether the AT1G52760 lysophospholipase interacted directly with lignin biosynthesis genes, the lysophospholipase was used as a bait to trap any interacting protein complexes using a tandem affinity purification system. Evaluation of the proteins co-purifying with the lysophospholipase by GC-MS revealed several potential lignin biosynthesis enzymes (data not shown). This suggests that the lysophospholipase influences lignin by some direct mechanism modulating lignin biosynthesis.

BLAST searches for orthologues in other species

Evaluation of AT1G52760 orthologues using BLAST searches of gene sequence data revealed several highly homologous sequences from *Populus trichocarpa*, *Vitis vinifera*,
20 *Glycine max*, *Medicago truncatula* and *Oryza sativa*, suggesting that the role of AT52760 is widely conserved in the plant kingdom (Figure 7).

Table 1: Cell wall and lignin amount and composition. ABSL lignin: lignin determined via the acetyl bromide soluble lignin (ABSL) protocol. Lignin composition was
25 determined via thioacidolysis. Numbers between brackets are standard deviations. * 0.01<p<0.05; ** 0.001<p<0.01; *** p<0.001. CWR: cell wall residue.

	Wild type Control for Mx12_7	Mx12_7	Difference Mx12_7/WT	Wild type Control for Gb9	Gb9	Difference Gb9/WT
CWR/dry weight (%)	82.9 (1.4)	79.0 (3.3)	-	79.8 (2.8)	72.9 (1.1)*	-9%
ABSL lignin/CWR (%)	16.2 (1.0)	13.4 (0.5)*	-17%	17.6 (0.5)	11.7 (0.6)***	-33%
cellulose/CWR (%)	45.0 (2.7)	42.2 (3.5)	-	59.7 (3.5)	43.5 (1.1)**	-27%
H units/CWR (μmol/g)	0.7 (0.1)	2.6 (0.4)**	+270%	0.4 (0.1)	8.2 (0.6)***	+1900%
G units/CWR (μmol/g)	67.4 (5)	41.1 (4.1)***	-39%	59.3 (4.5)	12.0 (1.4)***	-80%
S units/CWR (μmol/g)	20.7 (2.7)	15.3 (2.2)	-	31.4 (2.2)	10.4 (1.3)***	-67%
H+G+S/CWR (μmol/g)	88.7 (7.6)	59.0 (6.3)**	-33%	91.1 (6.4)	30.6 (3.2)***	-66%
H units/lignin (μmol/g)	5.0 (0.9)	22.3 (3.8)**	+350%	2.1 (0.4)	71.2 (14.7)***	+3300%
G units/lignin (μmol/g)	467.9 (35)	352.4 (35.3)*	-25%	342.3 (36.2)	104.8 (12.3)***	-69%
S units/lignin (μmol/g)	143.5 (18.8)	131.2 (18.5)	-	181.2 (19.4)	90.7 (10.8)**	-50%
H+G+S/lignin (μmol/g)	616.5 (53.1)	505.9 (53.8)	-	525.6 (54.6)	266.7 (28.3)**	-49%
%H	0.9 (0.2)	4.4 (0.6)***	+390%	0.4 (0.1)	27.1 (1.2)***	+6700%
%G	76.4 (1.1)	70.0 (1.6)**	-8%	65.0 (1)	39.1 (0.8)***	-40%
%S	22.7 (1.2)	25.6 (1.3)	-	34.5 (1)	33.8 (0.7)	-
S/G	0.30 (0.02)	0.37 (0.03)*	+23%	0.53 (0.02)	0.86 (0.02)***	+62%

5

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Claims

1. A plant exhibiting modulated expression of one or more lipase/esterase/thioesterase family gene(s), wherein said plant comprises an altered or modified lignin and/or lignin composition.

5

2. The plant of claims 1, wherein the lipase/esterase/thioesterase family gene encodes a lysophospholipase and/or esterase protein/enzyme.

10

3. The plant of claim 1 or 2, wherein the plant exhibits reduced expression of one or more lipase/esterase/thioesterase family gene(s) and/or lysophospholipase and/or esterase proteins/enzymes and comprises a modified lignin and/or lignin composition.

15

4. The plant of any preceding claim, wherein the lipase/esterase/thioesterase family gene is the lysophospholipase 2 gene (*LysoPL2*).

5. A plant exhibiting modulated expression, function and/or activity of one or more lipase/esterase/thioesterase family gene(s), the one or more lipase/esterase/thioesterase family gene(s) being selected from the group consisting of:

20

(i) a gene encoded by SEQ ID NO: 1 or a fragment thereof;

(ii) a gene having a degree of identity or homology with SEQ ID NO: 1 or a fragment thereof;

(iii) a gene encoded by any of the sequences designated SEQ ID NOS: 3, 5, 7, 9, 11, 13, 15 or 17 or a fragment thereof;

25

(iv) a gene having a degree of identity or homology with and of SEQ ID NOS: 3, 5, 7, 9, 11, 13, 15 or 17; and

(v) a gene encoding a protein functionally similar or equivalent to a protein encoded by any of the sequences designated SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15 or 17;

wherein said plant comprises an altered or modified lignin and/or lignin composition.

30

6. A plant exhibiting modulated expression, function and/or activity of one or more esterase/lysophospholipase enzyme(s), the esterase/lysophospholipase enzyme(s) being selected from the group consisting of:

(i) an esterase/lipophospholipase enzyme encoded by SEQ ID NO: 2 or a fragment thereof;

(vi) an esterase/lipophospholipase enzyme encoded by a protein having a degree of homology/identity with SEQ ID NO: 2 or a fragment thereof;

5 (vii) an esterase/lipophospholipase enzyme encoded by a protein having a sequence corresponding to a sequence designated SEQ ID NO: 4, 6, 8, 10, 12, 14, 16 or 18 or a fragment thereof;

(viii) an esterase/lipophospholipase enzyme encoded by a sequence having a degree of homology/identity with any of SEQ ID NOS 4, 6, 8, 10, 12, 14, 16 or 18; and

(ix) an esterase/lipophospholipase enzyme functionally similar or equivalent to a lipophospholipase enzyme encoded by any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16 or 18;

10 wherein said plant comprises an altered or modified lignin and/or lignin composition.

7. The plant of any preceding claim, wherein the plant is genetically modified so as to exhibit modulated expression of one or more lipase/esterase/thioesterase family gene(s) and/or a modulated level of esterase/lysophospholipase expression.

8. The genetically modified plant of claim 7, wherein the plant comprises a modified lipase/esterase/thioesterase family gene sequence(s).

25 9. The plant of any preceding claim, wherein the plant comprises one or more nucleic acid sequences selected from the group consisting of:

(i) Nucleic acid sequences complementary to a sequence derived from any one of SEQ ID NOS 1, 3, 5, 7, 9, 11, 13, 15 or 17;

(ii) one or more inverted repeat elements designed to silence one or more lipase/esterase/thioesterase family gene sequences; and

30 (iii) exogenous nucleic acid sequences which encode sections or parts of one or more lipase/esterase/thioesterase family gene(s).

