

SEQUENCE LISTING

<110> Bayer Bioscience N.V.
Bulone, Vincent
Guerriero, Gea

<120> Methods for manufacturing plant cell walls comprising chitin

<130> bcs10-2001

<150> EP10000712.9
<151> 2010-01-25

<150> US61/297,973
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<170> PatentIn version 3.5

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agcttgtaaa aggacgactt gggtggc 27

<210> 9

<211> 28
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer CHS1Rev1

 <400> 9
 ttccttggt cattgcagc gagggttc 28

<210> 10
 <211> 28
 <212> DNA
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 <220>
 <223> Primer CHS1Rev2

 <400> 10
 cagaacgtt ttgcaaacct tgcggagt 28

<210> 11
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 <220>
 <223> Primer CHSFwd1

 <400> 11
 tccggtcgac actccgcaag gtttgcaa 28

<210> 12
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 <212> DNA
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 <220>
 <223> Primer CHS1Fwd2

 <400> 12
 actacacggt cctcctcgat gttgggac 28

<210> 13
 <211> 24
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 <220>
 <223> Primer CHS2Fwd1

 <400> 13
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<210> 14
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer CHS2Fwd2

 <400> 14
 ttggctcta cggtgtgacg gact 24

<210> 15
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer CHS1FLFwd

 <400> 15
 caccatgccg cccaagcgac cgacgaccga 30

<210> 16
 <211> 28
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer CHS1FLRev

 <400> 16
 ctagcgcacg cggttgtagc gcgcttgg 28

<210> 17
 <211> 28
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> CHS2FLRev

 <400> 17
 ttagacttgt tggtaggcgc cgccgcgg 28

<210> 18
 <211> 2889
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> modified nucleotide sequence of Saprolegnia monoica chitin synthase 2 (codon optimized for expression in cotton)

<400> 18
 atgagtgatc aacttgacct cgcagcaagg ttacgagcat taagagaggg taatgcagct 60
 cctgctgatc ctgaagcacc tccacctaca caacctgctc cagcacccca ataccatcca 120
 cagagattac caccctcta tacacaagag agtctggagt ttggtgggac ttatgctact 180
 ggatctcctg tcggagctga agctgaagga tcctatagtc aagtcccggg gtggaaggat 240
 tctaaagaaa ctgctcgtc ttacttggat gatgagccaa ctccgcaacc acagtcactc 300
 attaacatgg caaacacctt ggtccagcgt caagcctcta atcaatcctt caggcgtcag 360
 catactgcta atttccgacc tctgccaac actgtcgaag agcttttgga cggaacacca 420
 acctacgaag gtgcttttcg attggttcaa cttgctgtgc agatggagca agatggcgac 480
 ccagggtgcag caattaacct ttacgtcgat gctggtacaa ctctcgttga agtcggtaaa 540
 cgtgaggtag accctctact tcagaagggt atccagcaga aggcctttga acttctgcaa 600
 cgtgctgaag agttggggac ttggatgaat actgttgccg aagaagcaag gaaagctgct 660

ttgccacctc aactcaagat tgctagaaca aacgtcccga ctgtcgaaca agcatggaaa	720
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tcgcttggtg tcgacgtgca gatgcacttg tttgagagca cactccaact ggttgagaac	1140
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 cgtaacctac ctcccgattg gcaaaccat tacaatgtcc agaaccaagc tgacggtcgg 2820
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 caagtctaa 2889

<210> 19
 <211> 69
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> MIT domain from Saprolegnia monoica chitin synthase 2 gene
 <400> 19

Thr Tyr Glu Gly Ala Phe Arg Leu Val Gln Leu Ala Val Gln Met Glu
 1 5 10 15

Gln Asp Gly Asp Pro Gly Ala Ala Ile Asn Leu Tyr Val Asp Ala Gly
 20 25 30

Thr Thr Leu Val Glu Val Gly Lys Arg Glu Val Asp Pro Leu Leu Gln
 35 40 45

Lys Gly Ile Gln Gln Lys Ala Phe Glu Leu Leu Gln Arg Ala Glu Glu
 50 55 60

Leu Gly Thr Trp Met
 65

<210> 20
 <211> 69
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> MIT domain from Saprolegnia monoica chitin synthase 1 gene
 <400> 20