10. The plant of any preceding claim, wherein the plant comprises one or more antisense sequences or inverted hairpin constructs which affect the expression, function and/or activity of one or more lipase/esterase/thioesterase family gene(s).
- 5 11. The plant of any preceding claim, wherein the plant is a crop and/or a grass species.
12. The plant of any preceding claim, wherein the plant is a species or hybrid belonging to any one of the *Saccharum*, *Zea*, *Triticum*, *Secale*, *Hordeum*, *Glycine*, *Oryza*,
10 *Sorghum*, *Lolium*, *Vitis* and *Medicago*, *Eucalyptus*, *Panicum* genera.
13. Use of the plant of any preceding claim or biomass therefrom in methods for accessing and/or processing carbohydrate polymers from plant matter and/or biofuel production.
- 15 14. The use of claim 13, wherein the biomass comprises the stems, leaves and/or roots of the plant.
15. A modified plant according to any one of claims 1-12 or material derived
20 therefrom, for use in methods of producing biofuels.
16. A method of producing a biofuel, the method comprising the steps of obtaining material from a plant according to any one of claims 1-12 and subjecting the material or
25 carbohydrates therefrom to a fermentation protocol.
17. A biofuel, animal feed, bioplastic, chemical, pulp or paper produced by the method of claim 16.
- 30 18. The method of claim 16 or biofuel of claim 17, wherein the biofuel is a bioethanol.
19. A method of modifying the lignin content of a plant, said method comprising the step of modifying the expression of one or more lipase/esterase/thioesterase family

gene(s) and/or the expression, function and/or activity of a lipase/esterase/thioesterase family protein(s).