Thr Ile Asp Asp Ala Phe Arg Ala Ile Glu Arg Ala Ile Gln Ala Glu
 1 5 10 15

Asn Glu Gly Arg Tyr Arg Glu Ala Leu Lys His Phe Leu Asp Gly Gly
 20 25 30

Glu Met Ile Val Thr Ala Ala Glu Lys Glu Ala Ser Gln Lys Val Arg
 35 40 45

Asn Leu Leu Leu His Lys Gly Lys Glu Val Leu Glu Trp Ala Glu His
 50 55 60

Leu Ala Glu Trp Ile
 65

<210> 21
 <211> 2676
 <212> DNA
 <213> *Saprolegnia parasitica*

<400> 21
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 gcgtccaacg tcccatccgc cgcctcgtcg tacgagtacg actacgagta caacatgatg 180
 ccgatgatgc aggcgcccgc caagtcgcag ccaaccttcc tctccaacat tgcgcccatac 240
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 ctcgagtggg ccgagcacct cgccgagtgg atcgagcgtc acaacacgtc gacgccgccg 540
 gtccgcacatg ccaagccgat ggccgtcgag gtcacgtacg accgcacgat gaactcgcca 600
 gacctcgacg aaaccgaggc gcgcatgatg ttttacacgc ccgtgtgctc gggcccgaag 660
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<210> 22
 <211> 891
 <212> PRT
 <213> *Saprolegnia parasitica*

<400> 22

Met Pro Pro Lys Arg Pro Thr Glu Ala Ser Gly Arg Arg Tyr Ala Pro
1 5 10 15

Pro Ala Gly Arg Pro Ser Asn Asn Ala Ala Asn Ala Lys Pro Arg Ala
20 25 30

Pro Arg Lys Gly Val Ser Ser Arg Ala Ser Asn Val Pro Ser Ala Ala
35 40 45

Ser Ser Tyr Glu Tyr Asp Tyr Glu Tyr Asn Met Met Pro Met Met Gln
50 55 60

Ala Pro Pro Lys Ser Gln Pro Thr Phe Leu Ser Asn Ile Ala Pro Ile
65 70 75 80

Ser Ala Lys Glu Ala Ser Met Lys Gly Ser Asn Ala Met Gln Leu Leu
85 90 95

Leu Gln Gly Thr Ser Phe Thr Ile Asp Asp Ala Phe Arg Ala Ile Glu
100 105 110

Arg Ala Ile Gln Ala Glu Asn Glu Gly Arg Phe Arg Glu Ala Leu Lys
115 120 125

His Phe Leu Asp Gly Gly Glu Met Ile Val Thr Ala Ala Glu Lys Glu
130 135 140

Ala Ser Gln Lys Val Arg Asn Leu Leu Leu His Lys Gly Lys Glu Val
 145 150 155 160
 Leu Glu Trp Ala Glu His Leu Ala Glu Trp Ile Glu Arg Tyr Asn Thr
 165 170 175
 Ser Thr Pro Pro Val Arg Ile Ala Lys Pro Met Ala Val Glu Val Thr
 180 185 190
 Tyr Asp Arg Thr Met Asn Ser Pro Asp Leu Asp Glu Thr Glu Ala Arg
 195 200 205
 Met Met Phe Tyr Thr Pro Val Cys Ser Gly Pro Lys Ala Phe Thr Glu
 210 215 220
 Thr Gly Tyr Arg Leu Gln Cys Ile Gln Ser Gly Arg Arg Pro Arg Leu
 225 230 235 240
 Met Val Val Ile Thr Met Tyr Asn Glu Asp Glu Asn Glu Leu Arg Ser
 245 250 255
 Thr Leu Arg Lys Val Cys Asn Asn Val Leu Tyr Leu Lys Gln His Ser
 260 265 270
 Leu Pro Gly Tyr Glu Gly Asp Asp Ala Trp Lys Gln Val Leu Val Val
 275 280 285
 Val Val Ser Asp Gly Arg Thr Lys Ala Asn Lys Gly Thr Leu Glu Trp
 290 295 300
 Leu Ala Asn Val Gly Leu Tyr Asp Glu Asp Val Met Asn Ile Thr Ser
 305 310 315 320
 Thr Gly Val Lys Val Gln Cys His Leu Phe Glu His Ser Leu Gln Met
 325 330 335
 Thr Lys Glu Asn Ser Ile Arg Phe Pro Pro Leu Gln Leu Asp Ser His
 340 345 350
 Leu Trp Tyr Phe Asp Ala Phe Ala Glu Gln Ile Met Pro Asp Tyr Thr
 355 360 365
 Val Leu Leu Asp Val Gly Thr Met Pro Thr Lys Ser Ser Phe Tyr Lys
 370 375 380
 Leu Leu Thr Ala Leu Glu Ile Asn Ala Gln Ile Gly Gly Val Cys Gly
 385 390 395 400
 Glu Ile Ala Val Asp Lys Pro Leu Pro Asn Met Cys Asn Trp Val Ile
 405 410 415