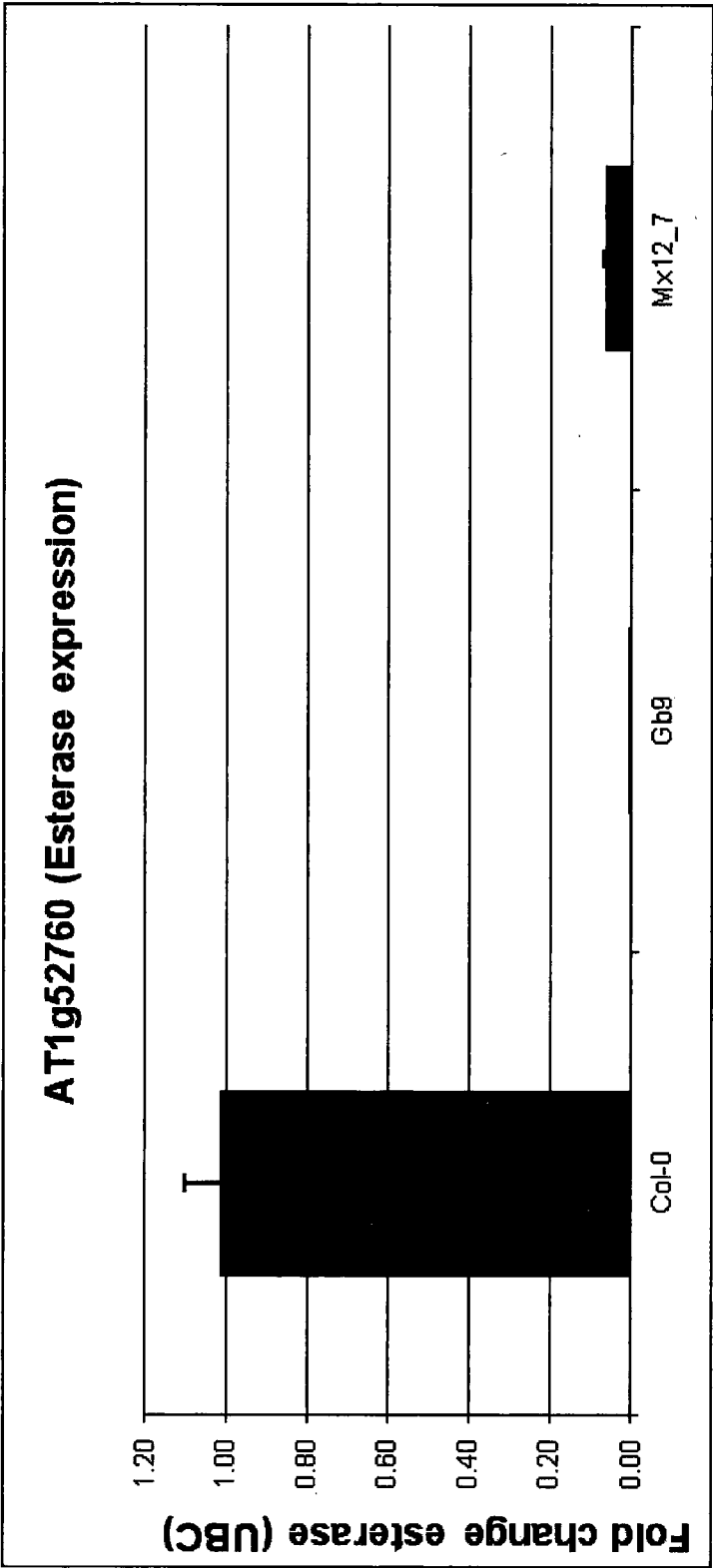


Figure 1A

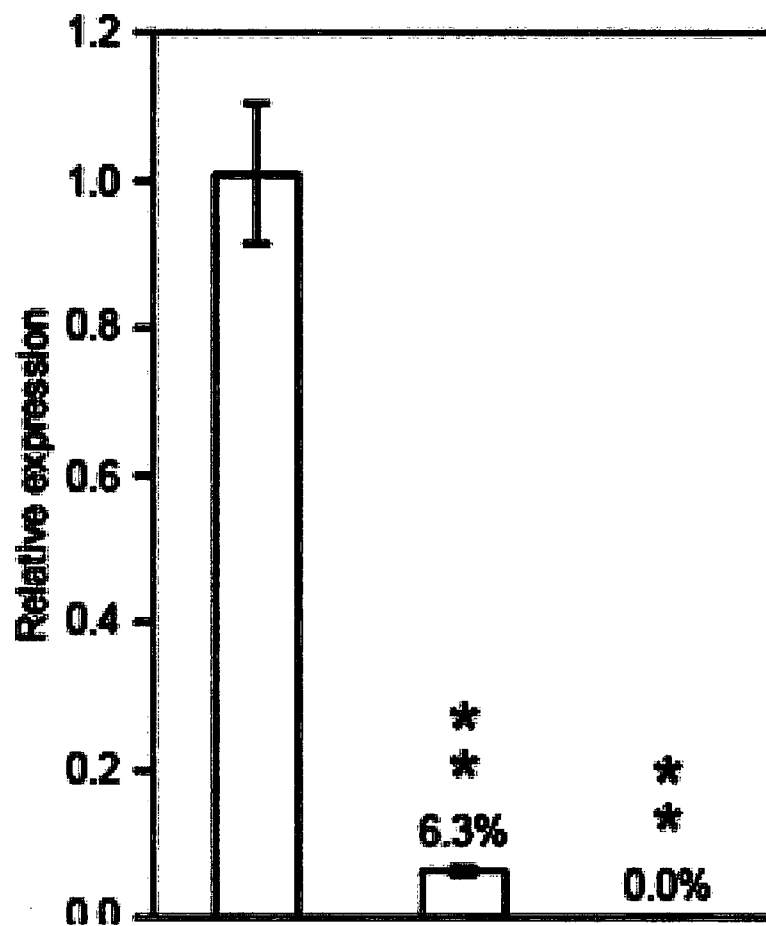


Figure 1B

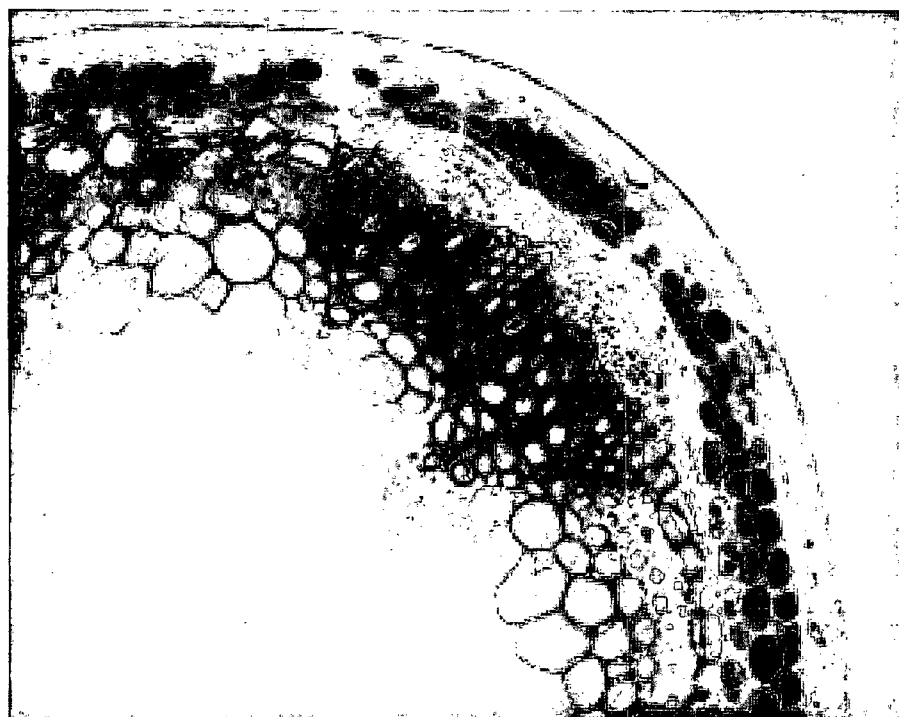


Figure 2A

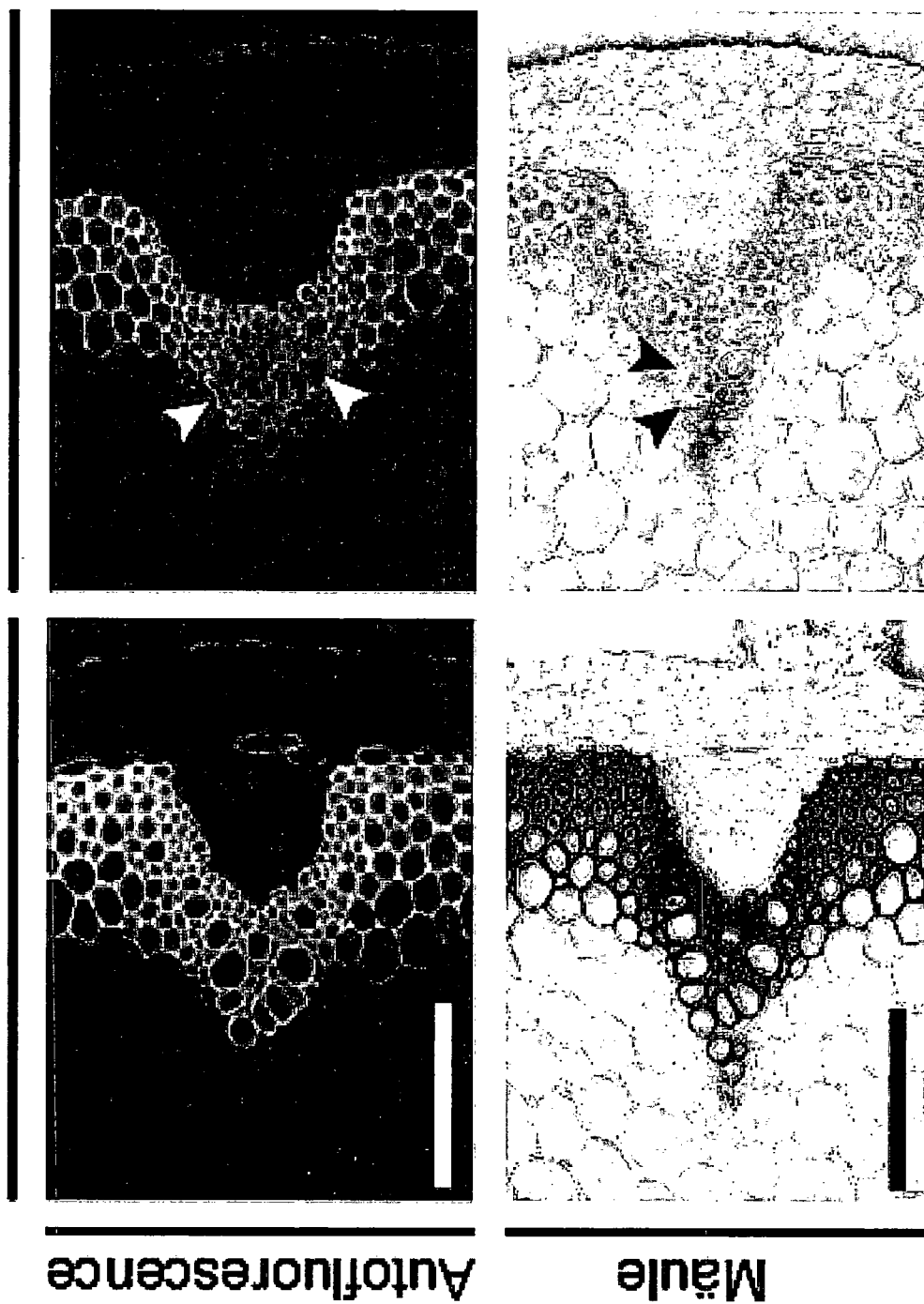


Figure 2B

5/15

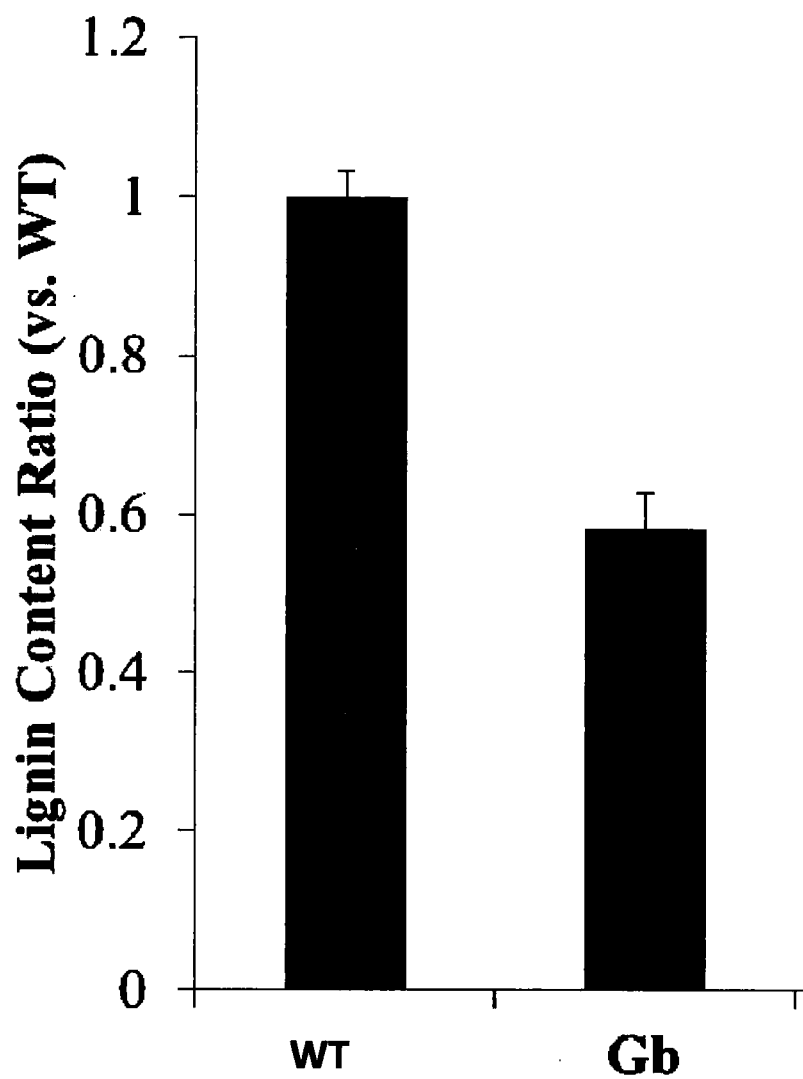


Figure 3A

6/15

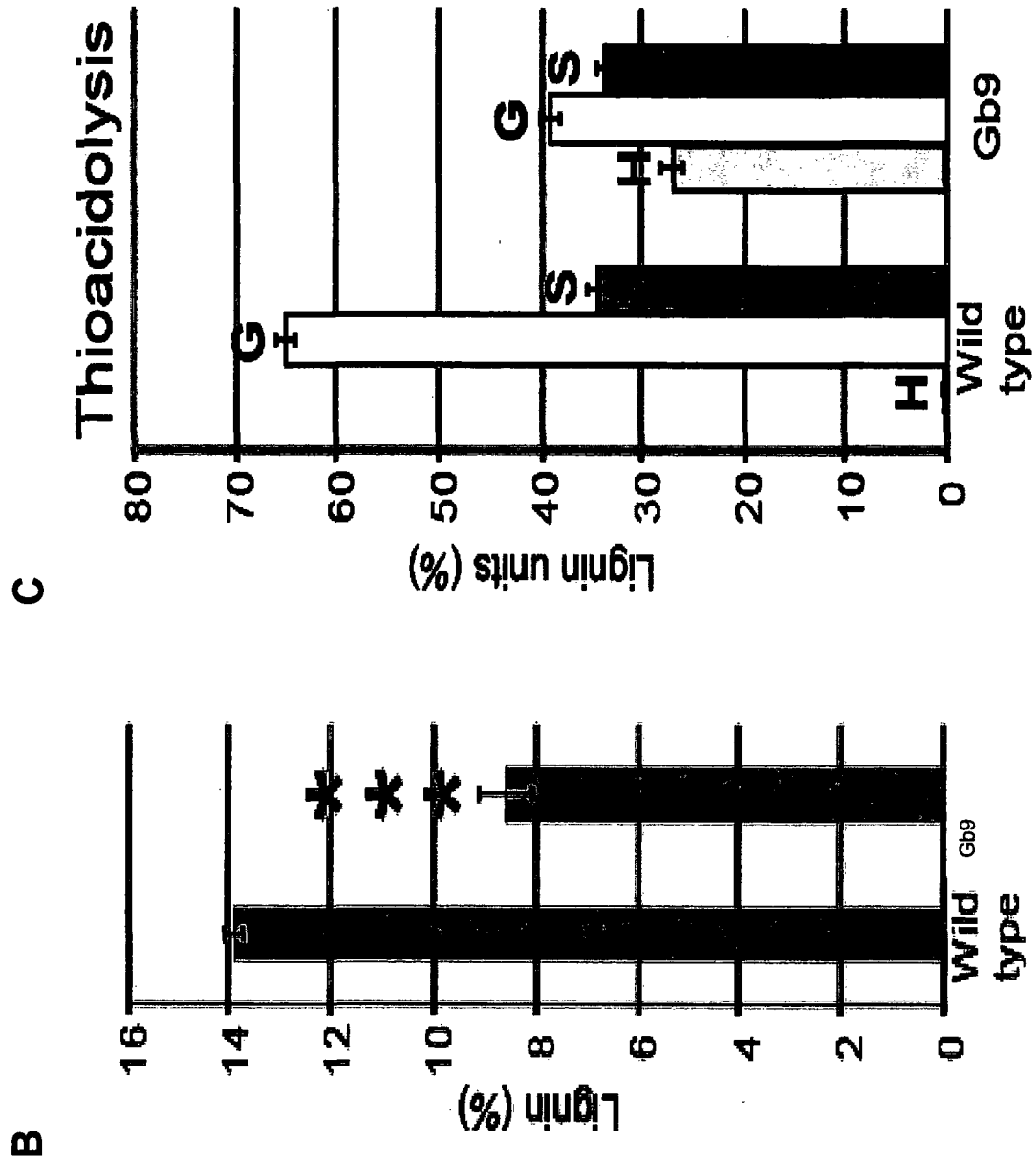


Figure 3B and C

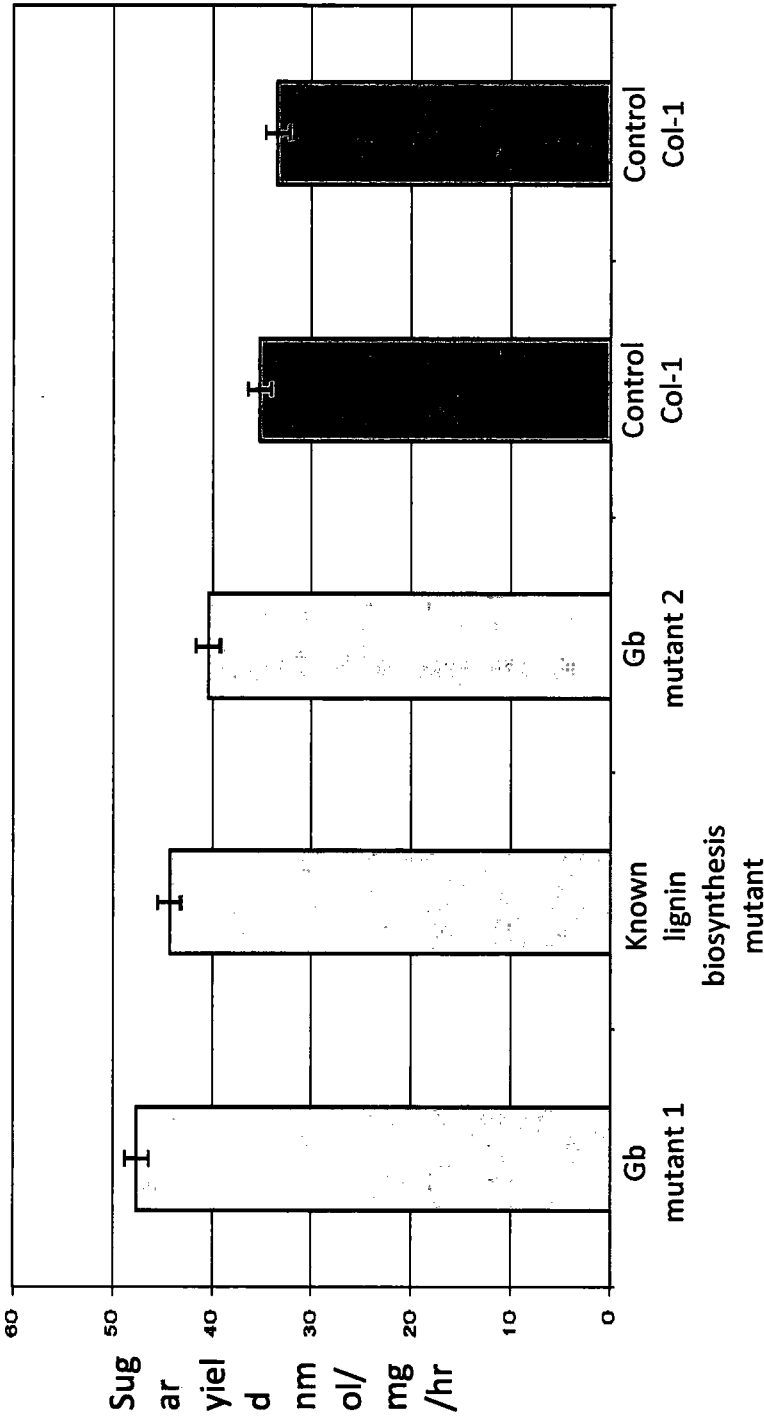