Ala Ala Gln His Phe Glu Tyr Lys Ile Ser Asn Ile Leu Asp Lys Ser
 420 425 430
 Leu Glu Ser Cys Phe Gly Phe Ile Ser Val Leu Pro Gly Ala Phe Ser
 435 440 445
 Ala Tyr Arg Tyr Lys Ala Ile Arg Gly Ala Pro Leu Gln Ala Tyr Phe
 450 455 460
 Lys Ser Leu Thr Thr Asp Met Ala Glu Leu Gly Pro Phe Ala Gly Asn
 465 470 475 480
 Met Tyr Leu Ala Glu Asp Arg Ile Leu Cys Phe Glu Leu Leu Ala Arg
 485 490 495
 Lys Asp Cys Asn Trp Thr Met His Tyr Val Lys Asp Ala Ile Ala Arg
 500 505 510
 Thr Asp Val Pro Thr Asn Leu Ile Asp Leu Val Gly Gln Arg Arg Arg
 515 520 525
 Trp Leu Asn Gly Ser Phe Phe Ala Thr Leu Phe Ala Ile Trp Asn Trp
 530 535 540
 Gly Arg Val Tyr Thr Glu Ser Asn His Ser Leu Thr Arg Lys Leu Ala
 545 550 555 560
 Leu Leu Val His Ala Leu Leu Gly Val Ser Ala Ala Asn Phe Tyr Leu
 565 570 575
 Ala Leu Tyr Phe Val Ile Phe Gln Gly Phe Arg Asp Asn Arg Trp Asn
 580 585 590
 Phe Ile Asp Thr Ser Glu Tyr Pro Gln Trp Val Leu Asp Gly Leu Pro
 595 600 605
 Thr Ala Phe Asn Val Phe Tyr Ala Val Thr Val Phe Thr Gln Val Thr
 610 615 620
 Ile Gly Leu Gly Asn Lys Pro Lys His Val Lys Gly Thr His Tyr Leu
 625 630 635 640
 Ile Ser Val Leu Phe Gly Leu Leu Met Leu Leu Ala Ser Gly Val Ala
 645 650 655
 Ile Val Ile Phe Ile Thr Ser Ser Lys Asp Ala Met Ala Ile Val Leu
 660 665 670
 Ala Val Leu Ile Leu Gly Thr Phe Phe Ile Gly Ser Ala Leu His Cys
 675 680 685

Glu Val His His Ile Val Leu Thr Phe Val Gln Tyr Thr Ala Leu Met
 690 695 700
 Pro Ser Phe Val Asn Ile Leu Met Val Tyr Ser Phe Cys Asn Leu His
 705 710 715 720
 Asp Leu Ser Trp Gly Thr Lys Gly Ile Asp Thr Gly His Glu Ala His
 725 730 735
 Lys Thr Glu Ala Val Gly Gln Tyr Lys Asp Ile Val Ala Arg Gln Lys
 740 745 750
 Ala Leu Glu Ala Lys Lys Ala Gln Asp Ala Arg Asn Gln Asp Glu Leu
 755 760 765
 Lys Lys Arg Phe Asp Ser Phe Arg Ser Asn Leu Leu Leu Val Trp Val
 770 775 780
 Met Ser Asn Met Ser Met Val Ile Ile Cys Val Asn Thr Val Gly Ala
 785 790 795 800
 Asp Ser Phe Leu Pro Phe Leu Tyr Ala Phe Val Ala Ala Phe Asn Gly
 805 810 815
 Ile Arg Leu Leu Gly Cys Ile Gly Tyr Leu Ile Tyr Tyr Ala Arg Gln
 820 825 830
 Phe Leu Leu Phe Asn Thr Leu Ser Ala Thr Gly Val Leu His Lys Arg
 835 840 845
 His Glu Ala Arg Lys His Lys Lys Ala Glu Asp Pro Asp Pro Ile Asp
 850 855 860
 Met Glu Leu Gly Thr Phe Asn Glu Pro Ala Thr Ser Glu Ile Gly Ala
 865 870 875 880
 Pro Met Met Gln Ala Pro Tyr Asn Arg Met Arg
 885 890

<210> 23
 <211> 2880
 <212> DNA
 <213> Saprolegnia parasitica

<400> 23
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 ttccgcgcgt tccggtcgac gttgctgctc tcgtggctca cgaccaacgg catttggtg 2580
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<210> 24
 <211> 959
 <212> PRT
 <213> *Saprolegnia parasitica*