Figure 4A

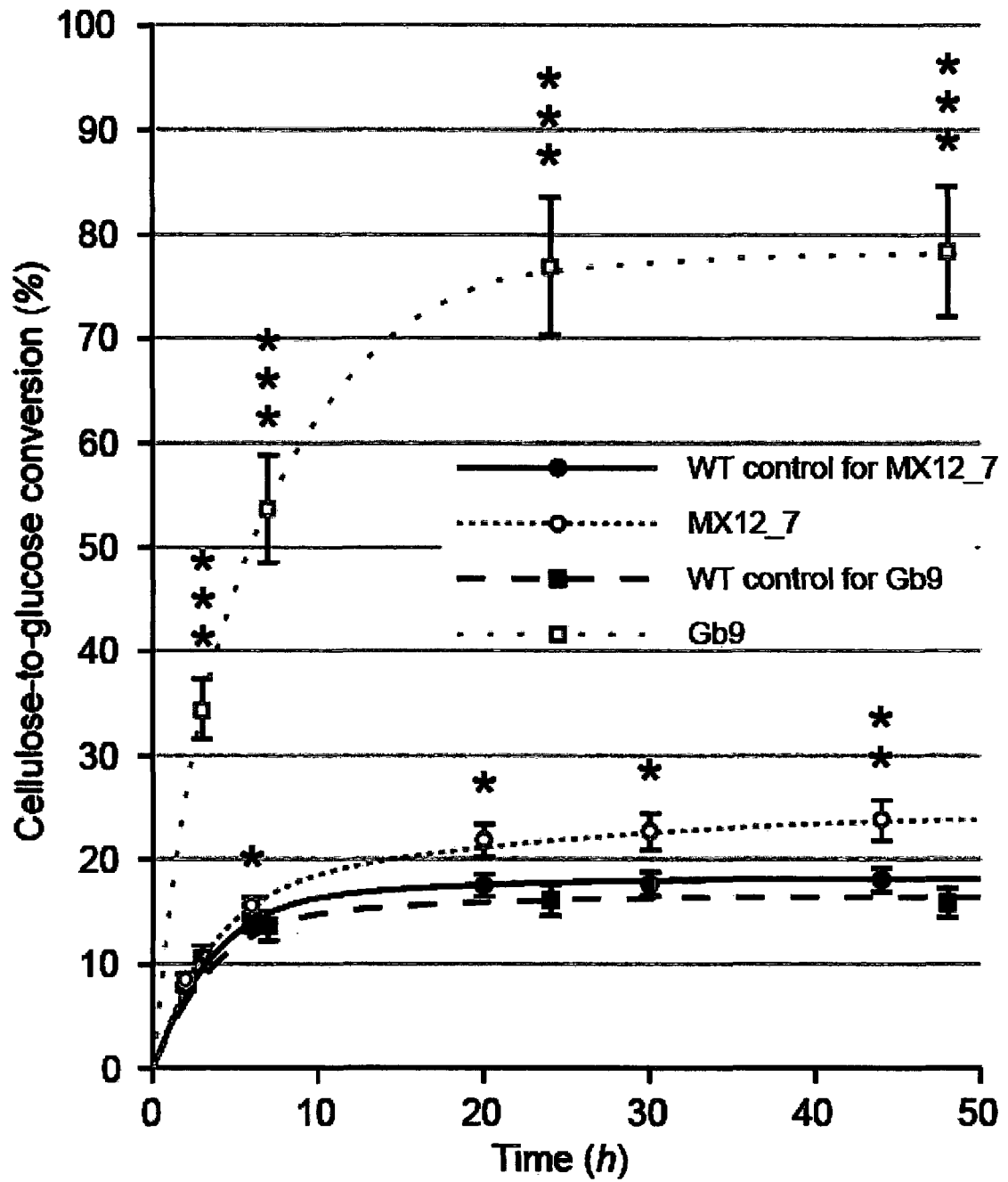


Figure 4B

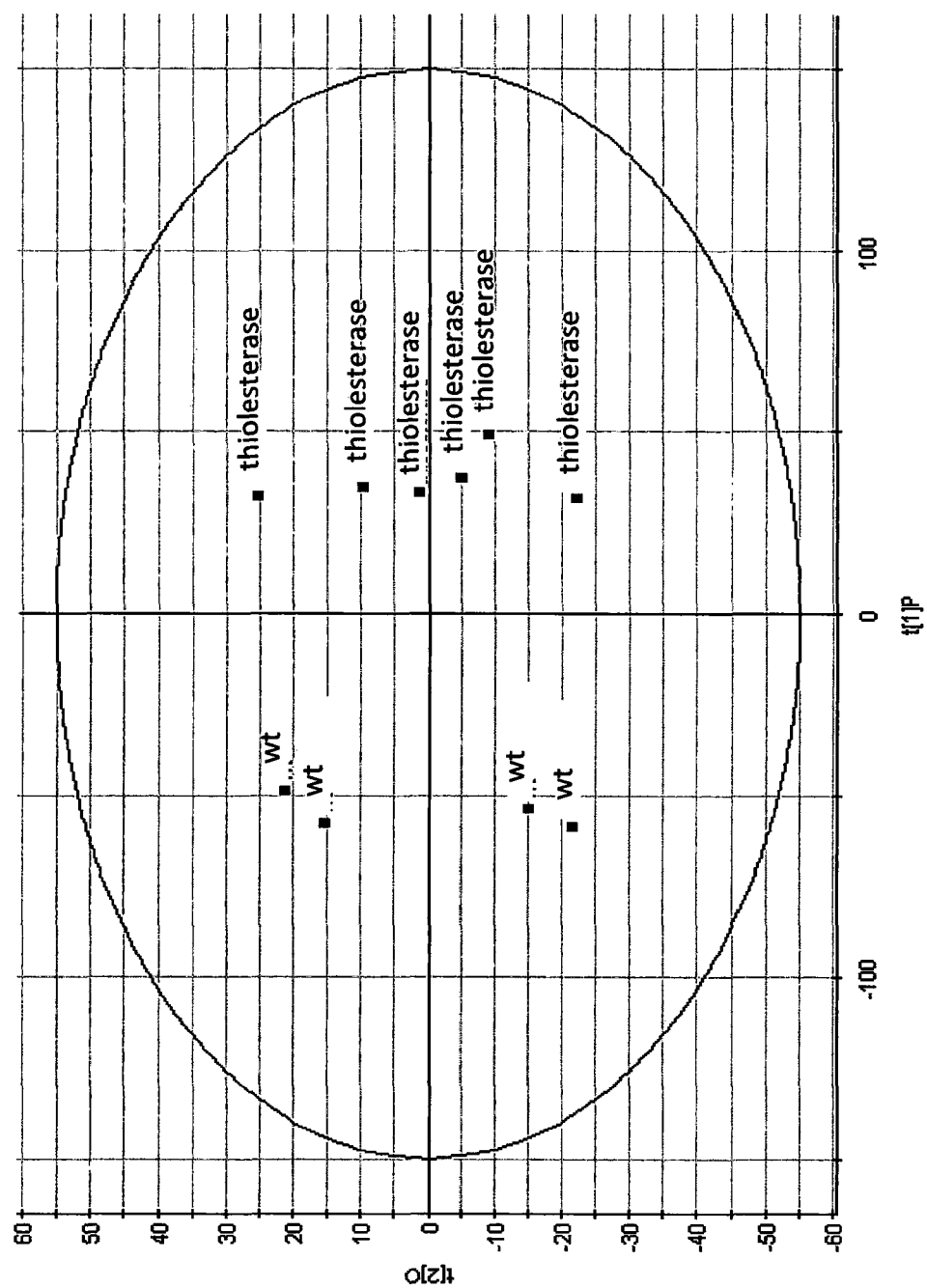


Figure 5A

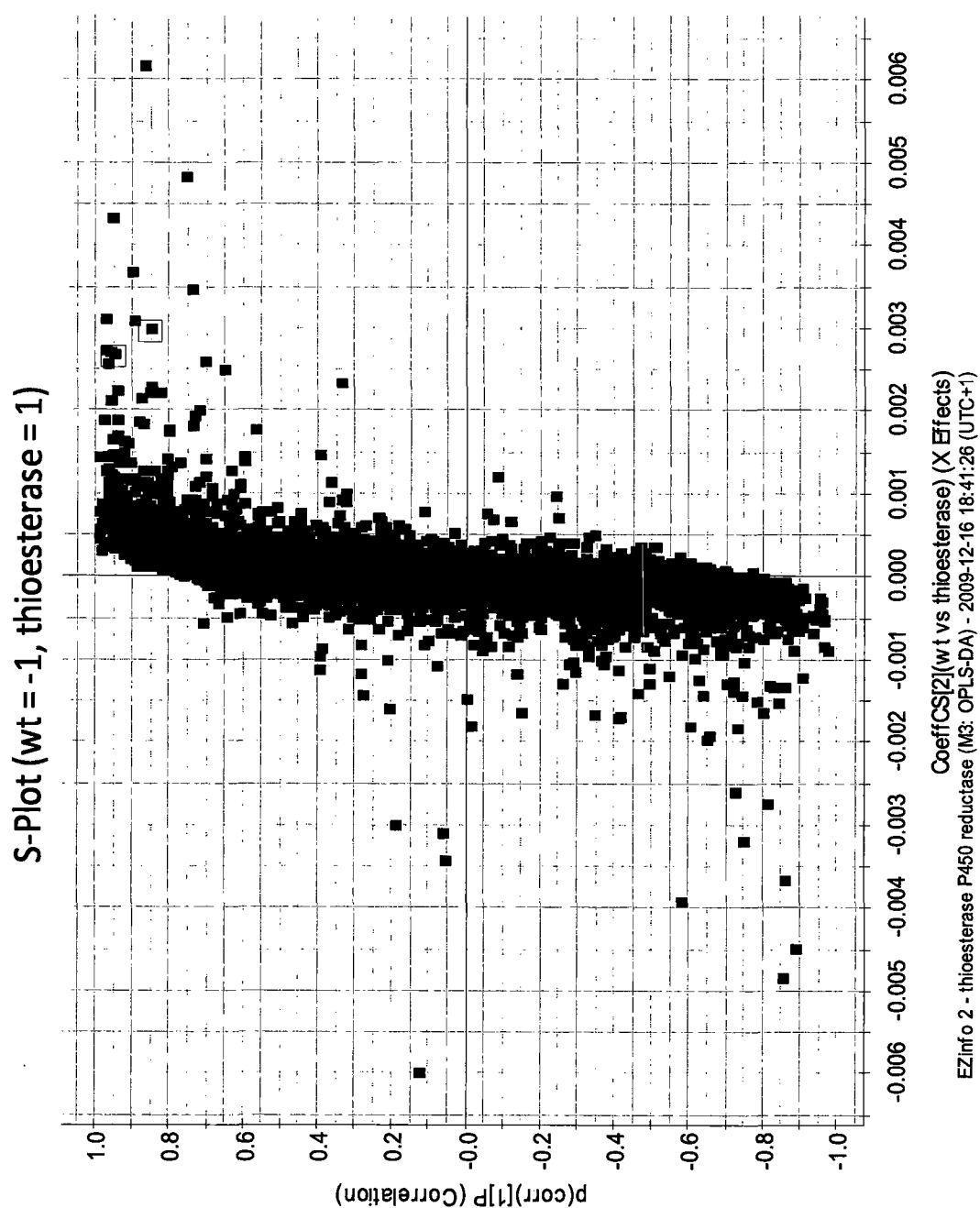


Figure 5B

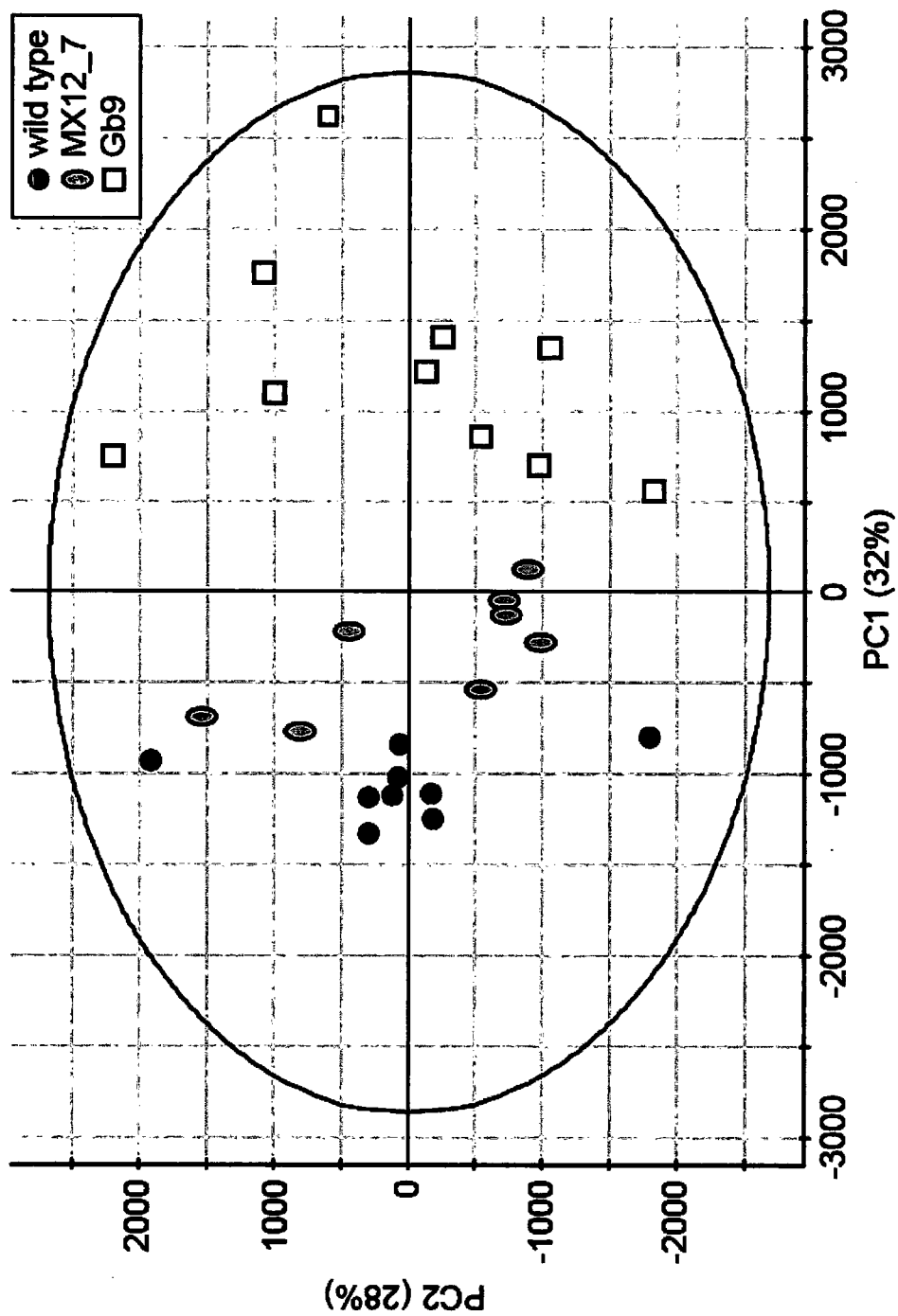
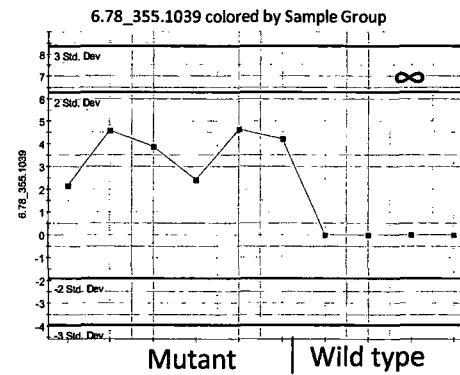
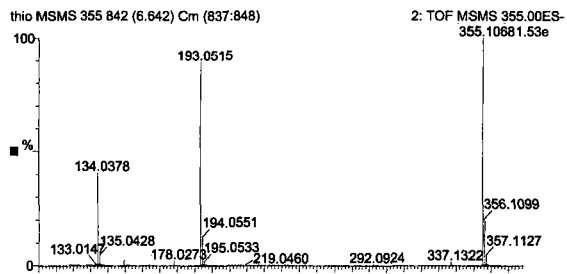
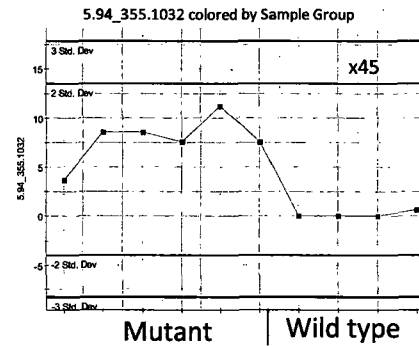
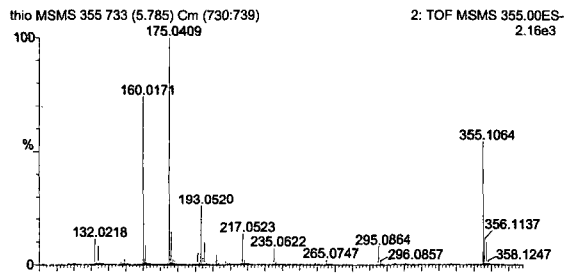