<400> 24

Met Ser Asp Ser Asn Leu Asp Leu Ala Ala Arg Leu Arg Ala Leu Arg
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Glu Gly Gly Ala Glu Pro Ala Pro Ala Pro Ala Pro Thr Pro Tyr Met
20 25 30

His Ser Pro Pro Ser Arg Thr Arg Pro Thr Pro Leu Tyr Thr Gln Glu
35 40 45

Ser Leu Glu Phe Gly Gly Thr Tyr Thr Thr Gly Ser Pro Val Gly Ala
50 55 60

Glu Ala Asp Gly Val Tyr Thr Gln Val Pro Val Trp Lys Asp Ser Lys
65 70 75 80

Glu Lys Thr Tyr Gly Tyr Leu Asp Asp Glu Pro Ala Pro Gln Ala Gln
85 90 95

Thr Leu Leu Asn Lys Ala Asn Asp Leu Val Gln Arg Gln Ala Ser Asn
100 105 110

Lys Ala Phe Arg Arg Gln His Thr Ala Ala Phe Arg Pro Leu Pro Asn
115 120 125

Thr Val Glu Glu Leu Leu Asp Gly Ser Pro Thr Tyr Glu Gly Ala Phe
130 135 140

Arg Leu Val Gln Leu Ala Val Gln Met Glu Gln Asp Gly Asp Pro Gln
145 150 155 160

Ala Ala Ile Asn Leu Tyr Ala Asp Ala Gly Ala Thr Leu Val Glu Val
165 170 175

Gly Arg Lys Glu Val Asp Pro Leu Leu Gln Lys Gly Ile Arg Gln Lys

180							185							190						
Ala	Gln	Glu	Leu	Leu	Gln	Arg	Ala	Glu	Asp	Leu	Glu	Ala	Trp	Met	Asn					
		195					200					205								
Gly	Val	Ala	Glu	Glu	Ala	Arg	Lys	Ala	Ala	Leu	Pro	Pro	Ser	Leu	Arg					
	210					215					220									
Ile	Ala	Arg	Thr	Asn	Val	Pro	Thr	Val	Glu	Gln	Thr	Trp	Ala	Gly	Arg					
225					230					235					240					
Pro	Pro	Pro	Phe	His	Asp	Ala	Asn	Glu	Phe	Lys	Leu	Met	Arg	Tyr	Thr					
				245					250					255						
Ala	Val	Ala	Thr	Lys	Asp	Pro	Ile	Gln	Phe	Ser	Asp	Asp	Gly	Tyr	Val					
			260					265					270							
Leu	Arg	Val	His	Glu	Leu	Gln	Arg	Pro	Ile	Lys	Val	Phe	Ile	Thr	Ile					
		275					280					285								
Thr	Met	Tyr	Asn	Glu	Glu	Gly	Ser	Glu	Ile	Lys	Gly	Thr	Leu	Thr	Gly					
	290					295					300									
Leu	Ala	Lys	Gly	Leu	Ala	Tyr	Met	Cys	Lys	Glu	Tyr	Gly	Asp	Asp	Phe					
305					310					315					320					
Trp	Gln	Gln	Val	Ala	Val	Ala	Ile	Val	Ser	Asp	Gly	Arg	Thr	Lys	Ala					
				325					330					335						
Ser	Lys	Thr	Cys	Leu	Glu	Tyr	Leu	Lys	Ala	Val	Gly	Ala	Phe	Asp	Glu					
			340				345						350							
Glu	Ile	Met	Thr	Val	Thr	Ser	Leu	Gly	Val	Asp	Val	Gln	Met	His	Leu					
		355					360					365								
Phe	Glu	Ser	Thr	Leu	Gln	Leu	Val	Glu	Asn	Gln	Asn	Phe	Glu	Ala	Tyr					
	370					375					380									
Tyr	Pro	Pro	Leu	Gln	Val	Ile	Tyr	Ala	Leu	Lys	Glu	Asn	Asn	Gly	Gly					
385					390					395					400					
Lys	Leu	Asn	Ser	His	Leu	Trp	Phe	Phe	Asn	Ala	Phe	Ser	Glu	Gln	Leu					
				405					410					415						
Asn	Pro	Lys	Tyr	Thr	Val	Leu	Val	Asp	Val	Gly	Thr	Ile	Pro	Ala	Glu					
			420					425					430							
Thr	Ser	Val	Phe	Arg	Leu	Ile	Arg	Ser	Met	Glu	Arg	Asn	Ala	Gln	Ile					
		435					440					445								
Gly	Gly	Val	Ala	Gly	Glu	Ile	Ala	Val	Glu	Ala	Pro	Asn	Phe	Phe	Asn					
	450					455					460									