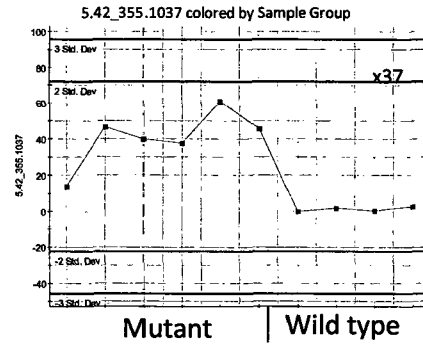
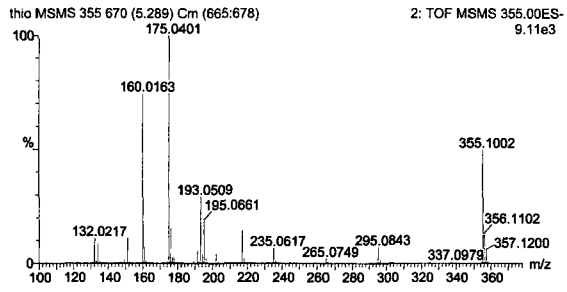


Figure 5C

Figure 6

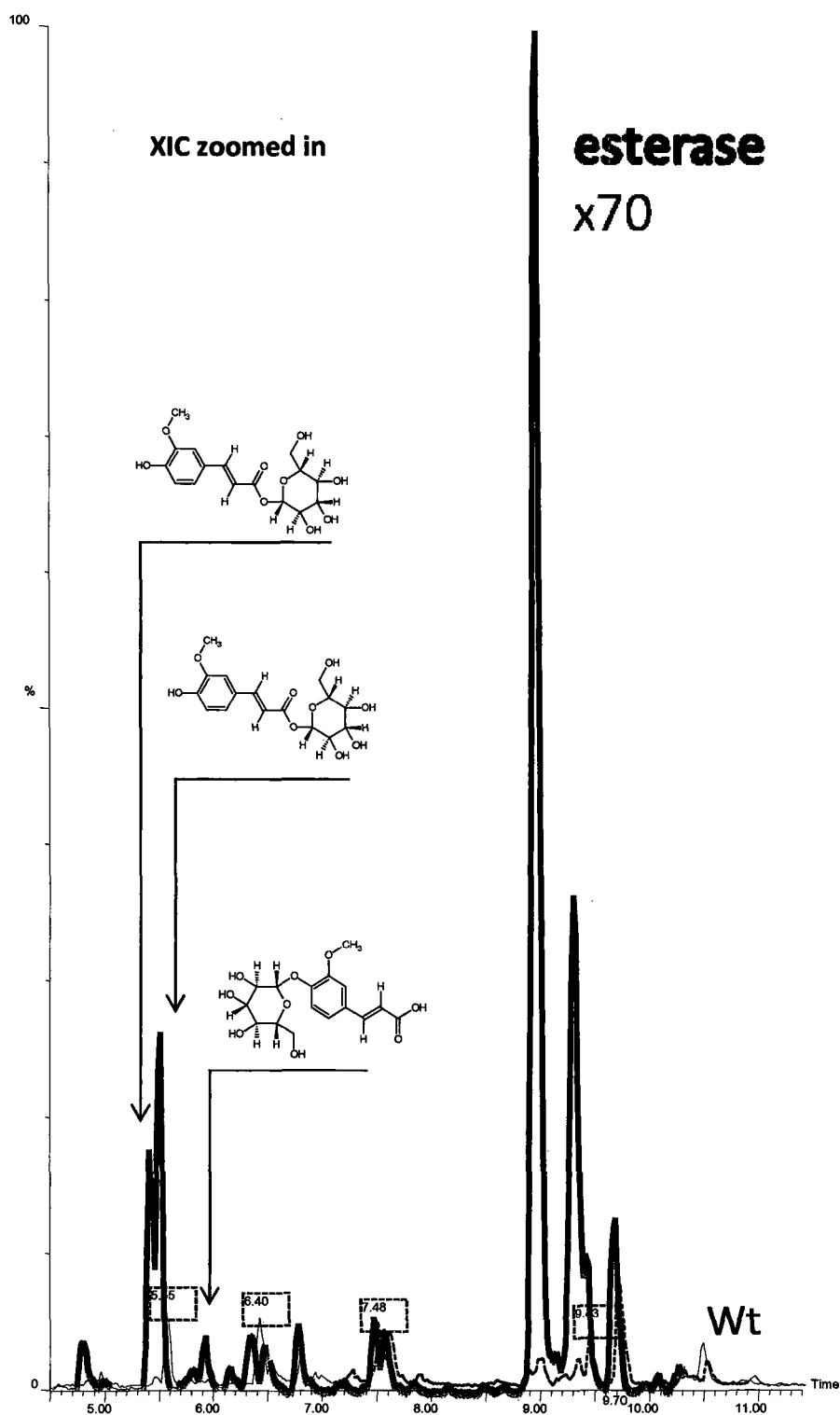
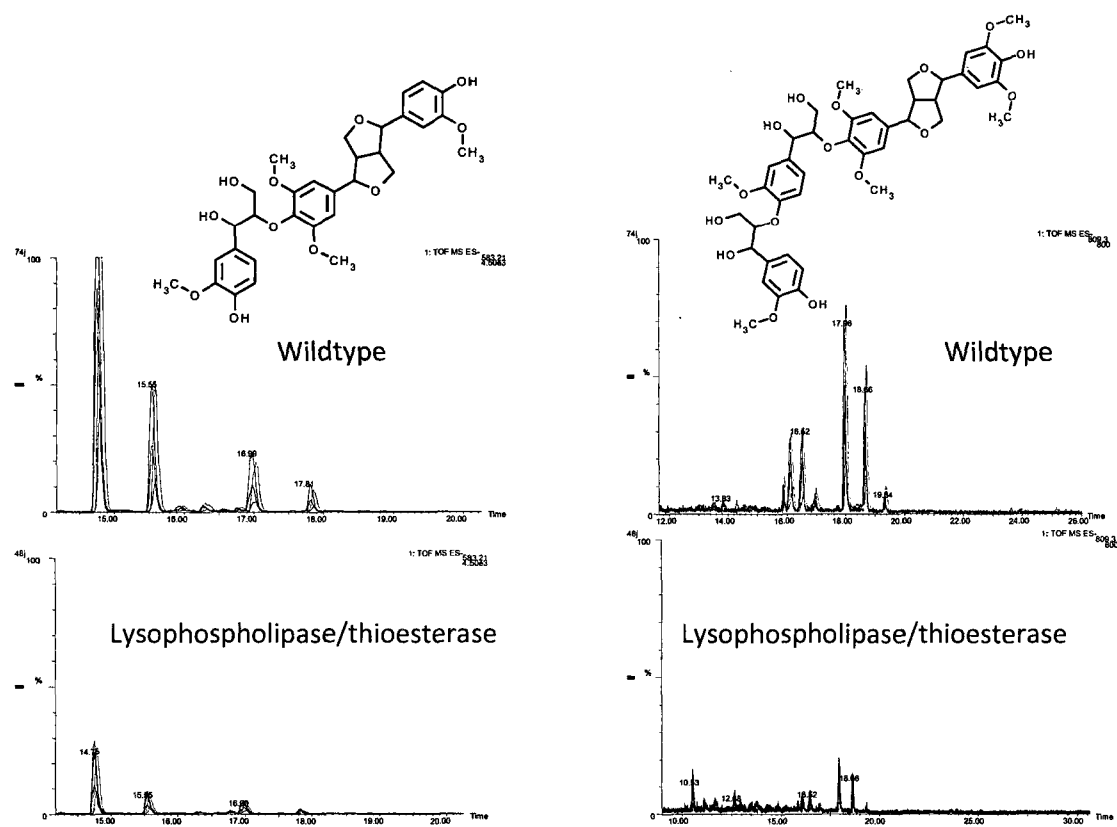