Pro Val Ile Ala Ala Gln His Phe Glu Tyr Lys Ile Ser Asn Ile Met
 465 470 475 480
 Asp Lys Ser Leu Glu Ser Val Phe Gly Phe Ile Ser Val Leu Pro Gly
 485 490 495
 Ala Phe Ser Ala Tyr Arg Tyr Glu Ala Ile Arg Ala Val Lys Gly Val
 500 505 510
 Gly Pro Leu Pro Glu Tyr Phe Lys Ser Leu Thr Ser Thr Thr Lys Glu
 515 520 525
 Leu Gly Pro Phe Gln Gly Asn Met Tyr Leu Ala Glu Asp Arg Ile Leu
 530 535 540
 Cys Phe Glu Leu Leu Ala Arg Lys Gln Arg Arg Trp Thr Met His Tyr
 545 550 555 560
 Val Lys Asp Ala Ile Ala Arg Thr Asp Val Pro Glu Thr Leu Val Asp
 565 570 575
 Leu Ile Lys Gln Arg Arg Arg Trp Leu Asn Gly Ser Phe Phe Ala Gly
 580 585 590
 Leu Phe Ala Ile Gly His Phe Gly Arg Val Trp Ser Gln Ser Ser His
 595 600 605
 Ser Phe Gly Arg Lys Leu Val Phe Thr Phe Gln Phe Val Tyr Leu Ala
 610 615 620
 Leu Gln Asn Leu Leu Ser Trp Phe Leu Leu Ser Asn Leu Phe Leu Thr
 625 630 635 640
 Phe Tyr Phe Val Leu Thr Leu Ala Phe Thr Glu Ser Ala Pro Ala Leu
 645 650 655
 Leu Gln Thr Met Leu Thr Val Tyr Leu Ala Ile Ile Gly Gly Leu Ile
 660 665 670
 Val Phe Ala Leu Gly Asn Lys Pro Glu Pro Arg Thr Ala Ser Phe Tyr
 675 680 685
 Leu Phe Ser Cys Leu Tyr Met Gly Ile Ile Met Leu Leu Val Thr Gly
 690 695 700
 Ile Ser Ile Tyr Gly Leu Ile Gly Lys Gly Thr Ser Ala Val Lys Asp
 705 710 715 720
 Pro Arg Thr Ile Thr Gly Ile Phe Ser Asn Cys Thr Val Ser Asp Ala
 725 730 735

Glu Leu Ala Gly Gly Val Ile Thr Ser Leu Gly Leu Ile Phe Leu Ser
 740 745 750
 Ala Phe Val His Gly Glu Phe Gly Ile Leu Leu Ser Phe Val Gln Tyr
 755 760 765
 Phe Phe Met Leu Pro Thr Phe Val Asn Val Leu Gly Ile Tyr Ala Tyr
 770 775 780
 Ser Asn Leu His Asp Leu Ser Trp Gly Thr Lys Gly Leu Glu Ser Gly
 785 790 795 800
 Gly Gly His Gly Pro Ala Lys Ala Gly Gly Gly Asn Val Lys Asp Val
 805 810 815
 Val Glu Gln Gln Lys Lys Ile Glu Ala Ala Arg Gln Ala Ala Ala Arg
 820 825 830
 Glu Lys Glu Asp Val Asp Asn Ser Phe Arg Ala Phe Arg Ser Thr Leu
 835 840 845
 Leu Leu Ser Trp Leu Thr Thr Asn Gly Ile Trp Leu Tyr Val Val Thr
 850 855 860
 Asp Tyr Met Ser Ser Gly Cys Tyr Leu Lys Gly Leu Ser Tyr Ile Val
 865 870 875 880
 Gly Phe Phe Asn Val Val Arg Phe Thr Gly Cys Val Val Phe Val Ile
 885 890 895
 Leu Arg Met Phe Arg Arg Phe Gly Cys Gly Ala Arg Ala Ser Arg Asp
 900 905 910
 Asn Tyr Gln Glu Ala Leu Pro Ala Glu Trp Gln Thr His Tyr Asn Val
 915 920 925
 Thr Asn Arg Thr Asp Gly Arg Val Ala Pro Pro Pro Lys His Ala Ala
 930 935 940
 Ser Met Asp Pro Thr Thr Pro His Gly Gly Val Tyr Gln Gln Val
 945 950 955