Figure 7

5 **Figure 8**

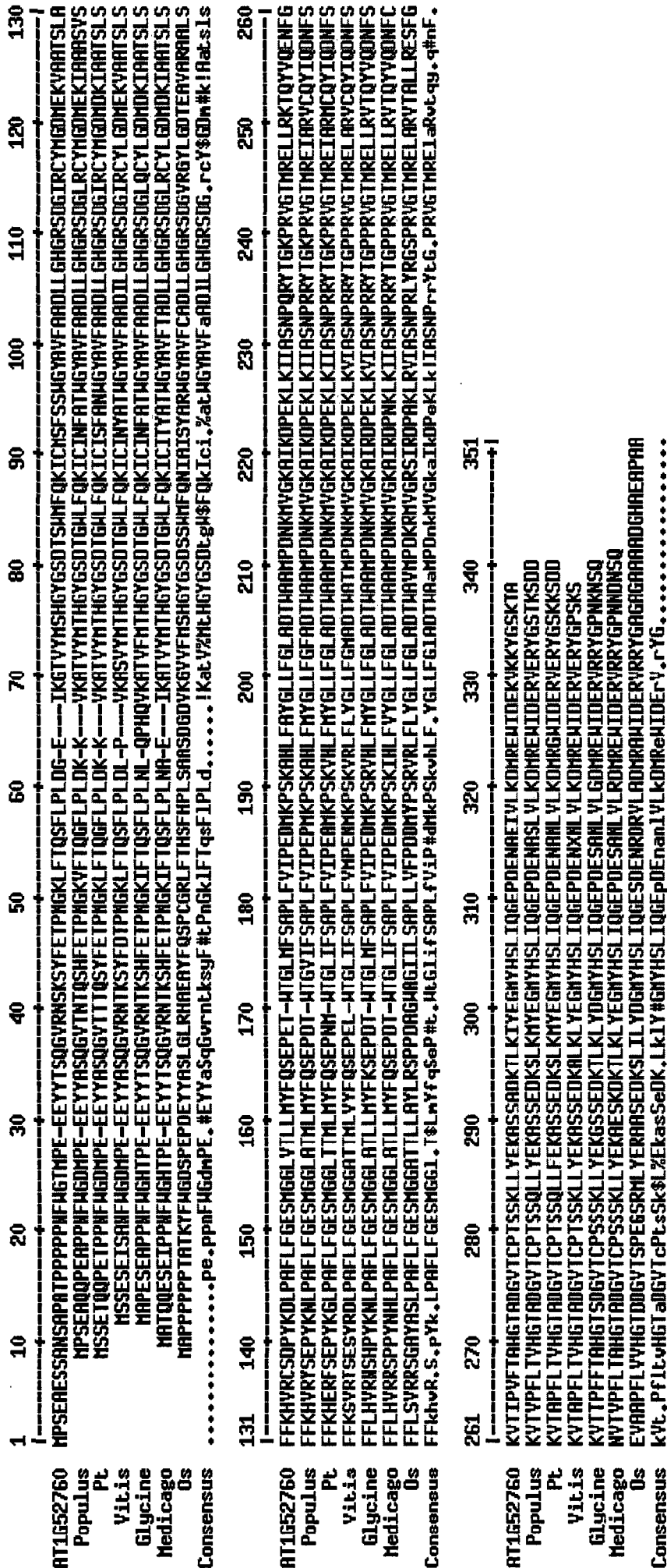


Figure 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/051206

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82
ADD.

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)
C12N

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

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

EPO-Internal, CHEM ABS Data, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUANAFINA MARCIA M DE O ET AL: "Targeting expression of a fungal ferulic acid esterase to the apoplast, endoplasmic reticulum or golgi can disrupt feruloylation of the growing cell wall and increase the biodegradability of tall fescue (Festuca arundinacea)", PLANT BIOTECHNOLOGY JOURNAL, BLACKWELL, OXFORD, GB, vol. 8, no. 3, 1 April 2010 (2010-04-01), pages 316-331, XP002689956, ISSN: 1467-7644 the whole document ----- -/-	1-3,5-19



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

5 August 2013

Date of mailing of the international search report

20/08/2013

Name and mailing address of the ISA/

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Authorized officer

Puonti-Kaerlas, J

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/051206

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	WO 2010/062240 A1 (SWETREE TECHNOLOGIES AB [SE]; HERTZBERG MAGNUS [SE]; SUNDBERG BJOERN []) 3 June 2010 (2010-06-03) the whole document -----	1-19
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International application No
PCT/GB2013/051206

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International application No

PCT/GB2013/051206

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